Molecular and Integrative Toxicology

Srikanth S. Nadadur
John W. Hollingsworth *Editors*

Air Pollution and Health Effects

Humana Press

Molecular and Integrative Toxicology

Series editor
Rodney R. Dietert, Department of Microbiology & Immunology,
Cornell University College of Veterinary Medicine, Ithaca, New York, USA

More information about this series at http://www.springer.com/series/8792

Srikanth S. Nadadur • John W. Hollingsworth
Editors

Air Pollution and Health Effects

Humana Press

Editors
Srikanth S. Nadadur
Division of Extramural Research and Training
National Institute of Environmental Health Sciences
Research Triangle Park, NC
USA

John W. Hollingsworth
Department of Internal Medicine
Division of Pulmonary, Allergy, Critical Care and Sleep Medicine
Ohio State University
Columbus, OH
USA

ISSN 2168-4219 ISSN 2168-4235 (electronic)
Molecular and Integrative Toxicology
ISBN 978-1-4471-6668-9 ISBN 978-1-4471-6669-6 (eBook)
DOI 10.1007/978-1-4471-6669-6

Library of Congress Control Number: 2015938579

Springer London Heidelberg New York Dordrecht

Printed on acid-free paper

Humana Press is a brand of Springer
Springer-Verlag London Ltd. is part of Springer Science+Business Media (www.springer.com)

Preface

The health effects of air pollution were recognized after the Industrial Revolution with a transition to manufacturing processes that utilize fuel burning and the emergence of large and densely populated cities. One of the first reports highlighting the health effects of air pollution described the experience in London in 1952 where increased coal burning for heat resulted in dense smog that engulfed the city (Logan 1953, 1956). During a brief period of time, approximately 12,000 excess deaths were associated with increased levels of smog. This incident represents one of the first reports linking level of ambient air pollution and excess mortality. This epidemiological observation contributed to the passing of the Clean Air Act in 1956 by the Parliament of the United Kingdom, which began the process of eliminating the burning of coal in homes and in factories in England. Numerous scientific reports have followed supporting the notion that adverse health effects are associated with exposure to ambient air pollution. In an effort to protect public health and welfare, the US Clean Air Act was passed in 1970, which mandated setting national ambient air quality standards for specific common and widespread pollutants based on the latest science. Similarly the World Health Organization currently provides recommendations with regard to ambient air quality standards in an effort to protect global health and well-being. The body of literature supporting the broad health effects of ambient air pollution is expansive. The overall goal of this book is to highlight the weight of scientific evidence supporting our current understanding of the impact of air pollution on human health.

Air pollution is emerging as a major contributing risk factor for the global burden of disease. With improved control of communicable diseases and population shifts, recent estimates from the Global Burden of Disease Study in 2010 place air pollution among the leading contributing risk factors for global disease burden. Ambient particulate matter air pollution, household air pollution from burning solid fuels, and tobacco smoke are among the top ten leading risk factors for disease. It was estimated that ambient particulate matter pollution accounted for 3.1 million deaths and household air pollution from solid fuels accounted for 3.5 million deaths in 2010 (Lim et al. 2012). A recent report from the World Health

Organization estimated an air pollution-related global mortality of about seven million people in 2012. This represents one in eight of total global deaths is related to air pollution, and these estimates identify air pollution as the single largest environmental health risk factor globally (*WHO press release March 25, 2014, http://www.who.int/mediacentre/news/releases/2014/air-pollution/en/*). This very high attributed mortality is, in part, based on the recognition that air pollution impacts cardiovascular disease. Recognition that exposure to particulate matter (PM) air pollution contributes to cardiovascular morbidity and mortality is supported by a scientific statement from the American Heart Association (Brook et al. 2004, 2010). While the health effects of air pollution were initially associated with pulmonary related morbidity and mortality, our current understanding of health impacts has considerably expanded to also include: cardiovascular, neuronal/cognitive, cancer, infectious disease, reproductive, and developmental effects. Together, growing evidence support that excess mortality related to air pollution is primarily associated with increased risks of ischemic heart disease, stroke, chronic obstructive pulmonary disease (COPD), lung cancer, and respiratory infections.

This book begins with an introduction to the chemistry and physics of ambient air pollutants. Topics focus on specific recognized health effects of air pollution including: reproductive function, pregnancy/preterm birth, asthma, COPD, potential mechanisms of action for known carcinogens in the ambient air, diabetes/metabolic syndrome, cardiovascular disease, central nervous system health, and immunological function. Authors highlight host genetic factors that are recognized to contribute to physiological responses to air pollution. Air pollution clearly presents a global health problem and there are dedicated chapters to discuss both global health and indoor air pollution in developing countries. The book concludes with a discussion of regulatory science. There is clearly an increased awareness of health effects of air pollution. Authors have made a sincere attempt to include as much discussion as possible on the contributions of air pollution morbidity in the context of regulatory guidelines and incremental increase in air quality. Detailed insight into the levels of evidence supporting the adverse consequence of exposure to air pollution in this book will provide insight for readers interested in furthering their knowledge and potential future research directions in translating basic to epidemiological and the clinical research continuum and will also serve as a resource book on the impact of the environment on human health.

An expanding body of literature supports a clear recognition of air pollution as a major risk factor for human disease. Environmental health science is challenged by the fact that many common exposures can impact multiple organ systems and either directly or indirectly contribute to numerous disease conditions. It is our goal that the reader will gain appreciation for the broad health consequences of air pollution covered by recognized experts in each chapter of this book. Improved understanding of the health consequences related to air pollution will both provide insight into disease pathogenesis and could provide new opportunities to beneficially impact human health.

Columbus, OH, USA — John W. Hollingsworth
Research Triangle Park, NC, USA — Srikanth S. Nadadur

References

Brook RD, Franklin B, Cascio W, Hong Y, Howard G et al (2004) Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655–2671

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A et al (2010) Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121:2331–2378

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224–2260

Logan WP (1953) Mortality in the London fog incident, 1952. Lancet 1:336–338

Logan WP (1956) Mortality from fog in London, January, 1956. Br Med J 1:722–725

Contents

Contributors

Jesus A. Araujo, M.D., Ph.D. Division of Cardiology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

John R. Balmes, M.D. Department of Medicine, Division of Occupational and Environmental Medicine, University of California, San Francisco, CA, USA

Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, Berkeley, CA, USA

Blanka Binkova, Ph.D. Department of Genetic Ecotoxicology, Institute of Experimental Medicine, AS CR, Prague 4, Czech Republic

Daniel L. Costa, Sc.D., DABT E205-09, Office of Research and Development, U.S. EPA, Research Triangle Park, NC, USA

Natália de Souza Xavier Costa, M.S. Laboratory of Experimental Air Pollution (LIM05), Department of Pathology, School of Medicine, University of São Paulo, São Paulo, São Paulo State, Brazil

Drew Day, B.A., B.S. Nicholas School of the Environment & Duke Global Health Institute, Duke University, Durham, NC, USA

Sharon Edwards, MStat School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI, USA

Alison Elder, Ph.D. Department of Environmental Medicine, University of Rochester, Rochester, NY, USA

Laís Fajersztajn, M.S. Laboratory of Experimental Air Pollution (LIM05), Department of Pathology, School of Medicine, University of São Paulo, São Paulo, São Paulo State, Brazil

Neal Fann, M.P.P. Office of Air Quality Planning and Standards, Office of Air and Radiation, C539-07, U.S. EPA, Research Triangle Park, NC, USA

Frank Gilliland, M.D., M.P.H., Ph.D. Division of Environmental Health, Department of Preventive Medicine, Keck School of Medicine, University of Southern California (USC), Los Angeles, CA, USA

Angela Haczku, M.D., Ph.D. Translational Lung Biology Center, Pulmonary, Critical Care and Sleep Medicine, University of California, Davis, CA, USA

John W. Hollingsworth, M.D. Department of Internal Medicine, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Ohio State University, Columbus, OH, USA

Philip K. Hopke, MA, Ph.D. Institute for a Sustainable Environment, Department of Chemical and Biomolecular Engineering, Clarkson University, Potsdam, NY, USA

Talat Islam, M.B.B.S., Ph.D. Division of Environmental Health, Department of Preventive Medicine, Keck School of Medicine, University of Southern California (USC), Los Angeles, CA, USA

Cuiqing Liu, Ph.D. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA

Department of Physiology, Medical College, Hangzhou Normal University, Hangzhou, Zhejiang, China

Pamela Maxson, Ph.D. School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI, USA

Marie Lynn Miranda, Ph.D. School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI, USA

Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA

Anuradha Mudipalli, Ph.D. National Center for Environmental Assessment, Office of Research and Development, US EPA, Research Triangle Park, NC, USA

Srikanth S. Nadadur, Ph.D. Division of Extramural Research and Training, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Günter Oberdörster, D.M.V., Ph.D. Department of Environmental Medicine, University of Rochester, Rochester, NY, USA

Elizabeth Oesterling Owens, Ph.D. National Center for Environmental Assessment, Office of Research and Development, U.S. EPA, Cincinnati, OH, USA

David B. Peden, M.D., M.S. Division of Allergy, Immunology & Rheumatology, Department of Pediatrics, Center for Environmental Medicine, Asthma and Lung Biology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Sanjay Rajagopalan, M.D. Division of Cardiovascular Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

Xiaoquan Rao, M.D., Ph.D. Division of Cardiovascular Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

Imre Redai, M.D., F.R.C.A. (deceased)

Michael E. Rosenfeld, Ph.D. Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA

Pavel Rossner Jr., Ph.D. Department of Genetic Ecotoxicology, Institute of Experimental Medicine, AS CR, Prague 4, Czech Republic

Andrea Rossnerova, Ph.D. Department of Genetic Ecotoxicology, Institute of Experimental Medicine, AS CR, Prague 4, Czech Republic

Jason D. Sacks, M.P.H. National Center for Environmental Assessment, Office of Research and Development, B243-01, U.S. EPA, Research Triangle Park, NC, USA

Paulo Hilário Nascimento Saldiva, M.D., Ph.D. Laboratory of Experimental Air Pollution (LIM05), Department of Pathology, School of Medicine, University of São Paulo, São Paulo, São Paulo State, Brazil

National Institute for Integrated Analysis of Environmental Risk, São Paulo, São Paulo State, Brazil

Nicole Sandberg, MURP School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI, USA

Joel Schwartz, Ph.D. Departments of Environmental Health and Epidemiology, Harvard School of Public Health, Boston, MA, USA

Radim J. Sram, M.D., D.Sc. Department of Genetic Ecotoxicology, Institute of Experimental Medicine, AS CR, Prague 4, Czech Republic

Robert M. Tighe, M.D. Department of Internal Medicine, Duke University Medical Center, Durham, NC, USA

Mariana Matera Veras, Ph.D. Laboratory of Experimental Air Pollution (LIM05), Department of Pathology, School of Medicine, University of São Paulo, São Paulo, São Paulo State, Brazil

National Institute for Integrated Analysis of Environmental Risk, São Paulo, São Paulo State, Brazil

Jennifer Wheeler, Ph.D. Department of Internal Medicine, Duke University Medical Center, Durham, NC, USA

Junfeng (Jim) Zhang, Ph.D. Nicholas School of the Environment & Duke Global Health Institute, Duke University, Durham, NC, USA

About the Editors

John W. Hollingsworth is a physician-scientist committed to the study of the impact of the environment on health. He completed his training at the University of Texas Medical Branch and the Duke University Medical Center. He remained on faculty at Duke University before relocating to Ohio State University. The overall goal of Dr. Hollingsworth's research program is to better understand the complex interaction between exposure to common environmental factors and host vulnerability. Laboratory work has included using animal models of human airway disease to better understand the complex relationship between the environment and health.

Srikanth S. Nadadur is a molecular toxicologist with over 25 years of research experience in environmental health. He graduated from Sri Venkateswara University, India, and obtained postdoctoral training in chemical carcinogenesis at Roswell Park Cancer Institute, Buffalo, NY. Dr. Nadadur's research on criteria air pollutants at US EPA was focused on integrating toxicogenomics efforts towards developing biomarkers of exposure. He is currently Program Director at the National Institute of Environmental Health Sciences, overseeing an extramural research program in air pollution cardiopulmonary health and nanotechnology environmental health and safety.

Chapter 1
Reactive Ambient Particles

Philip K. Hopke

1.1 Introduction

Oxidants are produced in the human body and are fundamental to life because they are required for many biological functions, e.g. immune system control and vascular smooth muscle function (Suzuki et al. 1997; Azzi et al. 2004). In the body, they also defend against environmental challenges such as unknown organisms (Kuo et al. 1998). However, they become damaging if too much oxidant exists by overproduction, lower depletion, inhalation, etc. (Gomes et al. 2005). They could cause the damage DNA and proteins (Bartold et al. 1984), lipid peroxidation, and enzymes oxidation (Varani et al. 1970; Chapple 1997). There are protective mechanisms such as antioxidants in lung fluids to protect the surface tissue from exposure to oxidants such as ozone in the inhaled air. Thus, it is important to have a balance between oxidants and antioxidants such that tissues that are damaged as a result of oxidant-based defenses are repaired. An imbalance leads to oxidative stress. Oxidative stress and the associated inflammation is hypothesized to play a major role in the manifestation of adverse health effects arising from the inhalation of airborne particles.

There has been considerable work characterizing the composition of airborne particles across a wide geographical area (Hopke and Rosser 2006; USEPA 2009). However, there are much more limited results on the relationship of airborne particles to the delivery of oxidants to the respiratory tract (Gurgueira et al. 2002; Hopke 2008). Ambient aerosol particles can represent a source of both exogenous and endogenous oxidants. These oxidants are generally reactive oxygen species (ROS). ROS can be constituents of the particles (exogenous) or formed by in situ

P.K. Hopke, MA, Ph.D. (✉)
Institute for a Sustainable Environment, Department of Chemical and Biomolecular Engineering, Clarkson University, Box 5708, Potsdam, NY 13699, USA
e-mail: phopke@clarkson.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_1

reactions with the particle after it is deposited in the lungs (endogenous). This chapter focuses on the formation chemistry of the oxidative species and the extent of the ROS associated with or formed by the presence of particles deposited in the lung.

1.2 Reactive Oxygen Species

In general, Reactive Oxygen Species (ROS) are chemical constituents with oxygen atoms that are highly reactive in the ambient air, can cause respiratory problems and produce adverse health effects in human beings. ROS has been defined to include oxygen-centered or related free radicals such as hydroxyl (·OH), hydroperoxyl (HOO·), alkoxyl (RO·) and organic peroxy (ROO·) radicals; ions such as superoxide $\left(O_2^-\right)$, hypochlorite (ClO^-), peroxynitrite ($ONOO^-$); and molecules such as hydroperoxides (ROOH) and organic peroxides (ROOR′).

The hydroxyl radical (OH) and hydroperoxyl radical (HO_2) collectively called HO_x are the most important and efficient oxidants in the atmosphere. HO_x radicals are known by-products of terpene ozonolysis (Kamens et al. 1999; Weschler and Shields 1996). Other important free oxygen radical species formed from terpene/ozone reactions are peroxy (ROO·) and alkoxy (RO·) radicals. ROO radicals are formed from reaction of alkyl (R·) radicals and O_2 and RO radicals from reaction of two ROO radicals (Kroll and Seinfeld 2008).

A major pathway of H_2O_2 formation is the self-reaction of HO_2 radicals (Finlayson-Pitts and Pitts 2000). H_2O_2 and hydroperoxides were reported by Li et al. (2002) to be formed by the ozonolysis of limonene with estimated yields ranging from 1.5 to 3.2 %.

Docherty et al. (2005) demonstrated that organic peroxides were the predominant compounds contributing to secondary organic aerosol (SOA) formed from the reactions of monoterpenes with ozone. Organic peroxides are formed from the reactions of peroxy radicals and self-reactions of large ROO· radicals.

1.3 Measurement Methods

1.3.1 Concentrations

The concentration of oxidative species is generally measured using fluorogenic probes. Typically a non-fluorescent species is mixed with the extract obtained from a particulate matter sample and the probe is oxidized to a fluorescent form. The resulting fluorescence intensity can then be compared to a calibration curve developed from known concentrations of a specific oxidant such as H_2O_2. Hasson and Paulson (2003) used the peroxidase enzyme catalyzed reaction of hydroperoxides with p-hydroxyphenylacetic acid (POHPAA) to produce a dimer that fluoresces

strongly under alkaline conditions at excitation and emission wavelengths of 320 and 400 nm, respectively, to quantify particle bound ROS in urban air. Another fluorescence technique is based on the oxidation of deacetylated (via NaOH) 2′-7′-dichlorodihydrofluorescein diacetate (DCFH) in a phosphate buffer containing the radical electron acceptor, horseradish peroxidase to its fluorescent product, 2′-7′-dichlorodihydrofluorescein (DCF) with excitation and emission wavelengths of 485 and 530 nm, respectively, (Hung and Wang 2001; Venkatachari et al. 2005, 2007). Cho et al. (2005) have developed an assay for PM redox activity utilizing the reduction of oxygen by dithiothreitol (DTT) that serves as an electron source. Recently Sameenoi et al. (2013) have produced a microfluidic method to implement the DTT analysis that may permit it to be more widely utilized in future studies.

Fairfull-Smith and Bottle (2008) reported a kind of novel, polyaromatic, profluorescent, isoindoline nitroxide synthesized probe which they described as a good choice for ROS detection. However, this kind of probe does not dissolve well in water, making it difficult to use to measure water soluble ROS. Venkatachari and Hopke (2008) compared the responses of DCFH-DA, POPHAA (p-hydroxyphenylacetic acid) and DTT (dithiothreitol) to surrogate ROS compounds in ambient air.

Based on DCFH-DA, an automated ROS monitor was developed (Venkatachari and Hopke 2008) and a modified version was deployed in Rochester, NY to provide a short record of semicontinuous ROS concentrations (Wang et al. 2011). However, the system proved hard to operate and not practical for even research monitoring. Further development of this technology was performed by King and Weber (2013) The instrument was deployed in urban Atlanta, Georgia, USA, and at a rural site during various seasons (Verma et al. 2012). Concentrations from the online instrument generally agreed well with those from an intensive filter measurement of ROS. The measured ROS concentrations made with this instrument were lower than reported in other studies, often below the instrument's average detection limit (0.15 nmol H_2O_2 equivalents m^{-3}). Mean ROS concentrations were 0.26 nmol H_2O_2 equivalents m^{-3} at the Atlanta urban sites and 0.14 nmol H_2O_2 equivalents m^{-3} at a rural site. Thus, it may be possible to obtain more detailed particle-bound ROS concentrations, but with a considerable effort to maintain its operation.

A non-fluorescent approach has been described by Mudway et al. (2004) in which they assess the oxidation of synthetic human respiratory tract lining fluid (depletion of ascorbate and reduced glutathione). This approach has been used in several subsequent studies (Ayres et al. 2008; Godri et al. 2010; Strak et al. 2012). Although these reports term this method as determining "oxidative potential," the method is really examining the presence and possible abiotic formation of ROS. The DTT assay can also provide similar information depending on how it is implemented. However, there is also the potential for cellular processes to produce oxidants and that is described in the following section.

There are very little data available on the concentrations of ROS in ambient PM. Hung and Wang (2001) determined the concentrations of particle-bound reactive oxidative species in various size fractions for Taipei aerosols and for particles in vehicular exhausts. Their results are summarized in Table 1.1. Hasson and

Table 1.1 Equivalent hydrogen peroxide concentrations in aerosol particles collected in Taiwan (Hung and Wang 2001)

	Equivalent H_2O_2 concentration (nM/m^3)			
Size interval	Sample size	Range	Mean	SD
3.2–10 (coarse) (μm)	32	0.006–0.138	0.064	0.033
1–3.2 (fine)	31	0.016–0.146	0.058	0.040
0.18–1 (very fine)	32	0.043–0.991	0.322	0.249
<0:18 (ultrafine)	32	0.026–0.592	0.163	0.155

Paulson (2003) used POPHAA to measure ambient gas- and aerosol-phase hydroperoxide levels in Los Angeles, CA. The values they measured were in the range 0.5–3:5 ppbv, and 0–13 ng m^{-3}, respectively. On average, about 40 % of aerosol-phase H_2O_2 was associated with fine particles.

Venkatachari et al. (2005) measured summer concentrations of ROS with DCFH in Rubidoux, CA while Venkatachari et al. (2007) measured winter values in New York City. In Rubidoux, which is on the eastern side of the L.A Basin, in close proximity to highway traffic, and having high sunlight intensity, leading to high PM concentrations and ozone production rates, it was found that the average nighttime ROS concentrations were comparable to average daytime concentrations. The trend in the average ROS concentrations showed the highest average total ROS concentrations, occurring during the daytime sampling intervals, at $6.11 \pm 1.39 \times 10^{-7}$ M/m^3 during the early afternoon sampling interval between 12–3 PM, at $5.96 \pm 1.16 \times 10^{-7}$ M/m^3 during the late afternoon sampling interval between 4–7 PM, and at $5.70 \pm 0.96 \times 10^{-7}$ M/m^3 during the early morning sampling interval of 8–11 AM. The average total nighttime ROS concentrations were found to be $5.19 \pm 0.83 \times 10^{-7}$ M/m^3. The intensity of photochemistry was found to be a moderate factor affecting the formation of ROS. Smaller particles were observed to have higher ROS concentrations, especially particles in the 10–56 nm range. The general magnitude of ROS concentrations was found to be at least an order of magnitude higher than measured by studies in Taipei (Hung and Wang 2001).

The ROS concentrations were relatively low, with values of the order of 10^{-7} M/m^3. However, they were found to be dominantly in the very fine and ultrafine particles, and therefore are capable of being deposited efficiently in the lower lung airways. A similar trend in diurnal variation was found in Flushing, NY although the magnitudes of the average total ROS concentrations were almost an order of magnitude less than the corresponding values in Rubidoux, as might be anticipated for winter conditions with lower sunlight intensity, lower ambient temperatures, and consequent lower ozone production rates.

The diurnal trends in Flushing, NY were, however, more pronounced. For the ambient aerosol in Flushing, the average total ROS concentrations expressed in terms of H_2O_2 concentrations were 0.919, 1.07, 0.941 and 0.845×10^{-7} M/m^3 during the sampling intervals between 8–11 AM, 12–3 PM, 4–7 PM and 9 PM–12 AM respec-

tively. Again, as in Rubidoux, the average nighttime concentrations were found to be comparable to the average daytime concentrations. The nighttime levels of particle-bound ROS suggest the presence of some long-lived ROS species, mostly organic peroxides (Docherty et al. 2005) and thus potential for transport (Friedlander and Yeh 1998). A positive correlation between the O_3 and the ROS concentrations was observed which indicated that the formation of ROS is promoted by enhanced photochemical activity. However, as in Rubidoux, intensity of photochemistry was found to be a moderate factor affecting the formation of particulate ROS in the daytime atmosphere. The absence of a more positive correlation in both these locations may be explained by the vertical mixing in the lower few kilometers, slow dry deposition to the surface, and local daytime photochemistry that destroys O_3 and produces HO_x.

1.3.2 Oxidative Potential

The methods described in the prior section detect the existence of ROS associated with the particles or the ability of the particles to abiotically induce the formation of ROS. The oxidative potential of PM extracts provides a measure of the production of ROS that could occur within a living system. It can be measured by in vitro exposure to rat alveolar macrophage (NR8383) cells using dichlorofluorescin diacetate (DCFH-DA) as the fluorescent probe (Landreman et al. 2008). DCFH-DA, a membrane permeable compound, is de-acetylated by cellular esterases generating dichlorodihydrofluorescein (DCFH). The oxidation of DCFH by ROS within the cell yields dichlorofluorescein (DCH), which is highly fluorescent and is monitored using a plate reader method.

Wang et al. (2012) investigated the sources of H_2O_2 generation in fine-mode aerosols by making a series of measurements on laboratory-generated particles, source materials, and ambient particles collected in the Los Angeles area. Ambient fine-mode H_2O_2 levels were associated with transition metals, and increased upon the addition of dithiothreitol. DTT is particularly a marker for quinone redox activity and may not fully represent the full range of ROS constituents as seen by Venkatachari and Hopke (2008). H_2O_2 levels were sensitive to the pH of the particle extraction solutions, peaking in the pH range of 2.5–5.5. They found that the initial rate of H_2O_2 generation by fine-mode ambient aerosols averaged 5.9 $(\pm 2.8) \times 10^{-9}$ $Mmin^{-1}$, similar to the initial rates of H_2O_2 generation by active quinones and hydroxyl radical generation by transition metals. The H_2O_2 concentrations persisted at nearly constant values for about a week in ambient fine-mode particles. Their laboratory experiments showed that SOA, diesel and biodiesel exhaust particles generated as much or more H_2O_2 than ambient particles (Table 1.2). Metals such as iron, zinc, and copper clearly played a role in the H_2O_2 formation in combination with sources that likely include quinones and other unidentified organics.

Table 1.2 Summary statistics of H_2O_2 generation by different types of source samples (Wang et al. 2012)

Type of sample	H_2O_2 per mass (ng/μg)		Percent of aerosol mass extracted mean (%)	H_2O_2 per extracted mass (ng/μg) mean
	Mean ± SD	N		
Ambient Fine, UCLA, 2005–2006[a]	0.42 ± 0.30	33	–	–
Ambient Fine, Downtown LA, 2005–2006[a]	0.58 ± 0.30	23	–	–
Ambient Fine, Riverside, UCR[b]	0.95 ± 0.69	35	–	–
Ambient Fine, Riverside, CRCAES[b]	0.49 ± 0.55	31	91	0.54
Ambient Fine, UCLA, 2009–2010	0.11 ± 0.07	33	52	0.18
Diesel Idle	0.19 ± 0.26	2	24	0.77
Diesel Load	0.06 ± 0.08	2	22	0.25
Biodiesel Idle	0.48 ± 0.17	8	70	0.69
Biodiesel Load	0.33 ± 0.16	9	23	1.40
α-pinene SOA	0.93 ± 0.36	19	99	0.94
B = pinene SOA	2.12 ± 1.48	11	101	2.09

[a]Arellanes et al. (2006)

[b]Samples were sporadically contaminated with Cu, Zn and Pb from Virtual Impactors and are thus likely elevated compared to the true ambient values

1.4 Formation Mechanisms

1.4.1 Atmospheric Gas-Phase Reactions

1.4.1.1 Biogenic Volatile Organic Compounds (BVOC)

Volatile organic compounds (VOCs) play a significant role in the generation of SOA especially those with biogenic origins as compared with anthropogenic VOCs (Andersson-Skold and Simpson 2001). Biogenic VOCs are emitted mainly by vegetation. Guenther et al. (2000) reported that vegetation contribute about 98 % of the total annual non-methane volatile organic compounds (NMVOC) emissions in North America. They also estimated total NMVOC flux of about 84×10^{12} g of carbon (Tg C) that is comprised mainly from isoprene (35 %), 19 other terpenoid compounds (25 %) and 17 non-terpenoid compounds (40 %). Although isoprene is the dominant biogenic VOC (BVOC) isoprene was generally not considered as a major producer of the SOA until Claeys et al. (2004a) found that natural particles collected in the Amazonian rain forest showed considerable quantities of previously unobserved polar organic compounds, which were identified as a mixture of two diastereoisomeric 2-methyltetrols: 2-methylthreitol and 2-methylerythritol. Laboratory and ambient results (Claeys et al. 2004b; Surratt et al. 2006; Xia and Hopke 2006) recognized isoprene as important component involved in SOA formation and since then isoprene was included in model predictions of SOA formation. Terpenoid compounds, especially monoterpenes ($C_{10}H_{16}$) are the most important precursors of SOA with α-pinene, β-pinene, sabinene, and limonene accounting for

40–80 % of the overall terpene emission on a global scale when isoprene is excluded (Seinfeld and Pankow 2003; Owen et al. 2001; Geron et al. 2000).

That monoterpene species contribution to particle formation has been demonstrated in many laboratory chamber experiments and ambient studies (Gao et al. 2004a, b, 2006; Odum et al. 1996; Docherty et al. 2005; Tolocka et al. 2004; Presto and Donahue 2006; Venkatachari and Hopke 2008; Chen and Hopke 2009a, b, 2010; Chen et al. 2011). Some of those experiments measured SOA yields and gas-phase kinetic rates and some analyzed SOA chemical composition. However, such chamber experiments often predict more or less SOA than is observed under the atmospheric conditions.

1.4.1.2 Isoprene Oxidation

Gaseous Reactions

Surratt et al. (2010) reviews the pathways for the formation of SOA in the gas-phase oxidation of isoprene. There are two pathways depending on the concentration of NOx. Figure 1.1 shows the low NOx pathways for SOA formation that would be

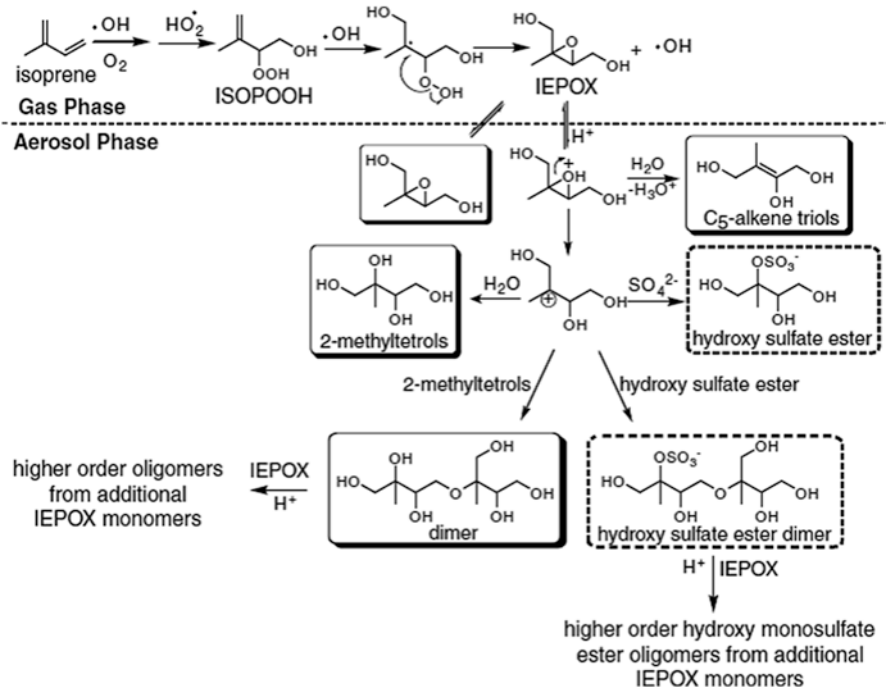

Fig. 1.1 Mechanism for SOA formation from isoprene under low-NOx conditions with the potential for enhancement due to aerosol acidity (Reprinted with permission from Surratt et al. (2010))

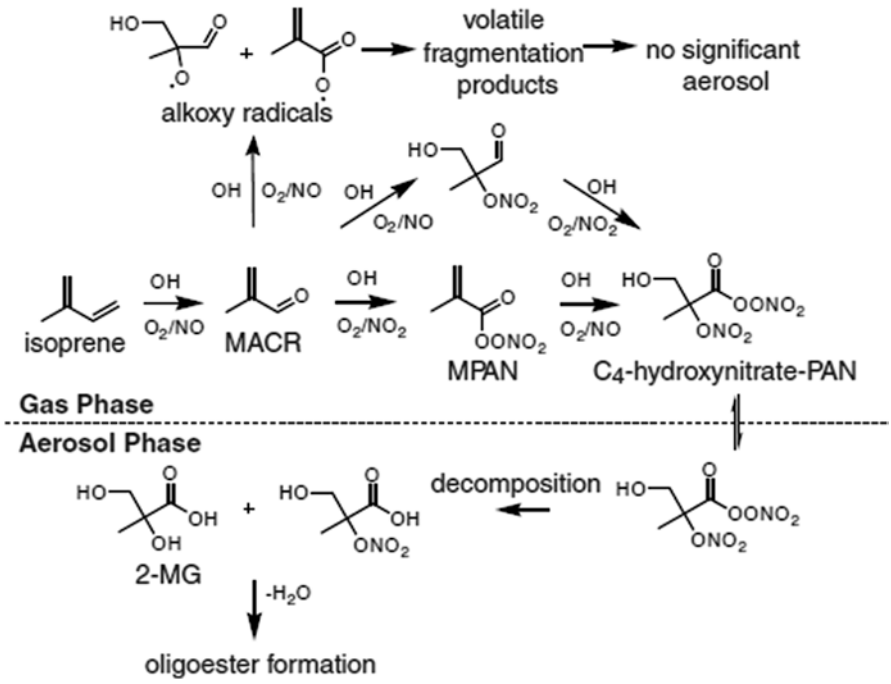

Fig. 1.2 Mechanism for SOA formation from isoprene under high-NOx conditions (Reprinted with permission from Surratt et al. (2010))

typically observed in rural locations such as the Amazon rain forests. The reactions lead to the formation of oligomeric species including sulfate containing compounds analogous to those first observed by Reemtsma et al. (2006). First-generation gas-phase oxidation products include hydroxycarbonyls, methyl-butenediols, hydroxy-hydroperoxides (ISOPOOH), methacrolein (MACR), and methyl vinyl ketone (MVK). Further reactions lead to the formation of epoxydiols of isoprene (IEPOX) and these in turn, lead to the identified SOA products of isoprene oxidation including the 2-methyltetrols that are used a marker species.

In the presences of high NO as would occur in urban locations, an alternative set of reactions occur as outlined in Fig. 1.2. In these reactions, the oxidation proceeds primarily through the methacrolein (MACR) that in the presence of NO becomes the major reaction product (25 %) of the isoprene oxidation. The further oxidation of MACR forms methacryloylperoxynitrate (MPAN) than can then react further to form SOA including nitrate containing oligomeric esters (Reemtsma et al. 2006).

Aqueous Reactions

It can be seen in these formation mechanisms that many of the initial products of isoprene oxidation are gas-phase species that may not partition to particles. However, they will be highly water soluble so that they can effectively partition into liquid

water droplets (cloud and fog) where additional reactions can occur (Ortiz-Montalvo et al. 2012). Aqueous phase hydroxyl (OH) radicals reacting with glycolaldehyde, glyoxal, methylglyoxal, pyruvate, acetate, acetone, methacrolein, and methyl vinyl ketone can form dicarboxylic acids and higher-molecular weight compounds (HMWCs) (e.g., oligomers) (Altieri et al. 2006, 2008; Carlton et al. 2006, 2007; El Haddad et al. 2009; Liu et al. 2009; Michaud et al. 2009; Perri et al. 2009; Tan et al. 2009, 2010, 2011; Poulain et al. 2010; Zhang et al. 2010). Although aqueous phase reactions will produce significant amounts of SOA, the processes will not result in the production of ROS species as is the case with the gas-phase reactions and thus, are not a source of exogenous ROS.

1.4.1.3 Monoterpene Oxidation

Monoterpene compounds are unsaturated and have one or two double bonds. Therefore, they can be rapidly oxidized in the atmosphere with ozone as well as oxidants such as OH and NO_3 radicals (Fig. 1.3). The relative importance of these reaction pathways for individual VOCs depends on the concentration of atmospheric oxidants and the reaction rate coefficients. The concentrations of OH, NO_3, and O_3 vary in time and space because of variations in pollutant concentrations and solar radiation (Calvert et al. 2000). The daylight and night-time average mixing ratios for an urban polluted environment are shown in Table 1.3 (from Calvert et al. 2000). The reaction rate coefficients may vary with location because of temperature and pressure differences.

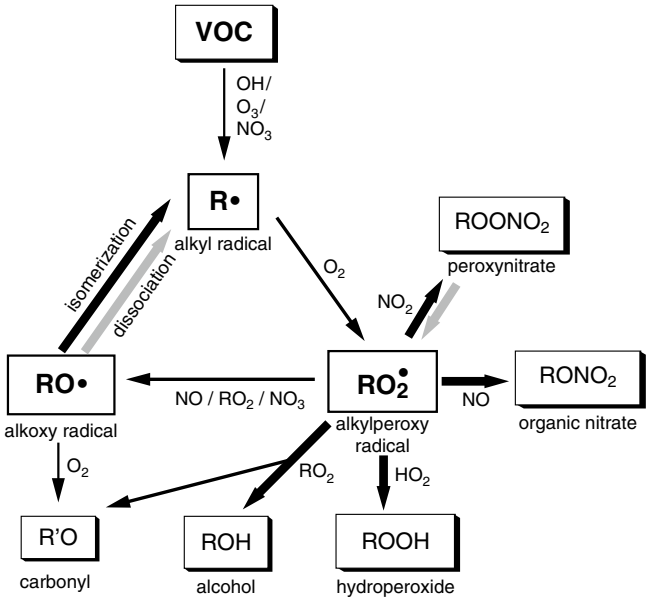

Fig. 1.3 Atmospheric oxidation mechanism for many VOCs, including monoterpenes (Reprinted from Kroll and Seinfeld (2008), with permission from Elsevier)

Table 1.3 Daylight and night-time average OH, NO_3, O_3 and $O(^3P)$ mixing ratios for conditions representative for a polluted urban atmosphere (From Calvert et al. 2000)

Species	Daylight average	Night-time average
OH	0.16 pptv	0.0007 pptv
O_3	110 ppbv	80 ppbv
NO_3	3 pptv	100 pptv
$O(^3P)$	0.003 pptv	0

Hydroxyl radical (OH·) is the most reactive and primary oxidant in the troposphere. The major mechanism for its formation in the upper troposphere is the reaction of singlet oxygen $O(^1D)$ with water molecule:

$$O\left(^1D\right) + H_2O \rightarrow 2OH\cdot \tag{1.1}$$

The HO· radicals present in the lower troposphere (surface HO radical) come from the photolysis of hydrogen peroxide or nitrous acid, Criegee intermediates (CI) decomposition, etc. Those intermediates are formed by the reaction of ozone (O_3) and unsaturated compounds –alkenes (Calvert et al. 2000):

$$R_1CH = CHR_2 + O_3 \rightarrow HO\cdot + \text{products} \tag{1.2}$$

Photolytic formation of hydroxyl radical require day-time conditions and sun light, while reaction (2) can occur at both, night- and day-time periods. OH reacts with most trace species in the atmosphere.

The key species in the ozone production are peroxyl (RO_2) and hydroperoxyl (HO_2) radicals. Those species can convert NO to NO_2 (reactions 3–4). Ozone formation occurs as a result of the photolysis of NO_2 at wavelengths < 420 nm (reactions 5–6). In the absence of RO_2 and HO_2 radicals, formed ozone (reaction 5) reacts with NO to regenerate NO_2 (reaction 7) and the net ozone production is zero. Reaction (7) is the main mechanism responsible for removal of ozone from the troposphere.

$$RO_2\cdot + NO \rightarrow RO\cdot + NO_2 \tag{1.3}$$

$$HO_2\cdot + NO \rightarrow HO\cdot + NO_2 \tag{1.4}$$

$$NO_2 + h\nu \rightarrow NO + O\left(^3P\right)\left(\lambda \le 420\,\text{nm}\right) \tag{1.5}$$

$$O\left(^3P\right) + O_2 + M \rightarrow O_3 + M\ \left(M = N_2, O_2\right) \tag{1.6}$$

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{1.7}$$

At night, NO_2 does not photolize (reaction 5), and in turn, it reacts with O_3 to produce the nitrate (NO_3) radical (reaction 8). Any NO present at night reacts rapidly with ozone to form NO_2 (Seinfeld and Pandis 2006).

Table 1.4 Calculated lower tropospheric lifetimes (at 298 K) for selected VOCs (Atkinson and Arey 2003)

VOC	OH	NO_3	O_3
n-octane	1.4 days	240 days	>4500 year
Isoprene	1.4 h	48 min	1.3 days
α-pinene	2.7 h	5.4 min	4.7 h
Benzene	9.5 days	>4 years	>4.5 years
Toluene	2.1 days	1.8 years	>4.5 years

$$NO_2 + O_3 \rightarrow NO_3 + O_2 \tag{1.8}$$

The highest nitrate radical concentrations one can expect in polluted regions, with high NO_x and O_3 levels, and under those conditions all NO will be converted to NO_2.

Calculated lower tropospheric lifetimes for some selected VOCs in the presence of three major atmospheric oxidants are shown in Table 1.4 (Atkinson and Arey 2003). Lifetimes are calculated using the following: for OH radical reactions, a 12-h daytime average of 2×10^6 molecule/cm^3; for NO_3 radical reactions, a 12-h night-time average of 5×10^8 molecule/cm^3; and for O_3, a 24-h average of 7×10^{11} molecule/cm^3. Longer chain alkanes (e.g. n-octane) and anthropogenic aromatic hydrocarbons (benzene and toluene) have the lowest reactivity and the longest lifetime. The OH radical reaction is the most dominant transformation process for these species. Biogenic VOCs, α-pinene and isoprene have the shortest lifetimes with respect to all of the atmospheric oxidants. In case of α-pinene, day-time reaction with hydroxyl radicals is faster than reaction with ozone (2.7 h versus 4.7 h). During the night-time, reaction with nitrate radicals occurs even faster (5.4 min).

1.4.1.4 Mechanism of Terpene Atmospheric Oxidation

Although reactions with OH radicals are often major day-time degradation pathway (have higher rate coefficients), reactions with ozone form many low-volatile products that contribute significantly to SOA formation (Calvert et al. 2000; Kroll and Seinfeld 2008).

The main products of monoterpene oxidation reactions are carbonyl compounds that include aldehydes, oxy-aldehydes, carboxylic acids, dicarboxylic acids, oxy-carboxylic acids, hydroxy-carboxylic acids, hydroxyketones, diols, etc. (Glasius et al. 1999, 2000; Winterhalter et al. 2003). The reaction pathways that lead to the addition of polar functional groups (hydroxyl, hydroperoxyl, nitrate and acid), with little or no fragmentation of the carbon skeleton, are those that lead to the formation of SOA (Kroll and Seinfeld 2008). Polar and low vapor pressure oxidation products can either create new fine organic particulate matter (homogeneous nucleation) or condense onto existing PM (Hoffmann et al. 1997). For homogeneous nucleation, the partial pressure of at least one of the reaction product must achieve its saturation value (Seinfeld and Pandis 2006).

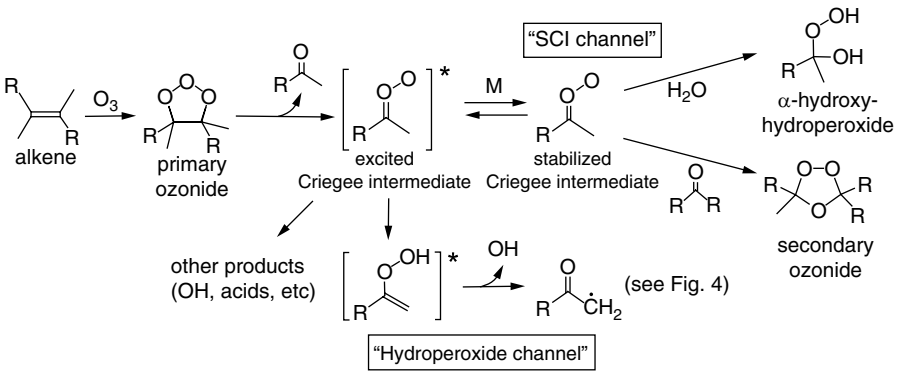

Fig. 1.4 Mechanism of alkene ozonolysis (Reprinted from Kroll and Seinfeld (2008), with permission from Elsevier)

In the case when the oxidation agent is a radical (OH, NO_3 or halogen atom), oxidation proceeds either by abstraction of a hydrogen atom or addition to a C-C double bond (Calvert et al. 2000; Atkinson and Arey 2003). The product of that reaction is the alkyl (R·) radical that then react with O_2 to form alkylperoxy (RO_2·) radical. Organic peroxyradicals react with NO, NO_2, HO_2, RO_2, NO_3 to form alkoxy (RO·) radicals and different carbonyl compounds (Fig. 1.3). Oxidation of monoterpenes with OH and NO_3 radicals result in less nucleation and this suggest that high volatile products are formed in those reactions (Hoffmann 2001).

In the case when the oxidation agent is O_3, oxidation proceeds by the addition of an O_3 molecule to the double C=C bond producing a primary ozonide. The ozonide decomposes into two fragments: a carbonyl and an excited biradical (excited Criegee Intermediate, CI*) (Hasson et al. 2001a, b). The intermediate can be stabilized in reaction with water or oxygenated organics ("SCI channel") or decomposes to form OH radical and alkyl radical ("Hydroperoxide channel HP"). The alkyl radical later reacts according to Fig. 1.3. The mechanisms of alkene oxidation by ozone are presented on Fig. 1.4 (Atkinson and Arey 2003; Jonsson et al. 2006; Kroll and Seinfeld 2008).

Reactions involved in α- and β-pinene/ozone oxidation are shown on Fig. 1.5 as adapted from Docherty et al. (2005). Pavlovic and Hopke (2011) showed that the radical species could be trapped with nitrone spin traps and their structures confirmed by tandem mass spectrometry.

Larsen et al. (1998) proposed a nomenclature for the oxidation products of monoterpene species. In the case of α-pinene, laboratory studies confirmed formation of all of the presented "1st generation" products in Fig. 1.5 (Glasius et al. 1999, 2000; Warscheid and Hoffmann 2001, 2002; Gao et al. 2004a, b; Docherty et al. 2005; Jang and Kamens 1999). The carboxylic acids are dominant products in the aerosol phase. Pinic acid is C_9 dicarboxylic acid with the highest yield found in SOA (Jang and Kamens 1999; Northcross and Jang 2007). During field studies, most of the polar α-pinene oxidation species are also found in the particulate phase (Warnke et al. 2006; Antilla et al. 2005; Yu et al. 1998; Gao et al. 2006).

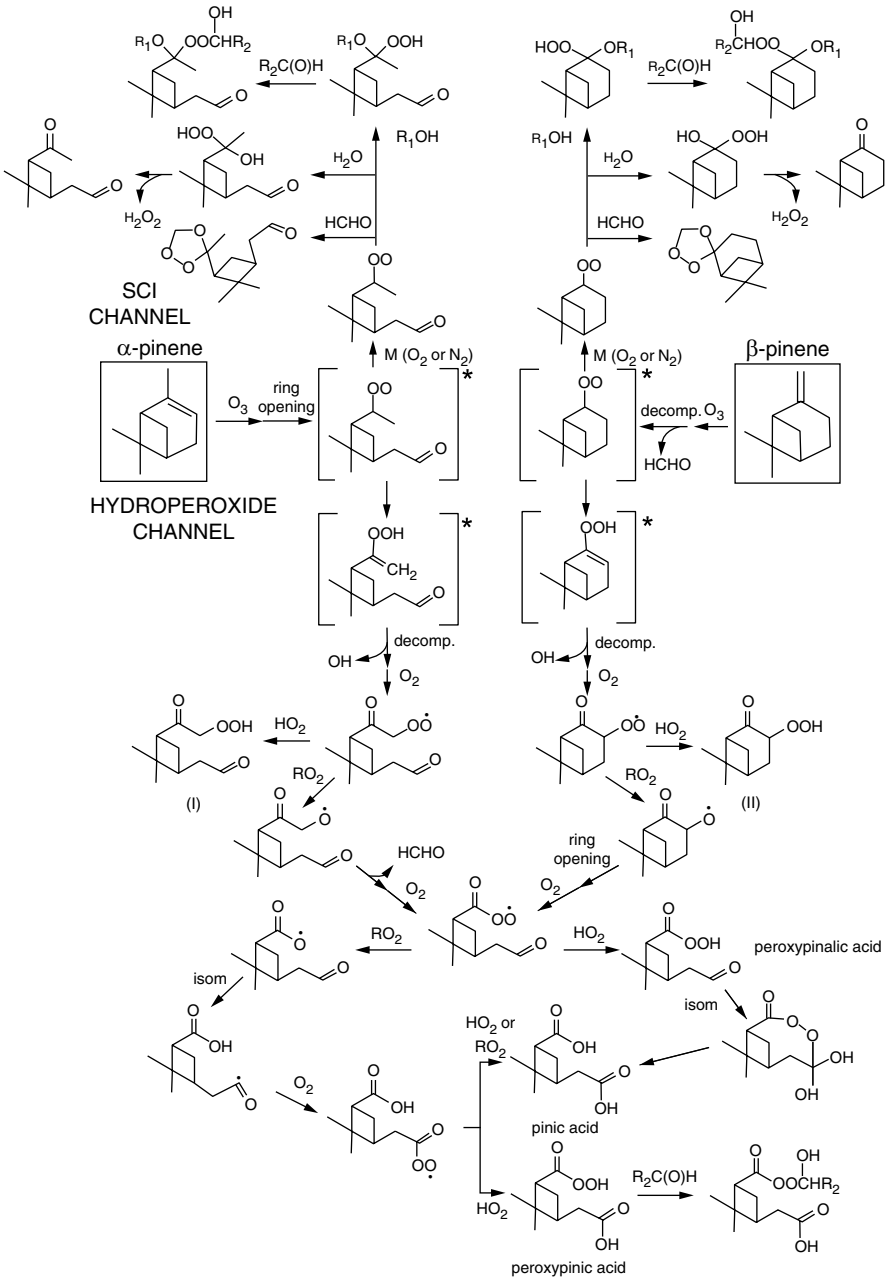

Fig. 1.5 Mechanism for α- and β-pinene/ozone oxidation (Adapted with permission from Docherty et al. (2005). American Chemical Society)

1.4.2 Mechanism of Aromatic Compound SOA Formation

The primary anthropogenic species that can form SOA and ROS are benzene and substituted aromatic compounds (e.g., toluene, xylenes, trimethyl benzene). These species are major components of gasoline so represent a major source of urban SOA. Aromatic compounds will not react with ozone and the oxidation occurs through reaction with hydroxyl radicals. Ng et al. (2006) provide information of the formation rates of SOA from these species. They found that the SOA yields (defined as the ratio of the mass of organic aerosol formed to the mass of parent hydrocarbon reacted) under low-NOx conditions are much larger than those under high-NOx conditions, suggesting the importance of peroxy radical chemistry in SOA formation. The mechanism for SOA formation from the oxidation of toluene by OH is outlined in Fig. 1.6.

1.4.3 Reactive Oxygen Species (ROS) in SOA

According to Fig. 1.4, hydroxyl radicals from the alkene (monoterpene)-ozone reactions are formed via the "hydroperoxide channel". Atkinson et al. (1995) and Atkinson and Aschmann (1993) reported OH radical yields close to a unit (1 molecule of OH· per 1 molecule of alkene reacted) for many different alkenes. Aschmann et al. (2002) measured OH· yields from O_3/terpene reactions for a series of terpenes and had results from 0.33 for sabinene to 0.86 for 3-carene. The same study measured OH radical formation yield for α-pinene to be 0.77 ± 0.10.

The hydroxyl radical does not react with the major constituents of the atmosphere (N_2, O_2, CO_2, H_2O) but reacts with most trace species such as CO, CH_4, VOCs including alkanes, alkenes, aromatics, aldehydes, ketones, alcohols etc. and these reaction pathways are presented in Fig. 1.1 (Seinfeld and Pandis 2006).

Once formed, OH· generates hydroperoxyl (HO_2·) radicals from the reaction of OH radicals and alkanes. The mechanism for HO_2· formation from ethane in the absence of NO_x is presented in reactions 9–12:

$$CH_3CH_3 + OH\cdot \rightarrow H_2O + CH_3CH_2\cdot \tag{1.9}$$

$$CH_3CH_2\cdot + O_2 \rightarrow CH_3CH_2OO\cdot \tag{1.10}$$

$$CH_3CH_2OO\cdot + ROO\cdot \rightarrow CH_3CH_2O\cdot + RO\cdot + O_2 \tag{1.11}$$

$$CH_3CH_2O\cdot + O_2 \rightarrow HO_2\cdot + CH_3C(O)H \tag{1.12}$$

It is always possible that the HO_2 radicals can be converted to OH radicals or form H_2O_2 molecule – sink for HO_x family (reactions 13–14)

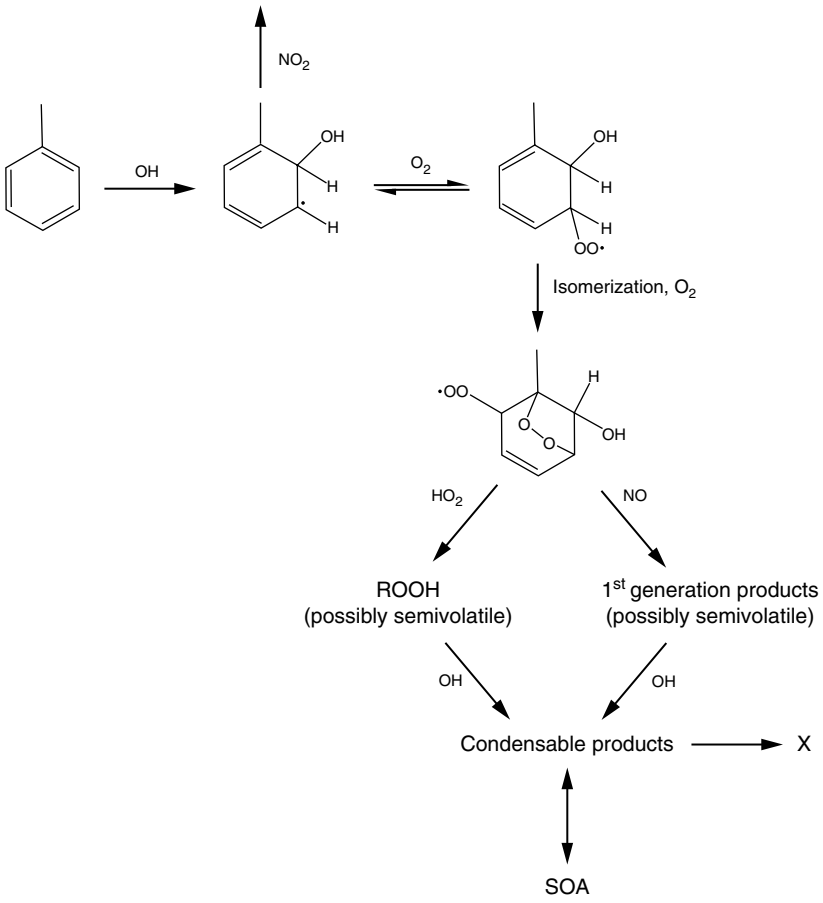

Fig. 1.6 Simplified SOA formation mechanism for the toluene-OH system (Image reprinted from *Atmos. Chem. Phys.*, 7, 3909–3922, 2007, www.atmos-chem-phys.net/7/3909/2007/ © Author(s) 2007. This work is licensed under a Creative Commons License)

$$HO_2 \cdot + O_3 \rightarrow OH + 2O_2 \tag{1.13}$$

$$HO_2 \cdot + HO_2 \cdot \rightarrow H_2O_2 + O_2 \tag{1.14}$$

Other important free oxygen radical species formed from monoterpene/ozone reactions are peroxyl (ROO·) and alkoxyl (RO·) radicals. Figure 1.1 shows that distribution of products formed from oxidation of VOCs is mainly governed by the reactions of these two radicals. At high-NO_x conditions, the chemistry tends to be dominated by radical propagation, in which RO_2 is efficiently converted to RO via

reaction with NO. At lower NO_x levels, the reactions of RO_2 with HO_2 and other RO2 radicals become more important and lead to hydroperoxide and alcohol formation (Kroll and Seinfeld 2008).

ROS molecules like organic hydroperoxides (ROOH) can be formed from: (1) reactions of stabilized biradicals with H_2O (reaction 15); (2) unimolecular isomerization of biradicals (reaction 16); (3) reactions of organic peroxyl radicals (ROO·) and hydroperoxyl (HO_2·) radicals (reaction 17) (Seinfeld and Pandis 2006):

1. $RC \cdot HOO \cdot + H_2O \rightarrow RCOOH + H_2O$ (1.15)
2. $\left(CH_3\right)_2 C \cdot OO. \rightarrow CH_3C\left(OOH\right) = CH_2$ (1.16)
3. $ROO \cdot + HO_2 \cdot \rightarrow ROOH + O_2$ (1.17)

Self-reactions of large ROO· radicals may form organic peroxides (ROOR) with very low volatility and high importance in SOA formation (Ziemann 2002).

1.5 In Situ ROS Formation

It is possible to form ROS in situ once the particle has deposited in the lungs. The ROS formation can be the result of biological activity in epithelial cells or can be abiotic. Much attention had been paid to endogenous ROS in prior studies. Different routes have been suggested through which ROS form after particle deposition in the respiratory tract.

Squadrito et al. (2001) hypothesized that $PM_{2.5}$ produces ROS continuously when deposited in the lung. About 10^{16}–10^{17} unpaired spins/g of free radicals that were stable for months are found in $PM_{2.5}$. These radicals share the same stability and spectral characteristics with semiquinone radicals. These organic radicals are hypothesized to have redox cycling during which tissue-reducing equivalents are consumed. Therefore, oxygen gets reduced and ROS is produced.

A chemisorbed substituted catechol was used to show the quidnoid redox cycling in Fig. 1.7. During this process, molecular oxygen is reduced by the semiquinone intermediate to the superoxide radical that could undergo dismutation and reduction reactions and produce hydrogen peroxide. Fenton's reactions could happen and generate the cytotoxic hydroxyl radicals when transition metals such as Fe^{2+} and Cu^{2+} exist with hydrogen peroxide. (Squadrito et al. 2001)

The dithiothreitol (DTT) assay was used to determine if ultrafine particles (UFP) have a greater ability to generate ROS than coarse and fine particles on a microgram basis (Li et al. 2003). Earlier studies have shown that UFPs have an increased capability to adsorb organic molecules and penetrate cellular targets because they have small sizes, relatively large number concentrations, and high surface area (Frampton 2001; Hei 2002; Nemmar et al. 2002; Oberdörster 1996; Utell and Frampton 2000; Verma et al. 2011). By comparing the formation of ROS by different size particles in vitro, Li et al. (2003) found UFPs had the highest redox activity. As shown in Fig. 1.3, UFPs have 21.7- and 8.6-fold larger redox cycling capacity than coarse and fine particles, respectively. Concentrated air particles (CAPs) have been shown to

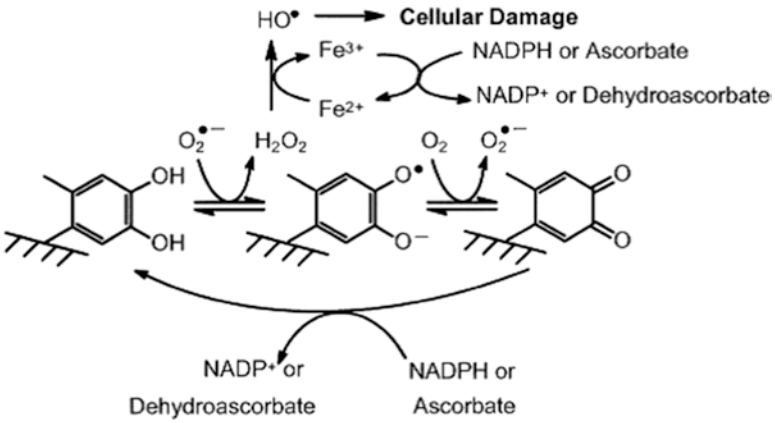

Fig. 1.7 Simplified mechanism using a chemisorbed substituted catechol as an example to explain quinoid redox cycling (Reprinted from Squadrito et al. (2001), with permission from Elsevier)

have the ability to transfer electrons. UFPs that have higher organic carbon fractions than other sized particles show greater biologic potency. This potency is related to polycyclic aromatic hydrocarbon (PAH) content (Li et al. 2003).

Metals have been suggested as playing a major role in inducing ROS formation and related oxidative stress (e.g., Kodavanti et al. 2002; Nadadur et al. 2009). Stohs et al. (1997) reports on in-situ ROS production by transition metals. It has been shown that metal ions, which have high concentration in tobacco smoke and other airborne particles, contribute to oxidative stress production (Faruque et al. 1995; Eiserich et al. 1995; Stohs and Bagchi 1995). The following equations express the generation process of ROS by redox cycling of metal ions:

$$\mathrm{M(X)} + O_2^{\cdot-} \rightarrow \mathrm{M(X-1)} + \mathrm{O_2}$$

$$2O_2^{\cdot-} + 2\mathrm{H^+} \rightarrow \mathrm{H_2O_2} + \mathrm{O_2}$$

$$\mathrm{M(X-1)} + \mathrm{H_2O} \rightarrow \mathrm{M(X)} + \mathrm{OH^{\cdot}} + \mathrm{OH^-}$$

Copper, iron, and chromium ions are found to undergo redox cycling. Cadmium, mercury, nickel, lead, arsenic and antimony are hypothesized to consume glutathione and protein-bound sulfhydryl groups that could lead to ROS generation, e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals (Stohs and Bagchi 1995; Aust 1989). Verma et al. (2010) found that several metals (Fe, Cr, Co and Mn) were significantly correlated with ROS as measured with the macrophage ROS assay. However, in the United States, there has been a substantial decline in the concentrations of these elements in fine particulate matter (Hopke and Rosser 2006; USEPA 2009) so their extent in oxidant formation is uncertain. Verma et al. (2009) have found that the DTT assay showed higher ROS in the afternoon in agreement with the earlier observations of Venkatachari et al. (2005) suggesting the role of

active particle formation mechanisms in forming ROS. Furthermore, Verma et al. (2011) found that DTT-measured ROS in quasi-ultrafine particles suggested a low role for metals and a much more important role for organic carbon and PAHs. However, these differences may be the result in differences in the sensitivity of the various assays to specific particulate constituents. Thus, there remains considerable uncertainty in the concentrations of both exogenous and endogenous oxidants associated with airborne particulate matter and further study will be required to fully assess the role of ROS in inducing observed adverse human health effects.

1.6 Conclusions

The formation of secondary particles results in chemically reactive species. The oxidation of SO_2 and NO_2 results in the formation of strong acids. However, the oxidation of organic compounds produces oxidative radicals and peroxide compounds. Oxidative stress is hypothesized to be a major factor in producing systemic inflammation and adverse cardiovascular effects. Thus, the presence of oxidative species in deposited particles coupled with the production of other oxidative species *in situ* by components of deposited particles may be an important driver of the observed impacts of ambient PM. Although there are limited measurements available showing the presence of ambient particle-bound ROS, ambient PM can both transport and catalyze the deposition of ROS to the lungs when those particles deposit in the respiratory tract. At the present time, the methods to make ROS measurements are crude and hard to use. Efforts to build automated systems have had limited success. There is thus a continuing need for better tools to assess both the ROS concentrations carried by the particles as well as their potential for inducing the formation of ROS once deposited in the respiratory system. Only then will it be possible to collect sufficient data to be able to assess the relationships between particle-bound ROS and particle-induced ROS with observed adverse health effects in an exposed population.

References

Altieri KE, Carlton AG, Lim HJ, Turpin BJ, Seitzinger SP (2006) Evidence for oligomer formation in clouds: reactions of isoprene oxidation products. Environ Sci Technol 40:4956–4960

Altieri KE, Seitzinger SP, Carlton AG, Turpin BJ, Klein GC, Marshall AG (2008) Oligomers formed through in-cloud methylglyoxal reactions: chemical composition, properties, and mechanisms investigated by ultra-high resolution FT-ICR mass spectrometry. Atmos Environ 42:1476–1490

Andersson-Skold Y, Simpson D (2001) Secondary organic aerosol formation in northern Europe: a model study. J Geophys Res 106:7357–7374

Antilla P, Rissanen T, Shimmo M, Kallio M, Hyotylainen T, Kulmala M, Riekkola ML (2005) Organic compounds in atmospheric aerosols from a Finnish coniferous forest. Boreal Environ Res 10:371–384

Arellanes C, Paulson SE, Fine PM, Sioutas C (2006) Exceeding of Henry's law by hydrogen peroxide associated with urban aerosols. Environ Sci Tech 40:4859–4866

Aschmann SM, Arey J, Atkinson R (2002) OH radical formation from the gas-phase reactions of O_3 with a series of terpenes. Atmos Environ 36:4347–4355

Atkinson R, Arey J (2003) Atmospheric degradation of volatile organic compounds. Chem Rev 103:4605–4638

Atkinson R, Aschmann SM (1993) Hydroxyl radical production from the gas-phase reactions of ozone with a series of alkenes under atmospheric conditions. Environ Sci Tech 27:1357–1363

Atkinson R, Tuazon EC, Aschmann SM (1995) Products of the gas-phase reactions of O_3 with alkenes. Environ Sci Tech 29:1860–1866

Aust SD (1989) Metal ions, oxygen radicals and tissue damage. Bibl Nutr Dicta 43:266–277

Ayres JG, Borm P, Cassee FR, Castranova V, Donaldson K, Ghio A et al (2008) Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential—a workshop report and consensus statement. Inhal Toxicol 20:75–99

Azzi A, Davies KJA, Kelly F (2004) Free radical biology: terminology and critical thinking. FEBS Lett 558:3–6

Bartold PM, Wiebkin OW, Thonard JC (1984) The effect of oxygen-derived free radicals on gingival proteoglycans and hyaluronic acid. J Periodontol 19:390–400

Calvert JG, Atkinson R, Keer JA, Madronich S, Moortgat GK, Wallington TJ, Yarwood G (2000) The mechanisms of atmospheric oxidation of the alkenes. Oxford University Press, New York

Carlton AG, Turpin BJ, Lim HJ, Altieri KE, Seitzinger S (2006) Link between isoprene and secondary organic aerosol (SOA): pyruvic acid oxidation yields low volatility organic acids in clouds. Geophys Res Lett 33, L06822

Carlton AG, Turpin BJ, Altieri KE, Seitzinger S, Reff A, Lim H-J et al (2007) Atmospheric oxalicacid and SOA production from glyoxal: results of aqueous photooxidation experiments. Atmos Environ 41:7588–7602

Chapple IL (1997) Reactive oxygen species and antioxidants in inflammatory diseases I. J Clin Periodontol 24:287–296

Chen X, Hopke PK (2009a) Secondary organic aerosol from α-pinene ozonolysis in dynamic chamber system. Indoor Air 19:335–345

Chen X, Hopke PK (2009b) A chamber study of secondary organic aerosol formation by linalool ozonolysis. Atmos Environ 43:3935–3940

Chen X, Hopke PK (2010) A chamber study of secondary organic aerosol formation by limonene ozonolysis. Indoor Air 20:320–328

Chen X, Hopke PK, Carter WPL (2011) Secondary organic aerosol from ozonolysis of biogenic volatile organic compounds: chamber studies of particle and reactive oxygen species formation. Environ Sci Technol 45:276–282

Cho AK, Sioutas C, Miguel AH, Kumagai Y, Schmitz DA, Singh M, Eiguren-Fernandez A, Froines JR (2005) Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. Environ Res 99:40–47

Claeys M, Graham B, Vas G, Wang W, Vermeylen R, Pashynska V, Cafmeyer J, Guyon P, Andreae MO, Artaxo P, Maenhaut W (2004a) Formation of secondary organic aerosols through photooxidation of isoprene. Science 303:1173–1176

Claeys M, Wang W, Ion AC, Kourtchev I, Gelencsér A, Maenhaut W (2004b) Formation of secondary organic aerosols from isoprene and its gas-phase oxidation products through reaction with hydrogen peroxide. Atmos Environ 38:4093–4098

Docherty KS, Wu W, Lim YB, Ziemann PJ (2005) Contributions of organic peroxides to secondary organic aerosol formed from reactions of monoterpenes with O_3. Environ Sci Tech 39:4049–4059

Eiserich JP, van der Vliet A, Handelman GJ, Halliwell B, Cross CE (1995) Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. Am J Clin Nutr 62(Suppl):1490–1500

El Haddad I, Yao L, Nieto-Gligorovski L, Michaud V, Temime-Roussel B, Quivet E et al (2009) In-cloud processes of methacrolein under simulated conditions – part 2: formation of secondary organic aerosol. Atmos Chem Phys 9:5107–5117

Fairfull-Smith KE, Bottle SE (2008) The synthesis and physical properties of novel polyaromatic profluorescent isoindoline nitroxide probes. Eur J Org Chem 32:5391–5400
Faruque MO, Khan MR, Rahman M, Ahmed F (1995) Relationship between smoking and antioxidant nutrient status. Br J Nutr 73:625–632
Finlayson-Pitts BJ, Pitts JN (2000) Chemistry of the upper and lower atmosphere: theory, experiments and applications. Academic, San Diego
Frampton MW (2001) Systemic and cardiovascular effects of airway injury and inflammation: ultrafine particle exposure in humans. Environ Health Perspect 109(suppl 4):529–532
Friedlander SK, Yeh EK (1998) The submicron atmospheric aerosol as a carrier of reactive chemical species: case of peroxides. Appl Occup Environ Hyg 13(6):416–420
Gao S, Keywood M, Ng NL, Surratt J, Varutbangkul V, Bahreini R, Flagan RC, Seinfeld JH (2004a) Low-molecular-weight and oligomeric components in secondary organic aerosol from the ozonolysis of cycloalkanes and α-pinene. J Phys Chem A 108:10147–10164
Gao S, Ng NL, Keywood M, Varutbangkul V, Bahreini R, Nenes A, He J, Yoo KY, Beauchamp JL, Hodyss RP, Flagan RC, Seinfeld JH (2004b) Particle phase acidity and oligomer formation in secondary organic aerosol. Environ Sci Tech 38:6582–6589
Gao S, Surratt JD, Knipping EM, Edgerton ES, Shahgholi M, Seinfeld JH (2006) Characterization of polar organic components in fine aerosols in the southeastern United States: identity, origin and evolution. J Geophys Res 111, D14314
Geron C, Rasmussen R, Arnts RR, Guenther A (2000) A review and synthesis of monoterpene speciation from forests in the United States. Atmos Environ 34:1761–1781
Glasius M, Duane M, Larsen BR (1999) Analysis of polar terpene oxidation products in aerosols by liquid chromatography ion trap mass spectrometry (MS^n). J Chromatogr 833:121–135
Glasius M, Lahaniati M, Calogirou A, Di Bella D, Jensen NR, Hjorth J, Kotzias D, Larsen BR (2000) Carboxylic acids in secondary aerosols from oxidation of cyclic monoterpenes by ozone. Environ Sci Tech 34:1001–1010
Godri KJ, Duggan ST, Fuller GW, Baker T, Green D, Kelly FJ et al (2010) Particulate matter oxidative potential from waste transfer station activity. Environ Health Perspect 118:493–498
Gomes A, Fernandes E, Lima JLFC (2005) Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Methods 65:45–80
Guenther A, Geron C, Pierce T, Lamb B, Harley P, Fall R (2000) Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. Atmos Environ 34:2205–2230
Gurgueira SA, Lawrence J, Coull B, Murthy GGK, Gonzales-Flecha B (2002) Rapid increase in the steady state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. Environ Health Prospect 110:749–755
Hasson AS, Paulson SE (2003) An investigation of the relationship between gas-phase and aerosol-bourne hydroperoxides in urban air. J Aerosol Sci 34:459–468
Hasson AS, Ho AW, Kuwata KT, Paulson SE (2001a) Production of stabilized criegee intermediate and peroxides in the gas phase ozonolysis of alkenes: 2. Asymmetric and biogenic alkenes. J Geophys Res D 106:34143–34152
Hasson AS, Orzechowska GE, Paulson SE (2001b) Production of stabilized criegee intermediate and peroxides in the gas-phase ozonolysis of alkenes: 1. Ethene, Trans-2-Butene and 2,3-Dimethyl-2-Butene. J Geophys Res D 106(24):34131–34142
HEI (2002) Understanding the health effects of components of the particulate matter Mix: progress and next steps, Health effects institute perspectives series. Health Effects Institute, Boston
Hoffmann T (2001) Final report of the 5th framework programme of the EU project OSOA (origin and formation of secondary organic aerosol), contract EVK2-CT-1999-00016. Available via the ftp: http://www.isas-dortmund.de/2002/e/staff/hoffmann/osoa/index.html
Hoffmann T, Odum JR, Bowman F, Collins D, Klockow D, Flagan RC, Seinfeld JH (1997) Formation of organic aerosols from the oxidation of biogenic hydrocarbons. J Atmos Chem 26:189–222
Hopke PK (2008) New directions: reactive particles as a source of human health effects. Atmos Environ 42:3192–3194

Hopke PK, Rosser A (2006) Exposure to airborne particulate matter in the ambient. Indoor and occupational environments. Clin Occup Environ Med 5:747–771

Hung HF, Wang CS (2001) Experimental determination of reactive oxygen species in Taipei aerosols. J Aerosol Sci 32:1201–1211

Jang M, Kamens RM (1999) Newly characterized products and compositions of secondary aerosols from reaction of α-pinene with ozone. Atmos Environ 33:459–474

Jonsson AM, Hallquist M, Ljungstrom E (2006) Impact of humidity on the ozone initiated oxidation of limonene, Δ^3-carene and α-pinene. Environ Sci Tech 40:188–194

Kamens R, Jang M, Chien C, Leach K (1999) Aerosol formation from the reaction of a-pinene and ozone using a gas phase kinetics aerosol partitioning model. Environ Sci Technol 33:1430–1438

King LE, Weber RJ (2013) Development and testing of an online method to measure ambient fine particulate reactive oxygen species (ROS) based on the 2', 7'-dichlorofluorescin (DCFH) assay. Atmos Meas Tech 6:1647–1658

Kodavanti UP, Schladweiler MC, Ledbetter AD, Hauser R, Christiani DC, McGee J, Richards JR, Costa DL (2002) Temporal association between pulmonary and systemic effects of particulate matter in healthy and cardiovascular compromised rats. J Toxicol Environ Health A 65:1545–1569

Kroll JH, Seinfeld JH (2008) Chemistry of secondary organic aerosol: formation and evolution of low-volatility organics in the atmosphere. Atmos Environ 42:3593–3624

Kuo M-L, Jee S-H, Chou M-H, Ueng T-H (1998) Involvement of oxidative stress in motorcycle exhaust particle-induced DNA damage and inhibition of intercellular communication. Mutat Res 413:143–150

Landreman AP, Shafer MM, Hemming JC, Hannigan MP, Schauer JJ (2008) A macrophage-based method for the assessment of the reactive oxygen species (ROS) activity of atmospheric particulate matter (PM) and application to routine (daily-24 h) aerosol monitoring studies. Aerosol Sci Tech 42:946–957

Larsen BR, Lahaniati M, Calogirou A, Kotzias D (1998) Atmospheric oxidation products of terpenes: a new nomenclature. Chemosphere 37:1207–1220

Li T-H, Turpin BJ, Shields HC, Weschler CJ (2002) Indoor hydrogen peroxide derived from ozone/d-limonene reactions. Environ Sci Technol 36:3295–3302

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley J, Froines J, Nel A (2003) Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111(4):455–460

Liu Y, El Haddad I, Scarfogliero M, Nieto-Gligorovski L, Temime-Roussel B, Quivet E et al (2009) In-cloud processes of methacrolein under simulated conditions – part 1: aqueous phase photooxidation. Atmos Chem Phys 9:5093–5105

Michaud V, El Haddad I, Liu Y, Sellegri K, Laj P, Villani P et al (2009) In-cloud processes of methacrolein under simulated conditions – part 3: hygroscopic and volatility properties of the formed secondary organic aerosol. Atmos Chem Phys 9:5119–5130

Mudway IS, Stenfors N, Duggan ST, Roxborough H, Zielinski H, Marklund SL, Blomberg A, Frew AJ, Sandstrom T, Kelly FJ (2004) An in vitro and in vivo investigation of the effects of diesel exhaust on human airway lining fluid antioxidants. Archives Biochem Biophys 423:200–212

Nadadur SS, Haykal-Coates N, Mudipalli A, Costa DL (2009) Endothelial effects of emission source particles: acute toxic response gene expression profiles. Toxicol In Vitro 23:67–77

Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF et al (2002) Passage of inhaled particles into the blood circulation in humans. Circulation 105:411–414

Ng NL, Kroll JH, Keywood MD, Bahreini R, Varubangkul V, Flagan RC, Seinfeld JH (2006) Contribution of first versus second generation products to secondary organic aerosols formed in the oxidation of biogenic hydrocarbons. Environ Sci Technol 40:2283–2297

Northcross AL, Jang M (2007) Heterogeneous SOA yield from ozonolysis of monoterpenes in the presence of inorganic acid. Atmos Environ 41:1483–1493

Oberdörster G (1996) Significance of particle parameters in the evaluation of exposure-dose–response relationships of inhaled particles. Inhal Toxicol 8 Suppl:73–89

Odum JR, Hoffmann T, Bowman F, Collins D, Flagan RC, Seifeld JH (1996) Gas/particle partitioning and secondary organic aerosol yields. Environ Sci Tech 30:2580–2585

Ortiz-Montalvo DL, Lim YB, Perri MJ, Seitzinger SP, Turpin BJ (2012) Volatility and yield of glycolaldehyde SOA formed through aqueous photochemistry and droplet evaporation. Aerosol Sci Tech 46:1002–1014

Owen SM, Boissard C, Hewitt CN (2001) Volatile organic compounds (VOCs) emitted from 40 Mediterranean plant species: VOC speciation and extrapolation to habitat scale. Atmos Environ 35:5393–5409

Pavlovic J, Hopke PK (2011) Detection of radical species formed by the ozonolysis of α-pinene. J Atmos Chem 66:137–155

Perri MJ, Seitzinger S, Turpin BJ (2009) Secondary organic aerosol production from aqueous photooxidation of glycolaldehyde: laboratory experiments. Atmos Environ 43:1487–1497

Poulain L, Katrib Y, Isikli E, Liu Y, Wortham H, Mirabel P et al (2010) In-cloud multiphase behaviour of acetone in the troposphere: gas uptake, Henry's law equilibrium and aqueous phase photooxidation. Chemosphere 81:312–320

Presto AA, Donahue NM (2006) Investigation of α-pinene + ozone secondary organic aerosol formation at low total aerosol mass. Environ Sci Tech 40:3536–3543

Reemtsma T, These A, Venkatachari P, Xia XY, Hopke PK, Springer A, Linscheid M (2006) Identification of fulvic acids and sulfated and nitrated analogues in atmospheric aerosol by electrospray ionization. Fourier transform ion cyclotron resonance mass spectrometry. Anal Chem 78(24):8299–8304

Sameenoi Y, Panymeesamer P, Supalakorn N, Koehler K, Chailapakul O, Henry CS, Volckens J (2013) Microfluidic paper-based analytical device for aerosol oxidative activity. Environ Sci Technol 47:932–940

Seinfeld JH, Pandis SN (2006) Atmospheric chemistry and physics: from air pollution to climate change. Wiley, Hoboken

Seinfeld JH, Pankow JF (2003) Organic atmospheric particulate material. Annu Rev Phys Chem 54:121–140

Squadrito GL, Cueto R, Dellinger B, Pryor WA (2001) Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. Free Radic Biol Med 31(9):1132–1138

Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radical Biol Med 18:321–336

Stohs SJ, Bagchi D, Bagchi M (1997) Toxicity of trace elements in tobacco smoke. Inhal Toxicol 9:867–890

Strak M, Janssen NAH, Godri KJ, Gosens I, Mudway IS, Flemming C, Cassee R, Lebret E, Kelly FJ, Harrison RM, Brunekreef B, Steenhof M, Hoek G (2012) Respiratory health effects of airborne particulate matter: the role of particle size, composition, and oxidative potential—the RAPTES project. Environ Health Perspect 120:1183–1189

Surratt JD, Murphy SM, Kroll JH, Ng NL, Hildebrandt L, Sorooshian A, Szmigielski R, Vermeylen R, Maenhaut W, Claeys M (2006) Chemical composition of secondary organic aerosol formed from the photooxidation of isoprene. J Phys Chem A 110:9665–9690

Surratt JD, Chan AWH, Eddingsaas HE, Chan M, Loza CL, Kwan AJ, Hersey SS, Flagan RC, Wennberg PO, Seinfeld JH (2010) Reactive intermediates revealed in secondary organic aerosol formation from isoprene. Proc Natl Acad Sci U S A 107:6640–6645

Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, Ueda O, Sakaguchi H, Higashi T, Suzuki T, Takashima Y, Kawabe Y, Cynshi O, Wada Y, Honda M, Kurihara H, Aburatani H, Doi T, Matsumoto A, Azuma S, Noda T, Toyoda Y, Itakura H, Yazaki Y, Horiuchi S, Takahashi K, Kruijt JK, van Berkel TJC, Steinbrecher UP, Ishibashi S, Maeda N, Gordon S, Kodama T (1997) A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature 386:292–296

Tan Y, Perri MJ, Seitzinger SP, Turpin BJ (2009) Effects of precursor concentration and acidic sulfate in aqueous glyoxal-OH radical oxidation and implications for secondary organic aerosol. Environ Sci Technol 43:8105–8112
Tan Y, Carlton AG, Seitzinger SP, Turpin BJ (2010) SOA from methylglyoxal in clouds and wet aerosols: measurement and prediction of key products. Atmos Environ 44:5218–5226
Tan Y, Lim YB, Altieri KE, Seitzinger SP, Turpin BJ (2011) Mechanisms leading to oligomers and SOA through aqueous photooxidation: insights from OH radical oxidation of acetic acid and methylglyoxal. Atmos Chem Phys 12:801–813
Tolocka MP, Jang M, Ginter JM, Cox FJ, Kamens RM, Johnston MV (2004) Formation of oligomers in secondary organic aerosol. Environ Sci Tech 38:1428–1434
U.S. EPA (2009) Integrated science assessment for particulate matter. National Center for Environmental Assessment, Office of Research and Development, Research Triangle Park
Utell MJ, Frampton MW (2000) Acute health effects of ambient air pollution: the ultrafine particle hypothesis. J Aerosol Med 13:355–359
Varani J, Phan SH, Gibbs DF, Ryan US, Ward PA (1970) H_2O_2-mediated cytotoxicity of rat pulmonary endothelial-cells – changes in adenosine-triphosphate and purine products and effects of protective interventions. Lab Invest 63:683–689
Venkatachari P, Hopke PK (2008) Development and laboratory testing of an automated monitor for the measurement of atmospheric particle-bound reactive oxygen species (ROS). Aerosol Sci Tech 42:629–635
Venkatachari P, Hopke PK, Grover BD, Eatough DJ (2005) Measurement of particle-bound reactive oxygen species in Rubidoux aerosols. J Atmos Chem 50:49–58
Venkatachari P, Hopke PK, Brune WH, Ren X, Lesher R, Mao J, Mitchell M (2007) Characterization of wintertime reactive oxygen species concentrations in Flushing, New York. Aerosp Sci Technol 41:97–111
Verma V, Ning Z, Cho AK, Schauer JJ, Shafer MM, Sioutas C (2009) Redox activity of urban quasi-ultrafine particles from primary and secondary sources. Atmos Environ 43:6360–6368
Verma V, Shafer MM, Schauer JJ, Siotas C (2010) Contribution of transition metals in the reactive oxygen species activity of PM emissions from retrofitted heavy-duty vehicles. Atmos Environ 44:5165–5173
Verma V, Pakbin P, Cheung KL, Cho AK, Schauer JJ, Shafer MM, Kleinman MT, Sioutas C (2011) Physicochemical and oxidative characteristics of semi-volatile components of quasi-ultrafine particles in an urban atmosphere. Atmos Environ 45:1025–1033
Verma V, Rico-Martinez R, Kotra N, King L, Liu J, Snell TW, Weber RJ (2012) Contribution of water-soluble and insoluble components and their hydrophobic/hydrophilic subfractions to the reactive oxygen species-generating potential of fine ambient aerosols. Environ Sci Technol 46:11384–11392
Wang Y, Hopke PK, Sun L, Chalupa DC, Utell MJ (2011) Laboratory and field testing of an automated atmospheric particle-bound reactive oxygen species sampling-analysis system. J Toxicol 2011:9. doi:10.1155/2011/419476
Wang Y, Arellanes C, Paulson SE (2012) Hydrogen peroxide associated with ambient fine-mode, diesel, and biodiesel aerosol particles in Southern California. Aerosol Sci Tech 46:394–402
Warnke J, Bandur R, Hoffmann T (2006) Capillary-HPLC-ESI-MS/MS method for the determination of acidic products from the oxidation of monoterpenes in atmospheric aerosol samples. Anal Bioanal Chem 385:34–45
Warscheid B, Hoffmann T (2001) Structural elucidation of monoterpene oxidation products by ion trap fragmentation using on-line atmospheric pressure chemical ionization mass spectrometry in the negative ion mode. Rapid Commun Mass Spectrom 15:2259–2272
Warscheid B, Hoffmann T (2002) Direct analysis of highly oxidized organic aerosol constituents by on-line ion trap mass spectrometry in the negative-ion mode. Rapid Commun Mass Spectrom 16:496–504
Weschler CJ, Shields HC (1996) Production of hydroxyl radicals in indoor air. Environ Sci Technol 30:3250–3258

Winterhalter R, Dingenen RV, Larsen BR, Jensen NR, Hjorth J (2003) LC-MS analysis of aerosol particles from the oxidation of α-pinene by ozone and OH-radicals. Atmos Chem Phys Discuss 3:1–39

Xia X, Hopke PK (2006) Seasonal variation of 2-methyltetrols in ambient aerosol samples. Environ Sci Tech 40:6934–6937

Yu J, Flagan RC, Seinfeld JH (1998) Identification of products containing –COOH, –OH and –C=O in atmospheric oxidation of hydrocarbons. Environ Sci Tech 32:2357–2370

Zhang X, Chen ZM, Zhao Y (2010) Laboratory simulation for the aqueous OH-oxidation of methyl vinyl ketone and methacrolein: significance to the in-cloud SOA production. Atmos Chem Phys 10:9551–9561

Ziemann PJ (2002) Evidence for low-volatility diacyl peroxides as a nucleating agent and major component of aerosol formed from reactions of O3 with cyclohexene and homologous compounds. J Phys Chem A 106:4390–4402

Chapter 2
Impacts of Air Pollution on Reproductive Health

Mariana Matera Veras, Natália de Souza Xavier Costa, Laís Fajersztajn, and Paulo Hilário Nascimento Saldiva

2.1 Introduction

Humans are continuously exposed to mixtures of environmental contaminants and a vast body of evidence now link exposure to these chemicals with an increased incidence of reproductive and developmental disorders (Woodruff and Walker 2008; Sadeu et al. 2010).

Since the second half of the twentieth century, the harmful effects of air pollution on human health have been the subject of many studies. Episodes of high levels of air pollution experienced by cities in Europe and the United States have enlightened both government agencies and the global community public about the harmful effects of air pollution on human health. Infamous examples include both the London Fog of 1952 and Donora Smog of 1948 that were associated with significantly elevated rates of hospital admissions and mortality (Logan 1953; Helfand et al. 2001). Similarly the more recent Bophal gas disaster (India, 1984) should also be remembered. In this tragic example, a methyl isocyanate gas leak killed 2,500 people in 5 days and many more were condemned to long-term morbidity including serious reproductive dysfunctions (Sriramachari 2005). Subsequent to these episodes, clean air legislations and other regulatory actions have significantly

M.M. Veras, Ph.D. • P.H.N. Saldiva, M.D., Ph.D. (✉)
Laboratory of Experimental Air Pollution (LIM05),
Department of Pathology, School of Medicine, University of São Paulo,
Av Dr Arnaldo 455, Room 1103 1st Floor, São Paulo, São Paulo State 01246-903, Brazil

National Institute for Integrated Analysis of Environmental Risk,
São Paulo, São Paulo State, Brazil
e-mail: pepino@usp.br

N. de S.X. Costa, M.S. • L. Fajersztajn, M.S.
Laboratory of Experimental Air Pollution (LIM05),
Department of Pathology, School of Medicine, University of São Paulo,
Av Dr Arnaldo 455, Room 1103 1st Floor, São Paulo, São Paulo State 01246-903, Brazil

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_2

reduced ambient air pollution in many regions of the world, especially in both North America and Europe (Chen et al. 2007). Despite successful regulatory oversight, air pollution in urban centers remains a substantial risk factor to global human health.

The respiratory tract is the first system to be in contact with the air pollutants. Respiratory and cardiovascular diseases are the most commonly observed effects associated with exposure to low levels of air pollution followed by neoplasia (Schwartz 2006; Pope et al. 2009; Dockery 2009). Recent studies support that common environmental air pollutants that we contact in our daily life and to which exposures are unavoidable could also affect both reproductive health and fetal development. Exposure to ambient air pollution appears to adversely impact fertility, pregnancy outcomes, and fetal health and development (Maisonet et al. 2004; Parker and Woodruff 2008).

Epidemiological studies suggest the association between exposures to air pollution and impairment of reproductive health. However, these studies also recognize and acknowledge many uncertainties with regard to this association (Slama et al. 2008), such as few evidence of plausible biological mechanisms, limited information on personal exposures, difficulties in linking particulate matter (PM) composition or single constituent to the biological effects (Pope 2000; Chen et al. 2008; Valavanidis et al. 2008; Ren and Tong 2008). Despite the adoption of different study designs and statistical evaluations and the potential of confounding variables (e.g. maternal smoking, gestational age, and socioeconomic factors), and lack of consistency in findings, these epidemiological investigations suggest a causal association.

Epidemiological studies are important to help guide further investigation. However, many unanswered questions remain with regard to the association between air pollution and reproductive health including;

- What are the mechanisms involved in decreased fertility due to the exposure to increased PM concentration?
- Which trimester of pregnancy is most relevant to the impairment of fetal development?
- Which component of the PM presents a higher risk for reduced birth weight?
- Are the mechanisms involved in decreased male fertility the same as in females?
- Could multigenerational exposure to ambient PM concentration present cumulative effects?

Future studies utilizing animal models and carefully designed human clinical investigation will help address the current gap in knowledge. Toxicological and clinical studies are necessary to confirm associations elucidate mechanisms involved, identify most susceptible subgroups, and to create ways to reduce these impacts on human health.

In this chapter, we will focus on the major epidemiological findings, current experimental evidence, and possible molecular mechanisms involved in the impairment of the reproductive process associated with exposure to common urban air pollution.

2.2 Urban Air Pollution

Air pollution is a widespread environmental contaminant. Expansion of industry and vehicular traffic has had a major impact on the overall air quality in urban areas over the last decades. This contributed to widespread contamination of the environment by thousands of harmful compounds derived from exhaust emissions. In a broad sense, air pollution can be defined as a mixture of gaseous, liquid, and solid substances containing many toxic components which include; CO, NO_2, SO_2, O_3, Pb, polycyclic aromatic hydrocarbons (PAH) and particulate matter (PM) (WHO 2005).

Because of the recognized health impacts, significant efforts have been placed on investigation and regulation of the particulate fraction of air pollution. As defined by Collbeck and Lazaridis (2010), particulate matter (PM) is a complex mixture of solid particles, liquid droplets, and liquid components contained in the solid particles constituted by many different chemical species originating from a wide variety of sources. Particles can be produced by combustion, suspension of soil material, and also from chemical reactions in the atmosphere. Nitrates, sulphates, elemental carbon, organic substances (VOC, PAH), metals (Cd, Pb) and mineral material (Al, SI, Fe and Ca) are major constituents of airborne PM (Colbeck and Lazaridis 2010; Heal et al. 2012). Particle size ranges vary considerably from a few nanometers (nm) to several micrometers (um), which strongly determine particle lifetime in the atmosphere and deposition in the respiratory tract.

The particles are classified according to their aerodynamic diameter, as PM_{10} [particles with an aerodynamic diameter smaller than 10 μm], $PM_{2.5}$ [aerodynamic diameter smaller than 2.5 μm] and $PM_{0.1}$ [aerodynamic diameter smaller than 0.1 μm] (Colbeck and Lazaridis 2010). The size and its composition are directly linked to negative health effects. The observed high toxicity of the smaller size fractions is believed to be due to the fact that they may reach the deeper portion of the lungs and may translocate into the circulation. Further these fractions contain higher concentrations of polycyclic aromatic hydrocarbons (PAH), semiquinones, metals, transition metals, and have a higher radical generating capacity (Squadrito et al. 2001; Kok et al. 2006).

2.3 Epidemiological Findings

The evidence for harmful effects at realistic urban air concentrations of air pollutants predominantly come from epidemiological studies (Slama et al. 2008; Stillerman et al. 2008). In this section, we will present recent reports that indicate that exposures to ambient levels of air pollutants (PM, NO_2 SO_2 and O_3), in addition to the recognized adverse cardio respiratory effect are associated with negative impacts on reproductive health.

Increased risks for low birth weight, prematurity, neonatal and post-neonatal mortality and congenital defects have been reported to be associated with exposures to ambient air pollution. Beyond the recognized adverse consequences on pregnancy outcomes, exposure to air pollution is associated with adverse impact on reproductive function including fertility. Furthermore, studies have shown that periods of elevated air pollution were significantly associated with changes in semen quality and damages in sperm DNA (Dejmek et al. 2000; Rubes et al. 2005).

2.3.1 Fertility and Fecundability

Very few studies have addressed the effects of ambient air pollution on human fertility. Available studies focus on the primarily impact on male fertility, probably due to readily accessible semen acquisition and analysis (Sokol et al. 2006). Detrimental changes in various semen parameters such as motility, sperm morphology, and DNA are reported, which may cause reduced fertility in males or miscarriage in females.

The positive association of the adverse effects of air pollution exposures on pregnancy outcomes has redirected the attention of the researchers to other important aspects of reproductive health including fertility. One of the first studies that investigated the effects of air pollution on fertility was conducted by Djemek et al. (2000). In this study, they have evaluated the impact of SO_2 on fecundability (the probability of conceiving during the menstrual cycle) in the first unprotected menstrual cycle (FUMC) of 2,585 parental pair in a heavily polluted region of Northern Bohemia. They found that adjusted odds ratios of conception in the FUMC may be reduced in couples exposed to mean SO_2 levels >40 pg/m^3 in the second month before conception. In a previous study with the same population, they have also found an association between PM_{10} and reduced conception rates (Dejmek et al. 1998). Slama et al. (2013) reanalyzed the data of the study by Dejmek et al. (2000) and defined the exposure window with respect of the start of the period of unprotected intercourse and considered the impact of $PM_{2.5}$, carcinogenic polycyclic aromatic hydrocarbons (c-PAH), ozone (O_3), nitrogen dioxide (NO_2) levels, in addition to SO_2 on fecundability. Their new results highlight that $PM_{2.5}$ and NO_2 levels in the 2 months before the end of the first month of unprotected intercourse were associated with decreased fecundability. This observation highlights the important concept of lag time between exposure and observed health consequences.

Selevan et al. (2000) showed that elevated period of air pollution were significantly correlated with changes in various semen parameters of young Czech men including; proportionately fewer motile sperm, less sperm with normal morphology or normal head shape, and proportionately more sperm with abnormal chromatin. Based on this preliminary findings, Rubes et al. (2005) monitored semen quality in a cohort of young Teplice residents over longer periods of time (periods of exposure to both low and high air pollution) and found a significant association between exposure to high levels of air pollution and decreased sperm chromatin integrity.

In the United States (Salt Lake City), Hammoud et al. (2010) demonstrated that $PM_{2.5}$ concentration was negatively correlated to sperm motility 2 months and 3 months after exposure, which coincides with the duration of spermatogenesis (72 days). However, a study of Hansen et al. (2010) did not support a consistent pattern of association between O_3 and $PM_{2.5}$. They performed analysis of several measures of semen quality and found only statistically significant adverse association between increased $PM_{2.5}$ averaged over the 0- to 90-day period before semen sampling and an increase in the percentage of sperm with abnormally shaped heads and the percentage of sperm with cytoplasmic droplets. However, after controlling for season and temperature results failed to reach statistical significance.

In the study of Sokol et al. (2006), there was a significant negative correlation between O_3 levels (70–90 days before collection) and average sperm concentration, which was maintained after correction for birth date, age at donation, temperature, and seasonality. These epidemiological results are in line with occupational exposures (De Rosa et al. 2003; Guven et al. 2008) and experimental studies of diesel exhaust inhalation and detrimental effects on sperm (Izawa et al. 2007).

There are very limited studies on the impact of air pollution on female fertility. We are aware of only two studies available in the literature on female fertility that were conducted by Perin et al. (2010a, b). These studies evaluated the impact of PM exposure during the follicular phase of the menstrual cycle or during the preconceptional period of women undergoing IVF/ET on early pregnancy loss and miscarriage. Both studies support an association between brief exposure to high levels of PM and the adverse gestational outcomes. This is an area that would likely benefit from additional studies.

Human fertility is declining in different parts of the world for unclear reasons. There are many hypothesis that have been used to explain this observation. Among the most supported causes of worldwide declining fertility are the delay of childbearing by modern women and other changes in social factors. However, there is also a growing incidence of impaired fecundity among young women and men, which cannot be easily explained by societal factors. Nutritional status, obesity, drugs, smoking habits, stress and increasing exposures to environmental pollutants, such as air pollution, are also plausible factors involved in human reduced fertility that deserve more attention.

2.3.2 Low Vitamin D and Immune System Alterations

Besides the "visible" effects of air pollution on fetal health, there is growing evidence of the impact of maternal exposure to urban air pollution on "non-visible" fetal outcomes. Serum vitamin D levels in the mother and fetus seems to be affected by air pollution. Vitamin D is scarce in natural food and the main source of vitamin D for humans is its synthesis from 7-dehydrocholesterol upon exposure to UVB solar radiation and conversion to circulating metabolite called 25-hydroxyvitamin D [25(OH)D] in the liver. Deficiencies in vitamin D are associated with different

bone diseases and more recently it has been involved in other diseases including cardiovascular (Kim et al. 2008) and autoimmune diseases (Lange et al. 2009).

In general, low intake of vitamin D and insufficient exposure to sunlight constitute the main causes of vitamin D deficiency. However, recent studies point out that exposure to air pollution may also contribute to low serum levels of vitamin D in women and in the unborn child as a consequence of maternal deficiency. In the study conducted by Baïz et al. (2012), maternal exposure to ambient urban levels of NO_2 and PM_{10} during the whole pregnancy was a strong predictor of low vitamin D status in newborns. Moreover, Kelishadi et al. (2013) have also found that air quality had an inverse and independent association with 25(OH)D levels of mothers and their neonates and Hosseinpanah et al. (2010) have shown that deficiency in vitamin D to be prior to pregnancy. In their study, they found that women living in polluted areas present with lower levels of serum 25-OH-D which is comparable to vitamin D deficiency.

Potential mechanisms that could explain this relationship are based on the impact of smoking on vitamin D status (Brot et al. 1999). It has been suggested that changes in inflammatory profile and oxidative stress caused by smoking affects liver function and hence vitamin D synthesis or affecting maternal intestinal absorption of vital vitamins and minerals (Need et al. 2002). Exposure to air pollutants may have similar impact on vitamin D status. Beyond observed differences in vitamin D, exposure to air pollution could result in changes of the immune system including; NK, T lymphocytes, and IgE content of umbilical blood (Herr et al. 2010, 2011). Maternal exposure to air pollution could have lasting effects of either gestational or pre-gestational exposures on their offspring. The consequences for the future health of these individuals remains unknown, but these effects could have far reaching impacts.

2.3.3 Gestational Outcomes

Studies conducted in different continents (Europe, Asia and Americas) consistently report that expectant mothers exposed to air pollution have greater risks of having negative gestational outcomes (Gouveia et al. 2004; Ha et al. 2001; Wang et al. 1997). However, it is not clear from the studies whether the effects are due to a specific pollutant or to the interactions of different pollutants and in which trimester exposures are more detrimental to fetal development. Negative gestational outcomes are predominant reproductive effects associated with exposures to ambient air pollution and are discussed in detail in Chap. 3. In this section, we will briefly highlight the gestational outcomes and its association with criteria pollutants in order to compare with experimental evidence.

The causes of the negative pregnancy outcomes are not well understood but it is clear that these outcomes have multifactorial causes and there are scientific evidence that environmental factor, such as air pollution, can contribute or aggravate these outcomes. Furthermore, there are groups within population that may be more susceptible to air pollution exposure. These groups include individuals with

preexisting circulatory and respiratory conditions and those socially and economically disadvantaged. In general people with low social and economic status live in more polluted areas, lack adequate health care, and spend more time near high traffic roads that may increase exposures to air pollutants and consequently increase the risks of adverse birth outcomes (Woodruff et al. 2003; O'Neill et al. 2003; Ponce et al. 2005; Gilbert et al. 2007; Ono et al. 2013).

2.3.3.1 Low Birth Weight

The prevalence of low birth weight (LBW), as defined by WHO as weight at birth of less than 2,500 g (5.5 lb) is estimated to be 15 % worldwide with a range of 3.3–38 % (http://www.who.int/nutrition/topics/lbw_strategy_background.pdf) and occurs mostly in developing countries; coincidentally these countries has elevated levels of air pollution (http://www.who.int/gho/phe/outdoor_air_pollution/phe_012.jpg). This topic is covered in more detail in Chap. 3.

Birth weight is an important indicator of subsequent health issues; low-birth-weight babies are more prone to develop hypertension, coronary heart disease, and non-insulin-dependent diabetes during adulthood (Osmond and Barker 2000). Evidence from studies conducted in developed and developing countries, China (Wang et al. 1997), Australia (Mannes et al. 2005), Chezec Republic (Dejmek et al. 1999); and USA (Ritz et al. 2000) point out that PM_{10}, $PM_{2.5}$ and SO_2 are the major pollutants associated with increased risks of LBW. However, other studies verified that exposures during pregnancy to CO, O_3, and NO_2 could also be associated with LBW (Morello-Frosch et al. 2010).

2.3.3.2 Preterm Birth, Intrauterine and Neonatal Mortality

Although less frequently reported, intrauterine and neonatal mortality and pre-term birth (PTB) (Glinianaia et al. 2004; Maisonet et al. 2001; Srám et al. 2005) are also outcomes associated with air pollution. Maternal first trimester exposure to ambient air pollutant exposure seems to be the critical period for PTB. However, Huynh et al. (2006) observed no association between PTB and ambient air $PM_{2.5}$ levels near the maternal residence during early pregnancy and late gestation.

In Australia, Hansen et al. (2006) observed that increased levels of O_3, NO_2 and SO_2 during 1st trimester increases the risk of PTB (OR = 1.26, 95 % CI 1.10–1.45). Another study from Korea (Leem et al. 2006) reported that increased levels of CO level during 1st trimester are responsible for the increased risk for PTB [OR = 1.26, 95 % CI 1.11–1.44, p-trend < .001]. Considering traffic proximity, the risk of PTB in Taiwan was elevated among women living close to a major freeway [<0.5 vs. 0.5–1.5 km, OR = 1.30, 95 % CI 1.03–1.65] (Yang et al. 2003). In Los Angeles, Wilhelm and Ritz (2003) found that there was a dose-response relationship between preterm birth and inverse-distance-weighted traffic density among women in their 3rd trimester [OR = 1.15, 95 % CI 1.05–1.26].

2.3.3.3 Fetal Growth and Developmental Abnormalities

Although evidence is less consistent, exposures accrued during gestation and sensitive periods of fetal organ development may be related to developmental abnormalities/congenital defects. Jedrychowski et al. (2004) observed that not only birth weight is affected; changes in other anthropometric measurements were observed, such as reduction in head circumference. Studies conducted by van den Hooven et al. (2012a) using ultrasound measurements observed that NO_2 levels were inversely associated with fetal femur length in the second and third trimester, and both PM_{10} and NO_2 levels both were associated with smaller fetal head circumference in the third trimester. Vrijheid et al. (2011) systematically reviewed epidemiologic studies on ambient air pollution and congenital anomalies and conducted a meta-analysis for a number of air pollutant–anomaly combinations. They conducted meta-analyses for 18 combinations of pollutants and cardiac anomaly groups and found that NO_2 and SO_2 exposures were related to increases in the risk of coarctation of the aorta and tetralogy of Fallot and PM_{10} exposure was related to an increased risk of atrial septal defects.

A large case-control study conducted in California reported a weak association between cleft lip/palate and ambient air O_3 levels near the maternal residence during the second month of gestation. Few studies evaluated if there were associations between maternal exposure to air pollutants and stillbirths. Results from these studies remain inconclusive. Pereira et al. (1998) found an association between daily counts of intrauterine mortality and NO_2, SO_2, and CO concentrations before ($\leq$5 days) delivery. Similarly, Faiz et al. (2013) reported that increased stillbirth is associated with increases in NO_2, SO_2, CO, and $PM_{2.5}$ concentrations in the immediate few days before delivery. In a study by Bobak and Leon (1999) stillbirth rates were not significantly associated with any indicator of air pollution.

2.3.3.4 Preeclampsia

Emerging evidence from a large European study and from three previous studies (Woodruff et al. 2008; Wu et al. 2009; Rudra et al. 2011; Olsson et al. 2013) further indicates that that there is also a positive associations between exposure to air pollution and risks of preeclampsia. However, the studies diverge to in terms of the pollutant associated with preeclampsia. In the largest study conducted in Europe, Olsson et al. (2013) found that exposure to O_3 in the first trimester of gestation are associated with increased risks of preeclampsia. Rudra et al. (2011) found a weak association between CO exposure during the first 7 months of pregnancy (per 0.1 ppm) and preeclampsia (OR = 1.07, 95 %, CI = 1.02–1.13). In another study conducted in the USA, Wu et al. (2009) reported odds ratios of 1.33 (95 %, CI = 1.18–1.49) and 1.42 (95 % CI = 1.26–1.59) for preeclampsia in the highest exposure quartiles for NO_x and $PM_{2.5}$. Increased blood pressure throughout pregnancy has also been observed in mothers who are exposed to air pollution (van den Hooven et al. 2012b; Lee et al. 2013) and both available studies agree that PM_{10} and O_3 are associated to this effect.

2.3.4 Secondary Sex Ratio

In the literature, there are many examples of studies suggesting the impact of both environmental pollution and occupational exposure to certain substances and changes in secondary sex ratio (SSR) (Terrell et al. 2011; Tragaki and Lasaridi 2009; Schnorr et al. 2001). Previous studies have shown changes in sex ratios of populations living near incinerators [lower sex ratio] (Williams et al. 1992) as well as in areas exposed to polluted air from steel foundries (higher sex ratio) (Lloyd et al. 1985). In urban areas with high levels of particulate pollution derived from traffic, there is only one study that investigated if the secondary sex ratio could be affected by air pollution. In this study, Miraglia et al. (2013) have found a significant negative association between SSR and PM_{10} concentration in São Paulo city, Brazil. Although the causality between environmental exposures and declines in secondary sex ration are still controversial, some authors suggest that the SSR as a sentinel indicator of reproductive injury (Davis et al. 1998).

2.3.5 Reproductive System Cancer

Little is known about the role of air pollution in cancers of the reproductive system cancer (e.g. prostate, ovarian and breast cancer). However, an increased risk of lung cancer associated with exposures to outdoor air pollution was consistently observed in studies from Europe, North America, and Asia (Fajersztajn et al. 2013). Thus it is reasonable to suspect that other types of cancer could be caused by long term exposure to air pollutants. The latest IARC release on cancer incidence mortality and prevalence worldwide predict a substantive increase to total 19.3 million new cancer cases per year by 2025, due to growth and ageing of the global population. However, environmental influences and lifestyle factors may also be implicated in this increase; and they highlight the fact that breast cancer incidence has increased by more than 20 %. Vehicular emission is the primary contributor to air pollution in urban areas, its composition include compounds that are recognized as carcinogens, such as diesel exhaust. Recently, the International Agency for Research on Cancer (IARC) of the World Health Organization has classified diesel and gasoline exhaust as carcinogenic to humans as possibly carcinogenic (Loomis et al. 2013).

Few studies have been conducted to evaluate if there is an association between reproductive system cancer and air pollution. These types of studies are limited by difficulties in the assessment of long term exposures, the presence of a myriad of confounding factors and co-exposures to other known carcinogens in food and water. Disinfection by products and even infections make it difficult the establishment strong associations between air pollution and cancer risk factors.

Prostate Cancer There are only two studies in the literature that found an association between prostate cancer and air pollution (Soll-Johanning and Bach 2004; Parent et al. 2013). Recently, a case–control study conducted in Montreal evaluated environmental risk factors for prostate cancer and found associations between

exposures to traffic related air pollution, assessed by exposures to NO_2, and increased risks of prostate cancer incidence. Limitations of the study include exposure assessment based on home address and time of exposure which was 25 years prior to the interview and thus which could misclassify an individual's exposure (Parent et al. 2013).

Breast Cancer In women, breast cancer has the highest incidence rate. Many risk factors have been pointed out such as genetic factors, lifestyle, reproductive history, smoking and alcohol consumption. Although many factors have been identified most of the cases remain with unknown etiology (Coyle 2004). The first evidence of a possible relationship between breast cancer and air pollution levels came from the observation that the incidence was higher in urban areas compared to rural areas (Bako et al. 1984; Hall et al. 2005; Reynolds et al. 2004). Human studies are limited, however there at least 30 substances present in urban air pollution that are known to be associated with increased mammary tumors in animals such as benzene (diesel exhaust) and polycyclic aromatic hydrocarbons (PAHs). Thus, it is plausible that traffic-related exposures may contribute to the incidence of breast cancer (reviewed by Rudel et al. 2007). In the USA, few studies assessed the relationship between air pollution exposure and breast cancer. Lewis-Michl et al. (1996) conducted a case-control interview study in New York (USA) and suggested a possible increased risk [OR = 1.29; 95 % CI: 0.77–2.15] of breast cancer among postmenopausal women who live near areas with high traffic density. Bonner et al. (2005) found that early-life exposures to relatively high concentrations of air pollution (i.e., >140 $\mu g/m^3$) were associated with an increased risk of developing postmenopausal breast cancer (OR = 2.42; 95 % CI, 0.97–6.09). In Canada, Crouse et al. (2010) found evidence of an association between the incidence of postmenopausal breast cancer and exposure to ambient concentrations of NO_2. In China, Huo et al. (2013) have shown that long-term air pollution exposure may contribute to the development of breast cancer.

Ovary Cancer In the literature, there are only two studies that have found an association between air pollution and ovarian cancer. In the first study published in 2005, Iwai et al. (2005) conducted a cross-sectional epidemiological study using the annual vital statistics and air pollution throughout Japan and found that breast, endometrial, and ovarian cancer showed significant increases in mortality rates in relation to particulate pollution. More recently in Taiwan, Hung et al. (2012) showed that individuals who resided in municipalities with higher $PM_{2.5}$ levels were at a significantly increased risk of death from ovarian cancer.

2.4 Experimental Evidence

All of the published studies acknowledged that there are many uncertainties on the association between adverse reproductive outcomes and air pollution (Pope 2000; Chen et al. 2008; Valavanidis et al. 2008; Ren and Tong 2008). Undoubtedly these

aspects point out that there is a need for further toxicological and clinical studies to confirm, to strengthen and elucidate the mechanism involved in this association, to identify most susceptible subgroups and to create ways to reduce these impacts on human health. The scientific literature is extremely scarce with regard to experimental studies conducted to evaluate reproductive efficiency using laboratory animals exposed to real urban air pollution. Available studies conducted on experimental animals, mainly mice, have corroborated human epidemiological data and have provided data showing additional effects not yet investigated in humans.

Studies using a multigenerational mouse model of exposure to "real world" ambient concentrations of air pollution, (i.e. mice mates and their litters were continuously exposed inside chambers to either filtered-clean air or non-filtered air-polluted air), found that urban air pollution compromises reproductive health in different ways across generations. This series of studies has shown that in the first generation (G1) of mice exposed to air pollution there were significant reductions in the number of viable fetuses, increased numbers of implantation failures, and a decreased male/female secondary sex ratio (Mohallem et al. 2005; Lichtenfels et al. 2007). In the second generation of mice (G2), females exposed to air pollution during gestation gave birth to litters with reduced birth weights, but no differences in litter size and viable fetuses were observed. Birth weight was significantly lower with a mean reduction of 21 % compared to fetuses from non-exposed females (Veras et al. 2009).

The negative effect of air pollution exposure during pregnancy and birth outcomes are increasingly recognized, but most epidemiological studies have focused only on exposure during the gestational period. Evidence from animal studies explored the effects of maternal exposure before pregnancy on fetal development and demonstrates that maternal exposure not only during pregnancy, but also before conception, adversely affected fetal birth weight (Veras et al. 2008; Rocha et al. 2008). In the same way, exposure to air pollutants during gestation and/or during the pre-gestational period was associated with increased post-implantation loss rates in exposed females (Veras et al. 2009). These results support the findings from Perin et al. (2010a, b) on the importance of the pre-gestational period on gestation establishment. Examination of the placenta from dams exposed to air pollution before and/or during pregnancy revealed that both pre-pregnancy and pregnancy periods of exposure to polluted air resulted in morphological changes in the placenta (Veras et al. 2008). Veras et al. (2008) found that decreases in fetal weight were accompanied by decreases in the volume of maternal blood spaces, in the mean diameter of maternal blood spaces, and in maternal:fetal surface ratio. These features were accompanied by increases in the surface area of fetal capillaries, the total diffusive conductance of the intervascular barrier, and the mass-specific conductance of that barrier. None of the studies conducted in human has evaluated whether placental changes are associated with adverse pregnancy outcomes in humans. Recently, van den Hooven et al. (2012a) using ultrasound measurements and markers of placental growth and function have shown that in human the placenta development, as observed in animals, is impaired by maternal exposure to air pollution.

Umbilical cords were also evaluated and exposures to air pollution were associated with thinner and less voluminous umbilical cords (loss of mucoid connective tissue and collagen content). Structural changes in umbilical arteries and veins and elevated immunoreactivity for 15-F2t-IsoP (oxidative stress), ETAR and ETBR (vascular tone) in their walls were found. Together these findings indicate compromised fetal development evidenced by reduced birth weight might be mediated by alteration in placental and umbilical structure and function as well as by imbalances in the endogenous regulators of vascular tone and oxidative stress (Veras et al. 2012).

The reproductive capacity of G2 nulliparous female mice was also examined and results have shown changes to estrous cyclicity and in ovarian follicle counts (decreased numbers of antral follicles) (Veras et al. 2009). Antral follicles represent the last stage in follicle development prior to ovulation and are the only follicle type capable of releasing an oocyte for fertilization and synthesizing estrogen (Hoyer and Sipes 1996; Hirshfield 1997). Increases in the rate of follicle depletion can potentially raise the possibility of premature ovarian failure and early menopause in the case of humans (Rowe 2006). Observed changes in estrous cyclicity are indicative of persistent estrus, which may reflect an impairment of ovulation as well as changes in the levels of circulating ovarian hormones (EPA 1996).

Further, in the second generation it was observed changes in couple-based outcomes. Couples exposed to air pollution presented decreased fertility indices, decreased pregnancy success and delayed onset of reproductive maturity, as evidenced by extended times to mating (Veras et al. 2009). In humans, air pollution exposures seemed to decrease conception rates (Dejmek et al. 1998). However, we still do not know if the effects are associated with impairments on female or male health.

It is important to point out that the mean concentration of $PM_{2.5}$ (24-h average concentration) used in some studies (27.5 μg/m^3, Veras et al. 2008, 2009), is less than the 35 μg/m^3 established by the U.S. National Ambient Air Quality Standards [US-NAAQS] (http://www.epa.gov/air/criteria.html) and, approximately equivalent to the World Health Organization (WHO) air quality guideline (25 μg/m^3; WHO 2005) raising the question of whether these proposed values are safe for reproductive health.

Two recent mechanistic experimental studies (*unpublished*) addressed the associations between air pollution exposure during the initial stages of pregnancy in mice and uterine response to embryo implantation. Scoriza et al. (2009) observed that in early pregnancy (6 and 8 GD) that reductions in the number of uterine natural killer (uNKcells) and mast cells could contribute to the increased rates of post implantation losses observed in mice. uNK cells are a subpopulation of lymphocytes that in normal mice promote decidual angiogenesis, trophoblast and placental cell growth, provide immunomodulation at the maternal-fetal interface for a healthy pregnancy (Bilinski et al. 2008). The role of mast cells in pregnancy is less known. However, during normal early pregnancy, the number of mast cells and their activation change (Gibbons and Chang 1972; Marx et al. 1999). These data suggest that components present in air pollution may indirectly interfere with or impair

embryonic development through changes to the maternal environment including maternal immune responses. In another study, nulliparous mice were exposed to two different doses of fine particulate air pollution ($PM_{2.5}$) for 45 days before pregnancy until gestational day 4 and expression of different uterine receptivity markers were evaluated (*Lif*, leukemia inhibitory factor; and *Muc1*, mucin, pinopods) as well as uterine histopathology. Histopathology revealed a decrease in the volume and thickness of the endometrium as well as changes in the diameter and thickness of the glandular and luminal epithelia. No significant alteration was observed in the expression (qPCR/IHC) of *Muc1* but there was significant suppression of *Lif* during the window of implantation. These findings suggest that air pollutants may affect the fine regulation of proliferation and differentiation of uterine stromal cells during decidualization via reduced LIF expression (Castro et al. 2013).

Evidences from experimental studies linking and chronic exposure to air pollution are in line with the epidemiological findings (Knottnerus et al. 1990; Peters et al. 1997; Pekkanen et al. 2000). However, the mechanisms involved in this association are not clearly known. There are many suggested potential mechanisms which include induction of p450 enzymes, DNA damage, and systemic alterations in hematocrit, blood viscosity, blood coagulation, endothelial dysfunction, oxidative stress, and inflammation (Baskurt et al. 1990; Sørensen et al. 2003; Andrysík et al. 2011). The mechanisms proposed are described in more detail in the next section.

2.5 Biological Mechanisms

The mechanisms, by which, air pollution could cause adverse health effects are characterized by their ability to directly act as pro-oxidants of lipids and proteins or as free radical generators, promoting oxidative stress, inflammatory responses and damage to mitochondrial function (Menzel 1994; Rahman and MacNee 2000; Li et al. 2003).

The first system to in contact with air pollution is the respiratory tract. Epidemiologic and experimental data show that the air pollution can cause pulmonary inflammation, decrease of pulmonary function, and aggravation of pre-existing pulmonary diseases such as asthma and bronchitis (Laumbach 2010; Saldiva et al. 2002; Seaton et al. 1995).

The bigger fraction of the PM gets trapped on the superior respiratory tract, and the smaller fraction can reach the lungs and these particles can be deposited (Amdur and Corn 1963; Amdur and Creasia 1966). In an attempt to remove such particles, alveolar macrophages phagocytize the particles, penetrating into the cellular interstitium, but part of these fine particles can be translocate across the air–blood barrier into circulation and towards secondary target organs, suggesting that the smaller the particle diameter, the greater the possibility of translocation to other organs (Takenaka et al. 1986; Ferin et al. 1992; Oberdorster and Utell 2002; Chen et al. 2006).

The majority of fine and ultrafine (<$PM_{0.1}$) particles found in the urban atmosphere derive from engine combustion. Ultrafine particles have very low mass typically with magnitudes higher particle numbers and therefore a high surface area relative to fine and coarse particles for adsorption of toxic species (Sioutas et al. 2005). Studies suggest that only a small fraction of PM can pass rapidly into systemic circulation, and that pulmonary inflammation seems to play a major role in enhancing the extra-pulmonary translocation of particles (Chen et al. 2006; Brown et al. 2002; Burch 2002; Mills et al. 2006; Wiebert et al. 2006a, b; Möller et al. 2008). Organic components of particles, which comprise a large proportion of freshly emitted exhaust and secondary aerosols, can induce a broad polyclonal expression of cytokines and chemokines in respiratory epithelium and this effect may be due to the action of PAHs, metals and related compounds that lead to the production of cytotoxic reactive oxygen species (ROS); and these inflammatory and oxidant stress responses are expected to occur at extra-pulmonary sites, as well (Sioutas et al. 2005; Ritz and Wilhelm 2008).

Several hypotheses have been proposed that air pollution can affect the reproductive system causing negative effects, such as impairment of male and female reproductive capacity, placental alterations and fetal health.

As we have previously described, exposure to air pollution is associated with detrimental pregnancy outcomes and these outcomes can be caused by a combination of maternal, fetal, and placental factors or a combination of them. . For example, air pollution can affect the utero-placental and umbilical cord flow and consequently the transport for glucose and oxygen though the placenta (Veras et al. 2008, 2012; Ritz and Wilhelm 2008; Vorherr 1982).

According to the review of Kannan and collaborators (Kannan et al. 2006, 2007), the particulate matter present on the air pollution can affect pregnancy outcomes due to:

- Increase in oxidative stress: an important mechanism of action PM can be the DNA damage induced by oxidative stress, also some metals in PM may inhibit the DNA repair enzymes.
- Inflammation: Inflammation could be associated with inadequate placental perfusion and impaired transplacental nutrient exchange, which may cause growth restriction in utero due to interference with some process or processes such as affecting nutrition of the fetus, reduced oxygenation of maternal blood, or both.
- Coagulation and blood pressure: PM exposures may increase any of the proteins of the clotting cascade, indicating a higher possibility for coagulation and may also lead to changes in hemoglobin, platelets, and white blood cells, which may potentially contribute to adverse fetal growth. PM exposure is also associated with elevations on the blood pressure in pregnant women and this could increase the risk of adverse outcomes, especially if there is preexisting hypertension (pregnancy-induced or not). Elevation of blood pressure in pregnant women has been associated with IUGR and preterm delivery.
- Hemodynamic responses: an impaired adaptation of maternal hemodynamic may lead to an impaired fetal growth. These changes may force the fetus to

adapt, down-regulate growth, and prioritize the development of essential tissues.

- Endothelial function: PM exposure may cause endothelial dysfunctions leading to vasoconstriction and could be considered as an intervening pathway in subsequent impact on fetal growth.

These pathways may or may not act independently, but it is more likely that the outcomes of the exposure to air pollution are an association among these pathways and they are probably related to the composition of the particulate matter (Saldiva et al. 2002).

There are evidences that the air pollution can affect not only the pregnancy but also the male and female reproductive fertility (Somers and Cooper 2009).

Based on epidemiological studies it was observed that exposure to air pollution affects fertility rates (Dejmek et al. 2000; Selevan et al. 2000) at different seminal parameters, motility and morphology and the sperm DNA (Rubes et al. 2005; Hansen et al. 2010; Jafarabadi 2007).

In a study conducted by Somers et al. (2002), a significant elevation in mutation frequency was reported in the offspring of animals exposed to air pollution; primarily through expanded simple tandem repeat (ESTR) DNA loci mutation events in the paternal germline.

In other studies conducted by Somers et al. (2004) and Yauk et al. (2008), they noted that ESTR mutation frequencies were also elevated the paternal germline of mice exposed to whole ambient air at the polluted industrial site, indicating that mutations were induced in spermatogonial stem cells. Maternal ESTR mutation frequencies were similar in all groups, and therefore unaffected by air pollution exposure (Somers et al. 2004). Bulky DNA adducts were not significant, suggesting that DNA reactive chemicals do not reach the germ line and cause ESTR mutation. In contrast, DNA strand breaks were elevated after 3 weeks of exposure, possibly resulting from oxidative stress arising from exposure to air pollution and its particulate matter (Yauk et al. 2008).

Sperm DNA in mice exposed to whole ambient air was globally hypermethylated compared to those exposed to filtered air. These methylation changes appeared early in the environmental exposure and were still present after 6 weeks without the air pollution exposure. Persistent changes in the methylation status of genes may have health implications for the next generation through altered gene expression (Somers et al. 2004; Yauk et al. 2008).

Environmental toxicants can alter the female reproduction by direct mechanisms (hormone disruptors) or indirect (immunological toxicants). Direct effects typically occur if an environmental chemical is structurally similar to a molecule capable of interacting with endogenous reproductive organs. Indirect effects can occur if a chemical interferes with the hormonal action. Natural hormones are critical for development, behavior, puberty beginning, sexual function, and gametogenesis. Some environmental chemicals can mimic or block the action of the natural hormone, thus negatively altering reproductive processes (McLachlan and Arnold 1996).

As steroid hormones, some environmental toxicants are lipo-soluble and cross the cell membrane by passive diffusion, thereby allowing access to any animal cell. Once these chemicals cross the cell membrane, which may interact with steroid receptors, access to the nucleus as a dimer hormone-receptor induces the activation or suppression of genes causing a biological response (McLachlan and Arnold 1996).

Several compounds present in air pollution (heavy metals, environmental oestrogens, diesel and PAHs) are able to suppress or interfere with the regulation of the hypothalamic-pituitary-gonadal axis; resulting in changes in growth and development of ovarian follicles and estrous cyclicity (Veras et al. 2009; Mamatsashvili 1970; Borgeest et al. 2004), affecting the whole process of pregnancy, including signaling pathways between the conceptus-mother or, the uterus preparation for implantation (Hoyer and Sipes 1996; Mattison and Thomford 1989; Tsukue et al. 2001; Takeda et al. 2004; Telisman et al. 2007). However, the specific contaminants that caused sperm damage and the potential impact on fertility or pregnancy outcomes were undetermined (Somers 2011).

PAHs in air pollution have the capacity to bind to steroid receptors, mimicking their action, and thus altering the production of these hormones, which can result in adverse consequences for the development and reproductive health (Kristensen et al. 1995; Wenger et al. 2009; Han et al. 2010). For example, Hood (2006) in his study suggests that exposure prior to conception, both female as male, can lead to a hormonal dysregulation causing direct damage to the reproductive organs and gametes.

Studies suggest that PAHs are able to cross the placenta and reach fetal organs causing adverse reproductive outcomes, including; stillbirths, reabsorptions, congenital abnormalities, and decreases in fetal weight. The exposure to PAHs may lead to increased DNA adducts, resulting to LBW and intrauterine growth restriction. Furthermore, the PM may bind receptors for placental growth factors leading to decreased fetal-placental exchange of oxygen and nutrients (Dejmek et al. 2000; Ritz and Wilhelm 2008).

2.6 Prevention

There are sufficient evidence of the harmful effects of exposures to environmental air pollution on reproductive health. Furthermore, it is clear that increased levels of air pollution are found in developing and underdeveloped countries in regions with high population density and higher fertility rates (Fig. 2.1). Although the risks associated with the negative reproductive outcomes tend to be small, the number of people that might be affected is significantly large. Furthermore, if we consider that negative influence on the initial stages of life (embryo/fetus) increases the risks of later life diseases, such as diabetes, metabolic syndromes and cardiovascular diseases (Osmond and Barker 2000; Gluckman and Hanson 2004) exposures to air pollution during pregnancy would have a profound impact in public health strategies to prevent most common health issues

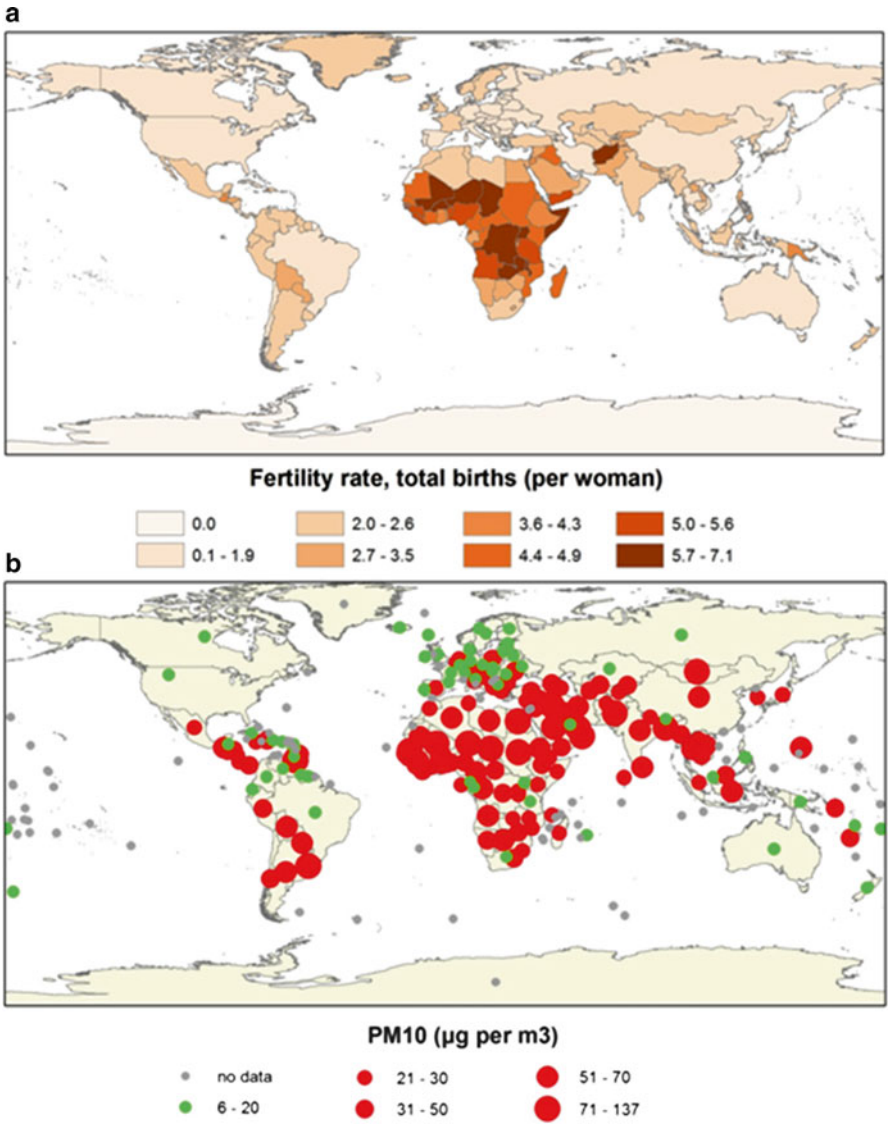

Fig. 2.1 Global scenario of gestational exposure to air pollution. The map compares the total births per women in 2009 (data from the World Bank) (**a**) (World Bank 2013a) with the annual mean concentration of particulate matter of 10 µm or less (PM10) in the same year (data from the World Bank) (**b**) (World Bank 2013b), showing that reproductive health consequences from air pollution exposure is a major concern in the developing nations where a growing population is being exposed to levels of particulate air pollution above the WHO recommended limits

The most effective way to reduce health risks associated with air pollution is to implement air quality management strategies and National Air Quality Standards (Thurston and Balmes 2012). The U.S. Environmental Protection Agency (EPA) projections that by 2020 adherence to the Clean Air Act standard for ozone alone will prevent 230 million premature deaths and 280 infant–mortalities (EPA 2011). Indeed, increasingly stringent (NAQS) already proved effectiveness in improving air quality in the United States and Western Europe in recent decades (Van Erp et al. 2008) and a global analysis concluded that flexible air quality standards lead to higher air pollution concentrations (Vahlsing and Smith 2012).

2.7 Concluding Remarks

Epidemiologic and experimental evidences converge to indicate that air pollution, at the current levels, play a deleterious role on reproductive function. In addition to the scientific questions raised from the aforementioned evidences presented in this chapter – which are the mechanisms, which are the most important pollutants or are the observed effects the result of a mixture of mixtures – some ethical considerations emerged at this moment. Indeed, the options of energy use, industrial production and mobility made by our and our preceding generations, may be affecting those that did not participate in the decision process. Moreover, there is a marked contrast in ambient air pollution concentrations, creating an uneven attributable risk across the globe. As a general rule, air pollution is the result of bad technologies, usually present in the less privileged countries. These points deserve serious consideration when translating knowledge into public policies aimed to protect children globally.

References

Amdur MO, Corn M (1963) The irritant potency of zinc ammonium sulfate of different particle sizes. Am Ind Hyg Assoc J 24:326–333

Amdur MO, Creasia DA (1966) The irritant potency of m-terphenyl of different particle sizes. Am Ind Hyg Assoc J 27(4):349–352

Andrysík Z, Vondráček J, Marvanová S, Ciganek M, Neča J, Pěnčíková K, Mahadevan B, Topinka J, Baird WM, Kozubík A, Machala M (2011) Activation of the aryl hydrocarbon receptor is the major toxic mode of action of an organic extract of a reference urban dust particulate matter mixture: the role of polycyclic aromatic hydrocarbons. Mutat Res 714(1–2):53–62

Baïz N, Dargent-Molina P, Wark JD, Souberbielle JC, Slama R, Annesi-Maesano I, EDEN Mother-Child Cohort Study Group (2012) Gestational exposure to urban air pollution related to a decrease in cord blood vitamin D levels. J Clin Endocrinol Metab 97(11):4087–4095

Bako G, Dewar R, Hanson J, Hill G (1984) Population density as an indicator of urban-rural differences in cancer incidence, Alberta, Canada, 1969–73. Can J Public Health 75(2):152–156

Baskurt OK, Levi E, Caglayan S, Dikmenoglu N, Kutman MN (1990) Hematological and hemorheological effects of air pollution. Arch Environ Health 45:224–228

Bilinski MJ, Thorne JG, Oh MJ, Leonard S, Murrant C, Tayade C, Croy BA (2008) Uterine NK cells in murine pregnancy. Reprod Biomed Online 16(2):218–226
Bobak M, Leon DA (1999) Pregnancy outcomes and outdoor air pollution: an ecological study in districts of the Czech Republic 1986–8. Occup Environ Med 56(8):539–543
Bonner MR, Han D, Nie J, Rogerson P, Vena JE, Muti P, Trevisan M, Edge SB, Freudenheim JL (2005) Breast cancer risk and exposure in early life to polycyclic aromatic hydrocarbons using total suspended particulates as a proxy measure. Cancer Epidemiol Biomarkers Prev 14(1):53–60
Borgeest C, Miller KP, Gupta R, Greenfeld C, Hruska KS, Hoyer P, Flaws JA (2004) Methoxychlor-induced atresia in the mouse involves Bcl-2 family members, but not gonadotropins or estradiol. Biol Reprod 70(6):1828–1835
Brot C, Jorgensen NR, Sorensen OH (1999) The influence of smoking on vitamin D status and calcium metabolism. Eur J Clin Nutr 53:920–926
Brown JS, Zeman KL, Bennett WD (2002) Ultrafine particle deposition and clearance in the healthy and obstructed lung. Am J Respir Crit Care Med 166(9):1240–1247
Burch WM (2002) Passage of inhaled particles into the blood circulation in humans. Circulation 106(20):141–142
Castro KR, Ribeiro R, Peres M, Saldiva P, Matsuda M, Veras M (2013) Murine uterine receptivity markers are affected by particulate air pollution in a dose response manner. Placenta 34(2):A10
Chen J, Tan M, Nemmar A, Song W, Dong M, Zhang G, Li Y (2006) Quantification of extrapulmonary translocation of intratracheal-instilled particles in vivo in rats: effect of lipopolysaccharide. Toxicology 222:195–201
Chen TM, Shofer S, Gokhale J, Kuschner WG (2007) Outdoor air pollution: overview and historical perspective. Am J Med Sci 333(4):230–234
Chen H, Goldberg MS, Villeneuve PJ (2008) A systematic review of the relation between long-term exposure to ambient air pollution and chronic diseases. Rev Environ Health 23(4):243–297
Colbeck I, Lazaridis M (2010) Aerosols and environmental pollution. Naturwissenschaften 97(2):117–131
Coyle YM (2004) The effect of environment on breast cancer risk. Breast Cancer Res Treat 84(3):273–288
Crouse DL, Goldberg MS, Ross NA, Chen H, Labrèche F (2010) Postmenopausal breast cancer is associated with exposure to traffic-related air pollution in Montreal, Canada: a case-control study. Environ Health Perspect 118(11):1578–1583
Davis DL, Gottlieb MB, Stampnitzky JR (1998) Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? JAMA 279(13):1018–1023
De Rosa M, Zarrilli S, Paesano L, Carbone U, Boggia B, Petretta M et al (2003) Traffic pollutants affect fertility in men. Hum Reprod 18(5):1055–1061
Dejmek J, Jelinek R, Benes I, Sram RJ (1998) Impact of air pollution on human fertility: acute effects of SO2, NOx and PM. Epidemiology 9(4):148
Dejmek J, Selevan SG, Benes I, Solanský I, Srám RJ (1999) Fetal growth and maternal exposure to particulate matter during pregnancy. Environ Health Perspect 107(6):475–480
Dejmek J, Jelínek R, Solansky' I, Benes I, Srám RJ (2000) Fecundability and parental exposure to ambient sulfur dioxide. Environ Health Perspect 108(7):647–654
Dockery DW (2009) Health effects of particulate air pollution. Ann Epidemiol 19(4):257–263
EPA (1996) Guidelines for reproductive toxicity risk assessment. http://www.epa.gov/raf/publications/pdfs/REPRO51.PDF. Accessed 15 June 2013
EPA (2011) Report to congress: the benefits and costs of the Clean Air Act 1990 to 2020. U.S. http://www.epa.gov/oar/sect812/feb11/fullreport_rev_a.pdf. Accessed 2 Aug 2013
Faiz AS, Rhoads GG, Demissie K, Lin Y, Kruse L, Rich DQ (2013) Does ambient air pollution trigger stillbirth? Epidemiol 24(4):538–544
Fajersztajn L, Veras M, Barrozo LV, Saldiva P (2013) Air pollution: a potentially modifiable risk factor for lung cancer. Nat Rev Cancer 13(9):674–678
Ferin J, Oberdörster G, Penney DP (1992) Pulmonary retention of ultrafine and fine particles in rats. Am J Respir Cell Mol Biol 6(5):535–542

Gibbons AF, Chang MC (1972) Number of mast cells in the rat uterus with special reference to its relation to hormonal treatment and decidual response. Biol Reprod 6:193–203
Gilbert WM, Young AL, Danielsen B (2007) Pregnancy outcomes in women with chronic hypertension: a population-based study. J Reprod Med 52(11):1046–1051
Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D (2004) Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiol 15:36–45
Gluckman PD, Hanson MA (2004) Maternal constraint of fetal growth and its consequences. Semin Fetal Neonatal Med 9(5):419–425
Gouveia N, Bremner SA, Novaes HM (2004) Association between ambient air pollution and birth weight in São Paulo, Brazil. J Epidemiol Community Health 58(1):11–17
Guven A, Kayikci A, Cam K, Arbak P, Balbay O, Cam M (2008) Alterations in semen parameters of toll collectors working at motorways: does diesel exposure induce detrimental effects on semen? Andrologia 40(6):346–351
Ha EH, Hong YC, Lee BE, Woo BH, Schwartz J, Christiani DC (2001) Is air pollution a risk factor for low birth weight in Seoul? Epidemiol 12(6):643–648
Hall SA, Kaufman JS, Millikan RC, Ricketts TC, Herman D, Savitz DA (2005) Urbanization and breast cancer incidence in North Carolina, 1995–1999. Ann Epidemiol 15(10):796–803
Hammoud A, Carrell DT, Gibson M, Sanderson M, Parker-Jones K, Peterson CM (2010) Decreased sperm motility is associated with air pollution in Salt Lake City. Fertil Steril 93(6):1875–1879
Han Y, Xia Y, Zhu P, Qiao S, Zhao R, Jin N, Wang S, Song L, Fu G, Wang X (2010) Reproductive hormones in relation to polycyclic aromatic hydrocarbon (PAH) metabolites among non-occupational exposure of males. Sci Total Environ 408(4):768–773
Hansen C, Neller A, Williams G, Simpson R (2006) Maternal exposure to low levels of ambient air pollution and preterm birth in Brisbane, Australia. BJOG 113(8):935–941
Hansen C, Luben TJ, Sacks JD, Olshan A, Jeffay S, Strader L, Perreault SD (2010) The effect of ambient air pollution on sperm quality. Environ Health Perspect 118(2):203–209
Heal MR, Kumar P, Harrison RM (2012) Particles, air quality, policy and health. Chem Soc Rev 41:6606–6630
Helfand WH, Lazarus J, Theerman P (2001) Donora, Pennsylvania: an environmental disaster of the 20th century. Am J Public Health 91:553
Herr CE, Dostal M, Ghosh R, Ashwood P, Lipsett M, Pinkerton KE, Sram R, Hertz-Picciotto I (2010) Air pollution exposure during critical time periods in gestation and alterations in cord blood lymphocyte distribution: a cohort of livebirths. Environ Health 9:46
Herr CE, Ghosh R, Dostal M, Skokanova V, Ashwood P, Lipsett M, Joad JP, Pinkerton KE, Yap PS, Frost JD, Sram R, Hertz-Picciotto I (2011) Exposure to air pollution in critical prenatal time windows and IgE levels in newborns. Pediatr Allergy Immunol 22(1):75–84
Hirshfield AN (1997) Overview of ovarian follicular development: considerations for the toxicologist. Environ Mol Mutagen 29(1):10–15
Hood RD (2006) Developmental and reproductive toxicology: a practical approach, 2nd edn. CRC Press, Boca Raton
Hosseinpanah F, Pour SH, Heibatollahi M, Moghbel N, Asefzade S, Azizi F (2010) The effects of air pollution on vitamin D status in healthy women: a cross sectional study. BMC Public Health 10:519
Hoyer PB, Sipes IG (1996) Assessment of follicle destruction in chemical-induced ovarian toxicity. Annu Rev Pharmacol Toxicol 36:307–331
Hung LJ, Chan TF, Wu CH, Chiu HF, Yang CY (2012) Traffic air pollution and risk of death from ovarian cancer in Taiwan: fine particulate matter (PM2.5) as a proxy marker. J Toxicol Environ Health A 75(3):174–182
Huo Q, Zhang N, Wang X, Jiang L, Ma T, Yang Q (2013) Effects of ambient particulate matter on human breast cancer: is xenogenesis responsible? PLoS One 8(10):e76609. doi:10.1371/journal.pone.0076609. eCollection 2013
Huynh M, Woodruff TJ, Parker JD, Schoendorf KC (2006) Relationships between air pollution and preterm birth in California. Paediatr Perinat Epidemiol 20(6):454–461

Iwai K, Mizuno S, Miyasaka Y, Mori T (2005) Correlation between suspended particles in the environmental air and causes of disease among inhabitants: cross-sectional studies using the vital statistics and air pollution data in Japan. Environ Res 99(1):106–117

Izawa H, Kohara M, Watanabe G, Taya K, Sagai M (2007) Diesel exhaust particle toxicity on spermatogenesis in the mouse is aryl hydrocarbon receptor dependent. J Reprod Dev 53(5):1069–1078

Jafarabadi M (2007) Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. Hum Reprod 22(12):3263

Jedrychowski W, Bendkowska I, Flak E, Penar A, Jacek R, Kaim I, Spengler JD, Camann D, Perera FP (2004) Estimated risk for altered fetal growth resulting from exposure to fine particles during pregnancy: an epidemiologic prospective cohort study in Poland. Environ Health Perspect 112(14):1398–1402

Kannan S, Misra DP, Dvonch T, Krishnakumar A (2006) Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. Environ Health Perspect 114(11):1636–1642

Kannan S, Misra DP, Dvonch JT, Krishnakumar A (2007) Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential. Cien Saude Colet 12(6):1591–1602

Kelishadi R, Sharifi-Ghazvini F, Poursafa P, Mehrabian F, Farajian S, Yousefy H, Movahedian M, Sharifi-Ghazvini S (2013) Determinants of hypovitaminosis D in pregnant women and their newborns in a sunny region. Int J Endocrinol 2013:1–6

Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ (2008) Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). Am J Cardiol 102:1540–1544

Knottnerus JA, Delgado LR, Knipschild PG, Essed GG, Smits F (1990) Haematologic parameters and pregnancy outcome: a prospective cohortstudy in the third trimester. J Clin Epidemiol 43:461–466

Kok TM, Driece HA, Hogervorst JG, Briedé JJ (2006) Toxicological assessment of ambient and traffic-related particulate matter: a review of recent studies. Mutat Res 613(2–3):103–122

Kristensen P, Eilertsen E, Einarsdóttir E, Haugen A, Skaug V, Ovrebø S (1995) Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. Environ Health Perspect 103(6):588–590

Lange NE, Litonjua A, Hawrylowicz CM, Weiss S (2009) Vitamin D, the immune system and asthma. Expert Rev Clin Immunol 5:693–702

Laumbach RJ (2010) Outdoor air pollutants and patient health. Am Fam Physician 81(2):175–180

Lee PC, Roberts JM, Catov JM, Talbott EO, Ritz B (2013) First trimester exposure to ambient air pollution, pregnancy complications and adverse birth outcomes in Allegheny County, PA. Matern Child Health J 17(3):545–555

Leem JH, Kaplan BM, Shim YK, Pohl HR, Gotway CA, Bullard SM, Rogers JF, Smith MM, Tylenda CA (2006) Exposures to air pollutants during pregnancy and preterm delivery. Environ Health Perspect 114(6):905–910

Lewis-Michl EL, Melius JM, Kallenbach LR, Ju CL, Talbot TO, Orr MF, Lauridsen PE (1996) Breast cancer risk and residence near industry or traffic in Nassau and Suffolk Counties, Long Island, New York. Arch Environ Health 51(4):255–265

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, Nel A (2003) Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111(4):455–460

Lichtenfels AJ, Gomes JB, Pieri PC, El Khouri Miraglia SG, Hallak J, Saldiva PH (2007) Increased levels of air pollution and a decrease in the human and mouse male-to-female ratio in São Paulo, Brazil. Fertil Steril 87(1):230–232

Lloyd OL, Smith G, Lloyd MM et al (1985) Raised mortality from lung cancer and high sex ratios of births associated with industrial pollution. Br J Ind Med 42(7):475–480

Logan WP (1953) Mortality in the London fog incident, 1952. Lancet 1:336–338

Loomis D, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Baan R, Mattock H, Straif K, on behalf of the International Agency for Research on Cancer Monograph Working Group IARC, Lyon, France, on behalf of the International Agency for Research on Cancer Monograph Working Group IARC, Lyon, France (2013) The carcinogenicity of outdoor air pollution. Lancet Oncol 14(13):1262–1263

Maisonet M, Bush TJ, Correa A, Jaakkola JJ (2001) Relation between ambient air pollution and low birth weight in the Northeastern United States. Environ Health Perspect 109(3):351–356

Maisonet M, Correa A, Misra D, Jaakkola JJ (2004) A review of the literature on the effects of ambient air pollution on fetal growth. Environ Res 95(1):106–115

Mamatsashvili MI (1970) Detrimental effect of carbon monoxide and sulfur dioxide on fertility of female rats. Hyg Sanit 35(4–6):277–279

Mannes T, Jalaludin B, Morgan G, Lincoln D, Sheppeard V, Corbett S (2005) Impact of ambient air pollution on birth weight in Sydney, Australia. Occup Environ Med 62(8):524–530

Marx L, Arck P, Kieslich C, Mitterlechner S, Kapp M, Dietl J (1999) Decidual mast cells might be involved in the onset of human first-trimester abortion. Am J Reprod Immunol 41:34–40

Mattison DR, Thomford PJ (1989) The mechanisms of action of reproductive toxicants. Toxicol Pathol 17(2):364–376

McLachlan JA, Arnold SF (1996) Environmental estrogens. Am Sci 84:452–461

Menzel DB (1994) The toxicity of air pollution in experimental animals and humans: the role of oxidative stress. Toxicol Lett 72(1–3):269–277

Mills NL, Amin N, Robinson SD, Anand A, Davies J, Patel D, de la Fuente JM, Cassee FR, Boon NA, Macnee W, Millar AM, Donaldson K, Newby DE (2006) Do inhaled carbon nanoparticles translocate directly into the circulation in humans? Am J Respir Crit Care Med 173(4):426–431

Miraglia SG, Veras MM, Amato-Lourenço LF, Rodrigues-Silva F, Saldiva PH et al (2013) Follow-up of the air pollution and the human male-to-female ratio analysis in Sao Paulo, Brazil: a times series study. BMJ Open 3(7). pii: e002552. doi:10.1136/bmjopen-2013-002552

Mohallem SV, de Araújo Lobo DJ, Pesquero CR, Assunção JV, de Andre PA, Saldiva PH, Dolhnikoff M (2005) Decreased fertility in mice exposed to environmental air pollution in the city of Sao Paulo. Environ Res 98(2):196–202

Möller W, Felten K, Sommerer K, Scheuch G, Meyer G, Meyer P, Häussinger K, Kreyling WG (2008) Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. Am J Respir Crit Care Med 177(4):426–432

Morello-Frosch R, Jesdale BM, Sadd JL, Pastor M (2010) Ambient air pollution exposure and full-term birth weight in California. Environ Health 28(9):44

Need AG, Kemp A, Giles N, Morris HA, Horowitz M, Nordin BE (2002) Relationships between intestinal calcium absorption, serum vitamin metabolites and smoking in postmenopausal women. Osteoporos Int 13:83–88

O'Neill MS, Jerrett M, Kawachi I et al (2003) Health, wealth, and air pollution: advancing theory and methods. Environ Health Perspect 111:1861–1870

Oberdorster G, Utell MJ (2002) Ultrafine particles in the urban air: to the respiratory tract – and beyond? Environ Health Perspect 110:440–441

Olsson D, Mogren I, Forsberg B (2013) Air pollution exposure in early pregnancy and adverse pregnancy outcomes: a register-based cohort study. BMJ Open 3(2):1–8

Ono Y, Takagi K, Seki H, Takai Y, Samejima K, Matsunaga S, Matsumura H (2013) Neonatal outcome in infants of chronically hypertensive mothers. J Obstet Gynaecol Res 39(6):1142–1146

Osmond C, Barker DJ (2000) Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ Health Perspect 108 Suppl 3:545–553

Parent MÉ, Goldberg MS, Crouse DL, Ross NA, Chen H, Valois MF, Liautaud A (2013) Traffic-related air pollution and prostate cancer risk: a case-control study in Montreal, Canada. Occup Environ Med 70(7):511–518

Parker JD, Woodruff TJ (2008) Influences of study design and location on the relationship between particulate matter air pollution and birthweight. Paediatr Perinat Epidemiol 22(3):214–227
Pekkanen J, Brunner EJ, Anderson HR, Tiittanen P, Atkinson RW (2000) Daily concentrations of air pollution and plasma fibrinogen in London. Occup Environ Med 57:818–822
Pereira LAA, Loomis D, Conceicao GMS et al (1998) Association between air pollution and intrauterine mortality in Sao Paulo, Brazil. Environ Health Perspect 106:325–329
Perin PM, Maluf M, Czeresnia CE, Januário DA, Saldiva PHN (2010a) Impact of short-term preconceptional exposure to particulate air pollution on treatment outcome in couples undergoing in vitro fertilization and embryo transfer (IVF/ET). J Assist Reprod Genet 27(7):371–382
Perin PM, Maluf M, Czeresnia CE, Nicolosi Foltran Januário DA, Saldiva PHN (2010b) Effects of exposure to high levels of particulate air pollution during the follicular phase of the conception cycle on pregnancy outcome in couples undergoing in vitro fertilization and embryo transfer. Fertil Steril 93(1):301–303
Peters A, Döring A, Wichmann HE, Koenig W (1997) Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349(9065):1582–1587
Ponce NA, Hoggatt KJ, Wilhelm M, Ritz B (2005) Preterm birth: the interaction of traffic-related air pollution with economic hardship in Los Angeles neighborhoods. Am J Epidemiol 162(2):140–148
Pope CA 3rd (2000) Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? Environ Health Perspect 108 Suppl 4:713–723
Pope CA 3rd, Ezzati M, Dockery DW (2009) Fine-particulate air pollution and life expectancy in the United States. N Engl J Med 360(4):376–386
Rahman I, MacNee W (2000) Oxidative stress and regulation of glutathione in lung inflammation. Eur Respir J 16(3):534–554
Ren C, Tong S (2008) Health effects of ambient air pollution-recent research development and contemporary methodological challenges. Environ Health 7:56
Reynolds P, Hurley S, Goldberg DE, Anton-Culver H, Bernstein L, Deapen D et al (2004) Regional variations in breast cancer among California teachers. Epidemiol 15(6):746–754
Ritz B, Wilhelm M (2008) Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. Basic Clin Pharmacol Toxicol 102(2):182–190
Ritz B, Yu F, Chapa G, Fruin S (2000) Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. Epidemiol 11(5):502–511
Rocha E, Silva IR, Lichtenfels AJ, Amador Pereira LA, Saldiva PH (2008) Effects of ambient levels of air pollution generated by traffic on birth and placental weights in mice. Fertil Steril 90(5):1921–1924
Rowe T (2006) Fertility and a woman's age. J Reprod Med 51:157–163
Rubes J, Selevan SG, Evenson DP, Zudova D, Vozdova M, Zudova Z, Robbins WA, Perreault SD (2005) Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. Hum Reprod 20(10):2776–2783
Rudel RA, Attfield KA, Schifano JN et al (2007) Chemicals causing mammary gland tumors in animals signal new directions for epidemiology, chemicals testing, and risk assessment for breast cancer prevention. Cancer 109:2635–2666
Rudra CB, Williams MA, Sheppard L, Koenig JQ, Schiff MA (2011) Ambient carbon monoxide and fine particulate matter in relation to preeclampsia and preterm delivery in western Washington State. Environ Health Perspect 119(6):886–892
Sadeu JC, Hughes CL, Agarwal S, Foster WG (2010) Alcohol, drugs, caffeine, tobacco, and environmental contaminant exposure: reproductive health consequences and clinical implications. Crit Rev Toxicol 40(7):633–652
Saldiva PHN, Clarke RW, Coull BA, Stearns RC, Lawrence J, Krishna Murthy GG, Diaz E, Koutrakis P, Suh H, Tsuda A, Godleski JJ (2002) Lung inflammation induced by concentrated ambient air particles is related to particle composition. Am J Respir Crit Care Med 165:1610–1617
Schnorr TM, Lawson CC, Whelan EA et al (2001) Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Health Perspect 109(11):1127–1132

Schwartz J (2006) Long-term effects of exposure to particulate air pollution. Clin Occup Environ Med 5(4):837–848

Scoriza J, Saldiva PHN, Caldini EG, Veras MM (2009) Effects of pre-gestational and gestational exposure to air pollution (particulate matter) on mast cell density in implantation sites of mice. In: Abstracts of the II symposium on reproductive immunology, Rio de Janeiro, 2009

Seaton A, Macnee W, Donald K, Godden D (1995) Particulate air pollution and acute health effects. Lancet 345:176–178

Selevan SG, Borkovec L, Slott VL, Zudová Z, Rubes J, Evenson DP, Perreault SD (2000) Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect 108(9):887–894

Sioutas C, Delfino R, Singh M (2005) Exposure assessment for atmospheric ultrafine particles (UFPs) and implications for epidemiologic research. Environ Health Perspect 113:947–955

Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, Glinianaia S, Hoggatt KJ, Kannan S, Hurley F, Kalinka J, Srám R, Brauer M, Wilhelm M, Heinrich J, Ritz B (2008) Meeting report: atmospheric pollution and human reproduction. Environ Health Perspect 116(6):791–798

Slama R, Bottagisi S, Solansky I, Lepeule J, Giorgis-Allemand L, Sram R (2013) Short-term impact of atmospheric pollution on fecundability. Epidemiology 24(6):871–879

Sokol RZ, Kraft P, Fowler IM, Mamet R, Kim E, Berhane KT (2006) Exposure to environmental ozone alters semen quality. Environ Health Perspect 114(3):360–365

Soll-Johanning H, Bach E (2004) Occupational exposure to air pollution and cancer risk among Danish urban mail carriers. Int Arch Occup Environ Health 77(5):351–356

Somers CM (2011) Ambient air pollution exposure and damage to male gametes: human studies and in situ 'sentinel' animal experiments. Syst Biol Reprod Med 57:63–71

Somers CM, Cooper DN (2009) Air pollution and mutations in the germline: are humans at risk? Hum Genet 125(2):119–130

Somers CM, Yauk CL, White PA, Parfett CLJ, Quinn JS (2002) Air pollution induces heritable DNA mutations. Proc Natl Acad Sci 99:15904–15907

Somers CM, McCarry BE, Malek F, Quinn JS (2004) Reduction of particulate air pollution lowers the risk of heritable mutations in mice. Science 304:1008–1010

Sørensen M, Daneshvar B, Hansen M, Dragsted LO, Hertel O, Knudsen L, Loft S (2003) Personal PM2.5 exposure and markers of oxidative stress in blood. Environ Health Perspect 111:161–166

Squadrito GL, Cueto R, Dellinger B, Pryor WA (2001) Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. Free Radic Biol Med 31(9):1132–1138

Srám RJ, Binková B, Dejmek J, Bobak M (2005) Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect 113(4):375–382

Sriramachari S (2005) Bhopal gas tragedy: scientific challenges and lessons for future. J Loss Prev Process Ind 18(4–6):264–267

Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ (2008) Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod Sci 15(7):631–650

Takeda K, Tsukue N, Yoshida S (2004) Endocrine-disrupting activity of chemicals in diesel exhaust and diesel exhaust particles. Environ Sci 11(1):33–45

Takenaka S, Dornhofer-Takenaka H, Muhle H (1986) Alveolar distribution of fly ash and of titanium dioxide after long-term inhalation by Wistar rats. J Aerosol Sci 17:361–364

Telisman S, Colak B, Pizent A, Jurasović J, Cvitković P (2007) Reproductive toxicity of low-level lead exposure in men. Environ Res 105(2):256–266

Terrell ML, Hartnett KP, Marcus M (2011) Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. Emerg Health Threats J 4:1–18

Thurston GD, Balmes JR (2012) Particulate matter and the environmental protection agency setting the right standard. Am J Respir Cell Mol Biol 47(6):727–728

Tragaki A, Lasaridi K (2009) Temporal and spatial trends in the sex ratio at birth in Greece, 1960–2006: exploring potential environmental factors. Popul Environ 30(3):114–128

Tsukue N, Toda N, Tsubone H, Sagai M, Jin WZ, Watanabe G, Taya K, Birumachi J, Suzuki AK (2001) Diesel exhaust (DE) affects the regulation of testicular function in male Fischer 344 rats. J Toxicol Environ Health A 63(2):115–126

Vahlsing C, Smith KR (2012) Global review of national ambient air quality standards for PM10 and SO2 (24 h). Air Qual Atmos Health 5:393–399

Valavanidis A, Fiotakis K, Vlachogianni T (2008) Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 26(4):339–362

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, Zandveld PY, Mackenbach JP, Steegers EA, Miedema HM, Jaddoe VW (2012a) Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. Environ Health Perspect 120(1):150–156

van den Hooven EH, Pierik FH, Kluizenaar Y, Hofman A, van Ratingen SW, Zandveld PY, Russcher H, Lindemans J, Miedema HM, Steegers EA, Jaddoe VW (2012b) Air pollution exposure and markers of placental growth and function: the generation R study. Environ Health Perspect 120(12):1753–1759

Van Erp AM, O'Keefe R, Cohen AJ, Warren J (2008) Evaluating the effectiveness of air quality interventions. J Toxicol Environ Health A 71:583–587

Veras MM, Damaceno-Rodrigues NR, Caldini EG, Maciel Ribeiro AA, Mayhew TM, Saldiva PH, Dolhnikoff M (2008) Particulate urban air pollution affects the functional morphology of mouse placenta. Biol Reprod 79:578–584

Veras MM, Damaceno-Rodrigues NR, Guimarães Silva RM, Scoriza JN, Saldiva PH, Caldini EG, Dolhnikoff M (2009) Chronic exposure to fine particulate matter emitted by traffic affects reproductive and fetal outcomes in mice. Environ Res 109(5):536–543

Veras MM, Guimarães-Silva RM, Caldini EG, Saldiva PH, Dolhnikoff M, Mayhew TM (2012) The effects of particulate ambient air pollution on the murine umbilical cord and its vessels: a quantitative morphological and immunohistochemical study. Reprod Toxicol 34(4):598–606

Vorherr H (1982) Factors influencing fetal growth. Am J Obstet Gynecol 142:577–588

Vrijheid M, Martinez D, Manzanares S, Dadvand P, Schembari A, Rankin J, Nieuwenhuijsen MJ (2011) Ambient air pollution and risk of congenital anomalies: a systematic review. Environ Health Perspect 119:598–606

Wang X, Ding H, Ryan L, Xu X (1997) Association between air pollution and low birth weight: a community-based study. Environ Health Perspect 105(5):514–520

Wenger D, Gerecke AC, Heeb NV, Schmid P, Hueglin C, Naegeli H, Zenobi R (2009) In vitro estrogenicity of ambient particulate matter: contribution of hydroxylated polycyclic aromatic hydrocarbons. J Appl Toxicol 29(3):223–232

WHO (2005) Air quality guidelines – global update 2005. http://www.euro.who.int/__data/assets/pdf_file/0005/78638/E90038.pdf. Accessed 10 July 2013

Wiebert P, Sanchez-Crespo A, Falk R, Philipson K, Lundin A, Larsson S, Möller W, Kreyling WG, Svartengren M (2006a) No significant translocation of inhaled 35-nm carbon particles to the circulation in humans. Inhal Toxicol 18(10):741–747

Wiebert P, Sanchez-Crespo A, Seitz J, Falk R, Philipson K, Kreyling WG, Möller W, Sommerer K, Larsson S, Svartengren M (2006b) Negligible clearance of ultrafine particles retained in healthy and affected human lungs. Eur Respir J 28(2):286–290

Wilhelm M, Ritz B (2003) Residential proximity to traffic and adverse birth outcomes in Los Angeles county, California, 1994–1996. Environ Health Perspect 111(2):207–216

Williams FL, Lawson AB, Lloyd OL (1992) Low sex ratios of births in areas at risk from air pollution from incinerators, as shown by geographical analysis and 3-dimensional mapping. Int J Epidemiol 21(2):311–319

Woodruff TK, Walker CL (2008) Fetal and early postnatal environmental exposures and reproductive health effects in the female. Fertil Steril 89:47–51

Woodruff TJ, Parker JD, Kyle AD, Schoendorf KC (2003) Disparities in exposure to air pollution during pregnancy. Environ Health Perspect 111(7):942–946

Woodruff TJ, Carlson A, Schwartz JM, Giudice LC (2008) Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary. Fertil Steril 89(2 Suppl):e1–e20

World Bank (2013a) Fertility rate (total births per women). http://data.worldbank.org/indicator/SP.DYN.TFRT.IN. Accessed 15 July 2013.

World Bank (2013b) PM10, country level (micrograms per cubic meter). http://data.worldbank.org/indicator/EN.ATM.PM10.MC.M3. Accessed 15 July 2013.

Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B (2009) Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. Environ Health Perspect 117(11):1773–1779

Yang CY, Chang CC, Chuang HY, Ho CK, Wu TN, Tsai SS (2003) Evidence for increased risks of preterm delivery in a population residing near a freeway in Taiwan. Arch Environ Health 58(10):649–654

Yauk CL, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ et al (2008) Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. Proc Natl Acad Sci 105:605–610

Chapter 3
Air Pollution and Pregnancy Outcomes

Sharon Edwards, Pamela Maxson, Nicole Sandberg, and Marie Lynn Miranda

3.1 Introduction

Adverse pregnancy outcomes – like low birth weight, preterm birth, intrauterine growth restriction, and small for gestational age – have long been recognized as a significant public health concern, with significant short and long term health, social, and economic consequences for the children, their families, and the community. Of additional concern, poor pregnancy outcomes are characterized by pronounced health disparities, with low income and racial/ethnic minority mothers having significantly worse outcomes. While the United States has worked aggressively to reduce poor pregnancy outcomes, especially through programs emphasizing universal access to good prenatal care, disparities remain. For example, in 2011, 16.75 % of births to non-Hispanic black mothers were preterm and 13.33 % were low birth weight, while 10.49 % of births to non-Hispanic white mothers were preterm and 7.09 % were low birth weight (Hamilton et al. 2012). Further, these disparities vary

The development of this chapter was supported by a grant from the U.S. Environmental Protection Agency (RD-83329301).

S. Edwards, MStat • P. Maxson, Ph.D. • N. Sandberg, MURP
School of Natural Resources and Environment, University of Michigan,
440 Church Street, Ann Arbor, MI 48109, USA

M.L. Miranda, Ph.D. (✉)
School of Natural Resources and Environment, University of Michigan,
440 Church Street, Ann Arbor, MI 48109, USA

Department of Pediatrics, University of Michigan, Ann Arbor, MI USA
e-mail: mlmirand@umich.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_3

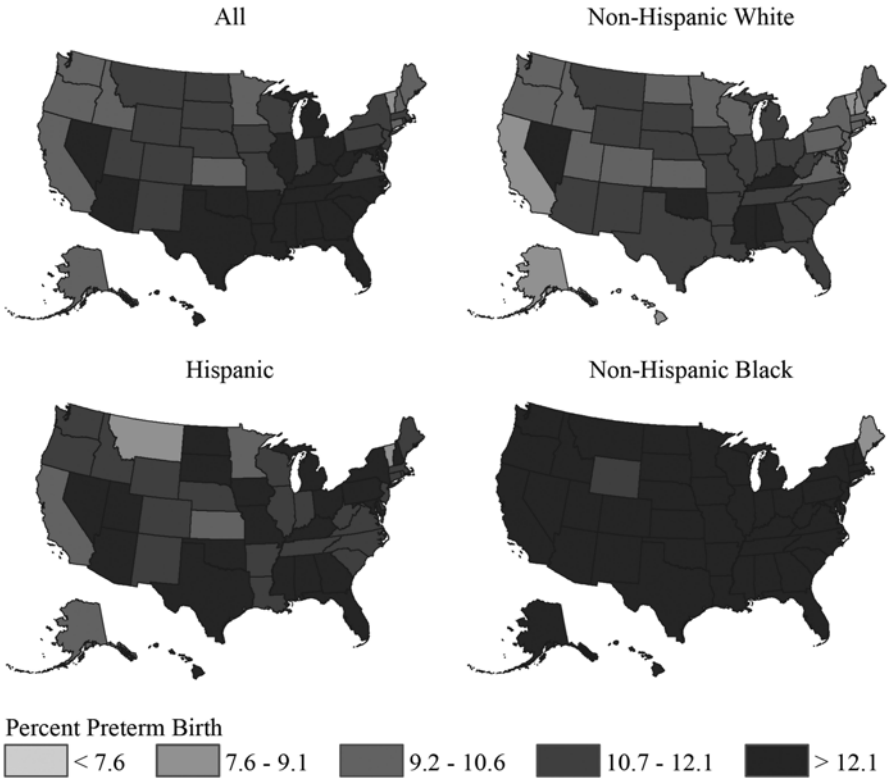

Fig. 3.1 US 2010 preterm birth rates by race. Note, 7.6 % was the Healthy People 2010 target (U.S.Centers for Disease Control and Prevention 2013)

regionally, as highlighted by the maps of state 2010 preterm birth rates displayed in Fig. 3.1 (U.S.Centers for Disease Control and Prevention 2013).

In trying to understand the causes of both poor pregnancy outcomes, patterns of disparities may be informative. Of note, many environmental exposures disproportionately affect low income and racial/ethnic minority populations. In 2010, the U.S. Environmental Protection Agency estimated that 123.8 million people were living in U.S. counties with concentrations of one or more air pollutants above the level of the National Ambient Air Quality Standards (NAAQS) (U.S.Environmental Protection Agency 2011). Based on estimates from 2006 to 2009, 26.2 % of Hispanics and 15.2 % of African Americans, compared to 9.7 % of Caucasians, live in counties failing to meet $PM_{2.5}$ standards and 48.4 % of Hispanics and 40 % of African Americans, compared to 32 % of Caucasians, live in counties failing to meet ozone standards (Yip et al. 2011). Figure 3.2 depicts the percentage of non-Hispanic whites and non-Hispanic blacks that live along the spectrum of air quality for $PM_{2.5}$ based on a grading methodology defined by the American Lung Association in which areas receiving an "A" grade have the best air quality and areas receiving an "F" grade have the poorest air quality (American Lung Association 2011).

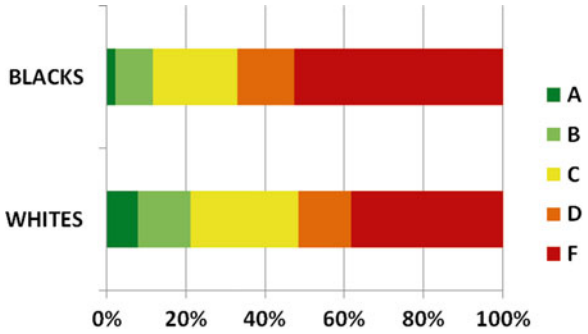

Fig. 3.2 For populations living in areas with a $PM_{2.5}$ air pollution grade from the American Lung Association, proportion living in areas with different air quality grades (American Lung Association 2011)

The disproportionate exposure burden borne by disadvantaged populations, coupled with persistent disparities in health outcomes, has led to extensive investigations into the relationship between environmental exposures and adverse health outcomes, including poor pregnancy outcomes. While previous research covers many different types of exposures and health endpoints, in this chapter, we focus on the impact that air pollution may have on poor pregnancy outcomes.

3.2 Long Term and Short Term Importance of Pregnancy Outcomes

Adverse pregnancy outcomes, including low birth weight, preterm delivery, and fetal growth restriction, have significant implications for affected children's morbidity and mortality. Preterm birth and low birth weight are leading causes of neonatal and infant mortality, as well as short and long term morbidity (Behrman and Butler 2007; Hack et al. 1995). Childhood health conditions associated with poor pregnancy outcomes include respiratory distress syndrome (Lemons et al. 2001), variable heart rate (Doussard-Roosevelt et al. 1997), and cerebral ventriculomegaly (Ment et al. 1999). Delays and decrements in development, including mental retardation (Lorenz et al. 1998; Crofts et al. 1998), vision and hearing difficulties (Avchen et al. 2001), learning disabilities (Saigal et al. 2000; Resnick et al. 1999), lower academic achievement and attainment (Swamy et al. 2008), increased special education needs (Petrou 2005), behavioral disabilities (Ross et al. 1990), and motor impairment (Osmond et al. 1993; Weiss et al. 2004), are also associated with adverse pregnancy outcomes. Long term, health consequences include increased risk of diabetes, obesity, cardiovascular disease, and other health problems in adulthood (Osmond et al. 1993; Weiss et al. 2004; Barker et al. 1993). Later reproductive ability and pregnancy outcomes in subsequent generations are also affected by pregnancy outcomes (Swamy et al. 2008).

These short and long term effects also generate substantial costs, including health care costs incurred by their families and communities. In the immediate term,

infants born prematurely or with sub-optimal fetal growth patterns experience longer birth hospitalizations (Wang et al. 2004) and increased medical system utilization (Stevenson et al. 1996) compared to healthy infants. In 2006, the estimated annual cost of preterm birth in the United States was roughly $26 billion, or approximately $51,600 per preterm infant; two-thirds of these costs were related to medical care (Behrman and Butler 2007). As these children grow, programs to address developmental delays and disabilities may also be necessary, costing both families and education systems significantly (Weiland et al. 2011; Lipkind et al. 2012; Robb et al. 2011). Finally, the increased risk of adult health issues associated with poor pregnancy outcomes have long term implications for health care costs (Heidenreich et al. 2011; Zhang et al. 2010; Wang et al. 2011). Thus, understanding and ameliorating the impact of air pollution on adverse pregnancy outcomes could contribute toward improving the overall health of current and future generations, as well as containment of health care costs.

3.3 A Review of Previous Research

There is a long history of research as to the effects air pollution may have on poor pregnancy outcomes (Glinianaia et al. 2004; Wang et al. 1997; Sram et al. 2005; Bobak and Leon 1999). We undertook a review of the recent literature on the topic in order to summarize the current state of understanding. The literature review was completed using the PubMed database maintained by the National Center for Biotechnology Information, located at the National Institutes of Health. We limited our review to a subset of ambient outdoor air pollutants and pregnancy outcomes. Pollutant keywords included: particulate matter, sulfur dioxide, ozone, total suspended particles, black smoke, toxic metals, nitrous oxide, nitrogen oxide, nitrogen dioxide, carbon monoxide, polycyclic aromatic hydrocarbons, traffic, emissions, proximity to road, and dioxin. Outcome keywords included: birth weight, preterm birth, small for gestational age, intrauterine growth restriction, and intrauterine growth retardation. Each possible combination of pollutant keywords and outcome keywords was searched in all fields (e.g. ozone and birth weight, ozone and preterm birth, etc).

After removing duplicates and restricting to publications released between January 1, 2000 and December 31, 2012, we were left with 398 articles. A title and abstract review eliminated 243 articles which studied indoor air pollution exposures, accidental/occupational air pollution exposures, tobacco-related exposures, or outcomes other than those focused on here. Upon further review, we excluded 21 articles that were unrelated, commentaries, not in English, or could not be accessed. After comparing our results to the references of selected review articles, we found an additional five articles that were not previously captured. This approach resulted in 139 articles, of which 16 were reviews.

These articles focus on exposures to criteria air pollutants (carbon monoxide [CO], particulate matter [PM], nitrogen dioxide [NO_2], sulfur dioxide [SO_2], and

ozone [O_3]), polycyclic aromatic hydrocarbons (PAHs), and traffic-related air pollution during pregnancy. PM is composed of many constituent components, and some studies attempt to differentiate among the species of particles; however, here we focus on studies utilizing the composite measures of PM_{10} (coarse PM; particles <10 μm in diameter) and $PM_{2.5}$ (fine PM; particles ≤2.5 μm in diameter). Similarly, traffic-related air pollution is composed of many chemicals, particulates, and air toxics. Studies focusing on traffic-related air pollution tend to use proxies or particular chemical markers for this mixture of pollutants.

We extracted information on six pregnancy outcome measures: continuous birth weight, low birth weight, continuous gestational age, preterm birth, small for gestational age, and term low birth weight. Continuous birth weight is most commonly measured in grams, and low birth weight is a binary classification indicating a birth weight of less than 2,500 g. Continuous gestational age is measured as the number of weeks of gestation that have been completed prior to delivery, and preterm birth is a binary classification indicating a birth at less than 37 weeks of gestation. The final two outcomes, small for gestational age and term low birth weight attempt to account for both birth weight and length of gestation by characterizing the rate of fetal growth. Small for gestational age is a binary classification indicating an infant weighing in the bottom 10 % of all infants born at the same gestational age. Term low birth weight is also a binary classification of fetal growth, indicating infants born at 37 or more weeks of gestation but failing to reach the expected birth weight range of at least 2,500 g. Low birth weight and preterm birth have a long history as public health indicators and are the most familiar pregnancy outcomes; however, all six outcomes have numerous short and long term risks to child health and development. Additionally, these outcomes are often correlated and do not occur in isolation. For example, birth weight and gestational age are highly correlated, even in the presence of growth restriction. Consequently, we cannot rank the pregnancy outcomes in terms of relative importance, but rather emphasize the overall importance of achieving the best possible outcomes across measures.

Table 3.1 provides the distribution of articles by pollutants and outcomes considered. Note that the article counts do not add up to the number of articles found in the literature review as most articles explored both multiple pollutants and pregnancy outcomes.

Table 3.1 Article count by pollutant and outcome

	Pollutant							
Outcome	CO	O_3	PM_{10}	$PM_{2.5}$	SO_2	NO_2	PAHs	Traffic-related
Birth weight	11	9	21	20	13	22	7	8
Low birth weight	9	5	9	4	10	9	1	3
Gestational age	2	1	2	3	0	0	1	1
Preterm birth	10	14	18	13	8	20	2	6
Small for gestational age	7	8	10	8	5	13	4	5
Term low birth weight	7	8	12	8	7	11	2	4

3.3.1 Air Pollution and Length of Gestation

3.3.1.1 Continuous Gestational Age

Gestational age is the number of completed weeks of gestation at the time of delivery. Current research offers a very limited understanding of the association between continuous measures of gestational age and air pollution, as relatively few studies have considered continuous gestational age as an outcome. One study based on birth registry data in New Jersey found that, when controlling for air pollution exposure during each of the three trimesters, exposure to higher levels of CO during the third trimester was associated with shorter gestations (Currie et al. 2009). This study did not observe associations between trimester exposures to PM_{10} or O_3 and length of gestation (Currie et al. 2009). A study in Sweden, however, observed decrements in length of gestation associated with second trimester and pregnancy length exposure to O_3 (Olsson et al. 2012). Exposure to SO_2 during early pregnancy was associated with reduced length of gestation among a cohort of women living near a Croatian coal-fired power plant (Mohorovic 2004). Road density, a metric for traffic-related air pollution, was also found to be negatively associated with continuous gestational age (Barnett et al. 2011). No association was found between continuous gestational age and $PM_{2.5}$ (Jedrychowski et al. 2004) or PAH (Masters et al. 2007) exposure during the second trimester of pregnancy, and our review did not find any studies of the effect of NO_2 on gestational age.

With only six studies specifically considering continuous gestational age as an outcome and little overlap of pollutants across these studies, we are unable to draw general conclusions regarding the effect of air pollution on continuous length of gestation. While the short and long term implications of preterm birth are well-documented, recent studies have described differences in long term health and development by gestational age at delivery even among children born at term ($\geq$37 weeks), highlighting the importance of understanding the relationship between air pollution and continuous gestational age. Further research in this area is necessary to better understand how air pollution may affect gestation across the full spectrum of gestational ages, even those extending into the term range.

3.3.1.2 Preterm Birth

Preterm birth (PTB; <37 week of gestation at delivery), in contrast to continuous gestational age, is a well-studied pregnancy outcome in the air pollution literature. Each of the criteria air pollutants, as well as traffic-related air pollution, has been associated with increased likelihood of PTB in studies varying in geographic location, pollution level, and exposure windows; however, other studies have also reported null findings between air pollution and PTB. Estimated increases in risk of PTB were generally small, but significant, especially among some subpopulations. PAH has also been associated with PTB; however, the evidence to date is quite limited.

Studies of the effect of CO exposure during pregnancy on the risk of PTB have generally focused on early and late pregnancy exposure windows and trimesters. One study did consider pregnancy-length exposure to CO, but did not find a relationship between exposure and PTB (Huynh et al. 2006). Early pregnancy exposure to CO, including exposures during the first month and first trimester of pregnancy, has been linked to increased likelihood of PTB (Ritz et al. 2000, 2007; Wilhelm and Ritz 2005). A study in Los Angeles, California, noted that first trimester CO exhibited a larger effect on the odds of PTB with high co-exposure to $PM_{2.5}$ (Ritz et al. 2007). On the other hand, several studies did not find associations between CO exposure during early pregnancy and PTB (Huynh et al. 2006; Jalaludin et al. 2007; Rudra et al. 2011). Inconsistent results were also observed for CO exposure later in pregnancy, with increases in PTB associated with CO during the last month (Liu et al. 2003; Wilhelm et al. 2011) and last 6 weeks (Ritz et al. 2000; Wilhelm and Ritz 2005) of pregnancy in some studies, but not in others (Huynh et al. 2006; Jalaludin et al. 2007; Rudra et al. 2011).

Increased risk of PTB has also been associated with O_3 exposure, primarily during early pregnancy. Higher levels of O_3 during the first 5 weeks (Huynh et al. 2006) and first trimester (Olsson et al. 2012; Jalaludin et al. 2007; Hansen et al. 2006; Lee et al. 2012; Kim et al. 2007) of pregnancy increased risk of PTB in some studies; however, results from a study in Sydney, Australia, were dependent on spatial scale, with the effect of first trimester O_3 significantly associated with PTB using a metro-wide exposure measure, but not significant for women living within 5 km of a monitor (Jalaludin et al. 2007). Exposure to O_3 during the last 3 months of pregnancy (Jalaludin et al. 2007; Hansen et al. 2006), 6 weeks prior to delivery (Kim et al. 2007; Lee et al. 2008), or the third trimester (Hansen et al. 2006) were generally not significantly associated with PTB in any of the studies reviewed. One study, conducted in Seoul, South Korea, did observe increased risk of PTB with increasing O_3 exposure during the third trimester (Kim et al. 2007), and another study, conducted in Los Angeles, CA, found no consistent relationship between ozone exposure during pregnancy and PTB (Ritz et al. 2007).

PTB has been linked with coarse particulate matter, PM_{10}, exposure throughout pregnancy in studies of varying populations and across varying levels of exposure. PM_{10} exposure during the first month (Ritz et al. 2000; Hansen et al. 2006), first trimester (Hansen et al. 2006), third trimester (Suh et al. 2008), last 6 weeks (Ritz et al. 2000), and last few days (Zhao et al. 2011) of pregnancy have been significantly associated with increased risk of PTB. Suh et al. found PM_{10} exposure during each trimester to increase the risk of PTB, with exposure during the first and third trimesters being of particular concern (Suh et al. 2009). Similar to other pollutants, results were not consistently significant across all studies (Wilhelm and Ritz 2005; Jalaludin et al. 2007; Hansen et al. 2006; Lee et al. 2008; Sagiv et al. 2005). These differences may be due to a variety of exposure assessment and study design conditions, as well as variations in study population vulnerability. For example, one study observed variations in genetic susceptibility to PM_{10} exposure, with third trimester exposure being differentially associated with PTB by genotype (Suh et al. 2008).

Fine particulate matter, $PM_{2.5}$, exposure throughout pregnancy has also been significantly associated with increased PTB. Higher levels of pregnancy-length exposure to $PM_{2.5}$ have been consistently associated with increased risk of PTB (Huynh et al. 2006; Wu et al. 2009; Chang et al. 2012; van den Hooven et al. 2012). In one study that included multi-pollutant models, these results were robust to control for co-pollutants (Huynh et al. 2006). Exposure to $PM_{2.5}$ during the first and second trimesters have also been linked to PTB in some (Lee et al. 2012; Chang et al. 2012), but not all studies (Jalaludin et al. 2007; Rudra et al. 2011; van den Hooven et al. 2012). The susceptibility of these results to study design is highlighted by a study in Sydney, Australia, which found a significant association between last 3 month $PM_{2.5}$ and PTB for women within 5 km of a monitoring site, but not in models using metro-wide exposure levels. Considering even smaller windows of exposure also revealed inconsistent results. One study observed associations between $PM_{2.5}$ and PTB during only the 4th through 22nd weeks of pregnancy (Warren et al. 2012), while other studies observed increased PTB associated with exposure during the first month and last 2 weeks of pregnancy (Huynh et al. 2006).

Fewer studies have considered NO_2 in relation to PTB, and results from these studies are unclear for all windows of exposure. Pregnancy-length exposure to NO_2 was associated with PTB in two studies (Wu et al. 2009; Llop et al. 2010), but not a third (Gehring et al. 2011a). While Llop et al. found increased risk of PTB with NO_2 exposure during the second and third but not the first trimester (Llop et al. 2010) in a Spanish population, Maroziene and Grazuleviciene found the opposite result for each trimester in a Lithuanian population (Maroziene and Grazuleviciene 2002). Two other studies found no associations between NO_2 exposure during any trimester and PTB (Ritz et al. 2007; Gehring et al. 2011a). NO_2 levels during the first 3 months and last 3 months of pregnancy were not found to be associated with PTB (Jalaludin et al. 2007; Hansen et al. 2006). Short term exposure windows in late pregnancy, such as the last month or last 6 weeks, however, were generally associated with higher risk of PTB (Wilhelm et al. 2011; Zhao et al. 2011; Satrell et al. 2012; Darrow et al. 2009), although one study did not find an association between PTB and NO_2 exposure during the last month of pregnancy (Jalaludin et al. 2007).

The effect of SO_2 on risk of PTB has also not been as well-studied as particulate matter, CO, and O_3; however, there is some evidence that SO_2 increases the risk of PTB. Bobak observed a significant association between pregnancy-length exposure to SO_2 and PTB (Bobak 2000). Shorter term exposures during late pregnancy, capturing just a few days before delivery, were observed to increase the risk of PTB (Zhao et al. 2011), while longer exposure windows in late pregnancy, such as the 6 weeks or 3 months before delivery, were not associated with PTB (Jalaludin et al. 2007; Sagiv et al. 2005; Darrow et al. 2009). One study did not find an association between SO_2 in the last month of pregnancy and PTB (Jalaludin et al. 2007); however, another study did observe a significant association between SO_2 levels in the last month of pregnancy and PTB, even when controlling for co-pollutant levels (Liu et al. 2003). Early pregnancy SO_2 exposures did not appear to be associated with an increased risk of PTB (Jalaludin et al. 2007; Darrow et al. 2009), although geographic scale seemed to impact the findings for first trimester SO_2 exposure

(Jalaludin et al. 2007). Further exploration of the relationship between PTB and SO_2 exposure is warranted.

Studies exploring the relationship between PTB and traffic-related air pollution fairly consistently observed an increased risk of PTB among women exposed to higher levels of traffic-related air pollution (Yang et al. 2003a; Ponce et al. 2005; Wilhelm and Ritz 2003; Miranda et al. 2013; Genereux et al. 2008). Results are difficult to compare across studies, since proximity to roadway and traffic density exposure metrics were constructed differently across studies. In a Taiwanese study, maternal residence within 500 m of a freeway was associated with a 30 % increase in the odds of PTB (Yang et al. 2003a), while residence with 250 m of a major road in North Carolina was associated with a 4 % increase in the odds of a PTB (Miranda et al. 2013). Two studies in Los Angeles, CA, found the relationship between traffic-related air pollution and PTB varied by season and socioeconomic status (Ponce et al. 2005; Wilhelm and Ritz 2003). One study in our review did not find a significant association between either traffic density or road proximity and risk of PTB (van den Hooven et al. 2009). Benzene exposure, which can also serve as a proxy for exposure to the mixture of pollutants comprising traffic-related air pollution (Slama et al. 2009), was also found to be associated with an increased risk of PTB (Wilhelm et al. 2011; Llop et al. 2010).

Finally, our review captured only two studies exploring the relationship between PTB and PAH exposure. In both studies, higher exposure to PAHs was associated with increased risk of PTB. Significant relationships were observed for PAH exposures (Wilhelm et al. 2011; Choi et al. 2008). Choi et al. explored disparities in vulnerability to PAHs, finding that third trimester PAH exposure increased the risk of PTB among African American women in New York City, but not among Dominican women in the same geographic area (Choi et al. 2008). Further exploration of the relationship between PTB and PAH exposure is warranted.

3.3.2 Air Pollution and Birth Weight

3.3.2.1 Continuous Birth Weight

There is an extensive research literature exploring the association between air pollution and birth weight. While not all studies have found higher air pollution levels to be linked with decrements in birth weight, there is evidence of small reductions in birth weight associated with each of the criteria air pollutants.

Numerous studies observed decrements in birth weight associated with CO exposure during pregnancy. These associations were noted for exposures measured for the entire pregnancy (Bell et al. 2007; Morello-Frosch et al. 2010), first trimester (Bell et al. 2007; Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Salam et al. 2005), second trimester (Mannes et al. 2005), and third trimester (Currie et al. 2009; Bell et al. 2007; Morello-Frosch et al. 2010; Mannes et al. 2005). A few studies did not find significant associations for some of these exposure windows (Currie

et al. 2009; Mannes et al. 2005; Geer et al. 2012). On the other hand, two studies reported increasing birth weight with increasing CO during the second or third trimester (Morello-Frosch et al. 2010; Medeiros and Gouveia 2005).

Most studies of O_3 did not observe changes in birth weight associated with exposure during various windows of pregnancy, including trimesters and months (Currie et al. 2009; Medeiros and Gouveia 2005; Mannes et al. 2005; Hansen et al. 2007). Three US studies, however, found O_3 exposure during the entire pregnancy or specific trimesters was associated with decrements in birth weight (Morello-Frosch et al. 2010; Salam et al. 2005; Geer et al. 2012).

Associations between $PM_{2.5}$ and birth weight have been well-studied, with higher levels of pregnancy-length $PM_{2.5}$ exposure associated with lower birth weight (Jedrychowski et al. 2004, 2009; van den Hooven et al. 2012; Bell et al. 2007; Morello-Frosch et al. 2010; Gray et al. 2010; Parker et al. 2005; Slama et al. 2010). Two studies, however, did not observe such associations (Parker and Woodruff 2008; Berrocal et al. 2011). Results from studies exploring trimester-length exposure are more varied. Exposures to $PM_{2.5}$ during each trimester have been associated with decrements in birth weight (Bell et al. 2007; Morello-Frosch et al. 2010; Gray et al. 2010), but studies have also reported null findings for each trimester (Mannes et al. 2005; Geer et al. 2012; Gray et al. 2010; Parker and Woodruff 2008). Information on shorter windows of exposure is more limited, with one study observing association between $PM_{2.5}$ during the last 30 and 90 days of pregnancy and birth weight (van den Hooven et al. 2012), and another study noting no association between $PM_{2.5}$ during the last month of pregnancy and birth weight (Mannes et al. 2005). Race (Bell et al. 2007), genotype (Slama et al. 2010), and components of $PM_{2.5}$ (Bell et al. 2010, 2012) may all affect the strength and/or existence of associations between birth weight and $PM_{2.5}$.

The relationship between PM_{10} exposure and birth weight is not as well-studied as that between $PM_{2.5}$ and birth weight, yet exposure to PM_{10} throughout pregnancy has generally been associated with lower birth weights (Bell et al. 2007; Morello-Frosch et al. 2010; Geer et al. 2012; Gray et al. 2010; Parker and Woodruff 2008). Two studies observed no such association (Gehring et al. 2011b; Rahmalia et al. 2012). Although some studies have reported no association between trimester exposures and birth weight (Currie et al. 2009; Geer et al. 2012; Hansen et al. 2007; Gehring et al. 2011b; Rahmalia et al. 2012), decrements in birth weight have been associated with PM_{10} exposure during the first (Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Parker and Woodruff 2008; Suh et al. 2007; Yang et al. 2003b), second (Morello-Frosch et al. 2010; Mannes et al. 2005; Parker and Woodruff 2008), and third trimesters (Bell et al. 2007; Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Salam et al. 2005; Gray et al. 2010; Parker and Woodruff 2008).

Pregnancy-length exposure to NO_2 has not been associated with birth weight (Gehring et al. 2011a, b; Geer et al. 2012; Iniguez et al. 2012; Estarlich et al. 2011; Kashima et al. 2011; Lepeule et al. 2010; Hitosugi et al. 2009), with the exception of two studies in higher pollution areas in California and New England, which noted

small decrements in birth weight with increasing NO_2 exposure (Bell et al. 2007; Morello-Frosch et al. 2010). Results are less consistent for trimester-length exposure. Some studies observed no associations between birth weight and NO_2 in any trimester (Gehring et al. 2011a; Geer et al. 2012; Hansen et al. 2007; Estarlich et al. 2011; Malmqvist et al. 2011). On the other hand, several studies found NO_2 during the first trimester to generally be associated with lower birth weight (Bell et al. 2007; Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Lepeule et al. 2010; Hitosugi et al. 2009), and two studies reporting no association in the same window of exposure (Mannes et al. 2005; Gehring et al. 2011b). Exposures in the second and third trimester were associated with decrements in birth weight (Mannes et al. 2005; Aguilera et al. 2009) in as many studies as reported null findings for these exposure windows (Medeiros and Gouveia 2005; Lepeule et al. 2010). As a further complication of our understanding of the relationship between NO_2 and birth weight, a study in Brazil observed higher birth weights associated with increasing NO_2 exposure during the third trimester of pregnancy (Medeiros and Gouveia 2005).

Several studies have explored the association between SO_2 and birth weight, revealing early pregnancy as a key window of exposure. Decrements in birth weight were consistently observed with higher SO_2 levels during the first trimester (Bell et al. 2007; Medeiros and Gouveia 2005; Yang et al. 2003b; Williams et al. 2007) and during the first 2 months of pregnancy (Medeiros and Gouveia 2005). Pregnancy-length exposure was generally not associated with birth weight (Bell et al. 2007; Rahmalia et al. 2012), although one study did note an association for pregnancy-length SO_2 (Geer et al. 2012). In contrast with most studies of air pollutants and birth weight, a couple studies observed increasing birth weight with exposure to SO_2 during the entire pregnancy (Morello-Frosch et al. 2010), as well as the second and third trimesters (Medeiros and Gouveia 2005).

Proximity to highways and major roadways, a proxy for traffic-related air pollution exposure, has not been significantly associated with reductions in birth weight (Barnett et al. 2011; Miranda et al. 2013; van den Hooven et al. 2009; Kashima et al. 2011; Yorifuji et al. 2012). Studies operationalizing traffic-related air pollution exposure using traffic density provided mixed results (van den Hooven et al. 2009; Kashima et al. 2011; Malmqvist et al. 2011; Zeka et al. 2008). Differences in findings across studies may be related to variation in definitions of road categories and mixture of vehicular traffic (Kashima et al. 2011).

Compared to the criteria air pollutants, studies of the effect of PAH exposure on birth weight are still limited. While some studies observed no association between exposure and birth weight (Masters et al. 2007; Tang et al. 2006; Perera et al. 2004), several studies have reported decrements in birth weight with increasing PAH levels (Choi et al. 2006, 2012; Perera et al. 2003; Choi and Perera 2012). The effect of PAH air pollution exposure varied by maternal characteristics, including race/ethnicity (Choi et al. 2006; Perera et al. 2003), obesity status, and food-related PAH exposures (Choi and Perera 2012). More studies with large sample sizes are needed to better understand relationships between birth weight and PAH exposure.

3.3.2.2 Low Birth Weight

Air pollution exposure and low birth weight (LBW; <2,500 g) is moderately well-studied in the literature. Many studies aimed at exploring LBW as an outcome, however, actually model term LBW (LBW at 37 weeks or later), which is one way to operationalize fetal growth as an outcome. In this section, we focus on the findings from studies of LBW across gestational ages, saving a description of the results for term LBW for Sect. 3.3, where we discuss air pollution and fetal growth. Thus, little evidence is available describing associations between LBW regardless of gestational age at delivery and the criteria air pollutants. Additionally, our review identified no studies exploring the relationship between LBW and PAH exposure during pregnancy.

In general, higher levels of exposure to CO during the entire gestational period were not significantly associated with risk of LBW (Bell et al. 2007; Lin et al. 2004a), although at least one study did report an increased risk of LBW (Hoggatt et al. 2009). Trimester CO exposures provided mixed results, with some studies suggesting an increased risk of LBW associated with first (Ha et al. 2001; Lee et al. 2003) and third trimester (Currie et al. 2009) CO, but other researchers reporting non-significant findings for trimester-length CO exposures (Currie et al. 2009; Lin et al. 2004a; Ha et al. 2001; Lee et al. 2003).

Relatively few studies were available on O_3 and LBW. Interestingly, results presented by Ebisu et al. indicated higher levels of O_3 during the first trimester may be protective against LBW (Ebisu and Bell 2012). Other studies generally found no effect of trimester or pregnancy-length O_3 exposure (Currie et al. 2009; Lin et al. 2004a; Ha et al. 2001), with the exception of one study reporting an increased risk of LBW from exposure in the third trimester (Ha et al. 2001).

PM_{10} and $PM_{2.5}$ were not widely studied in analyses modeling risk of LBW. For coarse particulate matter, PM_{10}, results trended toward positive associations between exposure and LBW; however, no studies reported significant effect estimates for PM_{10} exposure in any trimester (Currie et al. 2009; Kim et al. 2007; Bell et al. 2007; Lin et al. 2004a), 6 weeks before delivery (Kim et al. 2007), or the entire pregnancy period (Kim et al. 2007; Bell et al. 2007; Lin et al. 2004a). Pregnancy-length exposure to $PM_{2.5}$ was associated with LBW (Bell et al. 2007), as were levels of specific $PM_{2.5}$ component particles, including elemental carbon, titanium, zinc, potassium, and nickel (Bell et al. 2012; Ebisu and Bell 2012).

SO_2 exposures during the first month (Liu et al. 2003), first trimester (Bobak 2000; Ha et al. 2001), third trimester (Lin et al. 2004a), and entire pregnancy (Bobak 2000; Lin et al. 2004a) have been associated with elevated risk of LBW. However, contradictory results have also been reported, with some studies finding no associations and others finding results to be attenuated after adjustment for key covariates (Bobak 2000; Bell et al. 2007; Ha et al. 2001). The association between SO_2 exposure during the first month of pregnancy and LBW was robust to adjustment for exposures to co-pollutants (Liu et al. 2003). Similarly, the literature does not provide a consensus understanding of the association between NO_2 exposure during pregnancy and the risk of LBW. There is some evidence that higher levels of NO_2

exposures during the first trimester (Ha et al. 2001) and entire pregnancy (Bell et al. 2007) are associated with increased LBW, but other findings indicate a lack of association (Malmqvist et al. 2011; Lin et al. 2004a).

The relationship between traffic-related air pollution and LBW is being increasingly explored in the literature. Although one study did not find an association between LBW and traffic density (Malmqvist et al. 2011), results have generally shown increasing traffic-related air pollution increases risk of LBW. Maternal residence in close proximity to a highway or major roadway increased the odds of LBW in studies undertaken in Montreal, Canada (Genereux et al. 2008) and North Carolina, USA (Miranda et al. 2013). In addition, Genereux et al. noted that the effect of proximity to highways varied by maternal SES measures (Genereux et al. 2008). One study measured traffic-related air pollution using traffic density, observing increased risk of LBW associated with higher traffic density, particularly among births in the fall/winter (Wilhelm and Ritz 2003).

3.3.3 Air Pollution and Fetal Growth

Public health research has long focused on birth weight and gestational age, both categorically and to a somewhat lesser extent continuously, as these outcomes are straightforward to measure, commonly recorded in administrative datasets, linked to numerous long term outcomes, and easily understood by the public. Increasingly, however, the field is focusing on fetal growth trajectory during pregnancy. Bringing together the related outcomes of gestational age and birth weight, fetal growth metrics aim to describe if an infant is developing at the expected rate. Research exploring how fetal growth may be associated with maternal prenatal air pollution exposure is still fairly limited.

3.3.3.1 Growth Restriction

Small for gestational age (SGA), also referred to as intrauterine growth restriction (IUGR) or fetal growth restriction (FGR), is the clinical threshold of concern for fetal growth, identifying infants weighing in the bottom 10 % of all infants at a given gestational age. Studies of air pollution and SGA are sparse, but have generally focused on trimester exposures to criteria air pollutants, PAHs, and traffic-related air pollution.

Growth restriction has been associated with pregnancy-length exposure to $PM_{2.5}$ and CO (Liu et al. 2007). Increased risk of SGA with higher levels of $PM_{2.5}$ exposure in various trimesters has been observed, although specific trimesters of concern were not consistent across studies (Lee et al. 2012; Mannes et al. 2005; Liu et al. 2007; Rich et al. 2009). The risk of growth restriction or SGA was particularly elevated for higher CO exposures during early gestational windows, including the first two trimesters (Liu et al. 2003; Salam et al. 2005) and first month of pregnancy (Liu

et al. 2003). Results have not always been consistent, however, with some studies reporting no significant associations between trimester CO exposure and fetal growth outcomes (Mannes et al. 2005; Rich et al. 2009). The associations between CO exposure and thresholds of fetal growth were robust to adjustment for exposures to other air pollutants (Liu et al. 2003, 2007).

Pregnancy-length exposures to PM_{10}, O_3, and SO_2 have not been shown to increase the risk of SGA or growth restriction (Kim et al. 2007; Liu et al. 2007; Urbaniec et al. 2004). Evidence of a relationship between PM_{10} exposure and SGA is mixed, with a few studies finding small associations between SGA and trimester PM_{10} in select models (Kim et al. 2007; Mannes et al. 2005), but most studies finding no association between SGA and trimester (Lee et al. 2012; Mannes et al. 2005; Hansen et al. 2007), monthly (Hansen et al. 2007), or late pregnancy (Kim et al. 2007) PM_{10} levels. O_3 exposure during the third trimester of pregnancy was found to be associated with fetal growth restriction in a single study (Salam et al. 2005); however, no other studies have documented a relationship between fetal growth and O_3 exposure throughout pregnancy (Liu et al. 2007), during specific trimesters (Lee et al. 2012; Mannes et al. 2005; Hansen et al. 2007; Liu et al. 2007), or the last month of pregnancy (Mannes et al. 2005). On the other hand, there is evidence that shorter windows of exposure to SO_2, particularly in early pregnancy, may affect fetal growth, with some studies observing significant associations for exposures during the first two trimesters (Liu et al. 2003; Ballester et al. 2010) and first month (Liu et al. 2003) of pregnancy, even with adjustment for other air pollutants (Liu et al. 2003). More examination of the relationship between SO_2 and fetal growth is warranted, particularly with some studies observing no association for trimester length exposures (Liu et al. 2007; Rich et al. 2009).

Findings for NO_2 exposure and growth restriction are also highly varying, making interpretation and conclusions difficult. Many studies have observed no association between SGA and NO_2 levels during the entire pregnancy (Gehring et al. 2011a; Bobak 2000; Kashima et al. 2011), in any trimester (Gehring et al. 2011a; Bobak 2000; Mannes et al. 2005; Hansen et al. 2007; Kashima et al. 2011; Rich et al. 2009), or any month of pregnancy (Mannes et al. 2005; Hansen et al. 2007). However, one study found an association between SGA and second and third trimester exposures among women living within 5 km of an NO_2 monitor (Mannes et al. 2005). A study in Vancouver, Canada observed increased risk of growth restriction for women exposed to higher levels of NO_2 during the first month of pregnancy (Liu et al. 2003), and results of a third study found pregnancy-length NO_2 exposure was associated with fetal growth restriction (Liu et al. 2007).

There is limited data with which to understand the relationship between PAH exposure and SGA. A Czech study found PAH exposure during the first month of pregnancy to be associated with increased risk of growth restriction (Dejmek et al. 2000). Choi et al. found growth restriction to be associated with higher PAH levels (Choi et al. 2008, 2012; Choi and Perera 2012). These findings varied by maternal characteristics, with differences in associations by obesity status (Choi and Perera 2012) and race/ethnicity (Choi et al. 2008). While most studies of PAH exposure and health do not measure PAH levels in multiple exposure windows, Choi et al.

explored trimester exposure levels among a small sample of women, finding significant decrements in fetal growth associated with PAHs during the first, but not subsequent, trimesters (Choi et al. 2012).

Traffic-related air pollution, as characterized by either proximity to roadways or traffic density, was consistently not associated with risk of growth restriction (Miranda et al. 2013; Genereux et al. 2008; van den Hooven et al. 2009; Kashima et al. 2011).

3.3.3.2 Term Low Birth Weight

A number of studies operationalize the outcome of fetal growth by modeling the association between air pollution and LBW among term births only (term LBW). Since babies born at term should be in the normal birth weight range (i.e., not LBW), term LBW is often considered a marker for fetal growth restriction (Ritz and Wilhelm 2008; Woodruff et al. 2009). Maisonet et al., however, highlight a need for caution in interpreting term LBW as the same outcomes as SGA, since the growth trajectory of babies delivered before 37 weeks are excluded from consideration in term LBW analyses (Maisonet et al. 2004).

Term LBW has not been as widely studied in the air pollution literature as other pregnancy outcomes. No articles exploring PAHs and term LBW were found by our review. CO exposure during pregnancy (Ebisu and Bell 2012) and the third trimester (Maisonet et al. 2001) were associated with increased risk of term LBW, but other studies found no association between CO and term LBW (Medeiros and Gouveia 2005; Gouveia et al. 2004). Pregnancy-length SO_2 exposure (Ebisu and Bell 2012) and second trimester SO_2 (Maisonet et al. 2001) were also associated with term LBW, but other studies did not report significant (Medeiros and Gouveia 2005; Gouveia et al. 2004) or robust associations (Dugandzic et al. 2006). Associations between NO_2 and term LBW were generally non-significant (Medeiros and Gouveia 2005; Kashima et al. 2011; Gouveia et al. 2004). While most studies observed no associations between O_3 and term LBW (Medeiros and Gouveia 2005; Gouveia et al. 2004; Dugandzic et al. 2006), higher O_3 during the first trimester of pregnancy was found to be protective against term LBW in a Brazilian study (Ebisu and Bell 2012). Finally, one study did not report an association between traffic-related air pollution, characterized as either proximity to roadways or traffic density, and term LBW (Kashima et al. 2011); however, two other studies observed increased odds of term LBW as traffic density or modeled traffic-related pollution levels increased (Padula et al. 2012; Wilhelm et al. 2012).

Associations between term LBW and particulate matter are somewhat better studied. A meta-analysis (Dadvand et al. 2013) and a study attempting to implement a standardized study design across 14 populations (Parker et al. 2011) reported that higher levels of pregnancy-length PM_{10} and $PM_{2.5}$ were associated with increased odds of term LBW. Variability in the effect of particulate matter across the geographically dispersed study populations considered was noted (Parker et al. 2011). In the meta-analysis, although no associations were found between term LBW and

trimester $PM_{2.5}$ exposure, PM_{10} levels in each trimester were found to be associated with term LBW (Dadvand et al. 2013). Other studies investigating PM_{10} exposure and term LBW either did not find significant associations (Medeiros and Gouveia 2005; Maisonet et al. 2001; Gouveia et al. 2004) or associations were not robust to adjustment to key covariates (Dugandzic et al. 2006).

3.3.4 Summary of Findings by Pollutant

3.3.4.1 Carbon Monoxide

Overall, the research on CO has demonstrated an effect on pregnancy outcomes, but there are inconsistencies across studies and outcomes. CO has been associated with a decrease in birth weight (Currie et al. 2009; Bell et al. 2007; Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Salam et al. 2005; Mannes et al. 2005; Gouveia et al. 2004) and an increased risk of low birth weight (Currie et al. 2009; Bell et al. 2007; Morello-Frosch et al. 2010; Hoggatt et al. 2009; Ha et al. 2001; Lee et al. 2003; Lin et al. 2004b), with the most important windows of exposure appearing to be the first and third trimesters (Maisonet et al. 2001), although no effect (Currie et al. 2009; Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Mannes et al. 2005; Geer et al. 2012; Lin et al. 2004a; Ha et al. 2001; Seo et al. 2010), as well as a protective effect (Medeiros and Gouveia 2005) of CO on birth weight, has also been reported. CO has been associated with small effects on preterm birth, particularly for higher level exposures and certain geographic regions (Ritz et al. 2000, 2007; Wilhelm and Ritz 2005; Liu et al. 2003). Conversely, some studies find no effect of CO on preterm birth, even when considering early and late pregnancy windows and residences close to air quality monitoring stations (Huynh et al. 2006; Jalaludin et al. 2007; Rudra et al. 2011). Although there are few studies, CO exposure has not been found to be associated with decreased gestational age or increased risk of small for gestational age (Currie et al. 2009; Sagiv et al. 2005; Rich et al. 2009). However, CO exposure in each trimester and month has been associated with increased risk of intrauterine growth restriction (Liu et al. 2003, 2007; Salam et al. 2005).

3.3.4.2 Ozone

While most of the research on O_3 shows little to no association with ozone exposure and pregnancy outcomes (Currie et al. 2009; Ritz et al. 2007; Jalaludin et al. 2007; Hansen et al. 2006, 2007; Lee et al. 2008, 2012; Kim et al. 2007; Warren et al. 2012; Medeiros and Gouveia 2005; Mannes et al. 2005; Geer et al. 2012; Lin et al. 2004a; Ha et al. 2001; Dugandzic et al. 2006), a few studies find increased risk of adverse pregnancy outcomes. Reduced birth weight (Morello-Frosch et al. 2010; Salam et al. 2005; Geer et al. 2012) and increased risk of LBW (Ha et al. 2001; Lin et al. 2004b; Nascimento and Moreira 2009) have been associated with exposure to

O_3 in a few studies. One study found a protective effect of ozone exposure in the first trimester (Ebisu and Bell 2012). Early (Jalaludin et al. 2007; Hansen et al. 2006; Lee et al. 2012; Warren et al. 2012) and late (Jalaludin et al. 2007; Kim et al. 2007) pregnancy ozone exposure has been associated with increased risk of preterm birth (PTB); several studies, however, have found no effect of ozone on PTB (Ritz et al. 2007; Jalaludin et al. 2007; Hansen et al. 2006; Kim et al. 2007; Lee et al. 2008; Warren et al. 2012). O_3 exposure is associated with reduced gestational age (Olsson et al. 2012) in one study and with increased risk of IUGR in another (Salam et al. 2005).

3.3.4.3 Coarse Particulate Matter

The associations found between PM_{10} and adverse pregnancy outcomes are mixed. Many studies find no effect on birth weight from PM_{10} exposure during pregnancy (Currie et al. 2009; Medeiros and Gouveia 2005; Mannes et al. 2005; Geer et al. 2012; Hansen et al. 2007; Gray et al. 2010; Rahmalia et al. 2012). However, studies sometimes offer conflicting results, especially if they are comparing windows of exposure (Bell et al. 2007; Morello-Frosch et al. 2010; Medeiros and Gouveia 2005; Salam et al. 2005; Mannes et al. 2005; Gray et al. 2010, 2011; Parker and Woodruff 2008; Gehring et al. 2011b; Suh et al. 2007; Yang et al. 2003b; Gouveia et al. 2004). Results vary by region (Dadvand et al. 2013; Parker et al. 2011), and a few studies have even found PM_{10} exposure associated with increased birth weight (Medeiros and Gouveia 2005; Geer et al. 2012; Parker et al. 2011). Results are contradictory for LBW, with some studies finding exposure to PM_{10} to be associated with increased risk of LBW (van den Hooven et al. 2012; Bell et al. 2007; Lee et al. 2003; Lin et al. 2004b; Seo et al. 2010; Rogers and Dunlop 2006), with early pregnancy a potential window of vulnerability (Lee et al. 2003; Xu et al. 2011). A few studies have found no effect from PM_{10} exposure, even when looking at various windows of exposure (Currie et al. 2009; Kim et al. 2007; Lin et al. 2004a; Lee et al. 2003). A recent multi-national meta-analysis of term LBW found increased risk with increasing PM_{10} exposure, although significance varies by geographic region with no clear pattern (Dadvand et al. 2013; Parker et al. 2011). Other studies of PM_{10} and term LBW reported either increasing risk of term LBW with increasing PM_{10} exposure (Dugandzic et al. 2006; Lin et al. 2004b) or no significant association between exposure and outcome (Medeiros and Gouveia 2005; Lin et al. 2004a; Maisonet et al. 2001; Nascimento and Moreira 2009).

Studies find extremely mixed results when looking at PM_{10} exposure and risk of PTB, with some finding associations between increased risk of PTB and PM_{10} exposure during various windows (Wilhelm and Ritz 2005; Hansen et al. 2006; Zhao et al. 2011; Suh et al. 2009; van den Hooven et al. 2012; Kloog et al. 2012) and others studying similar windows finding no effects (Jalaludin et al. 2007; Hansen et al. 2006; Lee et al. 2008; Sagiv et al. 2005; Darrow et al. 2009). SGA/IUGR associations are similar (Currie et al. 2009; Lee et al. 2012; Kim et al. 2007; van den Hooven et al. 2012; Mannes et al. 2005; Hansen et al. 2007; Gehring et al. 2011b; Dejmek et al. 2000).

3.3.4.4 Fine Particulate Matter

$PM_{2.5}$ comprises smaller molecules than PM_{10}, with the potential for greater dissemination throughout the body. Many studies find exposure to $PM_{2.5}$ is associated with decreased birth weight (Jedrychowski et al. 2004, 2009; van den Hooven et al. 2012; Bell et al. 2007; Morello-Frosch et al. 2010; Gray et al. 2010, 2011; Parker et al. 2005, 2011; Slama et al. 2010; Parker and Woodruff 2008), with the effects varying by race (Bell et al. 2007), region (Parker and Woodruff 2008; Parker et al. 2011), and genotype (Slama et al. 2010). Many windows of vulnerability have been explored (van den Hooven et al. 2012; Bell et al. 2007; Gray et al. 2010), with mixed findings, with one study finding that exposure during pregnancy or the first trimester is associated with an increase in birth weight (Geer et al. 2012). Consistent relationships have been found between $PM_{2.5}$ exposure and increased risk of low birth weight (Bell et al. 2007; Morello-Frosch et al. 2010; Dadvand et al. 2013; Rossner et al. 2011; Slama et al. 2007), although results have been shown to vary by geographic region (Parker et al. 2011). Similar to PM_{10}, when looking at $PM_{2.5}$ exposure and risk of preterm birth, $PM_{2.5}$ exposure in early (Huynh et al. 2006; Lee et al. 2012; Chang et al. 2012), middle (Chang et al. 2012; Warren et al. 2012), late (Huynh et al. 2006; Jalaludin et al. 2007) and entire (Huynh et al. 2006; Wu et al. 2009; Chang et al. 2012; van den Hooven et al. 2012) pregnancy is associated with increased risk of preterm birth. Yet many other studies find no effects on preterm birth for these exposure windows (Ritz et al. 2007; Jalaludin et al. 2007; Rudra et al. 2011; Chang et al. 2012; van den Hooven et al. 2012; Warren et al. 2012; Darrow et al. 2009). For SGA/IUGR, $PM_{2.5}$ exposure has been associated with increased risk in various windows (Mannes et al. 2005; Parker et al. 2005; Liu et al. 2007; Rich et al. 2009); however, other studies have found no clear effect from exposure in similar windows (Jedrychowski et al. 2004; Lee et al. 2012; Mannes et al. 2005; Rich et al. 2009).

3.3.4.5 Nitrogen Dioxide

NO_2 and pregnancy outcomes have been studied in many windows of vulnerability. In general, these studies find conflicting results across pregnancy outcomes. Studies have observed decrements in birth weight (Bell et al. 2007; Medeiros and Gouveia 2005; Mannes et al. 2005; Gehring et al. 2011b; Lepeule et al. 2010; Hitosugi et al. 2009; Malmqvist et al. 2011; Aguilera et al. 2009) and increased risk of LBW (Bell et al. 2007; Morello-Frosch et al. 2010; Ha et al. 2001; Lee et al. 2003; Ebisu and Bell 2012; Lin et al. 2004b), PTB (Olsson et al. 2012; Zhao et al. 2011; Wu et al. 2009; Llop et al. 2010; Maroziene and Grazuleviciene 2002; Darrow et al. 2009), and growth restriction (Liu et al. 2003, 2007; Mannes et al. 2005; Gehring et al. 2011b; Rich et al. 2009) during certain windows. Yet other studies have observed no association between birth weight (Gehring et al. 2011a; Medeiros and Gouveia 2005; Mannes et al. 2005; Geer et al. 2012; Hansen et al. 2007; Iniguez et al. 2012; Estarlich et al. 2011; Kashima et al. 2011; Lepeule et al. 2010; Hitosugi et al. 2009), LBW (van den Hooven et al. 2012; Lin et al. 2004a; Ha et al. 2001; Seo et al. 2010),

PTB (Ritz et al. 2007; Jalaludin et al. 2007; Wilhelm et al. 2011; Hansen et al. 2006; Gehring et al. 2011a; Maroziene and Grazuleviciene 2002; Darrow et al. 2009), and growth restriction (Gehring et al. 2011a; Bobak 2000; Mannes et al. 2005; Hansen et al. 2007; Kashima et al. 2011; Rich et al. 2009) and NO_2 in similar windows.

3.3.4.6 Sulfur Dioxide

Sulfur dioxide has been associated with increased risk, no risk, and reduced risk for adverse pregnancy outcomes. Many of these studies vary by exposure window. While exposure during pregnancy, especially early in pregnancy, is associated with a decrease in birth weight (Mohorovic 2004; Bell et al. 2007; Medeiros and Gouveia 2005; Geer et al. 2012; Yang et al. 2003b; Williams et al. 2007; Gouveia et al. 2004), exposure has also been found to have no effect (Mohorovic 2004; Masters et al. 2007; Geer et al. 2012; Rahmalia et al. 2012), sometimes in the same study (Mohorovic 2004; Bell et al. 2007; Geer et al. 2012), but looking at shorter exposure windows. SO_2 exposure has also been associated with increases in birth weight (Morello-Frosch et al. 2010; Medeiros and Gouveia 2005). SO_2 exposure, particularly in early pregnancy, is associated with an increased risk of low birth weight (Liu et al. 2003; Bobak 2000; Bell et al. 2007; Lin et al. 2004a, b; Ha et al. 2001; Lee et al. 2003; Ebisu and Bell 2012; Dugandzic et al. 2006; Nascimento and Moreira 2009). Some studies, however, report no effect (Bell et al. 2007; Medeiros and Gouveia 2005; Seo et al. 2010; Dolk et al. 2000). Timing of exposure seems particularly important for PTB, with exposure during the last month (Liu et al. 2003; Zhao et al. 2011; Bobak 2000) and the whole pregnancy (Bobak 2000) being associated with increased risk. However, late pregnancy exposure was also found to have no effect (Sagiv et al. 2005) so clear exposure window definitions are important. Results vary for continuous gestational age, SGA and IUGR, reporting both increased risk (Mohorovic 2004; Liu et al. 2003; Ballester et al. 2010) and no effect (Mohorovic 2004; Bobak 2000; Liu et al. 2007; Rich et al. 2009).

3.3.4.7 Traffic-Related Air Pollutants

Traffic-related pollutants are often assessed in two ways – either distance to roadways or traffic density. Several studies find no association between proximity to major road and birth weight (Barnett et al. 2011; Miranda et al. 2013; Kashima et al. 2011), except for one which finds residence within 200 m to be associated with decreased birth weight (Yorifuji et al. 2012). We found no studies that support an association between proximity to major roads and pregnancy outcomes for gestational age, SGA, term low birth weight, or very preterm birth (Miranda et al. 2013; Genereux et al. 2008; Kashima et al. 2011). However, studies do find proximity to major roadway is associated with increased risk of low birth weight (Miranda et al. 2013; Genereux et al. 2008), preterm birth (Miranda et al. 2013; Genereux et al. 2008; Lewtas 2007) and late preterm birth (Miranda et al. 2013).

For traffic density or distance weighted traffic density, studies find an association between traffic density and reduced birth weight (Zeka et al. 2008), risk of low birth weight (Padula et al. 2012), reduced gestational age (Barnett et al. 2011), as well as no association (Kashima et al. 2011). Additionally, specific traffic-related pollutants (i.e. NO_2, benzene) are found to be associated with increased risk of preterm birth and term low birth weight (Ponce et al. 2005; Wilhelm et al. 2012). However, benzene, which is a proxy for traffic or combustion-related pollution, appears to have no association with birth weight as a result of exposure during the entire pregnancy or by trimester (Slama et al. 2009; Estarlich et al. 2011).

3.4 Biological Mechanisms

The biological mechanisms by which air pollution affects adverse pregnancy outcomes remain unclear. Air pollution may operate on pregnancy outcomes through developmental and functional toxicities, suppressing antioxidant defense systems, reducing oxygen flow to the fetus, or accumulating in the fetus as fetal elimination of pollutants is immature (Shah and Balkhair 2011). The mechanisms may depend on both the outcome of interest and the pollutant considered. Pregnancy outcomes are determined by multiple interacting host, social, and environmental factors. Figure 3.3 illustrates the influences these factors have on pregnant women and their developing fetuses. Birth weight at delivery, for example, is affected by many interacting factors, including maternal host factors (e.g., genetics, age, medical complications, and prenatal care) and environmental factors (e.g., air pollution). Similarly, pollutants can vary in how they affect pregnancy outcomes. For example, particulate matter can enter the lungs, be absorbed into the blood, and subsequently disperse to other organs, including the placenta, inducing inflammation (Liu et al. 2003; Ritz and Wilhelm 2008). PAHs, on the other hand, can interfere with fetal nourishment by increasing viscosity of the blood and reducing blood flow to the placenta and uterus (Liu et al. 2003; Ritz and Wilhelm 2008). An additional complexity is that the biological pathways by which pregnancy outcomes are affected by air pollution could be maternal, fetal, or both. Air pollution may affect maternal respiratory or general health, which can lead to oxidative stress, inflammatory responses, and disturbances to blood flow (Sram et al. 2005; Huynh et al. 2006; Bosetti et al. 2010). Fetal (or embryonic) pathways may include vulnerability of developing organ systems or inhibition of weight gain (Sram et al. 2005). Absorbed pollutants can affect placental functioning, potentially increasing fetal distress and inhibiting fetal development (Choi et al. 2006, 2008; Choi and Perera 2012). Further, the timing of the air pollution exposure likely drives the biological mechanism. For example, very early exposure may affect embryonic implantation and the formation of the placenta, while exposure during the third trimester may affect birth weight since significant fetal weight gain occurs during this period (Gouveia et al. 2004; Xu et al. 2011; Shah and Balkhair 2011). Exposures during both of these periods may influence birth weight, although through different mechanisms.

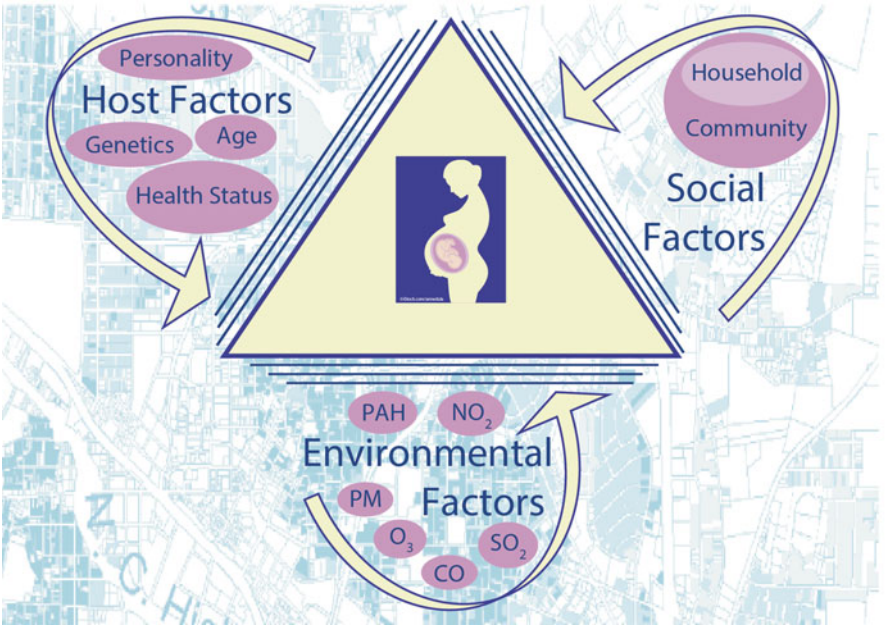

Fig. 3.3 Forces shaping pregnancy outcomes

Three main hypothesized biological mechanisms are found in the literature: oxidative stress, placental formation/function, and inflammation. **Oxidative stress** may act in the embryonic stage through disturbances in cord blood flow, as well as increased susceptibility to maternal infections (Olsson et al. 2012). Oxidative stress and subsequent inflammation may increase with exposure to air pollutants (Geer et al. 2012). Oxidative stress has been found to be influenced by exposure to PM (Ritz and Wilhelm 2008; Rossner et al. 2011), PAHs (Ritz and Wilhelm 2008), and O_3 (Olsson et al. 2012). **Placental formation and functioning** may be disrupted by air pollution exposure. In an animal model, PM exposure induced changes in the placenta, which were subsequently associated with a higher incidence of low birth weight (Veras et al. 2008). Minor alterations in placental structure, which can be induced by exposure to CO (Morello-Frosch et al. 2010; Ritz and Wilhelm 2008) and PAHs (Ritz and Wilhelm 2008), can impact fetal development (Backes et al. 2013), possibly by reducing nutrient and oxygen availability (Warren et al. 2012; Morello-Frosch et al. 2010; Ritz and Wilhelm 2008). **Inflammation**, which can result from exposure to pollutants, especially PM, may affect pregnancy outcomes in at least three ways (Rossner et al. 2011). When particulate matter enters the lungs, it can be absorbed into the blood and then dispersed into distant organs. This can lead to inflammation in the lungs and other organs, including the placenta, thereby increasing the susceptibility for preterm labor. Inflammation can also lead to systemic release of cytokines that may trigger preterm birth. Lastly, alveolar inflammation can lead to increased difficulties with blood flow, impacting placental function (Maisonet et al. 2004).

3.5 Methodological Issues

While the scientific literature clearly points to an association between maternal air pollution exposure during pregnancy and pregnancy outcomes, the literature does not yet provide a consensus understanding of the details of this association. Inconsistent findings for some pollutants and for some windows of exposure may be due to a variety of methodological inconsistencies and issues in the literature. Variations in study design, populations considered, and pollution levels make it hard to compare results across studies and draw general conclusions. Here we discuss some of the methodological issues that can make interpretation of air pollution and pregnancy outcome studies difficult to synthesize, noting why these issues arise, how they impact results, and if/how they may be addressed.

3.5.1 Geographic Region

Our review included studies of pregnancy outcomes and ambient outdoor air pollution exposure that were conducted with data on populations from Asia to North America to Europe to South America. The majority of studies focused on North America, Europe, Asia and Australia, with relatively few studies considering locations in South/Central America. Air pollution levels vary greatly depending on geographic location, with non-industrialized countries having higher exposures, as well as more vulnerable populations (Woodruff et al. 2009). Additionally, even among populations in industrialized countries, a study in one region may not be easily compared to a study in another region due to natural variation in populations and pollutants. Such variations may contribute to the contradictory or unclear patterns observed in the relationship between air pollutants and pregnancy outcomes. Even studies within the same large geographic region may involve populations with very different levels of air pollution exposure. For example, studies in California involve populations exposed to much higher levels of air pollutants than studies of populations in North Carolina. In an effort to address this, some reviews have combined or compared results across geographies, attempting to control for underlying heterogeneity; however, results remained unclear, with studies finding both consistent and inconsistent effects by region (Dadvand et al. 2013; Parker et al. 2011).

3.5.2 Exposure Assessment

Studies also vary greatly in their method of exposure assessment. This variation, in addition to complicating comparative analyses, can also lead to variable exposure misclassification, selection biases, and measurement error (Shah and Balkhair 2011). Exposure assessment methods differ in terms of data sources, data collection methods, frequency of pollutant level measurements, and exposure summary metrics.

Table 3.2 Article counts of source of air pollution data by pollutant being investigated

	Pollutant							
Data source	CO	O_3	PM_{10}	$PM_{2.5}$	SO_2	NO_2	PAHs	Traffic-related
Ambient monitors	32	29	43	31	23	39	3	1
Personal monitors	0	0	0	2	0	0	6	0
Modeled exposure surface	4	3	8	13	4	15	1	4
Road proximity/traffic density	4	3	7	9	1	12	2	12
Other	0	0	2	1	3	1	2	0

There are three main data collection methods: monitor-based, model-based, and proximity-based (Dadvand et al. 2013). See Table 3.2 for the count of articles utilizing data from each of these sources by pollutants investigated. Note that the article counts do not add up to the number of articles found in the literature review as some studies utilized multiple sources of air quality data and explored multiple pollutants.

Monitor-based assessments use either fixed ambient air quality monitoring stations (AQMS), which collect data at certain intervals, or passive samplers, which are movable monitors placed at a specific sites for a limited duration. A limitation of using AQMS data to assess exposure is that data collected by these monitors may only be indicative of pollution levels in the small area immediately surrounding the monitoring station, and the exact size of this area is unclear or may vary by pollutant. Furthermore, as shown in Fig. 3.4, AQMS are not randomly distributed; rather, monitoring sites tend to be located in more densely populated areas, leaving rural areas understudied due to a lack of monitoring data. Studies often extrapolate monitor data to larger areas, such as a cities or counties, which assumes exposures are homogenous across these larger areas, potentially assigning incorrect pollutant levels to individual residents, especially if only one monitor is present in the area (Glinianaia et al. 2004; Gouveia et al. 2004). To address this, studies often restrict their study populations to residents within varying buffers around each station, ranging from 1 to 20 km in size (Lepeule et al. 2010). Some studies, however, find that restricting the study area to smaller buffers does not produce significantly different results (Gray et al. 2010). Personal monitors, worn by participants during daily activities, are also occasionally used as a way of eliminating the fixed area bias of monitoring stations, but face other challenges addressed below.

Model-based methods combine ambient monitoring station or passive sampling monitor data with data on weather, space, time, and other factors that impact pollution levels. Modeled air pollution levels combine these data using geostatistics, dispersion models, land use regression (LUR), and other spatial analysis techniques to create estimates of air pollution levels across a surface. These models create interpolated spatial layers of estimated air pollutant concentrations, taking into account relevant environmental variables (Aguilera et al. 2009), while still utilizing monitoring station data patterns (Aguilera et al. 2009; Ritz and Wilhelm 2008; Gilliland et al. 2005). For example, LUR creates a smoothed exposure surface over a given

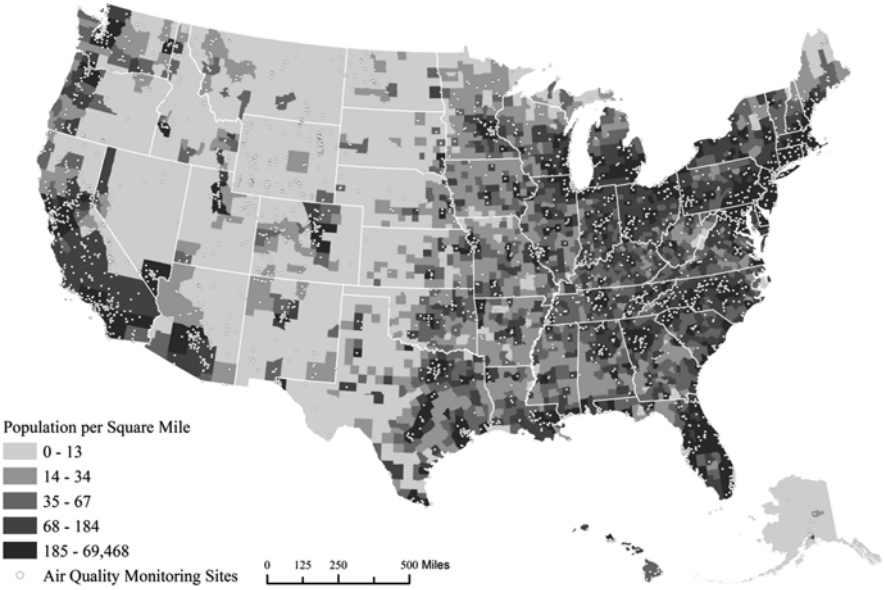

Fig. 3.4 Air quality monitoring stations and population density in the US (U.S.Census Bureau 2010; U.S.Environmental Protection Agency 2013)

geography and thus can avoid the issue of selection bias which may occur when limiting analysis to populations living near AQMS since these populations may be significantly different from populations not residing near monitors (Woodruff et al. 2009). However, LUR may focus on spatial variability in exposure, but neglect to account for temporal variation despite known seasonal variability in air pollution levels (Aguilera et al. 2009; Ritz and Wilhelm 2008). Some models, however, do adjust for both spatial and temporal variation concurrently, considering how effects change across at-risk windows (Chang et al. 2012). Some important considerations for modeled exposures include validation and calibration of model estimates and selection of appropriate geographic scale (Gilliland et al. 2005).

Given that traffic emissions contain a mix of NO_2, CO, volatile organic compounds, PM, PAHs, and other toxic substances (Boothe and Shendell 2008), many studies investigating traffic-related air pollution do not look at specific pollutants, rather they use proximity-based methods to proxy exposure to this mixture of pollutants. With proximity-based methods, exposure is measured in one of three ways: distance to a defined road type, summed traffic density within a defined distance, or distance-weighted traffic density within a defined distance. Studies have demonstrated that pollutant concentrations are higher near roadways, and diminish with increasing distance from roadways as a result of evaporation, dispersion, and coagulation (Boothe and Shendell 2008). While the general cutoff seems to take place around 150–300 m from roadways, many proximity-based studies either do not consider greater distances or only consider much smaller distances (Miranda et al. 2013; Genereux et al. 2008; van den Hooven et al. 2009; Kashima et al. 2011;

Yorifuji et al. 2012; Boothe and Shendell 2008; Wu et al. 2011; Brauer et al. 2008). Roads used in proximity-based analyses are classified by traffic counts or by local, state, or national function codes, which may or may not be indicative of traffic volume depending on the region. Given the variety of classification schemes worldwide and the rapid pace of development in some areas, it is not surprising that roads may be misclassified, traffic counts may be over- or underestimated, and road classifications from one region may not be comparable to those in other regions. Vehicle mix between cars and trucks may also vary from place to place, and is not specifically addressed in most studies. Additionally, like other methods, proximity-based methods have recognized limitations, including regional variation in pollutant components and exposure misclassification due to population mobility (Boothe and Shendell 2008).

Several studies have attempted to assess the utility of these three exposure data sources by implementing exposure assessments via each method on the same population (van den Hooven et al. 2012). One study comparing AQMS, LUR, air dispersion models, and traffic-density measures found that the size of effect estimates were smaller for air pollution exposures based on the relatively simple traffic density metric compared to other metrics; however, regardless of exposure assessment method used, effects were still quite similar (Wu et al. 2011). Another study compares exposures assessed using the nearest AQMS and using a temporally adjusted geostatistical (TAG) model. The TAG model combines smoothed, annual average estimates of pollutants over a region with a time-specific measure by exposure window using AQMS data. The two models tended to give similar results (Lepeule et al. 2010).

Even among studies using the same data collection method, there is still variation in exposure measurements and metrics, making cross-study comparisons challenging. Studies may measure pollutant levels with continuous, categorical, or scaled variables (Ritz and Wilhelm 2008), all requiring different statistical procedures and interpretation of results. Others choose which pollutants to include based only on availability of data, which may fail to account for important co-pollutant interactions (Ritz and Wilhelm 2008). Additionally, most studies use the peak or mean daily concentrations averaged across the exposure window (Glinianaia et al. 2004). With monitors and pollutants varying in measurement schedules, precision of such exposure assignments varies. Other studies use total pollutant levels measured during the exposure window or select the maximum concentration measured during the exposure window, and some studies even rely on annual air pollution estimates rather than estimates for specific pregnancy exposure windows (Glinianaia et al. 2004). Averaging can be problematic as consistent low exposure levels combined with occasional high exposures will lead to an average exposure assessment of "moderate" despite the fact that the biological impact of consistent moderate exposure may be different from the bimodal exposure described (Shah and Balkhair 2011). Errors can also easily occur if the exposure metric captures only a portion of the critical time window or extra-gestational time periods, necessitating a good understanding of the temporal trends of different air pollutants and monitoring data validity (Gilliland et al. 2005). It is unclear which, if any, metric or measurement is preferable.

3.5.3 *Personal Exposure Assessment*

Exposure assessments based on AQMS, modeled surfaces, and proximity provide an estimate of outdoor exposure based on an individual's residence. These estimates do not take into account personal characteristics that may influence an individual's level of air pollution exposure. Personal exposure assessments take into account personal activity patterns, indoor and outdoor exposures, and residential mobility. Given that people frequently travel to non-residential locations and do not spend all their time outside, personal exposure assessment can eliminate some exposure misclassification that results from simply using a residential address and ambient outdoor estimated air pollution levels to assign exposures (Ritz and Wilhelm 2008). This is especially important as shorter window estimates and pollutants with spatial heterogeneity over small areas may be more affected by personal activity patterns (Ritz and Wilhelm 2008). Additionally, indoor pollutant levels are not always similar to outdoor pollutant levels and personal exposure assessments can take both into account (Jedrychowski et al. 2009). Many AQMS, modeled surfaces, and proximity-based studies also assume a consistent maternal residence during gestation, ignoring maternal residential mobility (Bell et al. 2012; Shah and Balkhair 2011). One study attempting to understand the influence of residential mobility found that restricting the analysis to women who did not move during pregnancy resulted in stronger associations between air pollution exposures and adverse pregnancy outcomes compared to analyses that included women who moved during pregnancy (Ritz and Wilhelm 2008). However, at least one study reports that pregnant women are less mobile to begin with (Iniguez et al. 2012).

Two main methods are available for assessing personal exposure to air pollution: activity patterns and personal monitoring. Activity patterns can be used to understand how often individuals travel, where they spend their time, and how much time they spend indoors versus outdoors. This information is then used to better estimate exposure using data relevant to where individuals spend their time rather than simply where they live. Personal monitoring provides detailed data on actual air pollution levels to which individuals are exposed. Personal monitoring devices are typically worn by study participants 24 h a day for a short duration (2 days) and collect air samples at set intervals. Both methods require detailed personal data and can be difficult and expensive to implement on large populations. However, more accurately measuring personal exposure often strengthens study results. For example, three studies reported that in analyses only considering women who either did not work or who spent less than 2 h outdoors daily, stronger associations between air pollution levels and adverse pregnancy outcomes were observed compared to analyses with all women (Aguilera et al. 2009; Yorifuji et al. 2012; Ritz and Wilhelm 2008). These findings highlight the importance of considering individual behaviors which may be accounted for by using more personal exposure assessment methods.

Personal exposure measurement, however, does not eliminate measurement error and is not without limitations. Personal monitors are expensive, preventing research-

ers from conducting analyses with large study populations. They also require meaningful participant compliance to achieve accurate readings (Ritz and Wilhelm 2008). Given these cost and practicality limitations, it is ideal if researchers are able to specify their pollutant of interest and the key windows of exposure (Ritz and Wilhelm 2008). Personal monitoring is typically only conducted over such a short window, typically 2–7 days in the second or third trimester, and the resulting measures are often considered a proxy for trimester or entire pregnancy exposure. The accuracy of this assumption remains questionable (Jedrychowski et al. 2004, 2009; Slama et al. 2009; Perera et al. 2003; Choi and Perera 2012); however, studies that have compared short term personal exposure measurement with longer term measures have been encouraging. Using a subsample of study participants for a series of repeated measurements in each trimester, Jedrychowski et al. reported that although $PM_{2.5}$ exposure levels differed between trimesters, the second trimester had good agreement with the other trimesters and was an adequate reflection of other exposure periods (Jedrychowski et al. 2009). Other studies have found $PM_{2.5}$ to be relatively stable over pregnancy (Jedrychowski et al. 2004). These findings suggest that personal monitors, despite their short exposure window, may provide accurate pollutant measurements for other windows, depending on the pollutant of interest.

3.5.4 Spatial Resolution

Associations between different pollutants and pregnancy outcomes may depend on the spatial resolution of exposure measures. Air pollution may be measured at many different scales, the largest being multi-county or metropolitan areas, and the smallest being the individual person. In addition to political boundaries such as counties, zip codes, and cities, studies commonly use fixed distances radii from either AQMS or individual residences to define exposure (Glinianaia et al. 2004).

The spatial resolution of measurement is important for several reasons. Some pollutants, such as CO, vary over small spatial scales, while others, like PM, vary regionally. Thus, assigning exposure based on an areal unit that is larger or smaller than the pollutant's extent may lead to exposure misclassification, the magnitude of which will depend on the pollutant of interest (Bell et al. 2012; Woodruff et al. 2009). As a guideline, Gilliland et al. proposed use of variable spatial scales ranging from the household (<50 m) to regional (100–1,000 km) scale depending on the pollutant (Gilliland et al. 2005). Additionally, aggregating smaller geographic unit measurements to form larger units can result in overly smoothed measurements that hide varying exposures (Xu et al. 2011). Furthermore, a refined geographic scale may reduce some of the exposure misclassification associated with fixed AQMSs, as reliable exposure estimates may only be made within a certain distance from the AQMS (Lin et al. 2004a).

Studies confirm these key principles, finding that separate pollutants do not show consistent effects at similar buffer distances (Morello-Frosch et al. 2010). Several studies find that the effects of county-wide exposure estimates differ little from

effects within small radii from AQMS (Basu et al. 2004), but other studies find the reverse, with smaller areas yielding significantly different results (Wilhelm and Ritz 2005; Jalaludin et al. 2007; Darrow et al. 2009; Mannes et al. 2005; Basu et al. 2004). Hence, uncertainty remains as to which geographic scale best proxies personal exposures, or whether more spatially resolved studies produce more precise results. Often it is even difficult to discern what scale was used in studies, and inferences from studies using various approaches may not be comparable.

3.5.5 Windows of Vulnerability

Embryonic and fetal development varies significantly across pregnancy, with early pregnancy being particularly important due to implantation, placental attachment, and organogenesis, and later pregnancy being particularly important for fetal growth (Shah and Balkhair 2011). The prenatal period is characterized by rapid growth, organ growth and specialization, and brain development, among other things. This rapid development makes the fetus especially vulnerable to negative effects of air pollution. Thus, identifying potential windows of vulnerability to air pollution exposure is important in order to understand biological mechanisms (Aguilera et al. 2009; Woodruff et al. 2009) and possibly prevent adverse pregnancy outcomes. Identifying critical exposure windows is a research need, but is difficult because of variances in the mixture of pollutants across space and time, as well as possible different effects of specific pollutants during specific exposure periods (Woodruff et al. 2009; Bosetti et al. 2010; Salam 2008).

Studies vary in their designation of an exposure window. Table 3.3 presents the number of articles in our review that operationalized air pollution exposures during various windows by pollutants considered. Note that the article counts do not add up to the number of articles found in the literature review as many studies considered both multiple windows of exposure and multiple pollutants. While the majority measure exposure either during one or more trimesters (Wilhelm et al. 2011; Chang et al. 2012; Pereira et al. 2011) and/or the entire pregnancy term (Wilhelm et al.

Table 3.3 Article counts by windows of exposure and pollutants considered

	Pollutant							
Window of exposure	CO	O_3	PM_{10}	$PM_{2.5}$	SO_2	NO_2	PAHs	Traffic-related
Pregnancy	22	17	25	28	13	33	3	14
Trimesters	28	25	36	25	18	39	2	1
Months	6	6	7	4	5	8	1	0
Weeks	0	1	0	1	1	1	0	0
Lag before delivery (weeks, months)	10	10	12	9	5	11	0	0
First 1–3 months	3	3	3	2	2	3	0	0
Other	0	1	4	3	4	2	5	0

2011; Chang et al. 2012), others consider one or a combination of the following: day of birth (Zhao et al. 2011); selected weeks of pregnancy, including selected number of weeks past conception and/or prior to birth (Wilhelm and Ritz 2005; Wilhelm et al. 2011; Kim et al. 2007; Sagiv et al. 2005; Chang et al. 2012; Darrow et al. 2009; Iniguez et al. 2012; Rocha et al. 2008); all weeks of pregnancy (Warren et al. 2012); selected or all months of pregnancy (Jalaludin et al. 2007; Rudra et al. 2011; Hansen et al. 2006, 2007; Mannes et al. 2005; Liu et al. 2007; Dejmek et al. 2000; Wu et al. 2011; Brauer et al. 2008); and multi-year windows based on birth year (Rogers et al. 2000). Defining the most appropriate window of exposure is difficult, as there remains a dearth of toxicological evidence to drive the selection of these windows (Ritz and Wilhelm 2008). Further, the appropriate window may vary by outcome (Ritz and Wilhelm 2008; Woodruff et al. 2009). It is still unclear which exposure window is most critical or if consideration of smaller windows offers significant improvement in analyses. For this reason, some studies actually focus on identifying key windows of air pollution exposure (Warren et al. 2012).

Studies that compare full term or trimester windows to the corresponding monthly windows have found mixed results that are difficult to compare due to the variability of both pollutants and outcomes measured. While some show that monthly exposures differ from related trimester exposures (Hansen et al. 2006; Mannes et al. 2005), others find the results to be similar regardless of the window (Rudra et al. 2011; Hansen et al. 2006; Mannes et al. 2005; Liu et al. 2007; Wu et al. 2011). An additional challenge is the potential correlation of pollutants across time periods (Brauer et al. 2008). Some studies have found high correlations among the trimesters or across the pregnancy period (Brauer et al. 2008), making it difficult to delineate a particular window of vulnerability. Windows of exposure are also highly intertwined with the season of observation since it is well-documented that pollutant levels vary by season (Wilhelm and Ritz 2003), as does the correlation of pollutants across pregnancy (Aguilera et al. 2009). Strong negative correlations between first trimester and last month exposure for many pollutants, as well as strong positive correlations between second trimester and entire pregnancy averages, limit the ability to make conclusive statements regarding differences in risk across pregnancy periods (Wilhelm et al. 2011). Nearly all studies account for seasonal variation as a covariate or through other adjustments. Which trimester occurs during which season remains an important question, as the effect of the intensity of the pollutant exposure may interact with the prenatal development occurring during a particular trimester.

Some studies demonstrate that particular months have greater vulnerability (Hansen et al. 2007), while others argue that small exposure windows may not be relevant (Wu et al. 2011; Brauer et al. 2008). Similarly, findings by month sometimes mirror findings by trimester (Rudra et al. 2011; van den Hooven et al. 2012; Liu et al. 2007), and findings by trimester sometimes mirror findings for the entire pregnancy period (Morello-Frosch et al. 2010). Some studies find that trimester effects are stronger (Lee et al. 2003; Xu et al. 2011), while others find that the entire pregnancy period has the strongest effect (Morello-Frosch et al. 2010). In addition, an accumulative effect is likely to be present for some pollutants (Hansen et al. 2006).

Windows of exposure are also difficult to identify as the key windows may vary by both pollutant and outcome. For example, particular months or trimesters showed stronger effects for some pollutants (Olsson et al. 2012; Darrow et al. 2009; Wilhelm and Ritz 2003; Lee et al. 2003), but these results are inconsistent across studies, for both pollutants and outcomes. Some studies have found limited differences across exposure windows (Huynh et al. 2006; Wilhelm et al. 2011; Gray et al. 2011; Brauer et al. 2008). Similarly, the results vary by outcome. For preterm birth, some studies found early pregnancy to be an important window of vulnerability, as well as the last 6 weeks of pregnancy (Ritz et al. 2007; Wilhelm and Ritz 2005). For birth weight, fetal weight increases rapidly after 28 weeks, suggesting that later pregnancy may be most important (Maisonet et al. 2004). However, some studies found that exposure in the first 5 months of pregnancy had greater effects than later exposures on birth weight (Lee et al. 2003; Rossner et al. 2011). The particular findings, however, may be less important than the trajectory of the science. The methodology of examining windows of vulnerability using different time periods moves the field closer to understanding the chronological specificity of the effects of air pollution.

As the field progresses in determining the windows of greatest vulnerability, methodologies will likely become more refined in their definitions of windows of exposures and consistencies across studies may increase. However, pregnancy itself is a time of heightened vulnerability; therefore, regardless of which window brings with it the greatest vulnerability, a decrease in air pollution exposure during pregnancy for all women may subsequently decrease the risk of adverse pregnancy outcomes.

3.5.6 Multiple Pollutants

Multiple pollutant models are another area of growth in air pollution research. Currently, there is insufficient multi-pollutant work, and methodological challenges remain in this area (Ritz and Wilhelm 2008). Pollutants from the same source are correlated in both space and time, making it difficult to differentiate the effect of each pollutant and determine which pollutant may be most harmful (Shah and Balkhair 2011). In addition, pollutants are often not measured at the same monitoring stations, with the same accuracy, or on the same schedule, so measurement bias or differences may occur (Woodruff et al. 2009). Limiting sample size to individuals that have multi-pollutant data available may result in selection bias and limited power. Understanding how multi-pollutant exposures impact health is important, however, as most individuals are not exposed to single pollutants. Rather, air pollution is made up of many components, and there may be both accumulative and synergistic effects of these pollutants (Ritz and Wilhelm 2008).

Numerous studies have employed multi-pollutant models, with differing results (Olsson et al. 2012; Ritz et al. 2000, 2007; Liu et al. 2003; Lee et al. 2008; Zhao et al. 2011; van den Hooven et al. 2012; Bell et al. 2007; Geer et al. 2012; Estarlich et al. 2011; Ebisu and Bell 2012; Gouveia et al. 2004; Nascimento and Moreira 2009). These studies typically include pollutants that were significant in single pol-

lutant analyses (Gouveia et al. 2004) and that are not highly correlated (i.e., over .5) (Ebisu and Bell 2012). Some pollutants were robust to co-pollutant adjustment (Olsson et al. 2012; van den Hooven et al. 2012; Bell et al. 2007; Geer et al. 2012; Ebisu and Bell 2012), with some studies finding the multi-pollutant model to have stronger associations (Olsson et al. 2012; Ritz et al. 2007; van den Hooven et al. 2012), suggesting a synergistic effect of the pollutants. Other studies found that the associations of pollutants were not robust to multi-pollutant models (Ebisu and Bell 2012; Gouveia et al. 2004).

Pollutant exposures do not occur in isolation, and the combination of multiple air pollutants likely impacts pregnancy outcomes differentially, so multiple pollutant associations are important to consider. The high collinearity among such exposures make it difficult to identify which pollutant, in isolation, is the most deleterious (Shah and Balkhair 2011). Understanding how components of air pollution work synergistically, as well as in isolation, is an important advance to the study of the effects of air pollution on pregnancy outcomes (Woodruff et al. 2009). Studying combined air pollution exposures brings us closer to understanding air pollution in a real world context.

3.5.7 Interactions with Other Exposures and Risk Factors

As evidenced in this chapter, air pollution is associated with poor pregnancy outcomes. Little is known, however, about how this relationship works with other risk factors, such as poverty, minority status, or social stress. Vulnerable populations may have magnified responses to air pollution if it is concomitant with other risk factors (Shah and Balkhair 2011), such as health or neighborhood conditions. Elevated environmental exposures often occur in communities facing multiple social stressors like deteriorating housing, inadequate access to health care, poor schools, high unemployment, high crime, and high poverty – all of which may compound the effects of environmental exposures. This phenomenon is especially severe for low income and minority pregnant mothers, with significant health implications for the fetuses they carry.

The multiple interacting environmental, social, and host factors that influence pregnancy outcomes are depicted in Fig. 3.3 as three sides of an integrated triangle. Health disparities arise when the forces exerted by the triangle's sides are asymmetrical for different population groups. Given that minority and low-income women tend to be more systematically exposed to adverse environmental conditions (Brown 1995; Lopez 2002; Stretesky and Lynch 1999; Stretesky 2003; Wolff et al. 2003), air pollution may interact with adverse social environments (Stretesky and Lynch 1999), as well as host factors, to contribute to the observed poorer pregnancy outcomes among these women. Many monitoring stations are in areas where low-income, minority women live. Women who live within five miles of monitoring stations are often younger, more likely to be Hispanic, less educated, and more likely to be on government assistance (Wilhelm et al. 2011; Chang et al. 2012).

The differential impact of air pollution by race (Wilhelm and Ritz 2003; Choi et al. 2006, 2008) is of particular importance, especially since African American mothers are at higher risk of delivering low birth weight or preterm infants. This race effect may be due to baseline health conditions, socioeconomic status, or access to health care (Bell et al. 2012). Maternal characteristics, such as prenatal care adequacy, maternal medical complications, and neighborhood socioeconomic status measures (poverty, unemployment, home ownership, and educational status) are additional risk factors that may interact with air pollution (Morello-Frosch et al. 2010). Socioeconomic status has been associated with increased risk of adverse pregnancy outcomes, with air pollution disproportionately affecting lower socioeconomic neighborhoods (Ponce et al. 2005; Zeka et al. 2008).

Many studies obtain demographic and socioeconomic information from birth certificates. This information may not be sufficient to fully examine contextual risk factors for the association between air pollution and pregnancy outcomes (Woodruff et al. 2009). The impact of these contextual factors may additionally vary by geography, outcome, and pollutant (Woodruff et al. 2009). In addition to sociodemographic factors, there is limited evidence that males may be more vulnerable to air pollution adverse effects than females (Ebisu and Bell 2012; Woodruff et al. 2009; Ghosh et al. 2012).

3.6 Future Directions for Research and Conclusions

While the impact of air pollution on respiratory health has long been explored, increasingly researchers are turning their attention to potential impacts on pregnancy outcomes. The growing literature in this area provides strong evidence of a negative impact of air pollution exposure on pregnancy outcomes. In addition, the incongruous findings between some studies highlights important areas for future research, including critical gaps created by methodological differences across studies. Previous work tells us that spatial scale and particular windows of vulnerability both matter in analyzing the impact of air pollution on pregnancy outcomes. In addition, exposure levels vary substantially across existing studies, as do ways of measuring exposure.

In terms of key future research needs, we need to better understand the impact of air pollution at the low end of the exposure scale – which constitute the levels at which women are most commonly exposed. In addition, the field needs to investigate multiple pollutants simultaneously, rather than the more traditional one contaminant at a time approach. More modeled surfaces would lead to less selection bias and more overlap of estimates for different pollutants, allowing for more robust analysis of associations with adverse pregnancy outcomes.

Components of air pollution may also differ by geography. Particulate matter, for example, consists of several discrete particles of differing sizes that originate from varying sources. Depending on local and regional factors such as development, industry, agriculture, and the natural environment, particulate matter may be composed of

different particles, which may account, at least in part, for some of the contradictory or mixed findings in the literature. Some studies have already begun to examine the effects of different PM source components to tease out individual effects (Wilhelm et al. 2011; Bell et al. 2010, 2012; Ebisu and Bell 2012); this is an area that warrants additional research attention (Glinianaia et al. 2004; Backes et al. 2013).

Better monitoring technology, such as continuous portable monitors, is under development and will likely enable data collection over shorter time windows, as well as increased geographic coverage of air sampling. These improvements are especially important for urban areas with greater pollutant variability as well as for assessing short term peak exposures (Gilliland et al. 2005).

All these areas for future research are critical to developing a better understanding of the association between air pollution and pregnancy outcomes. However, while studies have established an association between air pollution and adverse pregnancy outcomes, these studies are inherently unable to establish a causal relationship. To truly understand pregnancy health impacts of air pollution, the results of epidemiological studies need to be coupled with animal studies that more directly and more tightly manipulate exposures and allow for exploration of mechanistic effects. While the results of animal studies are beyond the scope of this chapter, such approaches allow for identification of causal pathways, which in the end is most needed to establish the appropriate regulatory environment and protect prenatal and perinatal health.

References

Aguilera I, Guxens M, Garcia-Esteban R, Corbella T, Nieuwenhuijsen MJ, Foradada CM et al (2009) Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort. Environ Health Perspect 117(8):1322–1327

American Lung Association (2011) State of the air

Avchen RN, Scott KG, Mason CA (2001) Birth weight and school-age disabilities: a population-based study. Am J Epidemiol 154(10):895–901

Backes CH, Nelin T, Gorr MW, Wold LE (2013) Early life exposure to air pollution: how bad is it? Toxicol Lett 216(1):47–53

Ballester F, Estarlich M, Iniguez C, Llop S, Ramon R, Esplugues A et al (2010) Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environ Health 9:6

Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH (1993) Growth in utero and serum cholesterol concentrations in adult life. BMJ 307(6918):1524–1527

Barnett AG, Plonka K, Seow WK, Wilson LA, Hansen C (2011) Increased traffic exposure and negative birth outcomes: a prospective cohort in Australia. Environ Health 10:26

Basu R, Woodruff TJ, Parker JD, Saulnier L, Schoendorf KC (2004) Comparing exposure metrics in the relationship between PM2.5 and birth weight in California. J Expo Anal Environ Epidemiol 14(5):391–396

Behrman RE, Butler AS (2007) Preterm birth – causes, consequences and prevention. National Academies Press, Washington, DC

Bell ML, Ebisu K, Belanger K (2007) Ambient air pollution and low birth weight in Connecticut and Massachusetts. Environ Health Perspect 115(7):1118–1124

Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P et al (2010) Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Epidemiology 21(6):884–891

Bell ML, Belanger K, Ebisu K, Gent JF, Leaderer BP (2012) Relationship between birth weight and exposure to airborne fine particulate potassium and titanium during gestation. Environ Res 117:83–89

Berrocal VJ, Gelfand AE, Holland DM, Burke J, Miranda ML (2011) On the use of a PM(2.5) exposure simulator to explain birthweight. Environmetrics 22(4):553–571

Bobak M (2000) Outdoor air pollution, low birth weight, and prematurity. Environ Health Perspect 108(2):173–176

Bobak M, Leon DA (1999) Pregnancy outcomes and outdoor air pollution: an ecological study in districts of the Czech Republic 1986–8. Occup Environ Med 56(8):539–543

Boothe VL, Shendell DG (2008) Potential health effects associated with residential proximity to freeways and primary roads: review of scientific literature, 1999–2006. J Environ Health 70(8):33–36

Bosetti C, Nieuwenhuijsen MJ, Gallus S, Cipriani S, La VC, Parazzini F (2010) Ambient particulate matter and preterm birth or birth weight: a review of the literature. Arch Toxicol 84(6):447–460

Brauer M, Lencar C, Tamburic L, Koehoorn M, Demers P, Karr C (2008) A cohort study of traffic-related air pollution impacts on birth outcomes. Environ Health Perspect 116(5):680–686

Brown P (1995) Race, class, and environmental health: a review and systematization of the literature. Environ Res 69(1):15–30

Chang HH, Reich BJ, Miranda ML (2012) Time-to-event analysis of fine particle air pollution and preterm birth: results from North Carolina, 2001–2005. Am J Epidemiol 175(2):91–98

Choi H, Perera FP (2012) Sources of greater fetal vulnerability to airborne polycyclic aromatic hydrocarbons among African Americans. J Epidemiol Community Health 66(2):121–126

Choi H, Jedrychowski W, Spengler J, Camann DE, Whyatt RM, Rauh V et al (2006) International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. Environ Health Perspect 114(11):1744–1750

Choi H, Rauh V, Garfinkel R, Tu Y, Perera FP (2008) Prenatal exposure to airborne polycyclic aromatic hydrocarbons and risk of intrauterine growth restriction. Environ Health Perspect 116(5):658–665

Choi H, Wang L, Lin X, Spengler JD, Perera FP (2012) Fetal window of vulnerability to airborne polycyclic aromatic hydrocarbons on proportional intrauterine growth restriction. PLoS One 7(4):e35464

Crofts BJ, King R, Johnson A (1998) The contribution of low birth weight to severe vision loss in a geographically defined population. Br J Ophthalmol 82(1):9–13

Currie J, Neidell M, Schmieder JF (2009) Air pollution and infant health: lessons from New Jersey. J Health Econ 28(3):688–703

Dadvand P, Parker J, Bell ML, Bonzini M, Brauer M, Darrow LA et al (2013) Maternal exposure to particulate air pollution and term birth weight: a multi-country evaluation of effect and heterogeneity. Environ Health Perspect 121(3):267–373

Darrow LA, Klein M, Flanders WD, Waller LA, Correa A, Marcus M et al (2009) Ambient air pollution and preterm birth: a time-series analysis. Epidemiology 20(5):689–698

Dejmek J, Solansky I, Benes I, Lenicek J, Sram RJ (2000) The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. Environ Health Perspect 108(12):1159–1164

Dolk H, Pattenden S, Vrijheid M, Thakrar B, Armstrong B (2000) Perinatal and infant mortality and low birth weight among residents near cokeworks in Great Britain. Arch Environ Health 55(1):26–30

Doussard-Roosevelt JA, Porges SW, Scanlon JW, Alemi B, Scanlon KB (1997) Vagal regulation of heart rate in the prediction of developmental outcome for very low birth weight preterm infants. Child Dev 68(2):173–186

Dugandzic R, Dodds L, Stieb D, Smith-Doiron M (2006) The association between low level exposures to ambient air pollution and term low birth weight: a retrospective cohort study. Environ Health 5:3

Ebisu K, Bell ML (2012) Airborne PM2.5 chemical components and low birth weight in the northeastern and mid-Atlantic regions of the United States. Environ Health Perspect 120(12):1746–1752

Estarlich M, Ballester F, Aguilera I, Fernandez-Somoano A, Lertxundi A, Llop S et al (2011) Residential exposure to outdoor air pollution during pregnancy and anthropometric measures at birth in a multicenter cohort in Spain. Environ Health Perspect 119(9):1333–1338

Geer LA, Weedon J, Bell ML (2012) Ambient air pollution and term birth weight in Texas from 1998 to 2004. J Air Waste Manag Assoc 62(11):1285–1295

Gehring U, van Eijsden M, Dijkema MB, van der Wal MF, Fischer P, Brunekreef B (2011a) Traffic-related air pollution and pregnancy outcomes in the Dutch ABCD birth cohort study. Occup Environ Med 68(1):36–43

Gehring U, Wijga AH, Fischer P, de Jongste JC, Kerkhof M, Koppelman GH et al (2011b) Traffic-related air pollution, preterm birth and term birth weight in the PIAMA birth cohort study. Environ Res 111(1):125–135

Genereux M, Auger N, Goneau M, Daniel M (2008) Neighbourhood socioeconomic status, maternal education and adverse birth outcomes among mothers living near highways. J Epidemiol Community Health 62(8):695–700

Ghosh JK, Wilhelm M, Su J, Goldberg D, Cockburn M, Jerrett M et al (2012) Assessing the influence of traffic-related air pollution on risk of term low birth weight on the basis of land-use-based regression models and measures of air toxics. Am J Epidemiol 175(12):1262–1274

Gilliland F, Avol E, Kinney P, Jerrett M, Dvonch T, Lurmann F et al (2005) Air pollution exposure assessment for epidemiologic studies of pregnant women and children: lessons learned from the Centers for Children's Environmental Health and Disease Prevention Research. Environ Health Perspect 113(10):1447–1454

Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D (2004) Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiology 15(1):36–45

Gouveia N, Bremner SA, Novaes HM (2004) Association between ambient air pollution and birth weight in Sao Paulo, Brazil. J Epidemiol Community Health 58(1):11–17

Gray SC, Edwards SE, Miranda ML (2010) Assessing exposure metrics for PM and birth weight models. J Expo Sci Environ Epidemiol 20(5):469–477

Gray SC, Gelfand AE, Miranda ML (2011) Hierarchical spatial modeling of uncertainty in air pollution and birth weight study. Stat Med 30(17):2187–2198

Ha EH, Hong YC, Lee BE, Woo BH, Schwartz J, Christiani DC (2001) Is air pollution a risk factor for low birth weight in Seoul? Epidemiology 12(6):643–648

Hack M, Klein NK, Taylor HG (1995) Long-term developmental outcomes of low birth weight infants. Future Child 5(1):176–196

Hamilton BE, Martin JA, Ventura SJ (2012) Births: preliminary data for 2011. Natl Vital Stat Rep 61(5):1–19

Hansen C, Neller A, Williams G, Simpson R (2006) Maternal exposure to low levels of ambient air pollution and preterm birth in Brisbane, Australia. BJOG 113(8):935–941

Hansen C, Neller A, Williams G, Simpson R (2007) Low levels of ambient air pollution during pregnancy and fetal growth among term neonates in Brisbane, Australia. Environ Res 103(3):383–389

Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD et al (2011) Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation 123(8):933–944

Hitosugi M, Motozawa Y, Kawato H, Nagai T, Tokudome S (2009) Experimental analysis of the relationship between simulated low-velocity rear-end collisions and fetal outcomes of pregnant rats. Med Sci Law 49(3):213–217

Hoggatt KJ, Greenland S, Ritz BR (2009) Adjustment for response bias via two-phase analysis: an application. Epidemiology 20(6):872–879

Huynh M, Woodruff TJ, Parker JD, Schoendorf KC (2006) Relationships between air pollution and preterm birth in California. Paediatr Perinat Epidemiol 20(6):454–461
Iniguez C, Ballester F, Estarlich M, Esplugues A, Murcia M, Llop S et al (2012) Prenatal exposure to traffic-related air pollution and fetal growth in a cohort of pregnant women. Occup Environ Med 69(10):736–744
Jalaludin B, Mannes T, Morgan G, Lincoln D, Sheppeard V, Corbett S (2007) Impact of ambient air pollution on gestational age is modified by season in Sydney, Australia. Environ Health 6:16
Jedrychowski W, Bendkowska I, Flak E, Penar A, Jacek R, Kaim I et al (2004) Estimated risk for altered fetal growth resulting from exposure to fine particles during pregnancy: an epidemiologic prospective cohort study in Poland. Environ Health Perspect 112(14):1398–1402
Jedrychowski W, Perera F, Mrozek-Budzyn D, Mroz E, Flak E, Spengler JD et al (2009) Gender differences in fetal growth of newborns exposed prenatally to airborne fine particulate matter. Environ Res 109(4):447–456
Kashima S, Naruse H, Yorifuji T, Ohki S, Murakoshi T, Takao S et al (2011) Residential proximity to heavy traffic and birth weight in Shizuoka, Japan. Environ Res 111(3):377–387
Kim OJ, Ha EH, Kim BM, Seo JH, Park HS, Jung WJ et al (2007) PM10 and pregnancy outcomes: a hospital-based cohort study of pregnant women in Seoul. J Occup Environ Med 49(12):1394–1402
Kloog I, Melly SJ, Ridgway WL, Coull BA, Schwartz J (2012) Using new satellite based exposure methods to study the association between pregnancy PM(2). (5) exposure, premature birth and birth weight in Massachusetts. Environ Health 11:40
Lee BE, Ha EH, Park HS, Kim YJ, Hong YC, Kim H et al (2003) Exposure to air pollution during different gestational phases contributes to risks of low birth weight. Hum Reprod 18(3):638–643
Lee SJ, Hajat S, Steer PJ, Filippi V (2008) A time-series analysis of any short-term effects of meteorological and air pollution factors on preterm births in London, UK. Environ Res 106(2):185–194
Lee PC, Roberts JM, Catov JM, Talbott EO, Ritz B (2012) First trimester exposure to ambient air pollution, pregnancy complications and adverse birth outcomes in Allegheny County, PA. Matern Child Health J 17(3):545–555
Lemons JA, Bauer CR, Oh W, Korones SB, Papile LA, Stoll BJ et al (2001) Very low birth weight outcomes of the National Institute of Child health and human development neonatal research network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics 107(1):E1
Lepeule J, Caini F, Bottagisi S, Galineau J, Hulin A, Marquis N et al (2010) Maternal exposure to nitrogen dioxide during pregnancy and offspring birth weight: comparison of two exposure models. Environ Health Perspect 118(10):1483–1489
Lewtas J (2007) Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. Mutat Res 636(1–3):95–133
Lin CM, Li CY, Yang GY, Mao IF (2004a) Association between maternal exposure to elevated ambient sulfur dioxide during pregnancy and term low birth weight. Environ Res 96(1):41–50
Lin CM, Li CY, Mao IF (2004b) Increased risks of term low-birth-weight infants in a petrochemical industrial city with high air pollution levels. Arch Environ Health 59(12):663–668
Lipkind HS, Slopen ME, Pfeiffer MR, McVeigh KH (2012) School-age outcomes of late preterm infants in New York City. Am J Obstet Gynecol 206(3):222–226
Liu S, Krewski D, Shi Y, Chen Y, Burnett RT (2003) Association between gaseous ambient air pollutants and adverse pregnancy outcomes in Vancouver, Canada. Environ Health Perspect 111(14):1773–1778
Liu S, Krewski D, Shi Y, Chen Y, Burnett RT (2007) Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction. J Expo Sci Environ Epidemiol 17(5):426–432
Llop S, Ballester F, Estarlich M, Esplugues A, Rebagliato M, Iniguez C (2010) Preterm birth and exposure to air pollutants during pregnancy. Environ Res 110(8):778–785

Lopez R (2002) Segregation and black/white differences in exposure to air toxics in 1990. Environ Health Perspect 110(Suppl 2):289–295

Lorenz JM, Wooliever DE, Jetton JR, Paneth N (1998) A quantitative review of mortality and developmental disability in extremely premature newborns. Arch Pediatr Adolesc Med 152(5):425–435

Maisonet M, Bush TJ, Correa A, Jaakkola JJ (2001) Relation between ambient air pollution and low birth weight in the Northeastern United States. Environ Health Perspect 109(Suppl 3):351–356

Maisonet M, Correa A, Misra D, Jaakkola JJ (2004) A review of the literature on the effects of ambient air pollution on fetal growth. Environ Res 95(1):106–115

Malmqvist E, Rignell-Hydbom A, Tinnerberg H, Bjork J, Stroh E, Jakobsson K et al (2011) Maternal exposure to air pollution and birth outcomes. Environ Health Perspect 119(4):553–558

Mannes T, Jalaludin B, Morgan G, Lincoln D, Sheppeard V, Corbett S (2005) Impact of ambient air pollution on birth weight in Sydney, Australia. Occup Environ Med 62(8):524–530

Maroziene L, Grazuleviciene R (2002) Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environ Health 1(1):6

Masters ET, Jedrychowski W, Schleicher RL, Tsai WY, Tu YH, Camann D et al (2007) Relation between prenatal lipid-soluble micronutrient status, environmental pollutant exposure, and birth outcomes. Am J Clin Nutr 86(4):1139–1145

Medeiros A, Gouveia N (2005) Relationship between low birthweight and air pollution in the city of Sao Paulo, Brazil. Rev Saude Publica 39(6):965–972

Ment LR, Vohr B, Allan W, Westerveld M, Katz KH, Schneider KC et al (1999) The etiology and outcome of cerebral ventriculomegaly at term in very low birth weight preterm infants. Pediatrics 104(2 Pt 1):243–248

Miranda ML, Edwards SE, Chang HH, Auten RL (2013) Proximity to roadways and pregnancy outcomes. J Expo Sci Environ Epidemiol 23(1):32–38

Mohorovic L (2004) First two months of pregnancy–critical time for preterm delivery and low birthweight caused by adverse effects of coal combustion toxics. Early Hum Dev 80(2):115–123

Morello-Frosch R, Jesdale BM, Sadd JL, Pastor M (2010) Ambient air pollution exposure and full-term birth weight in California. Environ Health 9:44

Nascimento LF, Moreira DA (2009) Are environmental pollutants risk factors for low birth weight? Cad Saude Publica 25(8):1791–1796

Olsson D, Ekstrom M, Forsberg B (2012) Temporal variation in air pollution concentrations and preterm birth-a population based epidemiological study. Int J Environ Res Public Health 9(1):272–285

Osmond C, Barker DJ, Winter PD, Fall CH, Simmonds SJ (1993) Early growth and death from cardiovascular disease in women. BMJ 307(6918):1519–1524

Padula AM, Mortimer K, Hubbard A, Lurmann F, Jerrett M, Tager IB (2012) Exposure to traffic-related air pollution during pregnancy and term low birth weight: estimation of causal associations in a semiparametric model. Am J Epidemiol 176(9):815–824

Parker JD, Woodruff TJ (2008) Influences of study design and location on the relationship between particulate matter air pollution and birthweight. Paediatr Perinat Epidemiol 22(3):214–227

Parker JD, Woodruff TJ, Basu R, Schoendorf KC (2005) Air pollution and birth weight among term infants in California. Pediatrics 115(1):121–128

Parker JD, Rich DQ, Glinianaia SV, Leem JH, Wartenberg D, Bell ML et al (2011) The international collaboration on air pollution and pregnancy outcomes: initial results. Environ Health Perspect 119(7):1023–1028

Pereira G, Nassar N, Cook A, Bower C (2011) Traffic emissions are associated with reduced fetal growth in areas of Perth, Western Australia: an application of the AusRoads dispersion model. Aust N Z J Public Health 35(5):451–458

Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D et al (2003) Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect 111(2):201–205

Perera FP, Rauh V, Whyatt RM, Tsai WY, Bernert JT, Tu YH et al (2004) Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population. Environ Health Perspect 112(5):626–630

Petrou S (2005) The economic consequences of preterm birth during the first 10 years of life. BJOG 112(Suppl 1):10–15

Ponce NA, Hoggatt KJ, Wilhelm M, Ritz B (2005) Preterm birth: the interaction of traffic-related air pollution with economic hardship in Los Angeles neighborhoods. Am J Epidemiol 162(2):140–148

Rahmalia A, Giorgis-Allemand L, Lepeule J, Philippat C, Galineau J, Hulin A et al (2012) Pregnancy exposure to atmospheric pollutants and placental weight: an approach relying on a dispersion model. Environ Int 48:47–55

Resnick MB, Gueorguieva RV, Carter RL, Ariet M, Sun Y, Roth J et al (1999) The impact of low birth weight, perinatal conditions, and sociodemographic factors on educational outcome in kindergarten. Pediatrics 104(6):e74

Rich DQ, Demissie K, Lu SE, Kamat L, Wartenberg D, Rhoads GG (2009) Ambient air pollutant concentrations during pregnancy and the risk of fetal growth restriction. J Epidemiol Community Health 63(6):488–496

Ritz B, Wilhelm M (2008) Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. Basic Clin Pharmacol Toxicol 102(2):182–190

Ritz B, Yu F, Chapa G, Fruin S (2000) Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. Epidemiology 11(5):502–511

Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JK (2007) Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. Am J Epidemiol 166(9):1045–1052

Robb JA, Sibley MH, Pelham WE, Foster EM, Molina BSG, Gnagy EM et al (2011) The estimated annual cost of ADHD to the US education system. School Ment Health 3:169–177

Rocha E, Silva IR, Lichtenfels AJ, Amador Pereira LA, Saldiva PH (2008) Effects of ambient levels of air pollution generated by traffic on birth and placental weights in mice. Fertil Steril 90(5):1921–1924

Rogers JF, Dunlop AL (2006) Air pollution and very low birth weight infants: a target population? Pediatrics 118(1):156–164

Rogers JF, Thompson SJ, Addy CL, McKeown RE, Cowen DJ, Decoufle P (2000) Association of very low birth weight with exposures to environmental sulfur dioxide and total suspended particulates. Am J Epidemiol 151(6):602–613

Ross G, Lipper EG, Auld PA (1990) Social competence and behavior problems in premature children at school age. Pediatrics 86(3):391–397

Rossner P Jr, Tabashidze N, Dostal M, Novakova Z, Chvatalova I, Spatova M et al (2011) Genetic, biochemical, and environmental factors associated with pregnancy outcomes in newborns from the Czech Republic. Environ Health Perspect 119(2):265–271

Rudra CB, Williams MA, Sheppard L, Koenig JQ, Schiff MA (2011) Ambient carbon monoxide and fine particulate matter in relation to preeclampsia and preterm delivery in western Washington State. Environ Health Perspect 119(6):886–892

Sagiv SK, Mendola P, Loomis D, Herring AH, Neas LM, Savitz DA et al (2005) A time-series analysis of air pollution and preterm birth in Pennsylvania, 1997–2001. Environ Health Perspect 113(5):602–606

Saigal S, Hoult LA, Streiner DL, Stoskopf BL, Rosenbaum PL (2000) School difficulties at adolescence in a regional cohort of children who were extremely low birth weight. Pediatrics 105(2):325–331

Salam MT (2008) Air pollution and birth weight in Connecticut and Massachusetts. Environ Health Perspect 116(3):A106–A107

Salam MT, Millstein J, Li YF, Lurmann FW, Margolis HG, Gilliland FD (2005) Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: results from the Children's Health Study. Environ Health Perspect 113(11):1638–1644

Satrell E, Roksund O, Thorsen E, Halvorsen T (2012) Pulmonary gas transfer in children and adolescents born extremely preterm. Eur Respir J 22

Seo JH, Leem JH, Ha EH, Kim OJ, Kim BM, Lee JY et al (2010) Population-attributable risk of low birthweight related to PM10 pollution in seven Korean cities. Paediatr Perinat Epidemiol 24(2):140–148

Shah PS, Balkhair T (2011) Air pollution and birth outcomes: a systematic review. Environ Int 37(2):498–516

Slama R, Morgenstern V, Cyrys J, Zutavern A, Herbarth O, Wichmann HE et al (2007) Traffic-related atmospheric pollutants levels during pregnancy and offspring's term birth weight: a study relying on a land-use regression exposure model. Environ Health Perspect 115(9):1283–1292

Slama R, Thiebaugeorges O, Goua V, Aussel L, Sacco P, Bohet A et al (2009) Maternal personal exposure to airborne benzene and intrauterine growth. Environ Health Perspect 117(8):1313–1321

Slama R, Grabsch C, Lepeule J, Siroux V, Cyrys J, Sausenthaler S et al (2010) Maternal fine particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring birth weight. Reprod Toxicol 30(4):600–612

Sram RJ, Binkova B, Dejmek J, Bobak M (2005) Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect 113(4):375–382

Stevenson RC, McCabe CJ, Pharoah PO, Cooke RW (1996) Cost of care for a geographically determined population of low birthweight infants to age 8–9 years. I. Children without disability. Arch Dis Child Fetal Neonatal Ed 74(2):F114–F117

Stretesky PB (2003) The distribution of air lead levels across U.S. counties: implications for the production of racial inequality. Sociol Spectr 23(1):91–118

Stretesky PB, Lynch M (1999) Environmental justice and the predictions of distance to accidental chemical releases in Hillsborough County, Florida. Soc Sci Q 97(1 Suppl):289–310

Suh YJ, Kim BM, Park BH, Park H, Kim YJ, Kim H et al (2007) Cytochrome P450IA1 polymorphisms along with PM(10) exposure contribute to the risk of birth weight reduction. Reprod Toxicol 24(3–4):281–288

Suh YJ, Ha EH, Park H, Kim YJ, Kim H, Hong YC (2008) GSTM1 polymorphism along with PM10 exposure contributes to the risk of preterm delivery. Mutat Res 656(1–2):62–67

Suh YJ, Kim H, Seo JH, Park H, Kim YJ, Hong YC et al (2009) Different effects of PM10 exposure on preterm birth by gestational period estimated from time-dependent survival analyses. Int Arch Occup Environ Health 82(5):613–621

Swamy GK, Ostbye T, Skjaerven R (2008) Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. JAMA 299(12):1429–1436

Tang D, Li TY, Liu JJ, Chen YH, Qu L, Perera F (2006) PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort. Environ Health Perspect 114(8):1297–1300

Urbaniec N, Namyslowski G, Morawski K, Urbaniec P, Turecka L, Bazowska G (2004) Effects of intrauterine hypotrophy and perinatal hypoxia on cochlear function evaluated by click evoked otoacoustic emissions (TEOAE). Otolaryngol Pol 58(2):365–372

U.S.Census Bureau (2010) 2010 Census summary file 1. Ref type: Online source

U.S.Centers for Disease Control and Prevention (2013) National Center for Health Statistics. Vital Stats. US Birth Data 2010. 24 May 2013. Ref type: Online source

U.S.Environmental Protection Agency (2011) Our nation's air, status and trends through 2010

U.S.Environmental Protection Agency (2013) Air quality system site and monitor records. 24 May 2013

van den Hooven EH, Jaddoe VW, de Kluizenaar Y, Hofman A, Mackenbach JP, Steegers EA et al (2009) Residential traffic exposure and pregnancy-related outcomes: a prospective birth cohort study. Environ Health 8:59

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW et al (2012) Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. Environ Health Perspect 120(1):150–156

Veras MM, Damaceno-Rodrigues NR, Caldini EG, Maciel Ribeiro AA, Mayhew TM, Saldiva PH et al (2008) Particulate urban air pollution affects the functional morphology of mouse placenta. Biol Reprod 79(3):578–584

Wang X, Ding H, Ryan L, Xu X (1997) Association between air pollution and low birth weight: a community-based study. Environ Health Perspect 105(5):514–520

Wang ML, Dorer DJ, Fleming MP, Catlin EA (2004) Clinical outcomes of near-term infants. Pediatrics 114(2):372–376

Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M (2011) Health and economic burden of the projected obesity trends in the USA and the UK. Lancet 378(9793):815–825

Warren J, Fuentes M, Herring A, Langlois P (2012) Spatial-temporal modeling of the association between air pollution exposure and preterm birth: identifying critical windows of exposure. Biometrics 68(4):1157–1167

Weiland K, Neidell M, Rauh V, Perera F (2011) Cost of developmental delay from prenatal exposure to airborne polycyclic aromatic hydrocarbons. J Health Care Poor Underserved 22(1):320–329

Weiss JL, Malone FD, Emig D, Ball RH, Nyberg DA, Comstock CH et al (2004) Obesity, obstetric complications and cesarean delivery rate–a population-based screening study. Am J Obstet Gynecol 190(4):1091–1097

Wilhelm M, Ritz B (2003) Residential proximity to traffic and adverse birth outcomes in Los Angeles County, California, 1994–1996. Environ Health Perspect 111(2):207–216

Wilhelm M, Ritz B (2005) Local variations in CO and particulate air pollution and adverse birth outcomes in Los Angeles County, California, USA. Environ Health Perspect 113(9):1212–1221

Wilhelm M, Ghosh JK, Su J, Cockburn M, Jerrett M, Ritz B (2011) Traffic-related air toxics and preterm birth: a population-based case-control study in Los Angeles County, California. Environ Health 10:89

Wilhelm M, Ghosh JK, Su J, Cockburn M, Jerrett M, Ritz B (2012) Traffic-related air toxics and term low birth weight in Los Angeles County, California. Environ Health Perspect 120(1):132–138

Williams BL, Pennock-Roman M, Suen HK, Magsumbol MS, Ozdenerol E (2007) Assessing the impact of the local environment on birth outcomes: a case for HLM. J Expo Sci Environ Epidemiol 17(5):445–457

Wolff MS, Britton JA, Wilson VP (2003) Environmental risk factors for breast cancer among African-American women. Cancer 97(1 Suppl):289–310

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H et al (2009) Methodological issues in studies of air pollution and reproductive health. Environ Res 109(3):311–320

Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B (2009) Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. Environ Health Perspect 117(11):1773–1779

Wu J, Wilhelm M, Chung J, Ritz B (2011) Comparing exposure assessment methods for traffic-related air pollution in an adverse pregnancy outcome study. Environ Res 111(5):685–692

Xu X, Sharma RK, Talbott EO, Zborowski JV, Rager J, Arena VC et al (2011) PM10 air pollution exposure during pregnancy and term low birth weight in Allegheny County, PA, 1994–2000. Int Arch Occup Environ Health 84(3):251–257

Yang CY, Chang CC, Chuang HY, Ho CK, Wu TN, Tsai SS (2003a) Evidence for increased risks of preterm delivery in a population residing near a freeway in Taiwan. Arch Environ Health 58(10):649–654

Yang CY, Tseng YT, Chang CC (2003b) Effects of air pollution on birth weight among children born between 1995 and 1997 in Kaohsiung, Taiwan. J Toxicol Environ Health A 66(9):807–816

Yip FY, Pearcy JN, Garbe PL, Truman BI (2011) Unhealthy air quality – United States, 2006–2009. MMWR Surveill Summ 60(Suppl):28–32

Yorifuji T, Naruse H, Kashima S, Murakoshi T, Tsuda T, Doi H et al (2012) Residential proximity to major roads and placenta/birth weight ratio. Sci Total Environ 414:98–102

Zeka A, Melly SJ, Schwartz J (2008) The effects of socioeconomic status and indices of physical environment on reduced birth weight and preterm births in Eastern Massachusetts. Environ Health 7:60

Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J et al (2010) Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res Clin Pract 87(3):293–301

Zhao Q, Liang Z, Tao S, Zhu J, Du Y (2011) Effects of air pollution on neonatal prematurity in Guangzhou of China: a time-series study. Environ Health 10:2

Chapter 4
Air Pollution and Asthma

David B. Peden

4.1 Introduction

Asthma is a chronic inflammatory airway disease most commonly associated with an eosinophilic (TH2-type) inflammation, though chronic neutrophilic inflammation has also been reported and multiple variations (or endotypes) in this disease are being identified which are variably characterized by eosinophilic and neutrophilic inflammation (Anderson 2008; Belgrave et al. 2013; Busse and Lemanske 2001; Busse and Rosenwasser 2003; Custovic et al. 2013; Lotvall et al. 2011; Wenzel 2012). Airway pathophysiology of asthma is characterized by intermittent airway bronchoconstriction and mucus hypersecretion (and mucus plugging) with resultant decrease in airflow and symptoms such as cough, wheeze and breathlessness. In severe exacerbations, oxygenation is impaired and air trapping occurs. All of these events are linked to airway hyperresponsiveness, in which airway response to a wide variety of environmental stressors is increased, most notably increased response to cholinergic agonists and airway dehydration (Busse and Lemanske 2001; Busse and Rosenwasser 2003).

Environmental exposures are an important factor in the pathobiology of asthma. Allergic processes play a defining role in atopic asthma, with mite, cockroach and mammalian (both pest and pet) exposures linked to disease development (Matsui et al. 2008; Sharma et al. 2007; Zeldin et al. 2006). Occupational exposures lead to asthma genesis (Bernstein 2011; Cartier and Sastre 2011; Christiani et al. 2008; Malo and Vandenplas 2011; Redlich and Karol 2002; Szema 2012; Tarlo et al. 2008), and chronic exposure to ambient air pollutants and tobacco smoke have been associated with development of asthma (Eisner 2002; Jaakkola and Jaakkola 2002;

D.B. Peden, M.D., M.S. (✉)
Division of Allergy, Immunology & Rheumatology, Department of Pediatrics,
Center for Environmental Medicine, Asthma and Lung Biology, School of Medicine,
University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7310, USA
e-mail: David_Peden@med.unc.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_4

Etzel 2003; Kodgule and Salvi 2012; Accordini et al. 2012; Burke et al. 2012; Anto 2012). Acute exacerbation of asthma is triggered by environmental pollutants, including ozone (Balmes 1993; D'Amato et al. 2005; Hanna et al. 2011; Hernandez et al. 2010; McDonnell et al. 1999; Moore et al. 2008; Peden 2011; Peden et al. 1995; Romieu et al. 1997), particulate matter (Breysse et al. 2005; Diette et al. 2007; McConnell et al. 1999, 2003, 2010; Ostro et al. 2009; Peel et al. 2005; Schwartz et al. 1993; Silverman and Ito 2010; Zhang et al. 2002), biomass combustion products (e.g. wood smoke, tobacco smoke) (Kodgule and Salvi 2012; Accordini et al. 2012; Burke et al. 2012; Anto 2012; Agrawal 2012; Brims and Chauhan 2005; Bruce et al. 2000; Crain et al. 2002; David et al. 2005; Eisner et al. 2002; Gilliland et al. 2003; Jones et al. 2006; Mannino et al. 2002), and bioaerosols (Zeldin et al. 2006; Clapp et al. 1993; Eldridge and Peden 2000; Liu 2004; McConnell et al. 2006; Perzanowski et al. 2006; Rabinovitch et al. 2005; Thorne et al. 2005; Von Essen and Donham 1999). Additionally, other causes of acute asthma, including viral infection (Jaspers et al. 2005; Noah et al. 2012a, b) and acute response to allergen (Eldridge and Peden 2000; Bernstein et al. 2004; Boehlecke et al. 2003; Diaz-Sanchez et al. 1997, 2006; Gilliland et al. 2004, 2006; Holz et al. 2002; Jorres et al. 1996; Kehrl et al. 1999; Schaumann et al. 2008; Vagaggini et al. 2002), can be modified by environmental pollutant exposure. Thus, air pollutants impact both asthma development and exacerbation.

While environmental pollutants associated with asthma pathobiology seem somewhat disparate, they do have a number of features in common. First, each of these agents can induce an inflammatory response likely due to activation of innate immune processes (Hollingsworth et al. 2004, 2007a, b, 2010; Li et al. 2010). Second, increased oxidative stress is associated with many of these pollutants, suggesting molecular processes by which they can modulate innate immune responses (Peden 2005, 2011). Third, as outlined above many of these agents enhance response to other stimuli (most notably allergens) which may impact risk for disease exacerbation. Table 4.1 summarizes the actions of a number of NAAQS pollutants known to impact asthma.

We hypothesize that modulation of common biological processes accounts for many of the actions of air pollutants in asthma. These include activation of innate immune responses, induction of oxidative stress (and variation in antioxidant defense mechanisms), and impact on elements of IgE-mediated immunity. This

Table 4.1 Common acute actions of several pollutants which impact asthma

	NAAQS pollutant			
Physiologic effect	O_3	PM	NO_2	SO_2
Airway inflammation	++++	+++	++	+
Airway reactivity	+	+	+	+++
Allergen reactivity	+++	++++	+	+
Nociceptive decrease in lung function	++++	+	+	+

chapter will outline the effects of a number of pollutants in asthmatics, as well as potential individual and societal interventions by which the impact of pollutants may be mitigated.

4.2 Pollutants of Concern in Asthma

A number of outdoor (ambient) air pollutants are monitored as outlined in the Clean Air Act of 1970 (Bernstein et al. 2004; Peden 2008). The pollutants included in the Clean Air Act (so-called "criteria pollutants") include carbon monoxide, lead, sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), particulate matter ≤ 10 μm (PM 2.5–10) and 2.5 μm (PM 0.5–2.5) in diameter. Of these, SO_2, NO_2, O_3, PM (both PM2.5–10 and PM 0.5–2.5) have the most known impact in asthmatics. In indoor environments, second hand tobacco smoke, and other biomass related particles and bioaerosols (a common component of which is endotoxin) are known causes of asthma exacerbation. The epidemiology and actions of these agents in asthma are outlined below.

4.3 Particulate Matter

Particulate matter is a major cause of asthma exacerbation and wheeze in such varied locations as Asia, Oceania, Europe and the United States. Coarse mode PM (PM 2.5–10 μm) and fine mode PM (PM 0.5–2.5 μm) derive from a variety of sources, including point sources (power plants, factories) and mobile sources (automobiles, trucks). PM is composed of a variety of constituents, including metals, carbon black, polyaromatic hydrocarbons and other products of incomplete combustion and biological components (Bernstein et al. 2004; Peden 2008).

Traditional point sources, including power plants, steel mills, and other industrial sites are well known sources of PM and have been clearly linked with asthma exacerbation. One of the most intriguing demonstrations of the link between a point source and asthma exacerbation occurred in the 1980s in the Utah Valley. During this period, there was a year-long labor dispute at a local steel mill, resulting in a shutdown of the plant during this period. Examination of hospitalizations for respiratory diseases and PM revealed that during the strike year there was a marked decrease in both ambient air PM levels and hospital admissions for asthma and other respiratory diseases (Pope 1989, 1991). The strike not only associated with decreased PM levels, but the inflammatory action of PM recovered during the shutdown was decreased. Both epidemiologic studies and airway challenges suggest that metals modulate much of the pro-inflammatory effects of PM. This is likely due to oxidant activity associated with transition metal content of the particles (Ghio 2004; Schaumann et al. 2004). Exposure studies using concentrated air particulates (CAPS) or instilled particulates also demonstrate that ambient air particulates

(usually PM2.5) cause airway inflammation (Ghio et al. 2000, 2003, 2012; Ghio and Devlin 2001; Graff et al. 2009; Huang et al. 2012).

Vehicular traffic is also an important source of PM. Many studies suggest that PM levels within 500 m of a heavily traveled roadway are increased, as are exacerbations of asthma. One of the more common components of vehicular PM is diesel exhaust particles (DEP). One of the more intriguing aspects of DEP is the effect of these particles on TH2 inflammation. Numerous animal, in vitro and human studies have shown that DEP exerts are robust pro-TH2 effect (Diaz-Sanchez et al. 2003; Peden 2002; Riedl and Diaz-Sanchez 2005; Pandya et al. 2002). Numerous human challenge studies have examined the effect of diesel exhaust and DEP on airway inflammation and lung function.

Nasal challenge studies in humans have shown that DEP modulates IgE mediated responses. In the first of these studies, 4 atopic and 7 non-atopic volunteers underwent nasal challenge with increasing doses of DEP. 0.3 mg of DEP induced nasal IgE production (but not that of other immunoglobulin classes) 4 days after challenge (Diaz-Sanchez et al. 1994). The same group has observed that 0.3 mg DEP nasal challenge augments levels of Interleukin (IL)-4, IL-5, IL-6, and IL-10 mRNA in recovered airway cells as well as enhances ragweed specific IgE and IgG response to ragweed allergen when compared to challenge with ragweed alone (Diaz-Sanchez et al. 1996, 1997). This group also used topical immunization of the nasal mucosa with keyhole limpet hemocyanan (KLH) to examine the effect of DEP on primary response to a neoantigen in humans. They found that DEP promoted local IgE and IgG response to KLH, whereas control treatment with saline was associated with only an IgG response to KLH (Diaz-Sanchez et al. 1999). Taken together, these observations show that DEP is a pro-TH2 adjuvant in humans.

Another group of British and Swedish investigators examined inflammatory, cytokine and antioxidant airway responses of normal and allergic volunteers exposed to diesel exhaust with particles diluted to a concentration of 100–300 μg diesel particles/m^3 (Behndig et al. 2006; Mudway et al. 2004; Pourazar et al. 2004, 2005; Rudell et al. 1996; Salvi et al. 2000; Stenfors et al. 2004). Overall, responses to these exposures included increased non-specific airway reactivity, airway neutrophilia, IL-6, IL-8 and myeloperoxidase in BAL fluid. In bronchial tissue biopsies from these studies, mast cell and neutrophil numbers, ICAM-1, VCAM-1 and IL-13 expression and for phosphorylated p38, NF- kB (p65), and phosphorylated JNK is increased after diesel exposure.

Viral infection is also a significant cause of airway morbidity in asthma. Jaspers et al used live attenuated influenza virus infection as a model infection of the nasal airway to assess the effect of diesel exhaust exposure on LAIV infection. They found that diesel exposure was associated with augmented viral mRNA in nasal samples (Noah et al. 2012b). This effect was exaggerated in samples from allergic volunteers, and allergic volunteers also had evidence of increased eosinophilic inflammation. *In vitro* studies show that human epithelial cells exposed to diesel exhaust have increased expression of TLR3 and increased viral infection (Jaspers et al. 2005; Ciencewicki et al. 2006). These data suggest that diesel exhaust increases

susceptibility to viral infection with coincident enhancement of allergic airway inflammation.

An increasingly important source of PM in the United States and worldwide are wood smoke particles (WSP) generated by various fire events. Recent estimates indicate that landscape fires alone account for between 260,000 and 600,000 deaths worldwide annually (Johnston et al. 2012). The National Interagency Fire Center reports that in 2012 there were 67,774 wildfires involving 9,326,238 acres in the United States, demonstrating risk for WSP exposure from wildfires. Wood smoke particle (WSP) exposures also occur with use of wood for indoor heating or recreational activities, prescribed outdoor burns, and accidental structure fires. One example of WSP-induced adverse health outcomes were those observed during a wildfire in the eastern part of North Carolina in 2008 (Rappold et al. 2011). Studies of this event revealed an increased relative risk for asthma [1.65 (95 % confidence interval, 1.25–2.1)], chronic obstructive pulmonary disease [1.73 (1.06–2.83)], and pneumonia and acute bronchitis [1.59 (1.07–2.34)] associated with increased ambient air WSP exposure. Additionally, emergency department (ED) visits for cardiopulmonary symptoms [RR = 1.23 (1.06–1.43)] and heart failure [RR = 1.37 (1.01–1.85)] were also significantly increased in the exposed population. While both respiratory tract and systemic effects were seen in this study, the greatest impact was seen in asthmatics.

However, precise mechanisms by which WSP would impact asthma are largely unknown. A lack of controlled exposure studies of healthy or asthmatic volunteers results in a significant gap in knowledge of how WSP exposure impacts exposed populations (Naeher et al. 2007; Noonan and Balmes 2010; Zelikoff et al. 2002). To date, the majority of the few controlled exposure studies examining the effect of WSP on airway inflammation in volunteers have involved studies of healthy or atopic, but not asthmatic, volunteers (Ghio et al. 2012; Riddervold et al. 2012; Stockfelt et al. 2012; Barregard et al. 2006, 2008; Sallsten et al. 2006). Furthermore, the endpoints of these studies have primarily been symptoms, lung function, evidence of inflammation and a small number examining impact of cardiovascular responses. While the direct effect of acute WSP exposure seems modest, other effects, including increased either non-specific or allergen-specific bronchial reactivity has not been well studied. Regardless, the epidemiological data is compelling for biomass derived PM playing an important role in asthma exacerbation.

Side stream tobacco smoke (STS, side stream smoke from the burning end of the cigarette and exhaled mainstream smoke from the smoker) is also a major source respirable particles in indoor environments and is perhaps the most significant and remediable indoor air contaminant in the United States. An example of the impact of STS on indoor particulates was shown in a study of 11 restaurants in Paducah, Kentucky. In these locations the mean PM2.5 concentration in smoking areas was 177 $\mu g/m^3$ vs. 87 $\mu g/m^3$ in the non-smoking sections of these establishments. The PM 2.5 concentration of the non-smoking section was 29 times higher than that in smoke-free air and six times higher than local outdoor air in Paducah (Jones et al. 2006).

Early-life secondhand tobacco smoke (STS) exposure contributes to asthma development. A substantial percentage of children are subjected to perinatal tobacco smoke exposure or STS exposure during early childhood (Accordini et al. 2012; Gilliland et al. 2000; Lannero et al. 2006; Morgan and Martinez 1992). Effects of STS on DNA methylation and other epigenetic modifications through oxidative stress mechanisms are hypothesized to modify the lifelong effect of children exposed to tobacco smoke *in utero* or during early life (Lovinsky-Desir and Miller 2012). Tobacco smoke exposure also causes acute wheezing illnesses and asthma exacerbations in children. STS exposure increases urinary leukotriene E4 in children (a biomarker for atopic disease), which is associated with subsequent albuterol usage (Rabinovitch et al. 2008, 2011a, b). Although avoidance of home exposures have been shown to reduce asthma-related hospitalizations and emergency room visits, large-scale intervention studies thus far have showed modest to no changes in parental smoking habits. The evidence is overwhelming that STS is a cause of airway disease, with numerous reviews detailing the role of STS in asthma exacerbation and development of allergy (Etzel 2003; Gergen 2001; Gilmour et al. 2006; Gold 2000).

Diaz-Sanchez and colleagues in Southern California examined the effect of experimental exposure to STS on nasal responses to allergen in humans, They reported a remarkable increase in allergen-induced specific IgE and IgG4, increased IL-4, IL-5 and IL-13, and decreased levels of γ-interferon associated with STS exposure. There were also augmented amounts of histamine in nasal lavage fluid after allergen challenge associated with STS exposure (Diaz-Sanchez et al. 2006). It is notable that polyaromatic hydrocarbons, which mediate the TH2 promoting actions of DEP as previously discussed, are also found in STS. Taken together, these mechanistic studies support epidemiological reports demonstrating that STS exposure enhances development of atopy and asthma (Bernstein et al. 2004; Gilmour et al. 2006; Nel et al. 2001).

Bioaerosols are another type of airborne contaminant present in both indoor and outdoor airsheds. The most vigorously studied bioaerosol component is bacterial endotoxin, which has been found on recovered outdoor coarse and fine mode PM and indoor dust samples (Rabinovitch et al. 2005; Carty et al. 2003; Mueller-Anneling et al. 2004; Pacheco et al. 2002). With regard to development of asthma and allergy, endotoxin has two faces. On one hand, a substantial body of epidemiological and laboratory studies suggest that early life exposure to endotoxin may protect against the development of IgE responses to allergens (Liu 2002, 2004; Fuleihan 2002; Liu and Redmon 2001; Liu and Leung 2006; von Mutius 2000). This so-called "hygiene hypothesis" posits that as lifestyle changes have occurred in which immune response to microbial elements, including pathogen associated molecular patterns (PAMPS) is decreased, there has been increased development of TH2 responses. Such changes are widely thought to be multifactorial, including increased use of antibiotics, decreased exposure to mammals (be they pets, vermin, livestock or even other people) and the bacterial components which come from their skin and waste, increased general cleanliness, use of immunizations and almost certainly a host of other changes in human lifestyle which generally occurred

simultaneously over the past 100 years. However, contrasting with these studies is evidence that acute airway disease is exacerbated following increased exposure to endotoxin (Liu 2002, 2004; Thorne et al. 2005; Fuleihan 2002; Liu and Redmon 2001; Liu and Leung 2006; von Mutius 2000; Heederik and Sigsgaard 2005; Von Essen 1997; Von Essen et al. 1995a, b, 1998; Von Essen and McCurdy 1998). Occupational exposure to endotoxin (primarily agricultural) reveals that airway disease increases with increased exposure. In infants, wheezing events have been shown to correlate with levels of endotoxin present in household dust. In school age children with asthma, personal endotoxin exposure is correlated with increased asthma symptoms. Thus, in occupational, domestic and ambient settings, endotoxin in the airshed can cause exacerbation of airway disease.

Furthermore, while endotoxin exposure in early life (during sensitization) may protect against sensitization to allergen, such exposure may enhance response to allergen in already sensitized volunteers. We have reported that nasal challenge with endotoxin augments response to house dust mite allergen in the nasal airway (Eldridge and Peden 2000), and that exposure to low level particle bound endotoxin (250 ng/m^3) augments immediate phase response to inhaled mite allergen (Boehlecke et al. 2003). In studies of the effect of endotoxin instillation on response to instilled allergen in the bronchial airways, it was found that LPS augmented recovery of allergen-associated influx of lymphocytes, neutrophils, eosinophils, monocytes, and myeloid DCs, and as well as increased levels of lipopolysaccharide-binding protein, IL-1alpha, IL-6, and tumor necrosis factor-alpha in the bronchoalveolar lavage fluid compared with allergen alone (Schaumann et al. 2008).

Endotoxin also interacts with environmental agents other than allergen. In domestic settings, it has been shown that in environments in which airborne cotinine is increased, so too is disease morbidity linked to endotoxin exposure. In settings in which indoor NO_2 levels are increased, endotoxin exposure is associated with decreased symptoms. However, in low NO_2 settings, endotoxin is linked to increased disease (Matsui et al. 2013). In another study, having dogs associated with a specific domestic environment increased symptoms and responses to ambient air pollutants, most notably PM (McConnell et al. 2006). Overall, the environmental endotoxin appears to enhance response to pollutants, just as it enhances response to allergens. However, the precise nature of endotoxin exposure on response to specific environment agents remains incompletely understood.

4.3.1 Ozone

Of the various pollutants for which a National Ambient Air Quality Standard (NAAQS) has been established, ozone is the pollutant that the public most commonly encounters at levels that exceed its NAAQS limit (0.075 ppm O_3 over 8 h). Ozone is unquestionably associated with exacerbation of asthma, typically with a 1–2 day lag between exposure and exacerbation (Bernstein et al. 2004; Peden 2008). While levels above the NAAQS are associated with disease, it has also been shown

that asthma exacerbation can occur at levels as low as 0.06 ppm (Bernstein et al. 2004; Gent et al. 2003). In one recent study, it was shown that in asthmatic children aged 6-18, there was a 20 % increase in hospitalization admission and a 19 % increased risk for admission to the Intensive Care Unit for each 0.022 ppm increase in ozone (Silverman and Ito 2010). Other data suggest that ozone exposure might either cause, or at least unmask asthma. In a study in Southern California, children without asthma who played aerobic sports we assessed for presence of asthma over several years (McConnell et al. 2002). Those who lived in a higher ozone area were 3.5 fold more likely to have a new diagnosis of asthma identified than those who either lived in a low ozone area, or those who did not play outdoor aerobic sports.

The mechanism by which ozone causes asthma exacerbation is not entirely clear. In human challenge studies, ozone has been reported to cause a number of responses, the most avidly studied being including a pain (C-fiber) mediated immediate and short lived decrease in lung function and an acutely increased neutrophilic inflammation that includes increased PMNs (Bernstein et al. 2004; Barnes 1995; Bromberg and Koren 1995). Ozone exposure also causes influx of dendritic cells and macrophages with increased expression of innate immune and antigen presentation markers (Alexis et al. 2004; Lay et al. 2007), as well as increased airway permeability and bronchial reactivity (Kehrl et al. 1987; Que et al. 2011). It is important to note that as with epidemiology studies, several human challenge studies have shown that human airway responses to ozone can be initiated at levels of ozone as low as 0.06 ppm ozone (Adams 2006; Alexis et al. 2010, 2013; Kim et al. 2011).

Not all people express each response the same way. It has been shown that some people with a robust lung function effect of ozone have a modest (or absent) PMN response. Likewise, people with a muted lung function response to ozone may have increased airway reactivity. In short, each of these responses is likely mediated in whole or part by unique mechanisms (Hernandez et al. 2010, 2012a; Peden 2008, 2011; Bromberg and Koren 1995). Some pharmacologic studies support this idea. For instance, cyclooxygenase inhibitors, inhaled lidocaine, and opiate analgesics will inhibit or reverse the lung function effect of ozone, but have little effect on inflammation or airway reactivity (Alexis et al. 2000; Hazucha et al. 1989, 1996; Passannante et al. 1998; Schelegle et al. 1987; Ying et al. 1990). Likewise, inhaled corticosteroids and targeted anti-inflammatory agents will decrease the inflammatory response to ozone, but have no effect on the nociceptive lung function response (Alexis et al. 2008; Holz et al. 2005; Lazaar et al. 2011; Vagaggini et al. 2001).

While asthmatics have similar general responses to ozone as do non-asthmatics, they do have exaggerated innate immune/inflammatory responses to this gas. This includes increased PMNs in the airway, as well as increased IL-1 and IL-8 levels and increased expression of TLR4, antigen presentation markers on airway monocytes and macrophages (Hernandez et al. 2010, 2012b; Basha et al. 1994). Additionally, as atopic asthmatics and rhinitics have increased baseline TH2 inflammation, ozone exposure enhances airway eosinophilia and eosinophil cationic protein levels in airway fluids, whereas this is not seen in non-atopic volunteers (Peden et al. 1995, 1997; Bascom et al. 1990). Ozone challenge has also been shown to enhance immediate response to inhaled allergen (as shown by decreased PD15 or

PD20 levels) and increased allergen-induced eosinophil numbers following challenge (Peden et al. 1995; Holz et al. 2002; Jorres et al. 1996; Kehrl et al. 1999; Bascom et al. 1990; Chen et al. 2004; Molfino et al. 1991). It is not clear which ozone responses mediate increased responsiveness to allergen. However, we hypothesize that ozone-induced changes in airway inflammation or permeability contribute to enhanced response to allergen.

It is important to note that the effect of ozone on nociceptive decrements in lung function is influenced by the ambient air concentration, duration of exposure, and minute ventilation (McDonnell et al. 1983, 1993). Female gender and increased BMI may also be risk factors for increased lung function response to ozone (Bennett et al. 2007). Less is known about exposure/response characteristics for the inflammatory response to ozone. One recent study utilizing resting or exercise face-mask exposure to 0.4 ppm $^{-18}O_3$ again found that resting exposure did not impact lung function compared to exposure with exercise (Hatch et al. 1994). More intriguingly, ozonation products from the lower airway (in BAL fluid) were increased with exercise exposure, whereas they were increased in nasal lavage fluid with resting exposure. There was also increased airway inflammatory response reflected in BAL fluid associated with exercise exposure to ozone. Taken together, these observations support the hypothesis that the respiratory dose of ozone is a function of concentration, duration of exposure and ventilation. Indeed, even robust doses of ozone exert little effect on the lower respiratory tract with typical resting ventilation. Equally interesting, there is increase deposition and inflammation in the nasal airway at rest. To the extent that allergic rhinitics or airway infection might be impacted by ozone, this may be more likely at rest than with exercise.

4.3.2 Sulfur Dioxide

Ambient SO_2 can be encountered either as an acid aerosol (H_2SO_4) or as a gas phase pollutant. Increased ambient air exposure to SO_2 is associated with higher hospital admission rates and emergency room visits for respiratory disease (Bernstein et al. 2004; Health Effects of Outdoor Air Pollution 1996; Horstman et al. 1982, 1988; Koenig and Pierson 1991; Pierson et al. 1986; Schachter et al. 1984; Sunyer et al. 2003; Trenga et al. 2001). Decreased lung function in children and the risk of developing chronic asthma or obstructive lung disease likewise is associated with increased chronic exposure to SO_2 (Health Effects of Outdoor Air Pollution 1996; Sunyer et al. 2003; Delfino et al. 2003). However, it can be difficult to separate effects of sulfur dioxide from that of particulate air pollutants.

As outlined in previous reviews (Peden 1997, 2008), challenge studies with sulfur dioxide do not reveal marked inflammatory effects at relevant concentrations. However, this gas has potent bronchospastic effects in asthmatics. Normal volunteers are unaffected with concentrations as high as 0.6 ppm while in sensitive asthmatics the FEV1 can be reduced by as much as 60 % at concentrations of 0.25 ppm, though most asthmatics react to 0.50 ppm. Other SO_2-related symptoms in

asthmatics include wheezing, chest discomfort and dyspnea. The actions of SO_2 are usually observed within 2 min, becoming maximal within 5–10 min into exposure. Spontaneous recovery occurs within 30 min and exposed asthmatics are refractory to the effects of SO_2 for up to 4 h after initial exposure. Repeated exposures to SO_2 induce tachyphylaxis as well (Peden 1997, 2008).

Nasal breathing reduces the effect of SO_2 in asthmatics, due to absorption of this water-soluble gas by the nasal mucosa. As with ozone, exercise augments response in the lower airway. This is likely because exercise causes in a shift from nasal breathing to combined oral and nasal respiration, increasing the amount of SO_2 delivered to the bronchial airway. As asthmatics have a high occurrence of nasal co-morbidities (such as allergic rhinitics or sinusitis), this may increase the impact of SO_2 in this population.

4.3.3 Nitrogen Dioxide

Ambient air and indoor NO_2 (likely from gas stove use) has been shown to exert chronic and acute changes in lung function in numerous epidemiologic studies. It is a precursor for generation of ozone, but NO_2 has direct effects on inflammatory response. NO_2 has been shown to induce inflammatory cytokines secretion by epithelial cells *in vitro. In vivo* challenges of healthy volunteers have shown that NO_2 exposure causes an influx of airway PMNs. Higher levels of NO_2 (4.0 ppm) may impact airway function of asthmatics, but several investigators have reported that challenge with ambient levels of NO_2 did not modify non-specific airway reactivity (Peden 1997; Barnes 1994; Koenig 1999).

NO_2 does have a more noticeable effect on airway mucosal responsiveness to allergen in allergic asthmatics (Jenkins et al. 1999; Tunnicliffe et al. 1994; Wang et al. 1995a, b). Challenge with 0.4 ppm NO_2 for 4 h increases response to to inhaled allergen. Likewise, challenge to 0.2 ppm SO_2 and 0.4 NO_2 for 6 h increased immediate responses of mild asthmatics to inhaled allergen. Like ozone, NO_2 enhances late phase cellular responses of asthmatics to allergen. Another study found that exposure to 0.4 ppm NO_2 for 6 h increases allergen-induced eosinophil cationic protein in the nasal airways of allergic asthmatics. Taken together, these studies demonstrate that NO_2 augments acute response to allergen in atopic subjects.

4.4 Genetic Influences on the Response to Pollutants Relevant to Asthma

Large scale GWAS analysis of gene by environment interactions in respiratory disease has identified a number of candidate genes which modify risk for disease. However, many studies exploring gene by environment interactions in respiratory

and allergic diseases have been underpowered, and negative results are often underreported. Thus, a complete understanding of the genetic factors which likely modulate risks for adverse health effects to pollutants is elusive (London 2007). However, there are some genetic polymorphisms associated with oxidative stress and innate immune response have emerged as potential modulators of risk of environmentally-induced airway disease and are briefly reviewed below.

Exposure to most air pollutants promotes the radical formation and genetic variations that cause relative deficiencies of antioxidant defenses may allow for greater radical-induced airway inflammation and hyperreactivity following pollutant exposure. A number of antioxidant enzymes are regulated by master transcription factor nuclear factor (erythroid-derived 2)-like 2 (NRF2) regulate cellular and mucosal oxidant stress. Cells that encounter oxidative stress activate NRF2 binding to the Antioxidant Response Element (ARE), which initiates the transcription of a broad range of these antioxidant genes, protecting cells from the harmful effects of oxidative agents (Peden 2005; Kleeberger and Peden 2005).

Polymorphisms of several glutathione S-transferase (GST) genes have been examined due to the role of these enzymes in antioxidant defense. The glutathione-S-transferase Mu1 (GSTM1), null genotype has been associated with increased response to environmental agents. The GSTM1 null genotype (which results in no production of the GSTM1 protein) has been associated with an increased risks of wheezing in children exposed to tobacco smoke during the perinatal period and acute exacerbation of asthma following ambient air ozone exposure (Peden 2011; Gilliland et al. 2002; Romieu et al. 2004, 2006). In human challenge studies, the GSTMI null genotype increased have increased neutrophil influx to the airway 24 h following exposure to ozone at a level of 400 ppb as well as increased the risk of having a PMN response to 0.06 ppm O_3 by 13 fold (Peden 2011; Alexis et al. 2009, 2013). In human epithelial cells in culture, knockdown of GSTM1 led to enhanced IL-8 production from human bronchial epithelial cells exposed to ozone (Peden 2011; Alexis et al. 2009, 2013; Wu et al. 2011). Thus deletion of GSTM1 causes increased risk of response to ozone.

The GSTM1 null genotype has also been shown to augment response to particulates. Inhalational challenge to 20,000 endotoxin unit doses of endotoxin, characterized by increased PMN response as well as with elevated levels of IL-1β and TNFα in the sputum (Dillon et al. 2011). This level of endotoxin is similar to that to which a worker in an animal farming operation would be exposed to during an 8 h work shift. Genetic variation in both GSTM1 and GSTP1 modify the adjuvant effect of diesel exhaust particles on allergic inflammation. Subjects with the null genotype for GSTM1 and GSTP1 codon 105 variants have been reported to have enhanced nasal allergic responses in the presence of diesel exhaust particles (Gilliland et al. 2004). Subjects with these genetic variants also demonstrate larger responses to allergens with secondhand tobacco smoke with increases in nasal-allergen specific IgE and histamine (Gilliland et al. 2006).

The G-308A single nucleotide polymorphism of the promoter region of the gene for TNFα has also been associated with increased risk for pollutants induced disease in epidemiologic studies. Yang et al reviewed a number of studies examining the

impact of TNFα variations on response to pollutants (Yang et al. 2008). Human challenge studies have shown that this SNP modifies response to O_3 and SO_2. In one study of 51 participants exposed to ozone (Yang et al. 2005), a statistically significantly greater effect of O_3 on decrease in FEV1 was observed in those subjects with the TNF –308G/G genotype. This SNP is also a component of a specific haplotype thought to modulate response to O_3. The other elements of this haplotype include SNPs for lymphotoxin A (LTA) and two other regions of the TNF gene. It was found that the LTA +252G/TNF–1031 T/TNF–308A/TNF–238G haplotype conferred the smallest change in FEV1 with ozone exposure. It has also been shown that the TNF –308G/G genotype significantly increased the risk of being a SO_2 responder (>12 % decrease in FEV1 with SO_2) in a study of 51 asthmatics exposed to 0.5 ppm SO_2. In this study, all of the volunteers who met the definition of SO_2 responsiveness (n = 12) had this genotype (Yang et al. 2008).

The importance of interaction between antioxidant and pro-inflammatory genes was shown in an epidemiological study of 1,123 children. In this study, the TNF –308G/G genotype was associated with decreased risk of wheezing, especially in those living in low ozone areas. However, the protective effect of this TNF genotype was less in subjects with GSTM1-null and GSTP1 Ile/Ile genotypes, which are associated with decreased antioxidant defense. These are somewhat conflicting findings, as the TNF –308G/G genotype is associated with a greater fall in FEV1 with O_3 and SO_2, whereas this genotype was protective against wheezing in low ozone communities (Li et al. 2006).

One explanation for this apparent discrepancy may lie in the different effects of these genes on specific pollutant response phenotypes. For instance, O_3 evokes both a nociceptive response on lung function and inflammatory response in the airway. The GSTM1 null has no effect on the nociceptive response, but significant impact on airway inflammation. It is possible that in the population study, nociceptive effects on lung function may actually protect against disease due to inflammation. When coupled with GST SNPs which likely allow for increased inflammation, the TNF impact on wheeze may have been negated. These results highlight the need for continued definition of pollutant response phenotypes, functional confirmation of the biology of specific genetic factors on response to pollutants and understanding of gene X gene X environment interactions.

4.5 Potential Therapeutic Interventions for Pollutant-Induced Airway Disease

While specific agents targeting pollutant-induced asthma exacerbation are not currently clinically approved, understanding the potential mechanisms for pollutant-exacerbated asthma allows investigation into potential therapeutic interventions. Anti-inflammatory agents and antioxidants both have substantial potential to decrease the effects of pollutants on airway disease epithelial cells, as reviewed by

Table 4.2 Summary of early human studies of potential interventions to prevent pollutant effects

Intervention	Reported action
Inhaled corticosteroids	Prevents neutrophilic inflammation associated with O_3 and endotoxin
Cyclo-oxygenase inhibitors	Prevents nociceptive decrease in lung function following O_3 challenge
Ascorbic acid and alpha tocopherol	Associated with decrease effect of ozone in field studies, especially in GSTM1 deficient individuals
Sulforaphane	Shown to upregulate antioxidant enzymes in humans, proposed actions for PM, O_3 effects
Gamma tocopherol	Decreases neutrophilic response to inhaled endotoxin

Auerbach and Hernandez (2012). The effects of a number of potential interventions to mitigate the effect of pollutants is shown in Table 4.2

A number of anti-inflammatory agents have been demonstrated to decrease airway hyperreactivity in response to pollutants. Sodium cromoglycate inhibits LPS-induced bronchial obstruction in asthmatic subjects who were pre-treated with this agent (Michel et al. 1995). Corticosteroids may also be useful for acute pollution-exacerbated asthma. Inhaled corticosteroids reduce airway neutrophilia caused by ozone exposure in asthmatics and normal volunteers (Alexis et al. 2008; Vagaggini et al. 2001). Lazaar et al recently described a selective chemokine (C-X-C motif) receptor 2 (CXCR2) antagonist that inhibits chemokine (C-X-C motif) ligand 1 (CXCL1) -induced CD11b expression on peripheral blood neutrophils with resultant decrease in neutrophil activation and recruitment in ozone-induced airway neutrophilia, suggesting a potential role for this antagonist in neutrophil-predominant airway morbidity (Lazaar et al. 2011).

Antioxidant interventions have also received attention as potential interventions for pollutant-induced airway disease. Different research teams have reported that combination treatment with α-tocopherol and vitamin C reduces ozone-induced lung function decrements in asthmatics (Romieu et al. 1998, 2002; Romieu and Trenga 2001). A study in normal volunteers found that this combination was useful in healthy volunteers following a 3 week antioxidant depleted diet for 3 weeks (mimicking a state of poor antioxidant nutritional status) (Samet et al. 2001). Gamma tocopherol (another variant of vitamin E) has also been shown to mitigate endotoxin induced airway inflammation in both animal and human studies (Hernandez et al. 2013).

Oral supplementation with sulforaphane, an antioxidant compound recovered from specially bred broccoli, causes upregulation of NRF2-regulated Phase II enzymes (including GSTM1). Sulforaphane has been shown to induce phase II enzymes in B cells, effectively reducing diesel exhaust particle enhancement of IgE-production and in primary bronchial epithelial cells, reducing pro-inflammatory cytokine production by diesel (Wan and Diaz-Sanchez 2006). *In vivo* studies reveal that Phase II enzymes in nasal epithelial cells can be induced by 3 days of oral SFN

supplementation (Ritz et al. 2007). Further work is required to determine which antioxidant interventions may be best suited for particular pollutant exposures, the best route of administration, and which populations may benefit the most from particular interventions.

In addition to pharmaceutical/nutritional interventions, societal interventions can also decrease pollutant induced disease. One example of this was already outlined above in which a work stoppage at a steel mill in the Utah Valley resulted in decreased PM production associated with decreased respiratory disease (Pope 1989, 1991). In the 1996 Olympics in Atlanta, traffic patterns and use of major roadways were modified to accommodate the games (Friedman et al. 2001). These traffic interventions were associated with decreased ozone levels and fewer hospital and ER events related to asthma and respiratory disease compared to periods immediately before and after the games. Finally, it is very clear that removal of tobacco smoke from an indoor environment will markedly reduce airway disease. Thus, policy interventions can have significant impact on individual health outcomes.

4.6 Summary

While many ambient air pollutants contribute to development and exacerbation of asthma, some likely have greater impact than others. In the United States and other developed nations, the ambient levels of many NAAQS pollutants have decreased, to a great extent as a result of public policy decisions made over the past 30 years. However, despite these improvements, pollutant induced asthma continues to be a problem. This may be due to increased susceptibility of the population resulting from increases in atopy, obesity (which is increasingly recognized as a risk factor for response to pollutants) or patterns of exposure associated with socioeconomic status. Also, just as multiple lifestyle factors likely contribute to the increase in atopy, multiple simultaneous factors, many of which may currently be unknown, likely enhance response to pollutants. Yet to be determined is the full impact of modern diets, changes in mucosal microbiome, pharmacologic impacts on pollutants and simultaneous exposure to several pollutants.

Ozone and particulate matter appear to be of greater impact than other outdoor pollutants. As public policy changes have been helpful in causing a reduction in NAAQS pollutants, it is clear that further reductions in ambient air pollutants will be increasingly difficult to achieve. Further, levels of pollutants below the current NAAQS will cause responses in subsets of the populations (e.g. asthmatics, those genetically predisposed to pollutant effects). Thus, it will be important to understand how simultaneous pollutant exposures impact human biology, as regulatory efforts may require more nuanced efforts at multiple pollutants. It may be most effective to focus on the actions of ozone or PM with various pollutants to assess the potential for regulation of complex mixtures. It will also be important to identify interventions that individuals can employ to mitigate the impact of pollutant exposures when they known (or anticipated) for that individual. Identification of specific

risk factors may help to identify those who are most likely to benefit from interventions. Finally, it will be important to appreciate the impact of air pollutants on respiratory health in the developing world. In region where indoor biomass burden is increased due to biofuel burning cookstove use is prevalent, deploying cookstoves which vent resultant smoke outdoors may significantly decrease pollutant burden. Also, it seems likely that PM may prove to be even more important than ozone in these regions, and both policy and individual interventions should focus on the impact of PM.

References

Accordini S, Janson C, Svanes C, Jarvis D (2012) The role of smoking in allergy and asthma: lessons from the ECRHS. Curr Allergy Asthma Rep 12(3):185–191

Adams WC (2006) Comparison of chamber 6.6-h exposures to 0.04-0.08 PPM ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 18(2):127–136

Agrawal S (2012) Effect of indoor air pollution from biomass and solid fuel combustion on prevalence of self-reported asthma among adult men and women in India: findings from a nationwide large-scale cross-sectional survey. J Asthma 49(4):355–365

Alexis N, Urch B, Tarlo S, Corey P, Pengelly D, O'Byrne P et al (2000) Cyclooxygenase metabolites play a different role in ozone-induced pulmonary function decline in asthmatics compared to normals. Inhal Toxicol 12(12):1205–1224

Alexis NE, Becker S, Bromberg PA, Devlin R, Peden DB (2004) Circulating CD11b expression correlates with the neutrophil response and airway mCD14 expression is enhanced following ozone exposure in humans. Clin Immunol 111(1):126–131

Alexis NE, Lay JC, Haczhu A, Gong H, Linn W, Hazucha M et al (2008) Fluticasone propionate protects against ozone-induced airways inflammation and modified immune cell activation markers in normal volunteers. Environ Health Perspect 116(6):799–805

Alexis NE, Zhou H, Lay JC, Harris B, Hernandez ML, Lu TS et al (2009) The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 29

Alexis NE, Lay JC, Hazucha M, Harris B, Hernandez ML, Bromberg PA et al (2010) Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. Inhal Toxicol 22(7):593–600

Alexis NE, Lay JC, Zhou H, Kim CS, Hernandez ML, Kehrl H et al (2013) The glutathione-S-transferase mu 1 (GSTM1) null genotype and increased neutrophil response to low-level ozone (0.06 ppm). J Allergy Clin Immunol 131(2):610–612

Anderson GP (2008) Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 372(9643):1107–1119

Anto JM (2012) Recent advances in the epidemiologic investigation of risk factors for asthma: a review of the 2011 literature. Curr Allergy Asthma Rep 12(3):192–200

Auerbach A, Hernandez ML (2012) The effect of environmental oxidative stress on airway inflammation. Curr Opin Allergy Clin Immunol 12(2):133–139

Balmes JR (1993) The role of ozone exposure in the epidemiology of asthma. Environ Health Perspect 101(Suppl 4):219–224

Barnes PJ (1994) Air pollution and asthma. Postgrad Med J 70(823):319–325

Barnes PJ (1995) Air pollution and asthma: molecular mechanisms. Mol Med Today 1(3):149–155

Barregard L, Sallsten G, Gustafson P, Andersson L, Johansson L, Basu S et al (2006) Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal Toxicol 18(11):845–853

Barregard L, Sallsten G, Andersson L, Almstrand AC, Gustafson P, Andersson M et al (2008) Experimental exposure to wood smoke: effects on airway inflammation and oxidative stress. Occup Environ Med 65(5):319–324

Bascom R, Naclerio RM, Fitzgerald TK, Kagey-Sobotka A, Proud D (1990) Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. Am Rev Respir Dis 142(3):594–601

Basha MA, Gross KB, Gwizdala CJ, Haidar AH, Popovich J Jr (1994) Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest 106(6):1757–1765

Behndig AF, Mudway IS, Brown JL, Stenfors N, Helleday R, Duggan ST et al (2006) Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27(2):359–365

Belgrave DC, Custovic A, Simpson A (2013) Characterizing wheeze phenotypes to identify endotypes of childhood asthma, and the implications for future management. Expert Rev Clin Immunol 9(10):921–936

Bennett WD, Hazucha MJ, Folinsbee LJ, Bromberg PA, Kissling GE, London SJ (2007) Acute pulmonary function response to ozone in young adults as a function of body mass index. Inhal Toxicol 19(14):1147–1154

Bernstein DI (2011) Genetics of occupational asthma. Curr Opin Allergy Clin Immunol 11(2):86–89

Bernstein JA, Alexis N, Barnes C, Bernstein IL, Bernstein JA, Nel A et al (2004) Health effects of air pollution. J Allergy Clin Immunol 114(5):1116–1123

Boehlecke B, Hazucha M, Alexis NE, Jacobs R, Reist P, Bromberg PA et al (2003) Low-dose airborne endotoxin exposure enhances bronchial responsiveness to inhaled allergen in atopic asthmatics. J Allergy Clin Immunol 112(6):1241–1243

Breysse PN, Buckley TJ, Williams D, Beck CM, Jo SJ, Merriman B et al (2005) Indoor exposures to air pollutants and allergens in the homes of asthmatic children in inner-city Baltimore. Environ Res 98(2):167–176

Brims F, Chauhan AJ (2005) Air quality, tobacco smoke, urban crowding and day care: modern menaces and their effects on health. Pediatr Infect Dis J 24(11 Suppl):S152–S156, discussion

Bromberg PA, Koren HS (1995) Ozone-induced human respiratory dysfunction and disease. Toxicol Lett 82–83:307–316

Bruce N, Perez-Padilla R, Albalak R (2000) Indoor air pollution in developing countries: a major environmental and public health challenge. Bull World Health Organ 78(9):1078–1092

Burke H, Leonardi-Bee J, Hashim A, Pine-Abata H, Chen Y, Cook DG et al (2012) Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. Pediatrics 129(4):735–744

Busse WW, Lemanske RF Jr (2001) Asthma. N Engl J Med 344(5):350–362

Busse WW, Rosenwasser LJ (2003) Mechanisms of asthma. J Allergy Clin Immunol 111(3 Suppl):S799–S804

Cartier A, Sastre J (2011) Clinical assessment of occupational asthma and its differential diagnosis. Immunol Allergy Clin North Am 31(4):717–728, vi

Carty CL, Gehring U, Cyrys J, Bischof W, Heinrich J (2003) Seasonal variability of endotoxin in ambient fine particulate matter. J Environ Monit 5(6):953–958

Chen LL, Tager IB, Peden DB, Christian DL, Ferrando RE, Welch BS et al (2004) Effect of ozone exposure on airway responses to inhaled allergen in asthmatic subjects. Chest 125(6):2328–2335

Christiani DC, Mehta AJ, Yu CL (2008) Genetic susceptibility to occupational exposures. Occup Environ Med 65(6):430–436

Ciencewicki J, Brighton L, Wu WD, Madden M, Jaspers I (2006) Diesel exhaust enhances virus- and poly(I:C)-induced Toll-like receptor 3 expression and signaling in respiratory epithelial cells. Am J Physiol Lung Cell Mol Physiol 290(6):L1154–L1163

Clapp WD, Thorne PS, Frees KL, Zhang X, Lux CR, Schwartz DA (1993) The effects of inhalation of grain dust extract and endotoxin on upper and lower airways. Chest 104(3):825–830

Crain EF, Walter M, O'Connor GT, Mitchell H, Gruchalla RS, Kattan M et al (2002) Home and allergic characteristics of children with asthma in seven U.S. urban communities and design of an environmental intervention: the Inner-City Asthma Study. Environ Health Perspect 110(9):939–945

Custovic A, Lazic N, Simpson A (2013) Pediatric asthma and development of atopy. Curr Opin Allergy Clin Immunol 13(2):173–180

D'Amato G, Liccardi G, D'Amato M, Holgate S (2005) Environmental risk factors and allergic bronchial asthma. Clin Exp Allergy 35(9):1113–1124

David GL, Koh WP, Lee HP, Yu MC, London SJ (2005) Childhood exposure to environmental tobacco smoke and chronic respiratory symptoms in non-smoking adults: the Singapore Chinese Health Study. Thorax 60(12):1052–1058

Delfino RJ, Gong H Jr, Linn WS, Pellizzari ED, Hu Y (2003) Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111(4):647–656

Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A (1994) Diesel exhaust particles induce local IgE production in vivo and alter the pattern of IgE messenger RNA isoforms. J Clin Invest 94(4):1417–1425

Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A (1996) Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. J Allergy Clin Immunol 98(1):114–123

Diaz-Sanchez D, Tsien A, Fleming J, Saxon A (1997) Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. J Immunol 158(5):2406–2413

Diaz-Sanchez D, Garcia MP, Wang M, Jyrala M, Saxon A (1999) Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. J Allergy Clin Immunol 104(6):1183–1188

Diaz-Sanchez D, Proietti L, Polosa R (2003) Diesel fumes and the rising prevalence of atopy: an urban legend? Curr Allergy Asthma Rep 3(2):146–152

Diaz-Sanchez D, Rumold R, Gong H Jr (2006) Challenge with environmental tobacco smoke exacerbates allergic airway disease in human beings. J Allergy Clin Immunol 118(2):441–446

Diette GB, Hansel NN, Buckley TJ, Curtin-Brosnan J, Eggleston PA, Matsui EC et al (2007) Home indoor pollutant exposures among inner-city children with and without asthma. Environ Health Perspect 115(11):1665–1669

Dillon MA, Harris B, Hernandez ML, Zou B, Reed W, Bromberg PA et al (2011) Enhancement of systemic and sputum granulocyte response to inhaled endotoxin in people with the GSTM1 null genotype. Occup Environ Med 25

Eisner MD (2002) Environmental tobacco smoke and adult asthma. Clin Chest Med 23(4):749–761

Eisner MD, Yelin EH, Katz PP, Earnest G, Blanc PD (2002) Exposure to indoor combustion and adult asthma outcomes: environmental tobacco smoke, gas stoves, and woodsmoke. Thorax 57(11):973–978

Eldridge MW, Peden DB (2000) Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma. J Allergy Clin Immunol 105(3):475–481

Etzel RA (2003) How environmental exposures influence the development and exacerbation of asthma. Pediatrics 112(1 Pt 2):233–239

Friedman MS, Powell KE, Hutwagner L, Graham LM, Teague WG (2001) Impact of changes in transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air quality and childhood asthma. JAMA 285(7):897–905

Fuleihan RL (2002) The hygiene hypothesis and atopic disease. Curr Opin Pediatr 14(6):676–677

Gent JF, Triche EW, Holford TR, Belanger K, Bracken MB, Beckett WS et al (2003) Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. JAMA 290(14):1859–1867

Gergen PJ (2001) Environmental tobacco smoke as a risk factor for respiratory disease in children. Respir Physiol 128(1):39–46

Ghio AJ (2004) Biological effects of Utah Valley ambient air particles in humans: a review. J Aerosol Med 17(2):157–164

Ghio AJ, Devlin RB (2001) Inflammatory lung injury after bronchial instillation of air pollution particles. Am J Respir Crit Care Med 164(4):704–708

Ghio AJ, Kim C, Devlin RB (2000) Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. Am J Respir Crit Care Med 162(3 Pt 1):981–988

Ghio AJ, Hall A, Bassett MA, Cascio WE, Devlin RB (2003) Exposure to concentrated ambient air particles alters hematologic indices in humans. Inhal Toxicol 15(14):1465–1478

Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, Berntsen J et al (2012) Exposure to wood smoke particles produces inflammation in healthy volunteers. Occup Environ Med 69(3):170–175

Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Vora H, Rappaport EB et al (2000) Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. Thorax 55(4):271–276

Gilliland FD, Li YF, Dubeau L, Berhane K, Avol E, McConnell R et al (2002) Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. Am J Respir Crit Care Med 166(4):457–463

Gilliland FD, Berhane K, Li YF, Rappaport EB, Peters JM (2003) Effects of early onset asthma and in utero exposure to maternal smoking on childhood lung function. Am J Respir Crit Care Med 167(6):917–924

Gilliland FD, Li YF, Saxon A, Diaz-Sanchez D (2004) Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. Lancet 363(9403):119–125

Gilliland FD, Li YF, Gong H Jr, Diaz-Sanchez D (2006) Glutathione s-transferases M1 and P1 prevent aggravation of allergic responses by secondhand smoke. Am J Respir Crit Care Med 174(12):1335–1341

Gilmour MI, Jaakkola MS, London SJ, Nel AE, Rogers CA (2006) How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. Environ Health Perspect 114(4):627–633

Gold DR (2000) Environmental tobacco smoke, indoor allergens, and childhood asthma. Environ Health Perspect 108(Suppl 4):643–651

Graff DW, Cascio WE, Rappold A, Zhou H, Huang YC, Devlin RB (2009) Exposure to concentrated coarse air pollution particles causes mild cardiopulmonary effects in healthy young adults. Environ Health Perspect 117(7):1089–1094

Hanna AF, Yeatts KB, Xiu A, Zhu Z, Smith RL, Davis NN et al (2011) Associations between ozone and morbidity using the Spatial Synoptic Classification system. Environ Health 10:49

Hatch GE, Slade R, Harris LP, McDonnell WF, Devlin RB, Koren HS et al (1994) Ozone dose and effect in humans and rats. A comparison using oxygen-18 labeling and bronchoalveolar lavage. Am J Respir Crit Care Med 150(3):676–683

Hazucha MJ, Bates DV, Bromberg PA (1989) Mechanism of action of ozone on the human lung. J Appl Physiol 67(4):1535–1541

Hazucha MJ, Madden M, Pape G, Becker S, Devlin R, Koren HS et al (1996) Effects of cyclooxygenase inhibition on ozone-induced respiratory inflammation and lung function changes. Eur J Appl Physiol Occup Physiol 73(1–2):17–27

Health Effects of Outdoor Air Pollution (1996) Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society. Am J Respir Crit Care Med 153(1):3–50

Heederik D, Sigsgaard T (2005) Respiratory allergy in agricultural workers: recent developments. Curr Opin Allergy Clin Immunol 5(2):129–134

Hernandez ML, Lay JC, Harris B, Esther CR Jr, Brickey WJ, Bromberg PA et al (2010) Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. J Allergy Clin Immunol 126(3):537–544

Hernandez ML, Herbst M, Lay JC, Alexis NE, Brickey WJ, Ting JP et al (2012a) Atopic asthmatic patients have reduced airway inflammatory cell recruitment after inhaled endotoxin challenge compared with healthy volunteers. J Allergy Clin Immunol 130(4):869–876

Hernandez M, Brickey WJ, Alexis NE, Fry RC, Rager JE, Zhou B et al (2012b) Airway cells from atopic asthmatic patients exposed to ozone display an enhanced innate immune gene profile. J Allergy Clin Immunol 129(1):259–261

Hernandez ML, Wagner JG, Kala A, Mills K, Wells HB, Alexis NE et al (2013) Vitamin E, gamma-tocopherol, reduces airway neutrophil recruitment after inhaled endotoxin challenge in rats and in healthy volunteers. Free Radic Biol Med 60:56–62

Hollingsworth JW II, Cook DN, Brass DM, Walker JK, Morgan DL, Foster WM et al (2004) The role of toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 12

Hollingsworth JW, Maruoka S, Li Z, Potts EN, Brass DM, Garantziotis S et al (2007a) Ambient ozone primes pulmonary innate immunity in mice. J Immunol 179(7):4367–4375

Hollingsworth JW, Kleeberger SR, Foster WM (2007b) Ozone and pulmonary innate immunity. Proc Am Thorac Soc 4(3):240–246

Hollingsworth JW, Free ME, Li Z, Andrews LN, Nakano H, Cook DN (2010) Ozone activates pulmonary dendritic cells and promotes allergic sensitization through a toll-like receptor 4-dependent mechanism. J Allergy Clin Immunol 125(5):1167–1170

Holz O, Mucke M, Paasch K, Bohme S, Timm P, Richter K et al (2002) Repeated ozone exposures enhance bronchial allergen responses in subjects with rhinitis or asthma. Clin Exp Allergy 32(5):681–689

Holz O, Tal-Singer R, Kanniess F, Simpson KJ, Gibson A, Vessey RS et al (2005) Validation of the human ozone challenge model as a tool for assessing anti-inflammatory drugs in early development. J Clin Pharmacol 45(5):498–503

Horstman D, Hazucha M, Haak E, Stacy R (1982) Effects of submicronic sulfuric acid aerosol on human pulmonary function. Arch Environ Health 37(3):136–141

Horstman DH, Seal E Jr, Folinsbee LJ, Ives P, Roger LJ (1988) The relationship between exposure duration and sulfur dioxide-induced bronchoconstriction in asthmatic subjects. Am Ind Hyg Assoc J 49(1):38–47

Huang YC, Rappold AG, Graff DW, Ghio AJ, Devlin RB (2012) Synergistic effects of exposure to concentrated ambient fine pollution particles and nitrogen dioxide in humans. Inhal Toxicol 24(12):790–797

Jaakkola JJ, Jaakkola MS (2002) Effects of environmental tobacco smoke on the respiratory health of children. Scand J Work Environ Health 28(Suppl 2):71–83

Jaspers I, Ciencewicki JM, Zhang W, Brighton LE, Carson JL, Beck MA et al (2005) Diesel exhaust enhances influenza virus infections in respiratory epithelial cells. Toxicol Sci 85(2):990–1002

Jenkins HS, Devalia JL, Mister RL, Bevan AM, Rusznak C, Davies RJ (1999) The effect of exposure to ozone and nitrogen dioxide on the airway response of atopic asthmatics to inhaled allergen: dose- and time-dependent effects. Am J Respir Crit Care Med 160(1):33–39

Johnston FH, Henderson SB, Chen Y, Randerson JT, Marlier M, Defries RS et al (2012) Estimated global mortality attributable to smoke from landscape fires. Environ Health Perspect 120(5):695–701

Jones SC, Travers MJ, Hahn EJ, Robertson H, Lee K, Higbee C et al (2006) Secondhand smoke and indoor public spaces in Paducah, Kentucky. J Ky Med Assoc 104(7):281–288

Jorres R, Nowak D, Magnussen H (1996) The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. Am J Respir Crit Care Med 153(1):56–64

Kehrl HR, Vincent LM, Kowalsky RJ, Horstman DH, O'Neil JJ, McCartney WH et al (1987) Ozone exposure increases respiratory epithelial permeability in humans. Am Rev Respir Dis 135(5):1124–1128

Kehrl HR, Peden DB, Ball B, Folinsbee LJ, Horstman D (1999) Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone. J Allergy Clin Immunol 104(6):1198–1204

Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC et al (2011) Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183(9):1215–1221

Kleeberger SR, Peden D (2005) Gene-environment interactions in asthma and other respiratory diseases. Annu Rev Med 56:383–400

Kodgule R, Salvi S (2012) Exposure to biomass smoke as a cause for airway disease in women and children. Curr Opin Allergy Clin Immunol 12(1):82–90

Koenig JQ (1999) Air pollution and asthma. J Allergy Clin Immunol 104(4 Pt 1):717–722

Koenig JQ, Pierson WE (1991) Air pollutants and the respiratory system: toxicity and pharmacologic interventions. J Toxicol Clin Toxicol 29(3):401–411

Lannero E, Wickman M, Pershagen G, Nordvall L (2006) Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). Respir Res 7:3

Lay JC, Alexis NE, Kleeberger SR, Roubey RA, Harris BD, Bromberg PA et al (2007) Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals. J Allergy Clin Immunol 120(3):719–722

Lazaar AL, Sweeney LE, Macdonald AJ, Alexis NE, Chen C, Tal-Singer R (2011) SB-656933, a novel CXCR2 selective antagonist, inhibits ex-vivo neutrophil activation and ozone-induced airway inflammation in humans. Br J Clin Pharmacol 22

Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD (2006) Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. Am J Respir Crit Care Med 173(9):970–976

Li Z, Potts EN, Piantadosi CA, Foster WM, Hollingsworth JW (2010) Hyaluronan fragments contribute to the ozone-primed immune response to lipopolysaccharide. J Immunol 185(11):6891–6898

Liu AH (2002) Endotoxin exposure in allergy and asthma: reconciling a paradox. J Allergy Clin Immunol 109(3):379–392

Liu AH (2004) Something old, something new: indoor endotoxin, allergens and asthma. Paediatr Respir Rev 5(Suppl A):S65–S71

Liu AH, Leung DY (2006) Renaissance of the hygiene hypothesis. J Allergy Clin Immunol 117(5):1063–1066

Liu AH, Redmon AH Jr (2001) Endotoxin: friend or foe? Allergy Asthma Proc 22(6):337–340

London SJ (2007) Gene-air pollution interactions in asthma. Proc Am Thorac Soc 4(3):217–220

Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A et al (2011) Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 127(2):355–360

Lovinsky-Desir S, Miller RL (2012) Epigenetics, asthma, and allergic diseases: a review of the latest advancements. Curr Allergy Asthma Rep 12(3):211–220

Malo JL, Vandenplas O (2011) Definitions and classification of work-related asthma. Immunol Allergy Clin North Am 31(4):645–662, v

Mannino DM, Homa DM, Redd SC (2002) Involuntary smoking and asthma severity in children: data from the Third National Health and Nutrition Examination Survey. Chest 122(2):409–415

Matsui EC, Hansel NN, McCormack MC, Rusher R, Breysse PN, Diette GB (2008) Asthma in the inner city and the indoor environment. Immunol Allergy Clin North Am 28(3):665–686, x

Matsui EC, Hansel NN, Aloe C, Schiltz AM, Peng RD, Rabinovitch N et al (2013) Indoor pollutant exposures modify the effect of airborne endotoxin on asthma in urban children. Am J Respir Crit Care Med 188(10):1210–1215

McConnell R, Berhane K, Gilliland F, London SJ, Vora H, Avol E et al (1999) Air pollution and bronchitic symptoms in Southern California children with asthma. Environ Health Perspect 107(9):757–760

McConnell R, Berhane K, Gilliland F, London SJ, Islam T, Gauderman WJ et al (2002) Asthma in exercising children exposed to ozone: a cohort study. Lancet 359(9304):386–391
McConnell R, Berhane K, Gilliland F, Molitor J, Thomas D, Lurmann F et al (2003) Prospective study of air pollution and bronchitic symptoms in children with asthma. Am J Respir Crit Care Med 168(7):790–797
McConnell R, Berhane K, Molitor J, Gilliland F, Kunzli N, Thorne PS et al (2006) Dog ownership enhances symptomatic responses to air pollution in children with asthma. Environ Health Perspect 114(12):1910–1915
McConnell R, Islam T, Shankardass K, Jerrett M, Lurmann F, Gilliland F et al (2010) Childhood incident asthma and traffic-related air pollution at home and school. Environ Health Perspect 118(7):1021–1026
McDonnell WF, Horstman DH, Hazucha MJ, Seal E Jr, Haak ED, Salaam SA et al (1983) Pulmonary effects of ozone exposure during exercise: dose-response characteristics. J Appl Physiol 54(5):1345–1352
McDonnell WF, Muller KE, Bromberg PA, Shy CM (1993) Predictors of individual differences in acute response to ozone exposure. Am Rev Respir Dis 147(4):818–825
McDonnell WF, Abbey DE, Nishino N, Lebowitz MD (1999) Long-term ambient ozone concentration and the incidence of asthma in nonsmoking adults: the AHSMOG study. Environ Res 80(2 Pt 1):110–121
Michel O, Ginanni R, Sergysels R (1995) Protective effect of sodium cromoglycate on lipopolysaccharide-induced bronchial obstruction in asthmatics. Int Arch Allergy Immunol 108(3):298–302
Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA et al (1991) Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338(8761):199–203
Moore K, Neugebauer R, Lurmann F, Hall J, Brajer V, Alcorn S et al (2008) Ambient ozone concentrations cause increased hospitalizations for asthma in children: an 18-year study in Southern California. Environ Health Perspect 116(8):1063–1070
Morgan WJ, Martinez FD (1992) Risk factors for developing wheezing and asthma in childhood. Pediatr Clin North Am 39(6):1185–1203
Mudway IS, Stenfors N, Duggan ST, Roxborough H, Zielinski H, Marklund SL et al (2004) An in vitro and in vivo investigation of the effects of diesel exhaust on human airway lining fluid antioxidants. Arch Biochem Biophys 423(1):200–212
Mueller-Anneling L, Avol E, Peters JM, Thorne PS (2004) Ambient endotoxin concentrations in PM10 from Southern California. Environ Health Perspect 112(5):583–588
Naeher LP, Brauer M, Lipsett M, Zelikoff JT, Simpson CD, Koenig JQ et al (2007) Woodsmoke health effects: a review. Inhal Toxicol 19(1):67–106
Nel AE, Diaz-Sanchez D, Li N (2001) The role of particulate pollutants in pulmonary inflammation and asthma: evidence for the involvement of organic chemicals and oxidative stress. Curr Opin Pulm Med 7(1):20–26
Noah TL, Zhou H, Jaspers I (2012a) Alteration of the nasal responses to influenza virus by tobacco smoke. Curr Opin Allergy Clin Immunol 12(1):24–31
Noah TL, Zhou H, Zhang H, Horvath K, Robinette C, Kesic M et al (2012b) Diesel exhaust exposure and nasal response to attenuated influenza in normal and allergic volunteers. Am J Respir Crit Care Med 185(2):179–185
Noonan CW, Balmes JR (2010) Biomass smoke exposures: health outcomes measures and study design. Inhal Toxicol 22(2):108–112
Ostro B, Roth L, Malig B, Marty M (2009) The effects of fine particle components on respiratory hospital admissions in children. Environ Health Perspect 117(3):475–480
Pacheco KA, Rose CS, Thorne PS, O'Neill ME, McCammon C, Martyny J et al (2002) Airborne endotoxin is a significant determinant of symptoms in laboratory animal workers. J Allergy Clin Immunol 109(1):S281

Pandya RJ, Solomon G, Kinner A, Balmes JR (2002) Diesel exhaust and asthma: hypotheses and molecular mechanisms of action. Environ Health Perspect 110(Suppl 1):103–112
Passannante AN, Hazucha MJ, Bromberg PA, Seal E, Folinsbee L, Koch G (1998) Nociceptive mechanisms modulate ozone-induced human lung function decrements. J Appl Physiol 85(5):1863–1870
Peden DB (1997) Mechanisms of pollution-induced airway disease: in vivo studies. Allergy 52(38 Suppl):37–44
Peden DB (2002) Pollutants and asthma: role of air toxics. Environ Health Perspect 110(Suppl 4):565–568
Peden DB (2005) The epidemiology and genetics of asthma risk associated with air pollution. J Allergy Clin Immunol 115(2):213–219
Peden DB (2008) Air pollution: indoor and outdoor. In: Adkinson NF Jr, Busse W, Bochner B, Holgate S, Simons FE, Lemanske R (eds) Middleton's allergy: principles and practice, 7th edn. Mosby, Philadelphia, pp 495–508
Peden DB (2011) The role of oxidative stress and innate immunity in O(3) and endotoxin-induced human allergic airway disease. Immunol Rev 242(1):91–105
Peden DB, Setzer RW Jr, Devlin RB (1995) Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. Am J Respir Crit Care Med 151(5):1336–1345
Peden DB, Boehlecke B, Horstman D, Devlin R (1997) Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. J Allergy Clin Immunol 100(6 Pt 1):802–808
Peel JL, Tolbert PE, Klein M, Metzger KB, Flanders WD, Todd K et al (2005) Ambient air pollution and respiratory emergency department visits. Epidemiology 16(2):164–174
Perzanowski MS, Miller RL, Thorne PS, Barr RG, Divjan A, Sheares BJ et al (2006) Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood. J Allergy Clin Immunol 117(5):1082–1089
Pierson WE, Covert DS, Koenig JQ, Namekata T, Kim YS (1986) Implications of air pollution effects on athletic performance. Med Sci Sports Exerc 18(3):322–327
Pope CA III (1989) Respiratory disease associated with community air pollution and a steel mill, Utah Valley. Am J Public Health 79(5):623–628
Pope CA III (1991) Respiratory hospital admissions associated with PM10 pollution in Utah, Salt Lake, and Cache Valleys. Arch Environ Health 46(2):90–97
Pourazar J, Frew AJ, Blomberg A, Helleday R, Kelly FJ, Wilson S et al (2004) Diesel exhaust exposure enhances the expression of IL-13 in the bronchial epithelium of healthy subjects. Respir Med 98(9):821–825
Pourazar J, Mudway IS, Samet JM, Helleday R, Blomberg A, Wilson SJ et al (2005) Diesel exhaust activates redox-sensitive transcription factors and kinases in human airways. Am J Physiol Lung Cell Mol Physiol 289(5):L724–L730
Que LG, Stiles JV, Sundy JS, Foster WM (2011) Pulmonary function, bronchial reactivity, and epithelial permeability are response phenotypes to ozone and develop differentially in healthy humans. J Appl Physiol (1985) 111(3):679–687
Rabinovitch N, Liu AH, Zhang L, Rodes CE, Foarde K, Dutton SJ et al (2005) Importance of the personal endotoxin cloud in school-age children with asthma. J Allergy Clin Immunol 116(5):1053–1057
Rabinovitch N, Strand M, Stuhlman K, Gelfand EW (2008) Exposure to tobacco smoke increases leukotriene E4-related albuterol usage and response to montelukast. J Allergy Clin Immunol 121(6):1365–1371
Rabinovitch N, Silveira L, Gelfand EW, Strand M (2011a) The response of children with asthma to ambient particulate is modified by tobacco smoke exposure. Am J Respir Crit Care Med 184(12):1350–1357
Rabinovitch N, Reisdorph N, Silveira L, Gelfand EW (2011b) Urinary leukotriene E(4) levels identify children with tobacco smoke exposure at risk for asthma exacerbation. J Allergy Clin Immunol 128(2):323–327

Rappold AG, Stone SL, Cascio WE, Neas LM, Kilaru VJ, Carraway MS et al (2011) Peat bog wildfire smoke exposure in rural North Carolina is associated with cardiopulmonary emergency department visits assessed through syndromic surveillance. Environ Health Perspect 119(10):1415–1420

Redlich CA, Karol MH (2002) Diisocyanate asthma: clinical aspects and immunopathogenesis. Int Immunopharmacol 2(2–3):213–224

Riddervold IS, Bonlokke JH, Olin AC, Gronborg TK, Schlunssen V, Skogstrand K et al (2012) Effects of wood smoke particles from wood-burning stoves on the respiratory health of atopic humans. Part Fibre Toxicol 9(1):12

Riedl M, Diaz-Sanchez D (2005) Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol 115(2):221–228

Ritz SA, Wan J, Diaz-Sanchez D (2007) Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. Am J Physiol Lung Cell Mol Physiol 292(1):L33–L39

Romieu I, Trenga C (2001) Diet and obstructive lung diseases. Epidemiol Rev 23(2):268–287

Romieu I, Meneses F, Ruiz S, Huerta J, Sienra JJ, White M et al (1997) Effects of intermittent ozone exposure on peak expiratory flow and respiratory symptoms among asthmatic children in Mexico City. Arch Environ Health 52(5):368–376

Romieu I, Meneses F, Ramirez M, Ruiz S, Perez PR, Sienra JJ et al (1998) Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. Am J Respir Crit Care Med 158(1):226–232

Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Tellez-Rojo MM, Moreno-Macias H, Reyes-Ruiz NI et al (2002) Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 166(5):703–709

Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Moreno-Macias H, Reyes-Ruiz NI, Estela del Rio-Navarro B et al (2004) Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 59(1):8–10

Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ, Moreno-Macias H, Rio-Navarro BE, David G et al (2006) GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28(5):953–959

Rudell B, Ledin MC, Hammarstrom U, Stjernberg N, Lundback B, Sandstrom T (1996) Effects on symptoms and lung function in humans experimentally exposed to diesel exhaust. Occup Environ Med 53(10):658–662

Sallsten G, Gustafson P, Johansson L, Johannesson S, Molnar P, Strandberg B et al (2006) Experimental wood smoke exposure in humans. Inhal Toxicol 18(11):855–864

Salvi SS, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ et al (2000) Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. Am J Respir Crit Care Med 161(2 Pt 1):550–557

Samet JM, Hatch GE, Horstman D, Steck-Scott S, Arab L, Bromberg PA et al (2001) Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med 164(5):819–825

Schachter EN, Witek TJ Jr, Beck GJ, Hosein HB, Colice G, Leaderer BP et al (1984) Airway effects of low concentrations of sulfur dioxide: dose-response characteristics. Arch Environ Health 39(1):34–42

Schaumann F, Borm PJ, Herbrich A, Knoch J, Pitz M, Schins RP et al (2004) Metal-rich ambient particles (particulate matter 2.5) cause airway inflammation in healthy subjects. Am J Respir Crit Care Med 170(8):898–903

Schaumann F, Muller M, Braun A, Luettig B, Peden DB, Hohlfeld JM et al (2008) Endotoxin augments myeloid dendritic cell influx into the airways in patients with allergic asthma. Am J Respir Crit Care Med 177(12):1307–1313

Schelegle ES, Adams WC, Siefkin AD (1987) Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects. Am Rev Respir Dis 136(6):1350–1354

Schwartz J, Slater D, Larson TV, Pierson WE, Koenig JQ (1993) Particulate air pollution and hospital emergency room visits for asthma in Seattle. Am Rev Respir Dis 147(4):826–831

Sharma HP, Hansel NN, Matsui E, Diette GB, Eggleston P, Breysse P (2007) Indoor environmental influences on children's asthma. Pediatr Clin North Am 54(1):103–120

Silverman RA, Ito K (2010) Age-related association of fine particles and ozone with severe acute asthma in New York City. J Allergy Clin Immunol 125(2):367–373

Stenfors N, Nordenhall C, Salvi SS, Mudway I, Soderberg M, Blomberg A et al (2004) Different airway inflammatory responses in asthmatic and healthy humans exposed to diesel. Eur Respir J 23(1):82–86

Stockfelt L, Sallsten G, Olin AC, Almerud P, Samuelsson L, Johannesson S et al (2012) Effects on airways of short-term exposure to two kinds of wood smoke in a chamber study of healthy humans. Inhal Toxicol 24(1):47–59

Sunyer J, Atkinson R, Ballester F, Le TA, Ayres JG, Forastiere F et al (2003) Respiratory effects of sulphur dioxide: a hierarchical multicity analysis in the APHEA 2 study. Occup Environ Med 60(8):e2

Szema AM (2012) Work-exacerbated asthma. Clin Chest Med 33(4):617–624

Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D et al (2008) Diagnosis and management of work-related asthma: American College of Chest Physicians consensus statement. Chest 134(3 Suppl):1S–41S

Thorne PS, Kulhankova K, Yin M, Cohn R, Arbes SJ, Zeldin DC (2005) Endotoxin exposure is a risk factor for asthma - The National Survey of Endotoxin in United States Housing. Am J Respir Crit Care Med 172(11):1371–1377

Trenga CA, Koenig JQ, Williams PV (2001) Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. Arch Environ Health 56(3):242–249

Tunnicliffe WS, Burge PS, Ayres JG (1994) Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. Lancet 344(8939–8940):1733–1736

Vagaggini B, Taccola M, Conti I, Carnevali S, Cianchetti S, Bartoli ML et al (2001) Budesonide reduces neutrophilic but not functional airway response to ozone in mild asthmatics. Am J Respir Crit Care Med 164(12):2172–2176

Vagaggini B, Taccola M, Cianchetti S, Carnevali S, Bartoli ML, Bacci E et al (2002) Ozone exposure increases eosinophilic airway response induced by previous allergen challenge. Am J Respir Crit Care Med 166(8):1073–1077

Von Essen S (1997) The role of endotoxin in grain dust exposure and airway obstruction. Curr Opin Pulm Med 3(3):198–202

Von Essen S, Donham K (1999) Illness and injury in animal confinement workers. Occup Med 14(2):337–350

Von Essen SG, McCurdy SA (1998) Health and safety risks in production agriculture. West J Med 169(4):214–220

Von Essen SG, O'Neill DP, Olenchok SA, Robbins RA, Rennard SI (1995a) Grain dusts and grain plant components vary in their ability to recruit neutrophils. J Toxicol Environ Health 46(4):425–441

Von Essen SG, O'Neill DP, McGranaghan S, Olenchock SA, Rennard SI (1995b) Neutrophilic respiratory tract inflammation and peripheral blood neutrophilia after grain sorghum dust extract challenge. Chest 108(5):1425–1433

Von Essen SG, Scheppers LA, Robbins RA, Donham KJ (1998) Respiratory tract inflammation in swine confinement workers studied using induced sputum and exhaled nitric oxide. J Toxicol Clin Toxicol 36(6):557–565

von Mutius E (2000) The environmental predictors of allergic disease. J Allergy Clin Immunol 105(1 Pt 1):9–19

Wan J, Diaz-Sanchez D (2006) Phase II enzymes induction blocks the enhanced IgE production in B cells by diesel exhaust particles. J Immunol 177(5):3477–3483

Wang JH, Devalia JL, Duddle JM, Hamilton SA, Davies RJ (1995a) Effect of six-hour exposure to nitrogen dioxide on early-phase nasal response to allergen challenge in patients with a history of seasonal allergic rhinitis. J Allergy Clin Immunol 96(5 Pt 1):669–676

Wang JH, Duddle J, Devalia JL, Davies RJ (1995b) Nitrogen dioxide increases eosinophil activation in the early-phase response to nasal allergen provocation. Int Arch Allergy Immunol 107(1–3):103–105

Wenzel S (2012) Severe asthma: from characteristics to phenotypes to endotypes. Clin Exp Allergy 42(5):650–658

Wu W, Doreswamy V, Diaz-Sanchez D, Samet JM, Kesic M, Dailey L et al (2011) GSTM1 modulation of IL-8 expression in human bronchial epithelial cells exposed to ozone. Free Radic Biol Med 51(2):522–529

Yang IA, Holz O, Jorres RA, Magnussen H, Barton SJ, Rodriguez S et al (2005) Association of tumor necrosis factor-alpha polymorphisms and ozone-induced change in lung function. Am J Respir Crit Care Med 171(2):171–176

Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW (2008) Genetic susceptibility to the respiratory effects of air pollution. Thorax 63(6):555–563

Ying RL, Gross KB, Terzo TS, Eschenbacher WL (1990) Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. Am Rev Respir Dis 142(4):817–821

Zeldin DC, Eggleston P, Chapman M, Piedimonte G, Renz H, Peden D (2006) How exposures to biologics influence the induction and incidence of asthma. Environ Health Perspect 114(4):620–626

Zelikoff JT, Chen LC, Cohen MD, Schlesinger RB (2002) The toxicology of inhaled woodsmoke. J Toxicol Environ Health B Crit Rev 5(3):269–282

Zhang JJ, Hu W, Wei F, Wu G, Korn LR, Chapman RS (2002) Children's respiratory morbidity prevalence in relation to air pollution in four Chinese cities. Environ Health Perspect 110(9):961–967

Chapter 5
Air Pollution and Chronic Obstructive Airway Disease

Imre Redai and Angela Haczku

5.1 Introduction

Reigning in fire is regarded as one of the essential steps in the emergence of humans as the dominant species on Earth. Fire provides us with cooked, thus more easily digestible food. It is the source of heating that allowed us to spread to and continue to live at latitudes and altitudes with unfavorable climates. Until the invention of the light bulb, fire was the source of all light between sunset and dawn. Fire based energy fuelled the Industrial Revolution from the eighteen century onwards and allowed us to leave our planet and explore space. Inhaled fire-born pollutants affected and continue to affect the respiratory health of a large portion of humans. Preparing food with the use of open flames producing large quantities of environmental pollutants (lots of smoke that is) remains highly popular: think about backyard barbecues, a platter of smoked meats and cheeses, well roasted coffee beans, or just single malt whisky. On a different scale, industrial mining, chemical processing, emergence of mega-cities with continuously increasing needs in transportation resulted in permanent presence of pollutants like exhaust fumes and ground level ozone in the urban environment. Employees in a wide variety of professions are exposed to inhaled pollutants potentially damaging their lungs. All in all, development of civilization, as we define it, has been presenting a continuous non-physiological challenge for our respiratory tract.

Chronic obstructive airway disease (COPD), the focus of this chapter, is a chronic, progressive, irreversible condition characterized by exacerbations and remissions ultimately leading to respiratory failure and death. Exposure to air

I. Redai, M.D., F.R.C.A. (deceased)

A. Haczku, M.D., Ph.D. (✉)
Translational Lung Biology Center, Pulmonary, Critical Care and Sleep Medicine, University of California, 6510 GBSF, One Shields Avenue, Davis, CA 95616, USA
e-mail: haczku@ucdavis.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_5

pollutants not only aggravates pre-existing airway disease, but also increases the number of new cases, even in rural areas. Globally, air pollutants now rival tobacco smoke as the leading risk factor for COPD (Arbex et al. 2012). In this review, we discuss emerging epidemiologic evidence of the role of air pollution on COPD and will review the potential cellular-molecular basis of the pollutant-caused pathological changes.

5.2 Pollution and COPD: Historical Perspectives

While a vast body of COPD research has been focusing on the effects of cigarette smoke on the respiratory tract, data are also emerging on the pathogenic function of air pollution in this disease with the hope for prevention and treatment. These studies (initiated by catastrophic events in which a sudden deterioration of air quality resulted in dramatic respiratory effects) have been the driving force behind environmental plans for air quality monitoring and improvement. The lethal industrial haze that occurred in October 1948 in Donora, PA and the 5-day smog that enveloped London and killed close to 12,000 people within 3 months in December 1952 were two environmental disasters that were widely publicized and regarded as the major promoter for the study of air pollution epidemiology (Hunt et al. 2003; Davis et al. 2002; Bell and Davis 2001; Bates 2000). As a result, the US Congress passed the Clean Air Act Amendments in 1970, leading to the establishment of air quality standards.

The earliest study looking into the association of chronic environmental pollutants and morbidity and mortality related to COPD utilized sickness, retirement, and death statistics in the British Civil Service in the 1950s. Postmen delivering mail in areas with higher air pollution (increased incidence of urban fog) have had a significantly greater incidence of absence from work due to chronic bronchitis and were more likely to retire from the service early or die prematurely because of respiratory ailments. Prolonged repeated exposure with longer daily periods was associated with worse outcome on respiratory health: susceptible postmen (working outdoors delivering mail twice a day) on average developed COPD about 20 years earlier in their life than the civil servants who were confined indoors for most of their working hours. Even among civil servants working indoors the incidence of COPD related absence correlated with the air pollution rate of their area of residence (Fairbairn and Reid 1958).

The recognition of the effect of air pollutants on development of COPD took on a new dimension starting in the 1960s with research conducted among non-smokers using biomass fuel for cooking and heating in India, Papua-New Guinea, Central America and Sub-Saharan Africa. An estimated three billion people are today exposed to sustained daily indoor air pollution. Most of these people live either in low-income countries or form the low-income strata of wealthy nations and over 80 % of exposure to air pollutants in this population happens in their own homes (Ezzati and Kammen 2002).

In addition to the detrimental effects of chronic exposure to air pollutants, some of the most devastating outcomes of acute exposure to air pollution were observed in patients with preexisting obstructive airway disease (Zanobetti et al. 2008). In December 1991 Londoners experienced an air pollution episode with mortality sharply increasing for all causes (excluding accidents). In the elderly over 65, but not in children (0–14), the relative risk of hospital admission during this episode was increased for all respiratory diseases (1.19), but it was significantly greater for documented exacerbations of chronic obstructive lung diseases (1.43) (Anderson et al. 1995). In a different study, the effects of traffic related pollution on respiratory symptoms were delayed in children, but were more immediate in the elderly (Halonen et al. 2008). These studies suggested that the acute effects of air pollution are more severe in the elderly who are suffering from COPD. Indeed acute exposure to air pollution is now considered a main contributor to mortality in COPD patients (Cazzola et al. 2007).

5.3 The Role of Outdoor Air Pollutants in COPD

The major pollutants that are significantly associated with respiratory failure include NOx, SO_2, particulate matter (PM) and CO (Stieb et al. 2002). All of these are listed in the six criteria pollutants (nitrogen oxides, sulfur oxides, particulate matter, ozone, carbon monoxide, and lead) for which the US EPA established the National Air Quality Standards.

Nitrogen dioxide (NO_2) is a toxic gas that can be produced by fossil fuel combustion or industrial activities. The December 1991 air pollution incident in London came with record high nitrogen dioxide levels associated with black smoke. Elderly patients suffering from COPD showed a significantly increased sensitivity to this pollutant manifested in increased hospital admission (Anderson et al. 1995).

Particulate matter contains particles with size less than 10 μm in aerodynamic diameter (PM_{10}). These particles contain a mixture of inhalable material from dust, smoke, soot, and combustion, are small enough to reach the lower airways and induce/maintain chronic inflammation. Halonen et al. studied the effects of urban PM in air pollution on asthma and COPD. They found that traffic related $PM_{2.5}$ (≤2.5 μm in aerodynamic diameter) caused delayed respiratory effects (hospital emergency room visits for asthma) in children ≤15 years of age. Meanwhile, $PM_{2.5}$ accumulation was associated with acute respiratory effects (hospital emergency room visits for a combination of asthma and COPD) in adults (15–65) and the elderly (≥65 years of age) (Halonen et al. 2008). That elderly, COPD patients might have a heightened sensitivity to traffic related fine particles ($PM_{2.5}$) in comparison with healthy individuals has been confirmed by additional investigations (Zanobetti et al. 2008).

It is interesting to note that a single cigarette exposes COPD patients (the majority of whom have been smokers) to ~15,000–40,000 μg of PM of similar size and composition to what is found in traffic related pollution. Given that during an

average exposure the inhaled PM amount does not exceed 720 μg, the underlying mechanism of such sensitivity of the COPD patients to traffic related air pollution remains obscure and somewhat baffling. Nonetheless, to provide a greater awareness of the PM levels, the U.S. Environmental Protection Agency now includes levels of air pollution particles in an air quality index (Sint et al. 2008).

The role of outdoor, generally urban or traffic-related air pollution in triggering acute exacerbations and worsening symptoms in patients suffering from various degrees of COPD is well established (Sunyer 2001). In contrast, the association of chronic, life-long exposure to the same outdoor air pollution with the risk of developing COPD is far less clear and even recent studies have remained largely inconclusive (Schikowski et al. 2014). No single pollutant or pollutants have been identified as causative agents in traffic-related COPD. Measuring exposure to pollutants, like nitrogen oxides (NO_2 in particular), ozone (O_3), SO_2, and PM correlated (or not) with prevalence of COPD equally, to just measuring the distance of the patient's abode from a main road (Götschi et al. 2008). Long term continuous exposure to low-grade air pollution on the lungs however was shown to lead to increased mortality of COPD patients related to both cardiovascular and respiratory causes (Jerrett et al. 2009; Krewski 2009; Katsouyanni et al. 2009).

Whether chronic low-grade exposure (which perhaps maintains a low level activation of the inflammatory process in the airways) or intermittent high-level exposure to pollutants (causing exacerbation of symptoms and flare up of the inflammatory process) is the main contributor to the development of COPD (Arbex et al. 2009) remains unclear. Perhaps both have a role but their relative impact in the induction and natural progression of the disease will need to be clarified by future studies both in the laboratory and in the field. This problem is further confounded by the possible link of childhood exposure to environmental air pollution and delayed or incomplete lung development and its contribution to the emergence of COPD in later life (Gauderman et al. 2004).

5.4 Indoor Air Pollutants and COPD

Most of the research in COPD has been focusing on the association of this disease with chronic cigarette smoking and most of our understanding is based on clinical experience, animal models, diagnostic test, drugs, and therapeutic interventions all associated with cigarette smoke as a causative agent. Cigarette smoke exposes the smoker to high concentrations of airborne noxious agents for a short period of time in repeat intervals. On the other hand most chronic environmental airborne pollutants elicit their effect on the lung by sustained, low-grade exposure; sometimes this exposure is constant during almost the entire life of the subject. The most common current environmental pollutant with epidemiological link to COPD is biomass fuel smoke with about half the population of the planet being exposed to biomass fuel smoke on a daily basis. The studies on indoor air pollution started in the 1960s with research into household COPD in non-smokers and its relation to biomass fuel use

in India, Papua-New Guinea, Central America and Sub-Saharan Africa (Ezzati and Kammen 2002). Indoor air pollution is the result of a combination of burning biomass fuel (twigs, wood, animal dung, straw, peat and coal) with poor stove design (often open fire) and poor ventilation (lack of a chimney and often even windows). The smoke generated by these fires contains high concentrations of CO, nitrogen and sulfur oxides, aldehydes, and PM_{10}. Field studies have reported average concentrations of airborne particles and agents of several magnitudes above the recommended 150 $\mu g/m^3$ with inhabitants of these homes exposed to pollutants for long hours every day (Ezzati and Kammen 2002). Women and small children are disproportionately represented in the affected population and the worldwide prevalence of COPD in women and chronic respiratory ailments in small children is strongly associated with exposure to biomass fuel smoke (Torres-Duque et al. 2008). The importance of biomass smoke exposure is underscored by the finding that in women 30-years exposure to either biomass smoke or cigarette-smoking results in the same likelihood of developing COPD (Sezer et al. 2006). There are no significant differences in histopathological findings in the lungs (which include anthracosis, chronic bronchitis, centrolobular emphysema, bronchial squamous cell metaplasia and hypertrophic remodeling of the pulmonary vascular tree) between patients with wood smoke-associated lung disease and those of smokers at the time of death (Moran-Mendoza et al. 2008).

As of now, COPD and chronic bronchitis are the third highest cause of death in the World. The contribution of widespread exposure to biomass fuel smoke to COPD and ways to limit or eliminate it remains a significant public health issue in times of increasing energy costs (Po et al. 2011). A large fraction of this cost will affect the wider international community, as most of the affected inhabit low or very low-income countries already dependent on foreign aid and other forms of help.

5.5 Occupational Exposure and COPD

COPD associated with workplace exposure is notoriously difficult to detect for two reasons. Firstly, COPD is strongly associated with common non-occupational exposures like cigarette smoking often practiced in the same environmental setting ("cigarette break"). Secondly, the dose-response and time exposure relations with the disease are complex (Balmes et al. 2003). Inhalational exposure at the workplace consists of a mixture of airborne particles, fumes, and toxic gases. Some components like cadmium and vanadium are proven direct causative agents in emphysema and chronic bronchitis, respectively. Similarly to cigarette smoke (that contains over 400 potentially toxic components neither of which has been identified as a single most important causative agent in COPD), the etiological role of occupational exposure in COPD has been better ascertained by epidemiological studies than laboratory experiments.

Observing decline in FEV_1 over a period of years in workers employed in industries like coal and hard-rock mining, cement and concrete manufacturing,

construction (especially of tunnels), and agricultural activities has indicated the causative role of dust particles in occupational COPD (Becklake 1989). It is estimated that the population burden of occupational exposure for COPD is in the range of 15 % in developed countries (Balmes et al. 2003) with the related annual health expenditure at the beginning of this millennium in the United States alone of about $5 billion (Leigh et al. 2002). Excess occurrence of COPD among the general US population was further analyzed as part of the Third National Health and Nutrition Examination Survey (NHANES III) (Hnizdo et al. 2002). This study has found that not only workers in the 'traditional' COPD-related industries listed above but those in rubber, plastics, and leather manufacturing, utilities, office building services, textile mill products manufacturing, the armed forces, food products manufacturing, automotive repair services and gas stations, sales clerks, transportation and trucking, personal services (like hairdressers and cosmetologists), health care workers, record processing and distribution clerks, machine operators, and waitresses all have a higher than the average population prevalence of COPD. In fact the attributable portion of COPD among these workers was 19 % for the general population and 31 % for those who have never smoked (Hnizdo et al. 2002). It appears that workplace exposure to vapors, gas, dust, and fumes (VGDF) in common has the potential to induce and maintain the pathological process associated with COPD in susceptible individuals. In effect workplace exposure to VGDF more than doubled the odds of developing COPD later in life and accounted for almost a third of all cases in a population-based study (Blanc et al. 2009a). The interaction between occupational exposure and cigarette smoking on the development of COPD remains poorly understood. Studies observing a super additive increased risk (Blanc et al. 2009b) or a less than additive effect (Blanc et al. 2009a) have been published by the same group of investigators analyzing data collected from similar populations. This and other studies available suggest that factors beyond simple co-exposure to VGDF and cigarette smoke have to be dissected before we will have a clear understanding of their role in COPD.

5.6 Anatomical Considerations

COPD is a disease of the small airways and respiratory parenchyma. To better understand the underlying mechanisms, we first review the role of these constituents of the respiratory system. Air moves between the atmosphere and the alveoli via the upper (the nasal and oral cavity, the pharynx, the larynx) and lower (the trachea and the bronchi) airways. The flow of air depends on a variety of factors, most important of which is the diameter of the conductive pathway. The critical, flow-limiting elements in COPD are the small bronchi and bronchioli, the diameter of which are in the range of less than 1 mm to about 3.5 mm. Normal small bronchi with the size over 1 mm contain cartilaginous rings; these rings are replaced by smooth muscle fibers and connective tissue elements below the diameter of 1 mm (after about 11 divisions of the tracheobronchial tree). This feature results in an

important change in the response of the small airways to the alternating increase and decrease in intrathoracic pressure with the respiratory cycle. During inspiration the pressure in the thoracic cavity is reduced resulting in traction via elastic components on the alveolar walls and the airways. While this has negligible effect on airways containing cartilaginous rings (they are rigid in nature), those airways without cartilage get expanded and their diameter increases. During exhalation the reverse happens: these same airways get compressed. Under normal circumstances the air flow throughout these small airways when breathing at rest, and even during exercise is unrestricted. The total diameter of the airways is very large and the velocity of the flow is such that it remains laminar (Lumb 2000). However, with the pathological alterations present in patients with COPD this situation changes dramatically, resulting in critically reduced diameter and turbulent flow. There are three major underlying pathological mechanisms for air flow limitation due to decreased luminal diameter and increased airway resistance in the small airways:

1. *Accumulated material* within the lumen as a result of increased production and reduced clearance of mucus.
2. *Thickening of the wall of the passageway* due to increased deposition of connective tissue in the walls of the small airways resulting in constrictive remodeling.
3. *Increased pressure* (*compression*) *from outside* because the loss of alveoli and elastic matter causes reduced tethering of the small airways during expiration.

All three of these pathological features (the increased production and reduced clearance of mucus, deposition of connective tissue in the walls of the small airways and loss of alveoli and elastic matter) are simultaneously present in COPD. In the clinical manifestation of the disease (***1***) and (***2***) are characteristics of chronic bronchitis while (***3***) is commonly known as emphysema. Patients suffering from COPD present with a mixture of all three. Limited air flow during exhalation results in air trapping in the lung at end-expiration and the consequential alveolar hyperinflation further compresses the small airways (Barnes and Rennard 2009).

The limitation of air flow during exhalation can be quantified in patients by measuring the forced expiratory volume in the first second of expiration (FEV_1) and the Tiffeneau index which is the ratio of FEV_1 to FVC (forced vital capacity). Commonly used flow measurements are the peak expiratory flow rate and the maximum mid-expiratory flow (MMEF) rate which is the forced expiratory flow between the 25th and 75th percentiles of the FVC. While peak expiratory flow is effort sensitive, MMEF is effort independent and reflects the maximum flow rate permissible by the small airways. Lung development continues into early adulthood thus lung function steadily increases after birth until the third decade of life. This is important given that outdoor pollution and traffic-related air pollution can have an adverse effect on lung development in children aged 10–18 years (Gauderman et al. 2004; Ko and Hui 2012). In adults there is a constant decline of lung function resulting in reduced physiological reserve in the elderly. However, in a healthy individual even of very advanced age lung function provides adequate gas exchange at rest and during mild or moderate exercise (Sharma and Goodwin 2006). The effects of air pollution on the age-related decline in pulmonary physiology are not well understood but gender

differences have been reported in susceptibility. After controlling for demographic and socioeconomic factors, cigarette smoking and background air pollution, greater traffic density significantly correlated with reduced FEV1 and FVC in women but not men in a cross sectional community based study of 15,792 middle aged men and women (Ko and Hui 2012; Kan et al. 2007). The FEV1/FVC ratio however did not correlate with traffic exposure. While traffic-related air pollution was associated with the development of adult onset asthma among never-smokers (Kunzli et al. 2009), the relationship between air pollution and decline in lung function with subsequent development of COPD needs additional clarifications (Ko and Hui 2012).

The main effect of air pollution on the clinical progress of COPD is induced as chronic airway inflammation manifesting itself with wide variability between affected individuals. The quality, quantity and duration of exposure, the response of the various elements of the innate and adaptive immune systems, the still poorly understood genetic and epigenetic determinants regulating airway remodeling in COPD are focal points of ongoing research.

5.7 Cellular Pathways of Air Pollutant-Induced COPD

A wide variety of structural, immune and inflammatory cells have been implicated in the pathogenesis of COPD. The significance of specific cell types in mediating the effects of air pollution however remains the subject of intense investigations. The effects of acute inhalation of air pollutants are manifested in activation of signaling pathways and proinflammatory mediator release by resident epithelial cells and macrophages leading to recruitment of inflammatory cells, structural damage and exacerbations of the symptoms of COPD (Bates 2000; Barnes and Rennard 2009; Baraldo et al. 2012; Barnes 2004a, b, c; Becker et al. 2005). Chronic, prolonged or intermittent exposure to pollutants evokes more complex pathways including development of "adaptation" (Jakab et al. 1995) and tissue protection and repair (Burgel and Nadel 2004; Crosby and Waters 2010; Hogg et al. 2004; Leikauf et al. 2001; Sunil et al. 2012).

5.7.1 Macrophages

The most commonly found immune cells in the normal lung are the alveolar macrophages contributing to both the innate and acquired immune response. These cells clear inhaled particles, identify and destroy pathogens appearing in the terminal airways. Exposure of human alveolar macrophages to ultrafine carbon particles or diesel exhaust particles (DEP) *in vitro* reduced their binding and phagocytic activity via their scavenger, mannose, and complement receptors while binding and opsonization via the Fc receptor remained unchanged (Lundborg et al. 2006). Macrophage numbers in the lung may increase up to 15-fold in patients with COPD and their

numbers appear to correlate with disease severity (Barnes 2004a; Finkelstein et al. 1995). Macrophages, activated locally or recruited during the disease process can account for most of the known features of the disease (Barnes 2004a, b). In a healthy lung the majority of macrophages are made up by the "alveolar" and "interstitial" types. Under inflammatory conditions, however, these cells become highly heterogeneous and differentiate into several additional phenotypes classified as "migratory (or proinflammatory) vs. residential" or "M1 vs. M2" (Moreira and Hogaboam 2011). During inflammation in COPD, bone marrow derived monocytes migrate through the circulation and differentiate under the effects of macrophage colony stimulating factor (M-CSF) and epithelial derived cytokines, chemokines and other inflammatory mediators such as prostaglandins, leukotrienes and components of the complement cascade. Macrophages from COPD patients have increased levels of the transcription factor nuclear factor κB (NF-κB), that may be responsible for the increased secretion of elastolytic enzymes and inflammatory proteins (Di Stefano et al. 2004). Proinflammatory macrophages release a number of inflammatory mediators and chemokines (CXCL1, CXCL2, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, TNF-α, IL-1-β, IL-6, IL-18) (Barnes 2004a, b), elastolytic enzymes (matrix metalloproteinases (MMP) most important of which is MMP-9, cysteine and serine proteases) (Russell et al. 2002a), and oxidative radicals. Exposure to DEP suppressed CXCL8 expression as well as intracellular lysosomal and mitochondrial activity *in vitro* in monocyte-derived macrophages resulting in a reduced lifespan and impaired ability for translocation, differentiation, and cytokine response to lipopolysaccharide and heat killed bacteria (Chaudhuri et al. 2012). Phagocytosis of DEP by native primary alveolar macrophages induced apoptosis through generation of reactive oxygen species which triggered caspase and stress-activated protein kinase dependent mechanisms. This process was phagocytosis-dependent as non-phagocytic lung cells were not affected (Hiura et al. 1999). Released local factors are also important in the priming and skewing of these cells into the "classically activated" or "alternatively activated" subclasses. "Classically activated" M1 macrophages are present in severe inflammation. In response to LPS activation and exposure to IFN-γ M1 macrophages produce IFN-γ, TNF-α, IL-1β, IL-6, IL-12, IL-23, ROS, NO, GM-CSF, MIP-1α (CCL3), TARC (CCL17) and MCP-1 (CCL2) (Boorsma et al. 2013). The alternatively activated M2 macrophages are composed of several subclasses. M2a (after exposure to IL-4 or IL-13), M2b (in response to immune complexes in combination with IL-1β or LPS) and M2c (IL-10, TGFβ or glucocorticoids) has been described (Martinez et al. 2008). Depending on their differential expression of mediators, M2 cell subclasses support allergic airway inflammation; facilitate resolution of airway changes through a heightened phagocytic capability; contribute to airway remodeling; or provide immunosuppressive activities. M2 cells express CD206 (mannose receptor, MR) and display a unique mediator profile, that can include IL-4, IL-5, IL-10, IL-13 and IL-33, CCL18, CCL22, CCL24, HO-1, Arginase 1, chitinase-like Ym1, and Fizz1 (found in inflammatory zone-1) (Draijer et al. 2013; Martinez et al. 2013).

In patients with emphysema, macrophages accumulate in alveolar septae resulting in structural destruction and loss of breathing surface. This focal inflammation

is dominated by accumulation of macrophages and neutrophil cells in the lung parenchyma resulting in collagen deposition in the airways, goblet cell hyperplasia, and emphysema-like changes as documented in humans suffering of COPD as well as in a mouse model for NO_2-induced COPD (Wegmann et al. 2005). Macrophages derived CXCL1 and CXCL2 recruit circulating monocytes, while CXCL9, CXCL10 and CXCL11 attract T lymphocytes to the distal air spaces in COPD. Inhalation of aerosolized $PM_{2.5–10}$ resulted in macrophage activation, upregulation of inflammatory surface markers, increased phagocytosis and expression of TNF-α mRNA. Some but not all of this effect was mediated by surface bound endotoxin (Alexis et al. 2006). Alveolar macrophages isolated from bronchoalveolar lavage of healthy volunteers have shown proinflammatory response to coarse (PM_{10}) rather than fine ($PM_{2.5}$) particles and minimal oxidative stress response. These effects were mediated via TLR-4 (Becker et al. 2005). It is of note that alveolar macrophages, when exposed briefly to large quantities (20 % of LD50 dose) of PM *in vitro* exhibited reduced phagocytic and opsonizing capacities (Oberdorster et al. 1992; Becker and Soukup 1998). However, this may be attributed to the experimental setting rather than being a reflection of the physiological process in vivo where PM quantities are substantially smaller and exposure times are longer (Miyata and van Eeden 2011). Macrophages isolated from the lungs of patients with COPD exhibited reduced apoptosis and increased survival compared to those found in patients with normal lungs. This increased survival allows for sustained production of inflammatory mediators and increased destruction of lung parenchyma. Increased expression of $Bcl\text{-}X_L$, an antiapoptotic protein has been implicated in the extended survival of alveolar macrophages in COPD (Tomita et al. 2002). Macrophages from patients with COPD show resistance to corticosteroids, the most commonly used medication in obstructive airway diseases. This corticosteroid resistance is likely to be related to the reduced activity of histone deacetyase-2 (HDAC-2) an enzyme responsible the mediation of glucocorticoid effects on inflammatory gene transcription. Impaired suppression of inflammatory genes contributes to the sustained activation of macrophages in COPD (Ito et al. 2005). Normal alveolar macrophages have anti-inflammatory capacity expressed by secretion of TGF-β and inhibitors of MMPs. Macrophages in patients with COPD exhibit a limited capacity for anti-inflammatory activity which further shifts the inflammatory response towards tissue destruction (Pons et al. 2005).

5.7.2 Neutrophils

Normal, healthy lung parenchyma contains few if any neutrophil cells. The life span of neutrophils is short and these cells are capable of fast migration. A general acute effect of inhalation of toxic material (including the major pollutants NO_2, SO_2, PM_{10} [and more importantly $PM_{2.5}$] and ozone) is the influx of neutrophils into the distal air spaces. Although the exact mechanism of how such diverse agents can all elicit this rapid neutrophil recruitment is not clear, a shared pathway mediated by

oxidative stress and generation of reactive oxygen species may play an important role (Tao et al. 2003). Recruitment and translocation from the pulmonary circulation via chemotactic signaling from epithelial cells, activated macrophages and T-cells (via CXCL8, CXCL1, and leukotriene B_4) directs migration of neutrophils towards the airways. Adhesion molecules expressed on endothelial and epithelial cells mediate neutrophil migration with the MAC1/ICAM1 interactions being the most crucial. Smoker COPD patients have increased surface expression of MAC1 (CD11+/CD18+) on their neutrophils (reviewed in (Domagala-Kulawik 2008)). In addition to air pollution, the major causal agents in COPD exacerbations are usually considered bacterial or viral infections, or a combination of the two (Papi et al. 2006; Tsoumakidou and Siafakas 2006; Celli and Barnes 2007). Exacerbations represent an increase in the inflammation that is present in the stable state, with increased numbers of neutrophils, cytokines, chemokines and proteases in the airways, and some increased mediators in the blood with no reliable biomarkers to predict exacerbations (Celli and Barnes 2007). How exacerbations should be defined and graded are still debated today and the role of neutrophils in air pollutant-induced exacerbations of COPD remains complex (Tao et al. 2003; Papi et al. 2006; Tsoumakidou and Siafakas 2006; Celli and Barnes 2007; van Eeden et al. 2005; White et al. 2003).

Neutrophils can secrete a variety of proteases (including MMP, serine proteases, and cathepsin-G) and myeloperoxidase resulting in ROS formation. ROS accumulation in turn facilitates further extravasation of neutrophils in the inflamed airways (Rahman 2005). Oxidative stress also causes elevated concentration of cytokines and growth factors capable of activating and preventing apoptosis of neutrophils. This can lead to either increased survival or necrotic death of these cells. An important feature of the COPD lung is an increased number of dead cells due to necrotic cell death and a reduced ability of alveolar macrophages to perform their scavenger function. Neutrophil elastase was shown to cleave the phosphatidyl serine receptor on macrophages thereby disabling their apoptotic cell sensing and phagocytic capabilities (Vandivier et al. 2002).

Normally epithelial mucins serve the purpose of removing inhaled foreign materials by mucociliary transport and by coughing. In COPD however, excessive mucus is produced and is inadequately cleared. Some earlier studies correlated disease severity with sputum neutrophil numbers (Stanescu et al. 1996). It is becoming more accepted now that the main role of neutrophils in COPD is likely limited to goblet cell activation and increased mucus secretion rather than direct destruction of lung parenchyma. Indeed, the increased number of neutrophils in the airways during exacerbations of COPD manifests itself in the purulent nature of the expectorated sputum. Neutrophil proteases can stimulate mucin release by goblet cells. During oxidative stress neutrophils induce mucin production by activation of the epidermal growth factor receptor (EGFR). At present there is no specific treatment for mucus hypersecretion. However, the discovery that an EGFR related pathway is involved in mucin production by a wide variety of stimuli suggests that blockade may provide specific treatment for hypersecretory diseases (Burgel and Nadel 2004).

5.7.3 Dendritic Cells

Similarly to macrophages, dendritic cells are constitutive elements of the lung parenchyma and airways. In normal healthy lungs, dendritic cells are scattered throughout the respiratory mucosal wall. In resting state their primary role is to sample the environment and regulate immune homeostasis through communication with other cell types of the innate and adaptive immune system. Once dendritic cells are activated they will migrate to the regional lymph nodes and assume a T-cell–stimulatory phenotype. In the proximal and distal air spaces these cells are responsible for either initiating immune responses or maintaining immune tolerance. The fact that the respiratory surface of the average normal adult human lungs filters through over 11,000 l of air every day without a perpetual inflammatory state is in large part due to the collaborative action of dendritic cells and alveolar macrophages. The "hyperinflammatory" condition characterizing the COPD lung is associated with activation of different dendritic cell subpopulations, depending on their origins, migratory patterns, role and function (Haczku 2012; Botelho et al. 2012). The majority of the lung resident dendritic cells under resting conditions are made up by the tolerogenic plasmocytoid type (characterized by the cell surface marker profile 120G8^{high}/PDCA-1^{high}/Gr1^{high}/B220^{high}). During inflammation myeloid (CD11c^{high}/CD11b^{high}/MHC-II^{high}) and CD103^{+} cells migrate rapidly to the lung. These cells are mature and possess antigen presenting and CD4^{+} T cell stimulatory capabilities. Activated dendritic cell migration is propelled by the CCL19-CCR7, CXCL1-CXCR2, CCL2-CCR2/4 and CCL20-CCR6 chemokine ligand-receptor pathways (Demoor et al. 2009, 2011; Demedts et al. 2007). This process can amplify the chronicity of inflammation and can lead to destruction of the normal lung parenchyma. In a recent study, dendritic cells stimulated by cigarette smoke in mice, induced development of emphysema through auto-reactive CD8^{+} T cell activation and release of IL-17A (Shan et al. 2012). Unfortunately, there are no studies to date that investigated the role of these cells in mediating the effects of air pollution in COPD. There are however reports that looked at the effects of air pollutants on dendritic cell function and indicated a general tendency of these cells to lose their tolerogenic capabilities. For example, an association was seen between environmental tobacco smoke exposure and reduced dendritic cell interleukin 10 production during infancy (Gentile et al. 2004). Further, exposure to urban air extract, fine and ultrafine particles or DEP all induced dendritic cell activation leading to production of IL-1β or TNF-α (Gentile et al. 2004; Acciani et al. 2013; Bonisch et al. 2012; Kim et al. 2012; Karle et al. 2012; Myatt et al. 2011; Bezemer et al. 2011; Yoshida et al. 2010; Williams et al. 2008; de Haar et al. 2008). A recent study in mice demonstrated that IL-1R, TLR4, and TLR2 predominant receptors on activated dendritic cells, elicited inflammatory effects via MyD88 in response to wood or cow dung smoke derived PM suggesting that biomass fuel exposure elicits a persistent pulmonary inflammation largely through activation of TLR and IL-1R pathways (Sussan et al. 2014).

5.7.4 Lymphocytes

Lymphocyte accumulation in the pulmonary interstitium and peribronchial areas correlate with the severity of the symptoms of COPD and are considered to be part of the mechanism leading to exacerbation of symptoms brought on by air pollution or infections (Papi et al. 2006). Pollutants may have selective effects on lymphocytes. In a recent study lymphocyte redistribution was observed in NO_2 exposed volunteers (NO_2 levels were negatively correlated with peripheral blood lymphocyte count) but this effect was not seen in response to PM exposure (Steenhof et al. 2014). Lymphocytes organized in follicular structures with B lymphocyte containing germinal centers surrounded by $CD4^+$Th1-cells have been observed in clinically advanced cases of chronic bronchitis while increase in the numbers of $CD8^+$ cytotoxic Tc1 lymphocytes in the alveolar wall appears to be proportional with the severity of emphysema (Hogg et al. 2004).

Cytokine and chemokine signaling from epithelial cell injury, activated alveolar macrophages and antigen presenting dendritic cells may all contribute to the migration of $CD8^+$ and $CD4^+$T-lymphocytes to the lung in pollutant-induced COPD exacerbation. Interferon induced chemokines CXCL9, CXCL10, and CXCL11 elicit homing of CXCR3 positive T-cells while chemo-attractants for CCR4 positive Th2 cells (a predominant cell type in the pathogenesis of bronchial asthma) are not expressed in COPD (Grumelli et al. 2004). This feature is the main reason for the presence of T cells with cytotoxic characteristics in COPD with a relative lack of eosinophilic granulocytes (a hallmark of asthma). Th1 cells are $CD4^+$T-cells that express activated STAT-4 leading to interferon-γ (IFN-γ) secretion. Th1-cell help is required for $CD8^+$ cytotoxic Tc1 activation, survival (suppression of apoptosis) and maintenance of their immunological memory (Barnes and Rennard 2009). $CD8^+$ T-cells synthetize, store and release cytokines and cytotoxic substances like tumor necrosis factor-α (TNF-α), granzyme B, and perforins and their numbers inversely correlate with the FEV_1 of patients suffering of COPD (Freeman et al. 2010). It is of interest that patients with severe COPD of non-smoking origin do not have a Th1 cell inflammatory bias in their lung (Grumelli et al. 2004). CD8+ T cell activation and release of the above-mentioned cytotoxic substances however can be achieved by induction of Toll-like receptor (TLR)2 activation (Freeman et al. 2013). Indeed TLR2 was shown to play an important role in mediating the effects of biomass smoke derived PM (Sussan et al. 2014). Degradation of lung parenchyma may produce antigenic peptides (auto-antigens) that in the presence of chronic inflammation could induce a progressive self-perpetuating autoimmune inflammation (Cosio et al. 2002). The significance of lymphocytes and autoimmune pathways in air pollutant induced COPD needs clarification.

5.7.5 Epithelial Cells

Epithelial cells in the lung are highly specialized and form the first line of defense against inhaled air pollutants. The major role of type-1 alveolar cells is gas exchange: facilitation of the uptake of oxygen and release of carbon dioxide. Type-2 alveolar cells produce surfactant that reduces surface tension and allows alveoli of different diameters to remain open even at low lung volumes. The bronchial epithelium is composed of pseudostratified columnar cells that include basal, ciliated or secretory (Clara or goblet) cells. Ciliated bronchial epithelial cells transport foreign matter towards the larynx along the surface of the tracheobronchial tree. Goblet cells in the bronchi secret mucus, which enhances expulsion via cilial activity (Lumb 2000). In patients with COPD epithelial cells express high levels of inflammatory mediators (CXCL-8, IL-1-β, and GM-CSF) (Hellermann et al. 2002) and adhesion molecules (sICAM-1 (Hellermann et al. 2002) and E-selectin (Di Stefano et al. 1994)) thereby facilitating recruitment and translocation of inflammatory cells.

Cultured bronchial epithelial cells when exposed to airborne particulate matter respond with increased expression of CXCL8 and to a lesser and irritant specific extent by upregulation of a variety of chemokines (CXCL1, 3, 10, 11, CCL20) and proinflammatory factors (TNF-α, IL-6, LTβ) (Øvrevik et al. 2009). It is of note that expression of CXCL8, a chemokine mostly associated with neutrophilic granulocyte recruitment is associated with pollutants as diverse as fine carbon particles, 1-nitropyrene, aerosolized bacterial lipopolysaccharide and $ZnCl_2$ (Øvrevik et al. 2009). This common feature perhaps explains the epidemiological finding that exposure to a wide variety of inhaled airborne pollutants results in common clinical outcomes and disease progression (Schwarze et al. 2006). On the other hand, relatively similar chemical substances like various nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) may elicit either increased cytokine and chemokine expression or epithelial cell apoptosis (Øvrevik et al. 2010). Furthermore, seasonal variability in particulate matter composition produces distinct effects as shown from samples taken in Milan, Italy. The winter PM had higher levels of PAHs while in the summer, mineral dust elements have dominated. When cultured human epithelial cells were exposed to these PM samples the winter samples were more cytotoxic while the summer samples exhibited a higher proinflammatory potential (Gualtieri et al. 2010).

The mechanisms of interaction between airborne pollutants and epithelial cells, inflammatory cells and mesenchymal cells in the development of COPD are complex and remain to be fully elucidated. This complexity is increased by the fact that in addition to its pro-inflammatory function, the airway epithelium is also responsible for maintaining immune homeostasis and protecting against chronic inflammatory changes in the lung. The protective function of airway epithelial cells has been attributed to a constitutive production of the immune modulators, lung collectins: surfactant protein A and D. The majority of these molecules are produced by type II alveolar epithelial cells in the distal air spaces but Clara cells and goblet cells can also release collectins. SP-A and SP-D belong to a small family of structurally related Ca^{2+} dependent lectins that share collagen-like and lectin domains. Collectins are involved in pathogen binding (opsonization) and direct inhibition of

immune and inflammatory cell function (Rosseau et al. 1997). Collectin-specific interactions with lung cells can be mediated through CD14 (Sano et al. 2000), TLR2 (Ohya et al. 2006) and TLR4 (Ohya et al. 2006; Guillot et al. 2002) as well as through the signal inhibitory regulatory protein (SIRPα) (Gardai et al. 2003). This signal regulatory membrane protein uniquely carries an ITIM containing intracellular domain which is responsible for inhibiting functions of myeloid derived cells, particularly, macrophages and dendritic cells (Matozaki et al. 2009). Lung collectins regulate antigen presentation, T-cell stimulation (Brinker et al. 2003; Brinker et al. 2001; Hansen et al. 2007) and TNFα expression by antigen presenting cells (Hortobagyi et al. 2008).

Under normal conditions SP-A–/– mice display no overt pathological features. SP-D–/– mice on the other hand showed serious constitutive inflammatory alterations (Botas et al. 1998; Hawgood et al. 2002) resembling pathological characteristics of COPD indicating special importance of this molecule in regulating immune homeostasis and function of the innate immune cells (Crouch et al. 2000; Crouch 2000; Wright 2005; Haczku 2006, 2008). Studies on airway responses to allergen (Takeda et al. 2001) or O_3 (Kierstein et al. 2006) exposure showed that C57BL/6 mice were significantly more protected against developing inflammation than Balb/c mice and this protection was associated with a greater amount of SP-D in their airways (Kierstein et al. 2006; Atochina et al. 2003). Mice lacking this molecule due to genetic deletion were highly susceptible to allergen or O_3-induced airway inflammation and showed a prolonged recovery period (Haczku 2006; Kierstein et al. 2006). O_3-induced changes in sputum dendritic cell phenotype were associated with changes in serum SP-D levels in healthy volunteers (Alexis et al. 2008). Further, low SP-D levels in the lung correlated with disease severity in COPD patients (Sims et al. 2008).

Mouse models of low or no SP-D expression in different mouse strains and gene-manipulated mice mimic patients who have low SP-D levels in the lung due to genetic reasons or as a consequence of chronic inflammation. Locally administered SP-D has been protective in mouse models of airway inflammation. It is possible therefore that it will work in patients, too. SP-D is not only an anti-inflammatory agent but also an important opsonin that binds a wide range of common respiratory pathogens. Thus, unlike the traditional immunosuppressive therapies, an SP-D-based anti-inflammatory approach could have an advantage of a preserved host defense function.

5.8 Molecular Mechanisms of Air Pollutant-Induced Lung Damage in COPD

COPD is characterized by a progressive destruction of the alveolar structure of the lung parenchyma with associated fibrotic narrowing of small airways by the underlying inflammatory processes. In addition to cytokines and chemokines many inflammatory mediators were described and characterized in this process. The major

players include arachidonic acid derivatives, molecular products of oxidative stress, players of cell death, extracellular matrix destruction and repair (Barnes 2004c).

5.8.1 Arachidonic Acid Metabolites

Ozone exposure of human nasal mucosa *in vitro* induced heightened eicosanoid metabolism with the release of cyclooxygenase and lipoxygenase products into the culture supernatant including prostaglandin (PG)$F_2\alpha$, thromboxane B2 and leukotriene B4 (LTB4) (Schierhorn et al. 1997). In COPD however arachidonic acid metabolism follows a typical pattern in the lungs: There is an increase in prostaglandins PGE_2, $PGF_2\alpha$, and LTB_4 but not of thromboxane or cysteinyl leukotrienes (increase of which is characteristic for bronchial asthma) (Barnes and Rennard 2009). Increased expression of cyclooxygenase-2 (COX2) is responsible for increased production of these prostanoids.

Urban air collected in different zones in Mexico City, containing PM_{10} induced PGE_2 in cultured rat fibroblasts in a dose-dependent manner *in vitro* (Alfaro-Moreno et al. 2002). Further, nickel (Ni), a common component of urban PM enhanced IL-8 production by fibroblasts in the presence of PGE_2, suggesting a synergistic action (Brant and Fabisiak 2013).

Alveolar macrophages in patients with COPD express pro-inflammatory BLT_1-receptors and LTB_4 inactivating PPAR-α receptors. LTB_4 also recruits circulating $CD8^+$ T-cells and neutrophils to the lung parenchyma through high affinity BLT_1-receptors expressed on these cells (Marian et al. 2006). Investigation of exhaled breath condensate from ozone-exposed subjects (in comparison with air exposed ones) revealed elevated levels of 8-isoprostane and LTB4. These levels were significantly greater in subjects sensitive to ozone suggesting that sensitive subjects have elevated arachidonic acid metabolites in their exhaled breath condensates (Alfaro et al. 2007).

5.8.2 Role of Oxidative Stress

Reactive oxygen species (ROS) are generated in the pulmonary parenchyma as part of the first line defense mechanism by the innate immune system. Exogenous irritants and inflammatory mediators induce ROS production by epithelial cells, alveolar macrophages, and neutrophil cells in the lungs (Yao and Rahman 2011). These ROS overwhelm and destroy invading bacteria, fungi, and viruses in a non-specific manner. ROS are also produced, in smaller quantities, as by-products of signaling and energy generating intracellular processes. ROS also have a role in inducing and maintaining local inflammation (Rahman 2005). The enzyme NADPH oxidase generates superoxide anions (O_2^-) from molecular oxygen. This superoxide anion is relatively unstable and highly reactive and is converted by superoxide dismutase

(SOD) to hydrogen peroxide (H_2O_2). H_2O_2 can diffuse to farther distances than superoxide anion allowing for a larger radius of effect. In the extracellular space reaction of O_2^- with free iron or nitric oxide (NO) produces highly reactive hydroxyl radicals (OH^-) and peroxynitrite ($ONOO^-$), respectively. ROS interacting with lipids generate lipid radicals (lipid peroxides) leading to a redox chain reaction in lipid bilayers damaging cellular membranes or oxidize arachidonic acid leading to the formation of isoprostanes, a class of prostanoids; mediators implicated in bronchoconstriction and increased alveolar and capillary permeability. Both isoprostane levels (Garcia-Rio et al. 2011) and lipid peroxidation markers (Rahman et al. 2002) positively correlate with disease severity in patients with COPD. There is increased synthesis of NO in lung parenchyma and small airways due to chronic inflammation-induced increase in the expression of inducible NO synthase in epithelial cells and macrophages in COPD (Ricciardolo et al. 2005). Peroxynitrite reacts with tyrosine residues in proteins producing 3-nitrotyrosine. As tyrosine moieties often have a significant role in protein activation or inhibition these changes may result in significant alterations in the function of the given proteins. Reactive aldehydes or carbonylate proteins form aldehyde-protein adducts that alter or suspend protein function. Aldehyde-protein adducts may also induce immunogenicity and serve as targets for the adaptive immune system (Yao and Rahman 2011). ROS when reaching the cell nucleus may inflict direct damage on the DNA or interact with regulatory enzymes like histone deacetylase (HDAC and sirtuins) or histone methylation (HMT)/demethylation (HDM) enzymes leading to alterations in inflammatory gene expression (Sundar et al. 2013).

Inhalation of nitro-PAHs (a component of diesel exhaust particles) can lead to the generation of electrophilic metabolites including epoxides (Yamazaki et al. 2000; Andersson et al. 2009) or nitro-reductions [this later is catalyzed by enzymes like NOS, xanthine oxidase, aldehyde oxidase (Arlt et al. 2003) or NADPH:quinone oxidoreductase (Arlt et al. 2005)] resulting in generation of ROS (Øvrevik et al. 2010). On the other hand, sulfate conjugation or acetylation generates reactive nitrenium ions which then create protein and DNA adducts (Arlt et al. 2005). DNA damaging potential of various nitro-PAHs appears to be specific to their chemical structure. The most abundant 1-nitropyrene appears to have very mild DNA toxicity while 3-nitrobenzanthrone has considerable DNA damaging potential resulting in accumulation of cells in S-phase and marked increase in apoptosis in cultured bronchial epithelial cells (Øvrevik et al. 2010). Furthermore, the generation of ROS by nitro-PAHs is paralleled by a decrease in intracellular GSH suggesting depletion of protective antioxidant mechanisms (Park and Park 2009).

Bronchial epithelial cells obtained from airway mucosal biopsies of healthy volunteers show significant increase in hemoxygenase-1 expression, a marker of oxidative stress when exposed to coarse or fine PM. This response involves the TLR-2 signaling pathway (Becker et al. 2005). The body counters the effects of ROS on the self by producing antioxidants. These substances (glutathione, ascorbic acid [vitamin C], tocopherol [vitamin E], lactoferrin, and uric acid) protect the self against the action of ROS. Enzyme systems geared towards removing reactive aldehydes including aldehyde dehydrogenase and aldo-keto reductase are present in the lung

parenchyma in COPD (Pastor et al. 2013). When these antioxidant mechanisms are exhausted, cellular damage ensues (Repine et al. 1997). In the lung parenchyma the majority of antioxidants are found in the extracellular matrix while intracellular antioxidants are expressed at a low level (Rahman 2005). While oxidative stress has little effect on intracellular antioxidant levels extracellular antioxidant enzymes like glutathione peroxidase, glutathione-S-transferase M_1, and SOD3 markedly increase. Activation of the transcription factor Nrf2 (nuclear erythroid-related factor 2) has a key role in gene activation and transcription of protective antioxidant enzymes in lungs when exposed to oxidative stress (Ishii et al. 2005). Nrf2 levels are decreased in lungs of patients with emphysema and COPD (Goven et al. 2008). Posttranslational modification of Nrf2 results in decreased activity and limited nuclear translocation. A stabilizer of Nrf2, DJ-1 has been found to be downregulated in patients with COPD but not in unaffected smokers (Malhotra et al. 2008). Activation of Nrf2 and stabilizing DJ-1 can open potential protective pathways in patients susceptible to airborne-inhalant induced COPD (Sundar et al. 2013). The Nrf2 pathway is also one of the outlets of the UPR (unfolded protein response) an intracellular mechanism protective among others against oxidative stress (Kelsen et al. 2008).

Increased oxidative stress in the lung epithelium and parenchyma may play a significant role in disease progression in COPD by amplifying and perpetuating the inflammatory response and increasing tissue destruction. ROS upregulate transcription factors NF-κB and AP-1 in alveolar macrophages and structural cells inducing inflammatory mediator and cytokine production as described above. Oxidative stress also decreases corticosteroid responsiveness in patients with COPD. This is attributed to reduced activity and expression of histone deacetylase (HDAC). Histone acetylation by histone acetyltransferase leads to transcription of pro-inflammatory genes encoding IL-8 and TNF-α through NF-κB. HDAC2 activated by corticosteroid activated-glucocorticoid receptor inhibits this process in normal lungs. However, in COPD increased peroxynitrite production inactivates HDAC2 leading to glucocorticoid resistance (Ito et al. 2005). ROS also directly impair protease inhibitors like α_1-antitrypsine and SLPI contributing to the destruction of elastic elements of the lung parenchyma in COPD. Ozone-induced NF-κB activation correlated with expression of monocyte chemotactic protein-1, inducible NO synthase and cyclooxygenase-2 in alveolar macrophages (Sunil et al. 2012). Similarly, oxidative stress secondary to free radicals generated by PM_{10} exposure resulted in calcium mediated nuclear translocation and activation of NF-κB leading to decreased expression of HDAC2 (MacNee and Donaldson 2003). The ensuing increase in acetylation of histone residues could then allow for increased transcription factor binding and expression of proinflammatory genes (Gilmour et al. 2003).

5.8.3 Mechanisms and Mediators of Apoptosis and Autophagy

Mounting evidence suggests an imbalance between apoptotic cell loss and cell proliferation in lung tissue of patients suffering of COPD as well as in animal models of emphysema (Demedts et al. 2006). This imbalance affects alveolar epithelial

cells (Imai et al. 2005), alveolar endothelial cells (Segura-Valdez et al. 2000), and lung T-cells (Hodge et al. 2005) suggesting a complex interaction between vascular growth regulation (Kasahara et al. 2000; Kanazawa and Yoshikawa 2005), inflammation, oxidative stress (Aoshiba and Nagai 2003) and epithelial cell wellbeing (Calabrese et al. 2005) in the lungs of patients with COPD. It is also of note that increased apoptosis continues after cessation of exposure to noxious inhalants (Hodge et al. 2005). Disturbance in the regulation of cell proliferation and programmed cell loss extends beyond the lungs in patients with advanced emphysema and examples of this are found in skeletal muscle atrophy resulting in peripheral muscle weakness (Agusti et al. 2002) and loss of peripheral T-cells resulting in inability to mount an adequate defensive response to infections (Hodge et al. 2003a). Suggested mechanisms of increased apoptosis include depletion or inhibition of VEGF (Kasahara et al. 2000), elevated levels of IFN-γ (Ma et al. 2005), activation of caspase-3 (Aoshiba et al. 2003) and accumulation of ceramide in the lung parenchyma (Scarpa et al. 2013). Inflammatory cells (Saetta et al. 1999) and mediators present in COPD have also been associated with pulmonary epithelial and endothelial cell apoptosis (Hodge et al. 2003b). On the other hand the decreased ability of alveolar macrophages in eliminating apoptotic cells contributes to the sustained inflammation (Vandivier et al. 2002). Elastolytic activity resulting in loss of structural basal membrane depletes alveolar epithelial cells of vital cell-matrix contacts for survival signaling and promotes apoptosis (Aoshiba et al. 1997).

Cellular organelles, old and damaged mitochondria, structural cellular proteins are removed and recycled in a dynamic process called autophagy (Ryter et al. 2012). This is an inducible mechanism originally developed to recycle cellular material thus reducing need for nutrients in starvation. Oxidative stress accelerates autophagy as there is increased number of damaged cellular components to recycle. However, excessive autophagy is harmful and may lead to death of the cell. Lung tissue from patients with COPD show accelerated autophagy, which is attributed to reduction of SIRT1, a sirtuin class histone deacetylase. Decrease in SIRT1 levels allows transcription of the early growth response protein-1 (Egr-1) gene, an important transcription factor in autophagy (Chen et al. 2008). Increased extracellular levels of superoxide dismutase (SOD) attenuate intracellular Egr-1 levels (Nozik-Grayck et al. 2008) further illustrating the complex interaction between oxidative stress and cell organelle recycling. Ozone exposure increased levels of biomarkers representative of apoptosis and autophagy like cleaved capsase-9 and beclin-1 in alveolar macrophages. This air pollutant effect was associated with presence of matrix metalloproteinases (MMP)-2, MMP-9 and cellular debris in the bronchoalveolar lavage fluid (Sunil et al. 2012).

5.8.4 Proteinases and Extracellular Matrix (ECM) Interactions

Proteinases released from macrophages, cytotoxic T-cells and to a lesser extent, neutrophilic granulocytes contribute to degrading of pulmonary parenchymal connective tissue (Segura-Valdez et al. 2000). MMPs especially MMP-9 released from

alveolar macrophages have been implicated in elastin destruction and development of emphysema (Russell et al. 2002b). Macrophages isolated from patients with COPD contain higher amounts of MMP-9 and are resistant to suppression by glucocorticoids (Chana et al. 2014). Alveolar macrophages also synthetize and secrete proteinase inhibitors (tissue inhibitors of MP, TIMP). However, the amount and activity of these inhibitors is reduced in patients with COPD and is insufficient to neutralize the MMP activity (Russell et al. 2002b).

MMPs have also been implicated in regulation of programmed cell death in COPD. MMP-7 sheds and activates Fas ligand, an apoptosis signaling molecule, from alveolar epithelial cells (Powell et al. 1999). Chronic exposure of mice to ozone mimicked the inflammatory and pathological changes of COPD and increased caspase-3 mediated apoptosis and activity of MMPs (Triantaphyllopoulos et al. 2011). The imbalance between levels and activity of MMPs and proteinase inhibitors has been associated with histone deacetylation and deficiency of sirtuin 1 (SIRT1, a member of class III histone deacetylases). The protective role of SIRT1 was also supported in a study using exposure to ambient PM and gene deficient mice in which lack of SIRT1 resulted in aggravated lung vascular leakage and inflammation after PM exposure, which was correlated with increased NF-κB acetylation and activation (Wu et al. 2012). Pertinent to air pollution induced COPD, increasing expression of SIRT1 was shown to reestablish the balance between proteinases and inhibitors. This strategy may become a pharmacological approach against inhaled irritant induced COPD (Yao et al. 2013).

5.8.5 Repair Mechanisms

Post inflammatory repair markers (arginase-1, galectin-3, Ym-1) representing alveolar macrophage contribution to wound healing were expressed in the lung 24–72 h after exposure to ozone (Sunil et al. 2012). Transforming growth factor-α (TGF-α) is secreted by alveolar macrophages and is a potent stimulator alveolar epithelial cell proliferation and repair (Crosby and Waters 2010). In goblet cells, TGF-α increases mucus production thereby worsening the disease (Burgel and Nadel 2004). Interestingly, transgenic mice expressing TGF-α were rescued from $NiSO_4$ injury (they had diminished SP-B loss and increased survival time) (Leikauf et al. 2001).

TGF-β is a potent anti-inflammatory molecule in the lung (Yang et al. 2012) expressed in both airway epithelial cells and alveolar macrophages. TGF-β is a key factor in the development of peribronchial fibrosis in COPD. A complex interaction between mediators regulating production and destruction of the ECM is highlighted by the fact that the action of MMP-9 is necessary to activate TGF-β contributing to fibrotic remodeling of lung parenchyma in COPD (Chung 2005). Exposure to various dust particles was shown to increase gene expression of procollagen, TGF-β, and platelet-derived growth factor, and increased hydroxyproline in lung explants. Addition of TNF-α increased dust adhesion to, and ozone exposure increased dust

uptake by tracheal epithelial cells (Churg and Wright 2002). Further, Katre and colleagues demonstrated in a mouse model that O_3 exposure increased TGF-β expression and activated TGF-β signaling pathways leading to O_3-induced lung fibrotic responses *in vivo* (Katre et al. 2011).

5.9 Summary and Future Projections

The evidence for epidemiological association between occupational and environmental (outdoor and indoor) exposure to air pollutants and COPD is well established but it is also clear, that individuals respond differently to the same noxious stimuli. Although various inflammatory pathways have been implicated, the mechanisms by which air pollution affects development of COPD in susceptible individuals are not well understood. Recent investigations on subjects participating in the Normative Aging Study (a prospective cohort of aging established in 1963, enrolling men from the Greater Boston area), applied a novel genetic approach to investigate interactions between pathways relevant to chronic inflammation and air pollution. The authors found that among participants with higher genetic scores (i.e. a higher allelic risk profile) within the oxidative stress and metal processing pathways, there were significant associations between pollutant particle number and variants of the fibrinogen, C-reactive protein and ICAM-1 genes (Bind et al. 2014a). These data supported that in addition to the extent of exposure, disease development in exposed subjects is related to genetic predisposition affecting specific, inflammatory genes.

Further investigations by Lepeule et al. (2014) showed that sub-chronic (3-to 28-days, cumulated) but not acute exposure to black carbon, total and non-traffic $PM_{2.5}$, CO and NO_2 was associated with a 1–5 % decrease in FVC and FEV_1 in elderly, Normative Aging Study participants. These data suggested the importance of duration of exposure to air pollutants. Interestingly, in participants with a greater exposure (28-days cumulated), low methylation in the TLR2 and high methylation in the glucocorticoid receptor genes were observed. Thus, prolonged exposure to air pollution may lead to epigenetic alterations of genes related to inflammatory and immune regulatory pathways.

Indeed in another well characterized cohort of subjects from the Normative Aging Study (1999–2009) (Bind et al. 2014b) increase in air pollutant (ozone and components of fine particle mass) concentrations was significantly associated with hypomethylation of tissue factor-3, ICAM-1, and TLR-2 genes and hypermethylation of the IFN-γ and IL-6 genes. While these studies suggested that altered DNA methylation could reflect biological impact of air pollution, the significance and mechanisms of such epigenetic pathways need further clarifications.

In summary, today we have epidemiological evidence that supports a pathogenetic role of air pollutants in the development and course of COPD. We also understand that there is a great degree of heterogeneity in the air pollution effects: disease development involves only a lesser but significant proportion of the population where in addition to genetic factors, the extent and duration of exposure also play a

deterministic role. Thus, the physician and policy maker are both in quandary when the question is asked: who are the vulnerable and who are those not affected by the pollutants. Protecting all including those who are not affected would consume large financial resources, oftentimes not available for the individual or the society. Further research is needed to determine the most vulnerable populations and what actions might be successful in preventing or slowing the progress of disease. Emerging work in genetics (Bind et al. 2014a; Zhou et al. 2013) and epigenetics (Lepeule et al. 2014; Bind et al. 2014b; Sakao and Tatsumi 2011) of COPD provides hope in this direction of prevention and cure.

References

Acciani TH, Brandt EB, Khurana Hershey GK, Le Cras TD (2013) Diesel exhaust particle exposure increases severity of allergic asthma in young mice. Clin Exp Allergy 43:1406–1418

Agusti AG, Sauleda J, Miralles C, Gomez C, Togores B, Sala E, Batle S, Busquets X (2002) Skeletal muscle apoptosis and weight loss in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 166:485–489

Alexis NE, Lay JC, Zeman K, Bennett WE, Peden DB, Soukup JM, Devlin RB, Becker S (2006) Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers. J Allergy Clin Immunol 117:1396–1403

Alexis NE, Lay JC, Haczku A, Gong H, Linn W, Hazucha MJ, Harris B, Tal-Singer R, Peden DB (2008) Fluticasone propionate protects against ozone-induced airway inflammation and modified immune cell activation markers in healthy volunteers. Environ Health Perspect 116:799–805

Alfaro MF, Walby WF, Adams WC, Schelegle ES (2007) Breath condensate levels of 8-isoprostane and leukotriene B4 after ozone inhalation are greater in sensitive versus nonsensitive subjects. Exp Lung Res 33:115–133

Alfaro-Moreno E, Martinez L, Garcia-Cuellar C, Bonner JC, Murray JC, Rosas I, Rosales SP, Osornio-Vargas AR (2002) Biologic effects induced in vitro by PM10 from three different zones of Mexico City. Environ Health Perspect 110:715–720

Anderson HR, Limb ES, Bland JM, Ponce de Leon A, Strachan DP, Bower JS (1995) Health effects of an air pollution episode in London, December 1991. Thorax 50:1188–1193

Andersson H, Piras E, Demma J, Hellman B, Brittebo E (2009) Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmic reticulum stress in human endothelial cells. Toxicology 262:57–64

Aoshiba K, Nagai A (2003) Oxidative stress, cell death, and other damage to alveolar epithelial cells induced by cigarette smoke. Tob Induc Dis 1:219–226

Aoshiba K, Rennard SI, Spurzem JR (1997) Cell-matrix and cell-cell interactions modulate apoptosis of bronchial epithelial cells. Am J Physiol 272:L28–L37

Aoshiba K, Yokohori N, Nagai A (2003) Alveolar wall apoptosis causes lung destruction and emphysematous changes. Am J Respir Cell Mol Biol 28:555–562

Arbex MA, de Souza Conceicao GM, Cendon SP, Arbex FF, Lopes AC, Moyses EP, Santiago SL, Saldiva PH, Pereira LA, Braga AL (2009) Urban air pollution and chronic obstructive pulmonary disease-related emergency department visits. J Epidemiol Community Health 63:777–783

Arbex MA, Santos Ude P, Martins LC, Saldiva PH, Pereira LA, Braga AL (2012) Air pollution and the respiratory system. J Bras Pneumol 38:643–655

Arlt VM, Glatt H, Muckel E, Pabel U, Sorg BL, Seidel A, Frank H, Schmeiser HH, Phillips DH (2003) Activation of 3-nitrobenzanthrone and its metabolites by human acetyltransferases,

sulfotransferases and cytochrome P450 expressed in Chinese hamster V79 cells. Int J Cancer 105:583–592

Arlt VM, Stiborova M, Henderson CJ, Osborne MR, Bieler CA, Frei E, Martinek V, Sopko B, Wolf CR, Schmeiser HH, Phillips DH (2005) Environmental pollutant and potent mutagen 3-nitrobenzanthrone forms DNA adducts after reduction by NAD(P)H:quinone oxidoreductase and conjugation by acetyltransferases and sulfotransferases in human hepatic cytosols. Cancer Res 65:2644–2652

Atochina EN, Beers MF, Tomer Y, Scanlon ST, Russo SJ, Panettieri RA Jr, Haczku A (2003) Attenuated allergic airway hyperresponsiveness in C57BL/6 mice is associated with enhanced surfactant protein (SP)-D production following allergic sensitization. Respir Res 4:15

Balmes J, Becklake M, Blanc P, Henneberger P, Kreiss K, Mapp C, Milton D, Schwartz D, Toren K, Viegi G, Environmental and Occupational Health Assembly, American Thoracic Society (2003) American Thoracic Society Statement: occupational contribution to the burden of airway disease. Am J Respir Crit Care Med 167:787–797

Baraldo S, Turato G, Saetta M (2012) Pathophysiology of the small airways in chronic obstructive pulmonary disease. Respiration 84:89–97

Barnes PJ (2004a) Alveolar macrophages as orchestrators of COPD. COPD 1:59–70

Barnes PJ (2004b) Alveolar macrophages in chronic obstructive pulmonary disease (COPD). Cell Mol Biol 50:OL627–OL637, Online Pub

Barnes PJ (2004c) Mediators of chronic obstructive pulmonary disease. Pharmacol Rev 56:515–548

Barnes PJ, Rennard SI (2009) Pathophysiology of COPD. In: Barnes PJ, Drazen JM, Rennard SI, Thomson NC (eds) Asthma and COPD: basic mechanisms and clinical management, 2nd edn. Elsevier, Amsterdam

Bates DV (2000) Revisiting "respiratory function in emphysema in relation to prognosis". Can Respir J 7:271–279

Becker S, Soukup JM (1998) Decreased CD11b expression, phagocytosis, and oxidative burst in urban particulate pollution-exposed human monocytes and alveolar macrophages. J Toxicol Environ Health A 55:455–477

Becker S, Mundandhara S, Devlin RB, Madden M (2005) Regulation of cytokine production in human alveolar macrophages and airway epithelial cells in response to ambient air pollution particles: further mechanistic studies. Toxicol Appl Pharmacol 207:269–275

Becklake MR (1989) Occupational exposures: evidence for a causal association with chronic obstructive pulmonary disease. Am Rev Respir Dis 140:S85–S91

Bell ML, Davis DL (2001) Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution. Environ Health Perspect 109(Suppl 3):389–394

Bezemer GF, Bauer SM, Oberdorster G, Breysse PN, Pieters RH, Georas SN, Williams MA (2011) Activation of pulmonary dendritic cells and Th2-type inflammatory responses on instillation of engineered, environmental diesel emission source or ambient air pollutant particles in vivo. J Innate Immun 3:150–166

Bind MA, Coull B, Suh H, Wright R, Baccarelli A, Vokonas P, Schwartz J (2014a) A novel genetic score approach using instruments to investigate interactions between pathways and environment: application to air pollution. PLoS One 9:e96000

Bind MA, Lepeule J, Zanobetti A, Gasparrini A, Baccarelli A, Coull BA, Tarantini L, Vokonas PS, Koutrakis P, Schwartz J (2014b) Air pollution and gene-specific methylation in the Normative Aging Study: association, effect modification, and mediation analysis. Epigenetics 9:448–458

Blanc PD, Eisner MD, Earnest G, Trupin L, Balmes JR, Yelin EH, Gregorich SE, Katz PP (2009a) Further exploration of the links between occupational exposure and chronic obstructive pulmonary disease. J Occup Environ Med 51:804–810

Blanc PD, Iribarren C, Trupin L, Earnest G, Katz PP, Balmes J, Sidney S, Eisner MD (2009b) Occupational exposures and the risk of COPD: dusty trades revisited. Thorax 64:6–12

Bonisch U, Bohme A, Kohajda T, Mogel I, Schutze N, von Bergen M, Simon JC, Lehmann I, Polte T (2012) Volatile organic compounds enhance allergic airway inflammation in an experimental mouse model. PLoS One 7:e39817

Boorsma CE, Draijer C, Melgert BN (2013) Macrophage heterogeneity in respiratory diseases. Mediators Inflamm 2013:769214

Botas C, Poulain F, Akiyama J, Brown C, Allen L, Goerke J, Clements J, Carlson E, Gillespie AM, Epstein C, Hawgood S (1998) Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. Proc Natl Acad Sci U S A 95:11869–11874

Botelho FM, Nikota JK, Bauer CM, Morissette MC, Iwakura Y, Kolbeck R, Finch D, Humbles AA, Stampfli MR (2012) Cigarette smoke-induced accumulation of lung dendritic cells is interleukin-1alpha-dependent in mice. Respir Res 13:81

Brant KA, Fabisiak JP (2013) Role of hypoxia-inducible factor 1, alpha subunit and cAMP-response element binding protein 1 in synergistic release of interleukin 8 by prostaglandin E2 and nickel in lung fibroblasts. Am J Respir Cell Mol Biol 49:105–113

Brinker KG, Martin E, Borron P, Mostaghel E, Doyle C, Harding CV, Wright JR (2001) Surfactant protein D enhances bacterial antigen presentation by bone marrow-derived dendritic cells. Am J Physiol Lung Cell Mol Physiol 281:L1453–L1463

Brinker KG, Garner H, Wright JR (2003) Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells. Am J Physiol Lung Cell Mol Physiol 284:L232–L241

Burgel PR, Nadel JA (2004) Roles of epidermal growth factor receptor activation in epithelial cell repair and mucin production in airway epithelium. Thorax 59:992–996

Calabrese F, Giacometti C, Beghe B, Rea F, Loy M, Zuin R, Marulli G, Baraldo S, Saetta M, Valente M (2005) Marked alveolar apoptosis/proliferation imbalance in end-stage emphysema. Respir Res 6:14

Cazzola M, Donner CF, Hanania NA (2007) One hundred years of chronic obstructive pulmonary disease (COPD). Respir Med 101:1049–1065

Celli BR, Barnes PJ (2007) Exacerbations of chronic obstructive pulmonary disease. Eur Respir J 29:1224–1238

Chana KK, Fenwick PS, Nicholson AG, Barnes PJ, Donnelly LE (2014) Identification of a distinct glucocorticosteroid-insensitive pulmonary macrophage phenotype in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 133:207–216.e211

Chaudhuri N, Jary H, Lea S, Khan N, Piddock KC, Dockrell DH, Donaldson K, Duffin R, Singh D, Parker LC, Sabroe I (2012) Diesel exhaust particle exposure in vitro alters monocyte differentiation and function. PLoS One 7:e51107

Chen ZH, Kim HP, Sciurba FC, Lee SJ, Feghali-Bostwick C, Stolz DB, Dhir R, Landreneau RJ, Schuchert MJ, Yousem SA, Nakahira K, Pilewski JM, Lee JS, Zhang Y, Ryter SW, Choi AM (2008) Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. PLoS One 3:e3316

Chung KF (2005) Inflammatory mediators in chronic obstructive pulmonary disease. Curr Drug Targets Inflamm Allergy 4:619–625

Churg A, Wright JL (2002) Airway wall remodeling induced by occupational mineral dusts and air pollutant particles. Chest 122:306S–309S

Cosio MG, Majo J, Cosio MG (2002) Inflammation of the airways and lung parenchyma in COPD: role of T cells. Chest 121:160S–165S

Crosby LM, Waters CM (2010) Epithelial repair mechanisms in the lung. Am J Physiol Lung Cell Mol Physiol 298:L715–L731

Crouch EC (2000) Surfactant protein-D and pulmonary host defense. Respir Res 1:93–108

Crouch E, Hartshorn K, Ofek I (2000) Collectins and pulmonary innate immunity. Immunol Rev 173:52–65

Davis DL, Bell ML, Fletcher T (2002) A look back at the London smog of 1952 and the half century since. Environ Health Perspect 110:A734–A735

de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R (2008) Lung dendritic cells are stimulated by ultrafine particles and play a key role in particle adjuvant activity. J Allergy Clin Immunol 121:1246–1254

Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG (2006) Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. Respir Res 7:53

Demedts IK, Bracke KR, Van Pottelberge G, Testelmans D, Verleden GM, Vermassen FE, Joos GF, Brusselle GG (2007) Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 175:998–1005

Demoor T, Bracke KR, Vermaelen KY, Dupont L, Joos GF, Brusselle GG (2009) CCR7 modulates pulmonary and lymph node inflammatory responses in cigarette smoke-exposed mice. J Immunol 183:8186–8194

Demoor T, Bracke KR, Dupont LL, Plantinga M, Bondue B, Roy MO, Lannoy V, Lambrecht BN, Brusselle GG, Joos GF (2011) The role of ChemR23 in the induction and resolution of cigarette smoke-induced inflammation. J Immunol 186:5457–5467

Di Stefano A, Maestrelli P, Roggeri A, Turato G, Calabro S, Potena A, Mapp CE, Ciaccia A, Covacev L, Fabbri LM, Saetta M (1994) Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. Am J Respir Crit Care Med 149:803–810

Di Stefano A, Caramori G, Ricciardolo FL, Capelli A, Adcock IM, Donner CF (2004) Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview. Clin Exp Allergy 34:1156–1167

Domagala-Kulawik J (2008) Effects of cigarette smoke on the lung and systemic immunity. J Physiol Pharmacol 59(Suppl 6):19–34

Draijer C, Robbe P, Boorsma CE, Hylkema MN, Melgert BN (2013) Characterization of macrophage phenotypes in three murine models of house-dust-mite-induced asthma. Mediators Inflamm 2013:632049

Ezzati M, Kammen DM (2002) The health impacts of exposure to indoor air pollution from solid fuels in developing countries: knowledge, gaps, and data needs. Environ Health Perspect 110:1057–1068

Fairbairn AS, Reid DD (1958) Air pollution and other local factors in respiratory disease. Br J Prev Soc Med 12:94–103

Finkelstein R, Fraser RS, Ghezzo H, Cosio MG (1995) Alveolar inflammation and its relation to emphysema in smokers. Am J Respir Crit Care Med 152:1666–1672

Freeman CM, Han MK, Martinez FJ, Murray S, Liu LX, Chensue SW, Polak TJ, Sonstein J, Todt JC, Ames TM, Arenberg DA, Meldrum CA, Getty C, McCloskey L, Curtis JL (2010) Cytotoxic potential of lung CD8(+) T cells increases with chronic obstructive pulmonary disease severity and with in vitro stimulation by IL-18 or IL-15. J Immunol 184:6504–6513

Freeman CM, Martinez FJ, Han MK, Washko GR Jr, McCubbrey AL, Chensue SW, Arenberg DA, Meldrum CA, McCloskey L, Curtis JL (2013) Lung CD8+ T cells in COPD have increased expression of bacterial TLRs. Respir Res 14:13

Garcia-Rio F, Romero D, Lores V, Casitas R, Hernanz A, Galera R, Alvarez-Sala R, Torres I (2011) Dynamic hyperinflation, arterial blood oxygen, and airway oxidative stress in stable patients with COPD. Chest 140:961–969

Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, Henson PM (2003) By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. Cell 115:13–23

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J (2004) The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351:1057–1067

Gentile D, Howe-Adams J, Trecki J, Patel A, Angelini B, Skoner D (2004) Association between environmental tobacco smoke and diminished dendritic cell interleukin 10 production during infancy. Ann Allergy Asthma Immunol 92:433–437

Gilmour PS, Rahman I, Donaldson K, MacNee W (2003) Histone acetylation regulates epithelial IL-8 release mediated by oxidative stress from environmental particles. Am J Physiol Lung Cell Mol Physiol 284:L533–L540

Götschi T, Heinrich J, Sunyer J, Kunzli N (2008) Long-term effects of ambient air pollution on lung function: a review. Epidemiology 19:690–701

Goven D, Boutten A, Lecon-Malas V, Marchal-Somme J, Amara N, Crestani B, Fournier M, Leseche G, Soler P, Boczkowski J, Bonay M (2008) Altered Nrf2/Keap1-Bach1 equilibrium in pulmonary emphysema. Thorax 63:916–924

Grumelli S, Corry DB, Song LZ, Song L, Green L, Huh J, Hacken J, Espada R, Bag R, Lewis DE, Kheradmand F (2004) An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. PLoS Med 1:e8

Gualtieri M, Øvrevik J, Holme JA, Perrone MG, Bolzacchini E, Schwarze PE, Camatini M (2010) Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells. Toxicol In Vitro 24:29–39

Guillot L, Balloy V, McCormack FX, Golenbock DT, Chignard M, Si-Tahar M (2002) Cutting edge: the immunostimulatory activity of the lung surfactant protein-A involves Toll-like receptor 4. J Immunol 168:5989–5992

Haczku A (2006) Role and regulation of lung collectins in allergic airway sensitization. Pharmacol Ther 110:14–34

Haczku A (2008) Protective role of the lung collectins surfactant protein A and surfactant protein D in airway inflammation. J Allergy Clin Immunol 122:861–879, quiz 880–881

Haczku A (2012) The dendritic cell niche in chronic obstructive pulmonary disease. Respir Res 13:80

Halonen JI, Lanki T, Yli-Tuomi T, Kulmala M, Tiittanen P, Pekkanen J (2008) Urban air pollution, and asthma and COPD hospital emergency room visits. Thorax 63:635–641

Hansen S, Lo B, Evans K, Neophytou P, Holmskov U, Wright JR (2007) Surfactant protein D augments bacterial association but attenuates major histocompatibility complex class II presentation of bacterial antigens. Am J Respir Cell Mol Biol 36:94–102

Hawgood S, Ochs M, Jung A, Akiyama J, Allen L, Brown C, Edmondson J, Levitt S, Carlson E, Gillespie AM, Villar A, Epstein CJ, Poulain FR (2002) Sequential targeted deficiency of SP-A and -D leads to progressive alveolar lipoproteinosis and emphysema. Am J Physiol Lung Cell Mol Physiol 283:L1002–L1010

Hellermann GR, Nagy SB, Kong X, Lockey RF, Mohapatra SS (2002) Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. Respir Res 3:22

Hiura TS, Kaszubowski MP, Li N, Nel AE (1999) Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J Immunol 163:5582–5591

Hnizdo E, Sullivan PA, Bang KM, Wagner G (2002) Association between chronic obstructive pulmonary disease and employment by industry and occupation in the US population: a study of data from the Third National Health and Nutrition Examination Survey. Am J Epidemiol 156:738–746

Hodge SJ, Hodge GL, Reynolds PN, Scicchitano R, Holmes M (2003a) Increased production of TGF-beta and apoptosis of T lymphocytes isolated from peripheral blood in COPD. Am J Physiol Lung Cell Mol Physiol 285:L492–L499

Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M (2003b) Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. Immunol Cell Biol 81:289–296

Hodge S, Hodge G, Holmes M, Reynolds PN (2005) Increased airway epithelial and T-cell apoptosis in COPD remains despite smoking cessation. Eur Respir J 25:447–454

Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD (2004) The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 350:2645–2653

Hortobagyi L, Kierstein S, Krytska K, Zhu X, Das AM, Poulain F, Haczku A (2008) Surfactant protein D inhibits TNF-alpha production by macrophages and dendritic cells in mice. J Allergy Clin Immunol 122:521–528

Hunt A, Abraham JL, Judson B, Berry CL (2003) Toxicologic and epidemiologic clues from the characterization of the 1952 London smog fine particulate matter in archival autopsy lung tissues. Environ Health Perspect 111:1209–1214

Imai K, Mercer BA, Schulman LL, Sonett JR, D'Armiento JM (2005) Correlation of lung surface area to apoptosis and proliferation in human emphysema. Eur Respir J 25:250–258

Ishii Y, Itoh K, Morishima Y, Kimura T, Kiwamoto T, Iizuka T, Hegab AE, Hosoya T, Nomura A, Sakamoto T, Yamamoto M, Sekizawa K (2005) Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. J Immunol 175:6968–6975

Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, Barczyk A, Hayashi S, Adcock IM, Hogg JC, Barnes PJ (2005) Decreased histone deacetylase activity in chronic obstructive pulmonary disease. N Engl J Med 352:1967–1976

Jakab GJ, Spannhake EW, Canning BJ, Kleeberger SR, Gilmour MI (1995) The effects of ozone on immune function. Environ Health Perspect 103(Suppl 2):77–89

Jerrett M, Burnett RT, Pope CA 3rd, Ito K, Thurston G, Krewski D, Shi Y, Calle E, Thun M (2009) Long-term ozone exposure and mortality. N Engl J Med 360:1085–1095

Kan H, Heiss G, Rose KM, Whitsel E, Lurmann F, London SJ (2007) Traffic exposure and lung function in adults: the Atherosclerosis Risk in Communities study. Thorax 62:873–879

Kanazawa H, Yoshikawa J (2005) Elevated oxidative stress and reciprocal reduction of vascular endothelial growth factor levels with severity of COPD. Chest 128:3191–3197

Karle AC, Oostingh GJ, Mutschlechner S, Ferreira F, Lackner P, Bohle B, Fischer GF, Vogt AB, Duschl A (2012) Nitration of the pollen allergen bet v 1.0101 enhances the presentation of bet v 1-derived peptides by HLA-DR on human dendritic cells. PLoS One 7:e31483

Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, Waltenberger J, Voelkel NF (2000) Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. J Clin Invest 106:1311–1319

Katre A, Ballinger C, Akhter H, Fanucchi M, Kim DK, Postlethwait E, Liu RM (2011) Increased transforming growth factor beta 1 expression mediates ozone-induced airway fibrosis in mice. Inhal Toxicol 23:486–494

Katsouyanni K, Samet JM, Anderson HR, Atkinson R, Le Tertre A, Medina S, Samoli E, Touloumi G, Burnett RT, Krewski D, Ramsay T, Dominici F, Peng RD, Schwartz J, Zanobetti A, Committee HEIHR (2009) Air pollution and health: a European and North American approach (APHENA). Res Rep Health Eff Inst 142:5–90

Kelsen SG, Duan X, Ji R, Perez O, Liu C, Merali S (2008) Cigarette smoke induces an unfolded protein response in the human lung: a proteomic approach. Am J Respir Cell Mol Biol 38:541–550

Kierstein S, Poulain FR, Cao Y, Grous M, Mathias R, Kierstein G, Beers MF, Salmon M, Panettieri RA Jr, Haczku A (2006) Susceptibility to ozone-induced airway inflammation is associated with decreased levels of surfactant protein D. Respir Res 7:85

Kim H, Tse K, Levin L, Bernstein D, Reponen T, LeMasters G, Lummus Z, Horner AA (2012) House dust bioactivities predict skin prick test reactivity for children with high risk of allergy. J Allergy Clin Immunol 129:1529–1537.e1522

Ko FW, Hui DS (2012) Air pollution and chronic obstructive pulmonary disease. Respirology 17:395–401

Krewski D (2009) Evaluating the effects of ambient air pollution on life expectancy. N Engl J Med 360:413–415

Kunzli N, Bridevaux PO, Liu LJ, Garcia-Esteban R, Schindler C, Gerbase MW, Sunyer J, Keidel D, Rochat T (2009) Traffic-related air pollution correlates with adult-onset asthma among never-smokers. Thorax 64:664–670

Leigh JP, Romano PS, Schenker MB, Kreiss K (2002) Costs of occupational COPD and asthma. Chest 121:264–272

Leikauf GD, McDowell SA, Wesselkamper SC, Miller CR, Hardie WD, Gammon K, Biswas PP, Korfhagen TR, Bachurski CJ, Wiest JS, Willeke K, Bingham E, Leikauf JE, Aronow BJ, Prows DR (2001) Pathogenomic mechanisms for particulate matter induction of acute lung injury and inflammation in mice. Res Rep Health Eff Inst 105:5–58; discussion 59–71

Lepeule J, Bind MA, Baccarelli AA, Koutrakis P, Tarantini L, Litonjua A, Sparrow D, Vokonas P, Schwartz JD (2014) Epigenetic influences on associations between air pollutants and lung function in elderly men: the normative aging study. Environ Health Perspect

Lumb AB (2000) Nunn's applied respiratory physiology. Reed Educational and Professional Publishing Ltd., London

Lundborg M, Dahlen SE, Johard U, Gerde P, Jarstrand C, Camner P, Lastbom L (2006) Aggregates of ultrafine particles impair phagocytosis of microorganisms by human alveolar macrophages. Environ Res 100:197–204

Ma B, Kang MJ, Lee CG, Chapoval S, Liu W, Chen Q, Coyle AJ, Lora JM, Picarella D, Homer RJ, Elias JA (2005) Role of CCR5 in IFN-gamma-induced and cigarette smoke-induced emphysema. J Clin Invest 115:3460–3472

MacNee W, Donaldson K (2003) Mechanism of lung injury caused by PM10 and ultrafine particles with special reference to COPD. Eur Respir J 40 Suppl:47s–51s

Malhotra D, Thimmulappa R, Navas-Acien A, Sandford A, Elliott M, Singh A, Chen L, Zhuang X, Hogg J, Pare P, Tuder RM, Biswal S (2008) Decline in NRF2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1. Am J Respir Crit Care Med 178:592–604

Marian E, Baraldo S, Visentin A, Papi A, Saetta M, Fabbri LM, Maestrelli P (2006) Up-regulated membrane and nuclear leukotriene B4 receptors in COPD. Chest 129:1523–1530

Martinez FO, Sica A, Mantovani A, Locati M (2008) Macrophage activation and polarization. Front Biosci 13:453–461

Martinez FO, Helming L, Milde R, Varin A, Melgert BN, Draijer C, Thomas B, Fabbri M, Crawshaw A, Ho LP, Ten Hacken NH, Cobos Jimenez V, Kootstra NA, Hamann J, Greaves DR, Locati M, Mantovani A, Gordon S (2013) Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. Blood 121:e57–e69

Matozaki T, Murata Y, Okazawa H, Ohnishi H (2009) Functions and molecular mechanisms of the CD47-SIRPalpha signalling pathway. Trends Cell Biol 19:72–80

Miyata R, van Eeden SF (2011) The innate and adaptive immune response induced by alveolar macrophages exposed to ambient particulate matter. Toxicol Appl Pharmacol 257:209–226

Moran-Mendoza O, Perez-Padilla JR, Salazar-Flores M, Vazquez-Alfaro F (2008) Wood smoke-associated lung disease: a clinical, functional, radiological and pathological description. Int J Tuberc Lung Dis 12:1092–1098

Moreira AP, Hogaboam CM (2011) Macrophages in allergic asthma: fine-tuning their pro- and anti-inflammatory actions for disease resolution. J Interferon Cytokine Res 31:485–491

Myatt TA, Vincent MS, Kobzik L, Naeher LP, MacIntosh DL, Suh H (2011) Markers of inflammation in alveolar cells exposed to fine particulate matter from prescribed fires and urban air. J Occup Environ Med 53:1110–1114

Nozik-Grayck E, Suliman HB, Majka S, Albietz J, Van Rheen Z, Roush K, Stenmark KR (2008) Lung EC-SOD overexpression attenuates hypoxic induction of Egr-1 and chronic hypoxic pulmonary vascular remodeling. Am J Physiol Lung Cell Mol Physiol 295:L422–L430

Oberdorster G, Ferin J, Morrow PE (1992) Volumetric loading of alveolar macrophages (AM): a possible basis for diminished AM-mediated particle clearance. Exp Lung Res 18:87–104

Ohya M, Nishitani C, Sano H, Yamada C, Mitsuzawa H, Shimizu T, Saito T, Smith K, Crouch E, Kuroki Y (2006) Human pulmonary surfactant protein D binds the extracellular domains of Toll-like receptors 2 and 4 through the carbohydrate recognition domain by a mechanism different from its binding to phosphatidylinositol and lipopolysaccharide. Biochemistry 45:8657–8664

Øvrevik J, Låg M, Holme JA, Schwarze PE, Refsnes M (2009) Cytokine and chemokine expression patterns in lung epithelial cells exposed to components characteristic of particulate air pollution. Toxicology 259:46–53

Øvrevik J, Arlt VM, Øya E, Nagy E, Mollerup S, Phillips DH, Låg M, Holme JA (2010) Differential effects of nitro-PAHs and amino-PAHs on cytokine and chemokine responses in human bronchial epithelial BEAS-2B cells. Toxicol Appl Pharmacol 242:270–280

Papi A, Luppi F, Franco F, Fabbri LM (2006) Pathophysiology of exacerbations of chronic obstructive pulmonary disease. Proc Am Thorac Soc 3:245–251

Park EJ, Park K (2009) Induction of pro-inflammatory signals by 1-nitropyrene in cultured BEAS-2B cells. Toxicol Lett 184:126–133

Pastor MD, Nogal A, Molina-Pinelo S, Melendez R, Salinas A, Gonzalez De la Pena M, Martin-Juan J, Corral J, Garcia-Carbonero R, Carnero A, Paz-Ares L (2013) Identification of proteomic signatures associated with lung cancer and COPD. J Proteomics 89:227–237

Po JY, FitzGerald JM, Carlsten C (2011) Respiratory disease associated with solid biomass fuel exposure in rural women and children: systematic review and meta-analysis. Thorax 66:232–239

Pons AR, Sauleda J, Noguera A, Pons J, Barcelo B, Fuster A, Agusti AG (2005) Decreased macrophage release of TGF-beta and TIMP-1 in chronic obstructive pulmonary disease. Eur Respir J 26:60–66

Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM (1999) The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. Curr Biol 9:1441–1447

Rahman I (2005) Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. Cell Biochem Biophys 43:167–188

Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W, De Boer WI (2002) 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 166:490–495

Repine JE, Bast A, Lankhorst I (1997) Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am J Respir Crit Care Med 156:341–357

Ricciardolo FL, Caramori G, Ito K, Capelli A, Brun P, Abatangelo G, Papi A, Chung KF, Adcock I, Barnes PJ, Donner CF, Rossi A, Di Stefano A (2005) Nitrosative stress in the bronchial mucosa of severe chronic obstructive pulmonary disease. J Allergy Clin Immunol 116:1028–1035

Rosseau S, Guenther A, Seeger W, Lohmeyer J (1997) Phagocytosis of viable Candida albicans by alveolar macrophages: lack of opsonin function of surfactant protein A. J Infect Dis 175:421–428

Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, Fitzgerald M, Barnes PJ (2002a) Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases. Am J Physiol Lung Cell Mol Physiol 283:L867–L873

Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ (2002b) Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 26:602–609

Ryter SW, Nakahira K, Haspel JA, Choi AM (2012) Autophagy in pulmonary diseases. Annu Rev Physiol 74:377–401

Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, Cavallesco G, Tropeano G, Mapp CE, Maestrelli P, Ciaccia A, Fabbri LM (1999) CD8+ ve cells in the lungs of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160:711–717

Sakao S, Tatsumi K (2011) The importance of epigenetics in the development of chronic obstructive pulmonary disease. Respirology 16:1056–1063

Sano H, Chiba H, Iwaki D, Sohma H, Voelker DR, Kuroki Y (2000) Surfactant proteins A and D bind CD14 by different mechanisms. J Biol Chem 275:22442–22451

Scarpa MC, Baraldo S, Marian E, Turato G, Calabrese F, Saetta M, Maestrelli P (2013) Ceramide expression and cell homeostasis in chronic obstructive pulmonary disease. Respiration 85:342–349

Schierhorn K, Zhang M, Kacy M, Kunkel G (1997) Ozone-induced augmentation of eicosanoid metabolism in human nasal mucosa in vitro. Int Arch Allergy Immunol 113:312–315

Schikowski T, Mills IC, Anderson HR, Cohen A, Hansell A, Kauffmann F, Kramer U, Marcon A, Perez L, Sunyer J, Probst-Hensch N, Kunzli N (2014) Ambient air pollution – a cause for COPD? Eur Respir J 43(1):250–263

Schwarze PE, Øvrevik J, Låg M, Refsnes M, Nafstad P, Hetland RB, Dybing E (2006) Particulate matter properties and health effects: consistency of epidemiological and toxicological studies. Hum Exp Toxicol 25:559–579

Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M (2000) Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. Chest 117:684–694

Sezer H, Akkurt I, Guler N, Marakoglu K, Berk S (2006) A case–control study on the effect of exposure to different substances on the development of COPD. Ann Epidemiol 16:59–62

Shan M, Yuan X, Song LZ, Roberts L, Zarinkamar N, Seryshev A, Zhang Y, Hilsenbeck S, Chang SH, Dong C, Corry DB, Kheradmand F (2012) Cigarette smoke induction of osteopontin (SPP1) mediates T(H)17 inflammation in human and experimental emphysema. Sci Transl Med 4:117ra119

Sharma G, Goodwin J (2006) Effect of aging on respiratory system physiology and immunology. Clin Interv Aging 1:253–260

Sims MW, Tal-Singer RM, Kierstein S, Musani AI, Beers MF, Panettieri RA, Haczku A (2008) Chronic obstructive pulmonary disease and inhaled steroids alter surfactant protein D (SP-D) levels: a cross-sectional study. Respir Res 9:13

Sint T, Donohue JF, Ghio AJ (2008) Ambient air pollution particles and the acute exacerbation of chronic obstructive pulmonary disease. Inhal Toxicol 20:25–29

Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, Maestrelli P (1996) Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. Thorax 51:267–271

Steenhof M, Janssen NA, Strak M, Hoek G, Gosens I, Mudway IS, Kelly FJ, Harrison RM, Pieters RH, Cassee FR, Brunekreef B (2014) Air pollution exposure affects circulating white blood cell counts in healthy subjects: the role of particle composition, oxidative potential and gaseous pollutants – the RAPTES project. Inhal Toxicol 26:141–165

Stieb DM, Judek S, Burnett RT (2002) Meta-analysis of time-series studies of air pollution and mortality: effects of gases and particles and the influence of cause of death, age, and season. J Air Waste Manage Assoc 52:470–484

Sundar IK, Yao H, Rahman I (2013) Oxidative stress and chromatin remodeling in chronic obstructive pulmonary disease and smoking-related diseases. Antioxid Redox Signal 18:1956–1971

Sunil VR, Patel-Vayas K, Shen J, Laskin JD, Laskin DL (2012) Classical and alternative macrophage activation in the lung following ozone-induced oxidative stress. Toxicol Appl Pharmacol 263:195–202

Sunyer J (2001) Urban air pollution and chronic obstructive pulmonary disease: a review. Eur Respir J 17:1024–1033

Sussan TE, Ingole V, Kim JH, McCormick S, Negherbon J, Fallica J, Akulian J, Yarmus L, Feller-Kopman D, Wills-Karp M, Horton MR, Breysse PN, Agrawal A, Juvekar S, Salvi S, Biswal S (2014) Source of biomass cooking fuel determines pulmonary response to household air pollution. Am J Respir Cell Mol Biol 50:538–548

Takeda K, Haczku A, Lee JJ, Irvin CG, Gelfand EW (2001) Strain dependence of airway hyperresponsiveness reflects differences in eosinophil localization in the lung. Am J Physiol Lung Cell Mol Physiol 281:L394–L402

Tao F, Gonzalez-Flecha B, Kobzik L (2003) Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic Biol Med 35:327–340

Tomita K, Caramori G, Lim S, Ito K, Hanazawa T, Oates T, Chiselita I, Jazrawi E, Chung KF, Barnes PJ, Adcock IM (2002) Increased p21(CIP1/WAF1) and B cell lymphoma leukemia-x(L) expression and reduced apoptosis in alveolar macrophages from smokers. Am J Respir Crit Care Med 166:724–731

Torres-Duque C, Maldonado D, Perez-Padilla R, Ezzati M, Viegi G, E. Forum of International Respiratory Studies Task Force on Health Effects of Biomass (2008) Biomass fuels and respiratory diseases: a review of the evidence. Proc Am Thorac Soc 5:577–590

Triantaphyllopoulos K, Hussain F, Pinart M, Zhang M, Li F, Adcock I, Kirkham P, Zhu J, Chung KF (2011) A model of chronic inflammation and pulmonary emphysema after multiple ozone exposures in mice. Am J Physiol Lung Cell Mol Physiol 300:L691–L700

Tsoumakidou M, Siafakas NM (2006) Novel insights into the aetiology and pathophysiology of increased airway inflammation during COPD exacerbations. Respir Res 7:80

van Eeden SF, Yeung A, Quinlam K, Hogg JC (2005) Systemic response to ambient particulate matter: relevance to chronic obstructive pulmonary disease. Proc Am Thorac Soc 2:61–67
Vandivier RW, Fadok VA, Hoffmann PR, Bratton DL, Penvari C, Brown KK, Brain JD, Accurso FJ, Henson PM (2002) Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. J Clin Invest 109:661–670
Wegmann M, Fehrenbach A, Heimann S, Fehrenbach H, Renz H, Garn H, Herz U (2005) NO2-induced airway inflammation is associated with progressive airflow limitation and development of emphysema-like lesions in C57bl/6 mice. Exp Toxicol Pathol 56:341–350
White AJ, Gompertz S, Stockley RA (2003) Chronic obstructive pulmonary disease. 6: The aetiology of exacerbations of chronic obstructive pulmonary disease. Thorax 58:73–80
Williams MA, Rangasamy T, Bauer SM, Killedar S, Karp M, Kensler TW, Yamamoto M, Breysse P, Biswal S, Georas SN (2008) Disruption of the transcription factor Nrf2 promotes pro-oxidative dendritic cells that stimulate Th2-like immunoresponsiveness upon activation by ambient particulate matter. J Immunol 181:4545–4559
Wright JR (2005) Immunoregulatory functions of surfactant proteins. Nat Rev Immunol 5:58–68
Wu Z, Liu MC, Liang M, Fu J (2012) Sirt1 protects against thrombomodulin down-regulation and lung coagulation after particulate matter exposure. Blood 119:2422–2429
Yamazaki H, Hatanaka N, Kizu R, Hayakawa K, Shimada N, Guengerich FP, Nakajima M, Yokoi T (2000) Bioactivation of diesel exhaust particle extracts and their major nitrated polycyclic aromatic hydrocarbon components, 1-nitropyrene and dinitropyrenes, by human cytochromes P450 1A1, 1A2, and 1B1. Mutat Res 472:129–138
Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C (2012) Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. Allergy 67:1193–1202
Yao H, Rahman I (2011) Current concepts on oxidative/carbonyl stress, inflammation and epigenetics in pathogenesis of chronic obstructive pulmonary disease. Toxicol Appl Pharmacol 254:72–85
Yao H, Hwang JW, Sundar IK, Friedman AE, McBurney MW, Guarente L, Gu W, Kinnula VL, Rahman I (2013) SIRT1 redresses the imbalance of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-9 in the development of mouse emphysema and human COPD. Am J Physiol Lung Cell Mol Physiol 305:L615–L624
Yoshida T, Yoshioka Y, Fujimura M, Yamashita K, Higashisaka K, Nakanishi R, Morishita Y, Kayamuro H, Nabeshi H, Nagano K, Abe Y, Kamada H, Tsunoda S, Yoshikawa T, Itoh N, Tsutsumi Y (2010) Potential adjuvant effect of intranasal urban aerosols in mice through induction of dendritic cell maturation. Toxicol Lett 199:383–388
Zanobetti A, Bind MA, Schwartz J (2008) Particulate air pollution and survival in a COPD cohort. Environ Health 7:48
Zhou H, Wu Y, Jin Y, Zhou J, Zhang C, Che L, Jing J, Chen Z, Li W, Shen H (2013) Genetic polymorphism of matrix metalloproteinase family and chronic obstructive pulmonary disease susceptibility: a meta-analysis. Sci Rep 3:2818

Chapter 6
Airborne Carcinogens: Mechanisms of Cancer

Anuradha Mudipalli

6.1 Introduction

Both indoor and outdoor air pollution is a well-recognized contributory factor to the global health burden and accounts for approximately two million mortalities per year worldwide (World Health Organization (WHO) News release 2011). The exhaust emissions from automobiles, industrial vents, fossil fuel combustion, volatile organic compounds and environmental tobacco smoke (ETS) contribute to outdoor air pollution. The emissions from biomass-burning cooking stoves, radon, and tobacco smoke constitute the majority of indoor air pollution. Scientific evidence both from epidemiology and animal experimental studies over the past few decades has consistently linked exposure to air pollution, especially, the respirable particles to a wide range of health effects such as cardio-respiratory diseases, asthma and chronic obstructive pulmonary disease (COPD) (Narot et al. 2011; Shah et al. 2013; Schluger and Koppaka 2014; Ferkol and Schraufnagel 2014). Children, elderly and people with predisposed health conditions such as asthma are demonstrated to be especially vulnerable to air pollution-induced adverse health effects (Schluger and Koppaka 2014; Ferkol and Schraufnagel 2014; Zar and Ferkol 2014; Esposito et al. 2014).

Air pollution exposure related health effects aroused concern for a long time. The first large epidemiological cohort study of the American Cancer Society by Pope et al. (2002) observed association between ambient fine particulate matter and lung cancer and cardiopulmonary mortality. Current research points out to a general

A. Mudipalli, Ph.D. (✉)
National Center for Environmental Assessment, Office of Research and Development, US EPA, Research Triangle Park, NC, USA
e-mail: Mudipalli.anu@epa.gov

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_6

positive association between air pollution exposure and lung cancer etiology (Demetriou et al. 2012). Recently, the International Agency for Research on Cancer (IARC) has designated outdoor air pollution as a whole to be carcinogenic (IARC 2014).

A detailed characterization of the carcinogenic/mutagenic properties of air pollution constituents is beyond the scope of this chapter. Readers are referred to two of the recent reviews which provided an exhaustive review of literature on the genotoxicity of ambient air using in vitro assay systems (Claxton et al. 2004) and mutagenicity and rodent carcinogenicity of ambient air (Claxton and Woodall 2007). In brief, these reviews concluded that smaller particles (≤PM 2.5) contain higher percentage of extractable organic materials which were found to be more mutagenic compared to larger particles (>PM 2.5). In addition the carcinogenic activity of air was primarily attributed to its component constituents, such as polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds, whose levels may be subjected to change due to variations in atmospheric conditions (Claxton and Woodall 2007). A critical analysis of global epidemiological studies related to air pollution and air borne carcinogens is discussed elsewhere in this book (Chap. 7). The readers are also referred to Chap. 12 where genetic factors influencing disease outcomes related to air pollution exposure are outlined and to Chap. 13 where carcinogenic potential of air pollutants resulting from the recent advancements in the combustion technology, use of biofuels and engineered nanomaterial (both as additives and catalytic converters) are detailed. In addition, Chap. 13 also discusses the in vitro and in vivo studies pertaining to the carcinogenicity of emerging air pollutants.

Chemical carcinogens are believed to either initiate and/or promote carcinogenesis by a diverse number of mechanisms such as DNA adducts and DNA protein cross links formation, epigenetic alterations, regenerative cell proliferation, oxidative stress, inhibition of DNA repair and altered cell cycle regulation. Given the scope, the present chapter will attempt to provide readers with the current state of understanding on the carcinogenic mechanisms for a select set of chemicals, namely formaldehyde and benzene, and two representative mixtures – PAHs, and ETS (Environmental Tobacco Smoke), for which more detailed and significant mechanistic understanding of the carcinogenic process had been obtained over the past decade.

6.2 Formaldehyde

Formaldehyde was first described in 1855 and is ranked as one of the top 25 highest volume chemicals produced in the U.S (National Toxicology Program (NTP) 2002; Agency for Toxic Substances and Disease Registry (ATSDR) 1999; International Agency for Research on Cancer (IARC) 2006) for the past 21 years. Worldwide production of formaldehyde was estimated to be ~21.0 metric tons in the year 2000 (International Agency for Research on Cancer (IARC) 2006). The saturated aqueous solution of formaldehyde called as formalin, (37 % by weight or 40 % by

volume), stabilized with 10–15 % methanol, is used in consumer products such as laundry detergent and several general purpose cleaners, as tissue preservative and as preservative in food and cosmetic products (National Toxicology Program (NTP) 2011).

In the ambient air, major sources of exposure to formaldehyde are from anthropogenic emissions, primarily from motor vehicles, power plants, and plants that produce or use formaldehyde or substances such as adhesives and coatings which release formaldehyde from curing (Agency for Toxic Substances and Disease Registry (ATSDR) 1999; International Agency for Research on Cancer (IARC) 1995). Additional sources of emission are incineration, wood burning, tobacco smoke and cooking operations (Agency for Toxic Substances and Disease Registry (ATSDR) 1999; Salthammer et al. 2010; Li et al. 1994; Health and Environment Canada 2012). In addition, endogenous formaldehyde is produced in the body through normal cellular metabolism through enzymatic or nonenzymatic reactions, and also as a detoxification product of xenobiotics during cellular metabolism (National Toxicology Program (NTP) 2011)

Population exposure to formaldehyde can occur via inhalation, ingestion and dermal routes. Ambient levels of formaldehyde in outdoor air are often significantly lower than those measured in the indoor air of workplaces or residences (National Toxicology Program (NTP) 2011; Salthammer et al. 2010). Improved manufacturing processes and construction practices have contributed to reduction in the levels of indoor formaldehyde emissions since the early to mid-1980s, yet, indoor levels of exposure of the population to formaldehyde remains a level which is still of concern to public health (National Toxicology Program (NTP) 2011).

6.2.1 Absorption, Distribution, Metabolism and Excretion (ADME)

A majority of the daily intake of formaldehyde results from inhalation exposure. Animal studies using labeled formaldehyde indicated differential ADME kinetics from oral, dermal and inhalation routes of exposure (Buss et al. 1964; Heck et al. 1983). In dogs, a majority of inhaled formaldehyde was found to be absorbed in the upper respiratory tract (Egle 1972), but deposition differed due to variability of scrubbing behavior across species (Kimbell et al. 2001). The computational modeling efforts for realistic representation of the human nasal airways from a single individual have demonstrated that approximately 90 % of inhaled formaldehyde was predicted to be absorbed in the nose at resting inspiration. This fraction decreased to about 70 % at light exercise and to 58 % at heavy exercise conditions (Kimbell et al. 2001).

Free formaldehyde exists in equilibrium (The ratio of methanediol to free formaldehyde 99.1–0.1 %) with its hydrated form, methanediol (CH_2OH_2) ($Kd = 5.5 \times 10^{-4}$) at physiological temperature and pH in the body (Fox et al. 1985). When free formaldehyde is removed from aqueous solution through covalent binding to small

peptides or cellular components (Metz et al. 2004), the equilibrium is reestablished by dehydration of a fraction of methanediol to free formaldehyde for maintaining the ratio. Thus, a small pool of free formaldehyde can potentially be sustained in biological systems due to reversible nature of this hydration reaction (Fox et al. 1985).

Glutathione (GSH)-dependent formaldehyde dehydrogenase (FALDH) and mitochondrial aldehyde dehydrogenase 2 (ALDH2) are the primary enzymes that metabolize formaldehyde. Numerous studies now recognize FALDH as a member of the alcohol dehydrogenase (ADH) family, specifically ADH3 (Thompson et al. 2009; Hedberg et al. 2003; Deltour et al. 1999). Essentially, two principle pathways operate in the metabolism of formaldehyde to formate by oxidation. This involves mitochondrial ALDH2 (minor pathway) and a two-enzyme systems that involves ADH3 (major pathway) that converts glutathione-conjugated formaldehyde or S-hydroxymethyl glutathione (HMGSH) to the intermediate S-formylglutathione and subsequently metabolized to formate and GSH by S-formylglutathione hydrolase. The pharmacokinetics of formate involves: (A) formate undergoing adenosine triphosphate (ATP)-dependent addition to tetrahydrofolate (THF), by carrying either one or two; one-carbon groups. (B) Formate conjugating with THF to form N10-formyl-THF and its isomer N5-formyl-THF, both of which getting converted to N5-, N10-methenyl-THF, which undergo further metabolism. Decreased metabolisms of formaldehyde in ADH-knockout mouse studies have demonstrated that deletion of ADH3 increases the sensitivity of mice to formaldehyde (Deltour et al. 1999). Formate, the main product of formaldehyde clearance is further metabolized to CO_2 and water, or incorporated into the one-carbon pool, or eliminated in the urine (National Toxicology Program (NTP) 2011).

6.2.2 Carcinogenic Effects of Formaldehyde

The International Agency for Research on Cancer (IARC) categorized formaldehyde as "known" human carcinogen based on the conclusion that nasopharyngeal cancer (NPC) was etiologically associated with formaldehyde exposure (International Agency for Research on Cancer (IARC) 2006) and that it is also a "known cause" for leukemia (International Agency for Research on Cancer (IARC) 2009). This classification is based on NCI cohort data (Beane Freeman et al. 2009), occupational exposure data on embalmers (Hauptmann et al. 2009) and studies in myeloid progenitor cells of exposed workers (Zhang et al. 2010) and meta-analysis of the epidemiology data by Zhang et al. (Zhang et al. 2009a). The conclusions of these assessments and studies are part of an on-going scientific debate which is beyond the scope of this chapter. The focus in this chapter is on a critical analysis of data from animal studies that indicate potential mechanisms of carcinogenesis.

Long-term inhalational exposure studies in diverse rat and mouse strains clearly demonstrated formaldehyde-induced tumors in nasal tissues: primarily squamous

cell carcinomas, papillomas, polyploid adenoma, adenocarcinoma and fibro sarcoma (Sellakumar et al. 1985; Morgan et al. 1986; Monticello et al. 1996; Kamata et al. 1997; Soffritti et al. 1989; Soffritti et al. 2002; National Toxicology Program (NTP) 2009).

6.2.3 Carcinogenic Mechanisms

The available experimental evidence from in vitro and limited in vivo studies suggest that several mechanisms such as induction of mutations at the site of entry, decreased DNA repair function and enhanced cell proliferation are possibly involved in the formaldehyde-induced carcinogenesis process. What is known at present about how these mechanisms contribute individually or together in the development of cancer is reviewed in the following paragraphs.

6.2.3.1 Mutations

Studies have demonstrated formaldehyde-induced DNA mutations under different experimental conditions. (Snyder and Van Houten 1986; Dillon et al. 1998; Speit and Merk 2002). These mutations may occur during repair of DNA adducts, DNA-protein cross links (DPXs), and as single strand breaks (SSBs) formed through replication errors during mitosis. Investigations using diverse bacterial mutagenicity assays under varied experimental conditions such as reverse and forward mutation assays as well as use of specific strains for detecting deletions, insertions point mutations either with or without metabolic activation have clearly demonstrated formaldehyde-induced mutations (Liber et al. 1989; Speit et al. 2000; Dillon et al. 1998; Speit and Merk 2002). Formaldehyde has also been shown to induce primary DNA damage in E. coli in the form of excisions which are repairable via the R-factor plasmid to mediate the repair process. Studies in several bacterial strains indicated that AT base pairs are the primary reversion sites for the induction of mutations by formaldehyde (Dillon et al. 1998).

Studies investigating the mutagenic potential of formaldehyde in the mammalian cell culture system suggested that covalent alteration of DNA bases was not responsive to excision repair mechanisms (Liber et al. 1989; Speit et al. 2000). One study using the hypoxanthine phosphoribosyl transferase locus with high concentrations of formaldehyde reported point mutations showing a preferential AT to CG transversions (Liber et al. 1989). Speit et al. (2000) observed increased susceptibility of the repair-deficient cell lines to formaldehyde-induced micronuclei (MN) induction and proposed that more than one repair pathway may be involved in the repair of crosslinks. Also, observations in the L5178Y cell mouse lymphoma system suggest that exposure of formaldehyde in workers resulted in increased mutant P53 protein in peripheral blood lymphocytes indicating potential compromised function of p53.

6.2.3.2 Cell Proliferation

In vitro studies suggest that formaldehyde may induce cell proliferation at concentrations well below those causing cytotoxicity (Tyihák et al. 2001; Reuzel et al. 1990). Induction of this proliferation capability may be responsible for the transient cell proliferation observed in rat nasal passages and trachea following formaldehyde exposure (Ura et al. 1989; Nelson et al. 1986). Acute or short-term exposure studies in rodents indicate that the observed cell proliferation is dose-and time-dependent and varies by species and location of exposure in the nose (Cassee and Feron 1994; Monticello et al. 1990, 1991). The increased staining for proliferating cell nuclear antigen observed in respiratory epithelium in formaldehyde exposed rats may be indicative of nasal hyperplasia and squamous metaplasia (Monticello et al. 1996). Taken together accumulated scientific evidence suggests genotoxic, mutagenic and possibly cell proliferation (portal of entry) mechanisms to contribute in the development of carcinogenic effects.

6.2.3.3 Genotoxicity Mechanisms

A wide range of experimental studies, from cell-free systems to single cells and in vivo animal exposures, suggest that formaldehyde directly reacts with DNA. Initial studies using cell free systems demonstrated that direct interaction of formaldehyde with DNA results in multiple forms of DNA adducts: N^6-hydroxymethyldeoxyadenosine, N^4-hydroxymethyldeoxycytidine, and N^2-hydroxymethyldeoxyguanosine as detected by high performance liquid chromatography (Beland et al. 1984; Kennedy et al. 1996; Cheng et al. 2003). The observation of aforementioned adducts in human nasal epithelial cells in vitro (Zhong and Que Hee 2004) further confirms the direct interaction of formaldehyde with DNA and DNA adduct formation. In vivo studies using labeled formaldehyde by a dual-isotope ($^3H/^{14}C$) method found that formaldehyde binds to DNA and protein resulting in DPXs and GSH depletion in the nasal mucosa of F344 rats. In addition, it also leads to oxidative stress by GSH depletion (Casanova and Heck 1987).

In vitro studies using cytotoxic concentrations of formaldehyde report that formaldehyde can induce clastogenic effects – increased MN, chromosomal aberrations, and sister chromatid exchanges (Miyachi and Tsutsui 2005; Hikiba et al. 2005). Similar observation from studies that used whole blood cultures exposed to cytotoxic concentrations (200 μM) of formaldehyde (Schmid and Speit 2007) suggest that clastogenic effects may also be implicated in the formaldehyde-induced carcinogenic process.

The initial observation of formaldehyde-induced DNA-histone cross links in isolated chromatin samples (Ohba et al. 1979) led to several in vitro studies demonstrating induction of DNA-protein crosslinks (DPX) by formaldehyde in bacteria (Wilkins and Macleod 1976), yeast (Magana-Schwencke and Ekert 1978) and

mammalian cells, including diverse animal and human cells (Environment Canada 2012). In addition to DPXs, formaldehyde at higher concentrations was also found to induce SSBs, DNA-DNA cross links (DDXs) and scheduled and unscheduled DNA synthesis (UDS) in multiple mammalian cell culture systems (Speit et al. 2000; Shaham et al. 2003; Li et al. 2004; Bermudez and Allen 1984; Bermudez et al. 1989; McQueen et al. 1989). Although the in vivo experimental data points out that DPX occur at lower concentration compared to DDX, a single in vitro experiment in buccal cells reported formaldehyde induced DNA strand breaks at low concentration, and DDXs and DPXs at higher concentrations (Li et al. 2004). UDS, which represents DNA repair activity following excision of DNA damage, was reported in formaldehyde exposed nasal epithelial cells of F344 rats (Bermudez and Allen 1984), rat hepatocytes (Bermudez et al. 1989; McQueen et al. 1989) Syrian hamster embryo cells (Hamaguchi and Tsutsui 2000) and in HeLa cells (Martin et al. 1978); however, UDS was not found in human peripheral blood cells (Doolittle et al. 1985; Grafstrom et al. 1984). DNA repair inhibition was reported in human bronchial epithelial cells, skin fibroblasts or keratinocytes exposed to formaldehyde (Grafstrom et al. 1984; Emri et al. 2004). Additional studies that utilized DNA repair proficient and deficient cell lines (Liteplo and Meek 2003) observed inhibition of DNA repair at a concentration range of 0.125 mM to 10 mM. In vitro studies to understand the potential confounding interactions between formaldehyde and UV irradiation in human keratinocytes indicated DNA repair inhibition by UVB, UVC, but not UVA. The incomplete repair of irradiation-induced DNA damage in the cells that were exposed to formaldehyde post irradiation suggests that formaldehyde may enhances UV-induced carcinogenesis (Emri et al. 2004).

The experimental evidence presented above suggest that formaldehyde-induced carcinogenic effects to be mediated by multiple pathways that appear to be usually initiated by a genotoxic mechanism and associated downstream events. Though a majority of the studies in laboratory animal models were carried out in high exposure doses, they do provide biological plausibility for the multiple types of cancers associated with exposure to formaldehyde.

6.3 Benzene

Benzene, also known as benzol, is a colorless liquid with a sweet odor. Benzene evaporates into air very quickly and dissolves slightly in water. Benzene is highly flammable and ranks in the top 20 for high production volume chemicals in the United States (Agency for Toxicological Substances Disease Registry (ATSDR) 2007). It is an industrial solvent with wide use in the production of resins, synthetic fibers, polymers and in industries such as printing, shoe making, chemical manufacturing, coal-based coke production, and as a component in gasoline (Galbraith et al. 2010). Early studies in 1800 to 1900s reported adverse health effects from occupational exposure to benzene, causing significant reduction of blood elements

associated with exposure in a dose and duration dependent manner (Hamilton 1931). The first comprehensive review on the acute and chronic benzene poisoning was associated with incidence of leukemia (Mallory et al. 1939).

Occupational exposure is the major route of high level human exposure to benzene; however, gasoline, cigarette smoke, gas emissions from volcanoes and forest fires also contribute to environmental benzene exposure. In general, the concentrations of benzene in ambient air have decreased steadily since 1900s and the use in consumer products has fallen off significantly (Galbraith et al. 2010; Williams et al. 2008). However, human exposure to benzene still continues to occur from ambient air due to burning of crude fossil oils, gasoline, and environmental cigarette smoke. The general population is exposed to benzene mainly through inhalation of contaminated air, particularly in areas of heavy traffic, around gas stations, and through inhalation of tobacco smoke from both active and passive smoking. It is reported that smokers have higher body burden (6–10 times) of benzene compared to non-smokers (Wallace 1996). People living in cities or industrial areas are generally exposed to higher levels of benzene in air than those living in rural areas. Measured levels of benzene in U.S in outdoor air have ranged from 0.02 to 34 ppb. Benzene levels in the home are usually higher than outdoor levels primarily because of tobacco smoke or materials stored in garages. As the average level of benzene is very low (0.1 ppb) in drinking water in the US, exposure to benzene through food, beverages, or drinking water is not a major concern, while exposure by inhalation is a major concern. Benzene exposure worldwide is of a significant concern as there are no regulatory standards for permissible exposure concentrations in gasoline in many countries.

6.3.1 Absorption, Distribution, Metabolism and Excretion (ADME)

Ingestion, inhalation and dermal routes are major routes of benzene exposure. About half of the benzene exposed by inhalation or ingestions routes was found to pass through the epithelial lining of lung and intestine and enter the blood stream. Once in the blood stream, benzene traverses throughout the body and is temporarily stored in adipose tissue and bone marrow (Agency for Toxicological Substances Disease Registry (ATSDR) 2007). Pathways of benzene metabolism are largely similar between rodents and primates. However significant differences exist in the type and quantity of the metabolites produced; indicating the species specific differences in metabolism. Primary metabolism of benzene occurs in liver and lung with secondary metabolism occurring in bone marrow.

Principally, the initial step involves cytochrome P-450 mediated oxidation of benzene to benzene oxide catalyzed by Cyp2E1. Benzene oxide, by and large, exists in equilibrium with its tautomer, oxepin, and can also spontaneously rearrange to phenol (PH), one of the intermediary metabolic products. Benzene oxide can also

be hydrolyzed to catechol 1, 2 benzoquinone via benzenedihydrol, which reacts with cellular glutathione to produce S-phenyl mercapturic acid. Another toxic metabolite, hydroquinone is converted to highly reactive 1, 2, 4-benzene triol by CYP2E1. Oxepin on the other hand, also can open up its aromatic ring, yielding highly reactive muconaldehydes and E-E muconic acid. The electrophilic benzene oxide, muconaldehydes and benzoquinones react with cellular components such as proteins and interfere with normal cellular processes. Muconaldehyde is a known hematotoxicant in mice. Peroxidases also play a major role in the oxidation of benzene to produce phenolic metabolites such as quinones and semiquinones, which can bind to macromolecules and generate oxygen radicals. Benzoquinone, the major carcinogenic metabolite of benzene, is produced by catalytic activation of hydroquinone in bone marrow by myeloperoxidase (Smith et al. 1989). Recent studies demonstrate CYP2E1 and CYP2A13-, as major metabolizing enzymes, active at concentrations <1 ppm in lung, indicating that lung may be primary active site of metabolism at low exposure levels (Rappaport et al. 2009).

6.3.2 Carcinogenic Effects of Benzene

Benzene exposure, mostly occupational has been associated with various types of lympho hematopoietic cancers – acute non-lymphocytic leukemia/myelogenous leukemia and chronic myelogenous leukemia, non Hodkins lymphoma, multiple myeloma, neoplasms of kidney and cancers of lung. However, there is a large degree of variation in the association reported in many of these epidemiological studies due to multiple factors such as study design, exposure duration and dose, presence of confounders, cohort size and statistical methods employed (Galbraith et al. 2010; International Agency for Cancer Research (IARC) 2010; Sorahan 2011; Snyder 2012).

A few epidemiological studies demonstrated associations between unmetabolized benzene as a potential biomarker for exposure to benzene in gasoline attendants, urban policemen and bus drivers (Farmer et al. 2005; Fustinoni et al. 2005; Waidyanatha et al. 2001). Urinary phenol measurements have been used for monitoring occupational exposure to benzene (Smith et al. 1989), trans-, trans-muconic acid and S-phenylmercapturic acid levels have also been found to be correlated with occupational exposure to benzene (Inoue et al. 1989, 2000; Ruppert et al. 1997; Qu et al. 2005; Bechtold and Henderson 1993; Bechtold et al. 1992a). Additionally, hemoglobin and albumin adducts of the benzene metabolites, benzene oxide and 1, 4-benzoquinone, have been used as biomarkers of exposure to benzene (Bechtold et al. 1992b; Smith and Rothman 2000; Yeowell-O'Connell et al. 1998, 2001). However, 'muconic acid' and 'phenyl mercapturic acid' 'have often shown correlation with environmental benzene exposure' (Inoue et al. 1989, 2000; Ruppert et al. 1997).

6.3.3 Carcinogenic Mechanisms

Studies in laboratory animals (rats and mice) of both sexes either by oral or inhalational routes of exposure to benzene have been found to induce cancers such as Zymbal gland carcinomas of oral cavity, fore stomach lesions and liver adenomas in female rats (Maltoni et al. 1982, 1983, 1985, 1989), lung adenomas, squamous cell carcinomas of the Preputial and Zymbal glands in male mice (Snyder et al. 1988; Farris et al. 1993). In both sexes of mice, increased incidence of lympho- and hematopoietic cancers were observed (Snyder et al. 1980; Cronkite et al. 1984). In mice exposed by the oral route, papillomas and carcinomas of the oral cavity, skin of the lip, and papillomas of the palate were reported (National Toxicology Program (NTP) 1989). Two year cancer bioassay studies carried out by NTP also observed an increased incidence of hepato-carcinomas, pheochromocytomas, harderian gland adenomas and preputial gland squamous cell carcinomas in male mice (National Toxicology Program (NTP) toxicology and carcinogenesis studies of benzene 1989).

Several in vitro studies have demonstrated that oxy radicals generated in the metabolism of benzene induce gene mutations, DNA strand breaks and homologous recombination (Mullin et al. 1995; Rothman et al. 1995; Win 2003). In vitro and limited in vivo studies using the benzene metabolites, hydroquinone, phenol and 1, 2, 4-benzene triol observed increased levels of 8-OH-2′-deoxy guanosine (8-OH-dG) adducts (Mullin et al. 1995; Win 2003; Shen et al. 2006). Studies from several human cell lines as well as yeast also demonstrate that benzene metabolites are capable of initiating genetic recombination and defective DNA damage repair resulting in chromosomal aneuploidy (Mullin et al. 1995; Kolachana et al. 1993; Luo et al. 2008; Andreoli et al. 2012; Gowans et al. 2005; Sheltzer et al. 2011; Solomon et al. 2011). The genomic instability induced by hydroquinone was found to be similar to that resulting from ionizing radiation in the bone marrow of susceptible mice (Gowans et al. 2005).

The RecQ family of helicase genes, which play a role in the maintenance of genomic stability, are also implicated with Werner syndrome (WS), a rare autosomal premature aging syndrome associated with a predisposition to cancer (Oshima 2000). The gene coding for Werner syndrome WRN(a RecQ helicase family gene) also plays a role in sensing oxidative DNA damage caused by oxy radicals. In studies using RNA interference approach to silence endogenous WRN in HeLa cells, subsequently exposed to hydroxy quinone, a benzene metabolite an increase in the amount of DNA double-strand breaks and an elevated DNA damage was demonstrated (Ren et al. 2009). Lan et al. (2009) observed a significant association between single nucleotide polymorphisms in WRN and other genes involved in DNA repair, genomic stability and increased incidence of hematotoxicity in workers exposed to benzene. This supports the role for DNA recombination pathways in benzene carcinogenesis.

Results from human cell culture studies on cells obtained from individuals exposed to high levels of benzene and its metabolites observed chromosomal abnormalities similar to chromosomal changes observed in acute myeloid leukemia. Investigations that used conventional classic cytogenetic analysis as well as modern techniques such as chromosome wide aneuploidy have also unequivocally established benzene as a clastogen capable of inducing aneuploidy (Zhang et al. 1998). Recent pilot study that utilized in situ hybridization for chromosome wide aneuploidy analysis on peripheral lymphocytes of benzene exposed subjects demonstrated both monosomy on Chromosomes 5–7 and 10 and trisomy on Chromosomes 5–8, 10, 14, 16 and 21–22. When this study was extended to larger sample size, these increases were found to be dose-dependent and statistically significant (Zhang et al. 2005, 2011).

Several DNA repair mechanisms such as base excision, double strand repair and nucleotide excision repair continuously monitor and fix DNA adducts and apurinic sites (Ishihama et al. 2008). In vitro studies where cells are exposed to benzene metabolites have demonstrated an increased expression and nuclear translocation of the catalytic sub unit of DNA dependent protein kinase c, which regulates the non-homologous end joining in double strand DNA repair (Bi et al. 2010). This increased expression of DNA repair enzymes observed in the hematopoietic stem cells treated with benzene metabolites has been implicated in cell survival, transformation and proliferation of leukemia phenotype (Alexander and Wagner 2010).

Recent findings from the studies in patients with chronic benzene exposure observed reduced activity of Topo isomerase II (Topo II) expression due to altered histone acetylation and methylation of Topoi-alpha promoter region (Yu et al. 2011). These findings provide supportive experimental evidence for interactions of benzene metabolites with Topo II-alpha inhibition elucidated in quantitative structure activity relationship modeling studies (Eastmond et al. 2001). Further Xing et al. (2010) also reported reduced expression of P15 and 16 through DNA methylation in gas station workers exposed to low levels of benzene indicating epigenetic effects.

In summary, the experimental evidence accumulated to date suggests that benzene-induced carcinogenic effects may begin by oxy radicals generated from metabolites of benzene, which in turn elicit DNA damage and a cascade of events associated with this process. The multiple mechanisms as published and suggested to be involved in benzene-induced toxicity and cancer are depicted in Fig. 6.1.

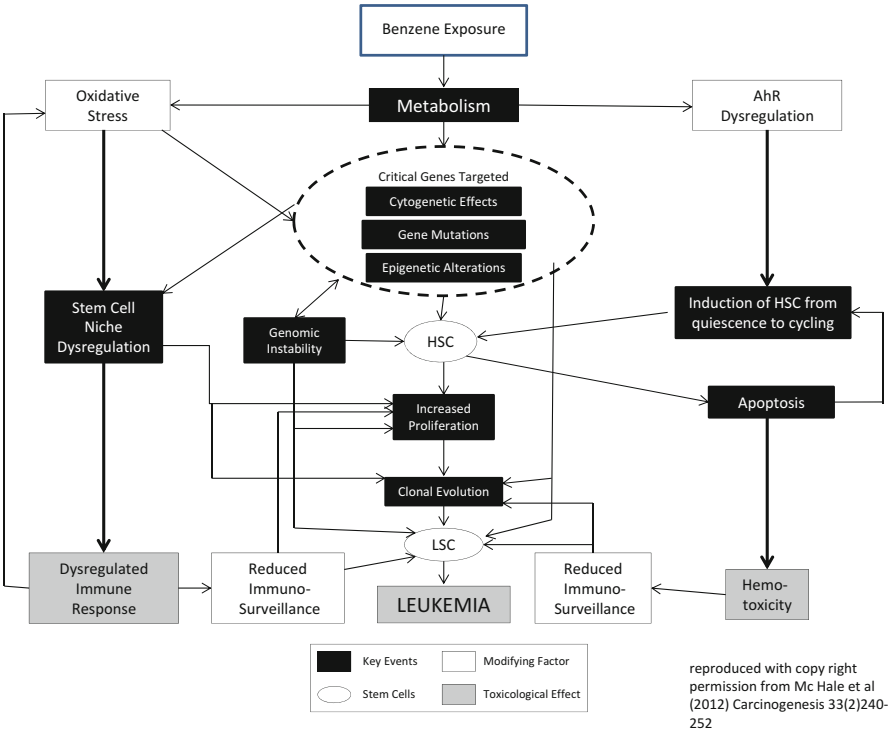

Fig. 6.1 Multiple modes of benzene-induced toxicity and leukemia (Reproduced with copyright permission from Mc Hale et al. (2012))

6.4 Polycyclic Aromatic Hydrocarbons (PAHs)

The PAHs consist of three or more fused benzene rings containing only carbon and hydrogen. A complex mixture of liquid and solid PAHs are produced in coal gasification plants, municipal incinerators, smokehouses, and some aluminum production facilities. In addition, cigarette smoking and environmental tobacco smoke, forest fires and volcanoes are additional sources of PAHs. There are approximately 100 different known PAHs in air, soil, food and water (Skupińska et al. 2004). The US EPA has designated several PAH compounds as priority pollutants (US 2009). The IARC has classified some of these compounds as carcinogenic (group 1) or likely carcinogenic (group 2A) to humans (IARC monographs on the evaluation of carcinogenic risks to humans 2010). ATSDR has grouped 17 PAHs in the ambient air as toxicants for their discussions in their toxicological profile (Agency for Toxic Substances and Disease Registry (ATSDR) 1995). Once emitted to the atmosphere, weight influences the fate of the gaseous PAH mixtures. The heavier PAHs (more than four rings) were found to adsorb to particulate matter, while lighter PAHs (less than four rings) tend to remain gaseous until removed via precipitation (Boström

et al. 2002; Jenkins et al. 1996a; Table II (exposure media and potential for Children's exposure) of USEPA BaP summary 2007; Jenkins et al. 1996b). PAHs are weakly soluble in water and this leads to their accumulation in sediments and aquatic organisms. Once in the atmosphere the PAHs are known to undergo chemical reactions with nitrogen radicals (•NO_3), hydroxide radicals (•OH) and ultraviolet light and generate more active derivatives such as hydroxylated PAHs and PAH quinones (Boström et al. 2002; Jenkins et al. 1996a, b).

The most studied PAHs [e.g., benzo [a] pyrene B[a]P] is a byproduct of incomplete combustion or burning of organic material (e.g., cigarettes, gasoline, and wood). B[a]P is a ubiquitous compound in ambient (outdoor) air due to releases from multiple sources and is also a common indoor air pollutant, particularly in homes where people smoke (Table II (exposure media and potential for Children's exposure) of USEPA BaP summary 2007). A wide range of PAHs have been identified based on chemical characterization and bioassay-directed fractionation in the particulates from diverse sources of emissions. Analysis of combustion source-specific differences in the PAHs content of emissions (Boström et al. 2002) indicated that gasoline vehicles without catalytic converters have the highest PAH emission rate for B[a]P compared to the ones with the converters. Diesel vehicles in general have lower PAH emissions than gasoline vehicles due to nitration of PAHs to nitro-PAHs. However, many of the nitro-PAHs are more carcinogenic than the parent compounds (Cook et al. 2007).

Residential combustion of wood has been estimated to be the largest source of PAH in Sweden and some areas of US (Agency for Toxic Substances and Disease Registry (ATSDR) 1995; Boström et al. 2002). Emission factors for PAH species have a wide range depending on the burning conditions. Several independent studies have reported a wide range of PAH emissions from biomass (cereal crop residues and wood fuels) burned in a combustion wind tunnel to simulate open burning. In a first study of four different cereal crop residues and four different wood fuels, the concentration range of 19 PAHs have been found to vary from 120 to 4,000 mg/kg fuel. Weakly flaming and spreading fires in the burning of cereal stubbles were observed to produce higher levels of heavier PAHs with greater partitioning of PAHs to the particulate phase (Dhammapala et al. 2007). Individual PAH species concentrations appeared correlated well within groups based primarily on molecular weight, but no single PAH species was observed to correlate with all others to serve as an indicator of PAH emission strength (Jenkins et al. 1996b). In general, these studies indicate that total PAH emissions, particle phase concentrations and fraction of PAH on particles were more strongly influenced by burning conditions than fuel type. Rogge et al. (1993) have conducted analysis of organic aerosols from road dust, tire, debris and organometallic break lining dust and reported PAHs of several species as well.

The fine particles, organic aerosols and organic carbon species generated during the cooking process make significant contributions to both indoor and outdoor air pollution. Multiple studies have reported emission rates for about 150 organic compounds emitted during residential cooking to include PAHs (Nolte et al. 1999; Kleeman et al. 1999). Though the PAHs emitted from cooking sources may be a

small fraction in the urban ambient air, studies from Taiwan demonstrated that a higher proportion of carcinogenic B[a]P was emitted from cooking compared to traffic sources (Li et al. 2003). Tobacco smoke, both mainstream and secondhand contains about 50 or more carcinogens which includes PAHs and other nitrogen containing Azarenes, N-nitrosoamines, aromatic amines and heterocyclic aromatic amines (Hecht 1999).

6.4.1 Absorption, Metabolism and Excretion of PAHs

Clear qualitative similarities have been found in the distribution of certain PAHs across tissues upon inhalation exposure (IARC monographs on the evaluation of carcinogenic risks to humans polycyclic aromatic heterocarbons 2010).

The PAHs are chemically less reactive lipophilic compounds and on absorption (inhalation, ingestion, dermal) are activated to highly reactive electrophilic compounds by the classic Phase I and Phase II drug/xenobiotic enzyme systems. The metabolic activation of PAHs by Phase I enzymes result in the formation of radical cations, diol epoxides, and electrophilic and redox-active o-quinones. This is handled by three main routes: (1) Metabolic oxidation mediated by Cytochrome peroxidases leading to the formation of cationic PAH radicals; (2) Dihydrodiol dehydrogenase-catalyzed oxidation and formation of PAH-O-quinones; and (3) CYP mediated dihydrodiol epoxide formations involving epoxide hydrolase. The key cytochrome P450s involved in these metabolic conversions includes CYPs1A1, 1A2, 1B1 and 3A4. Cytochrome enzymes also play a vital role in PAH interactions with aryl hydrocarbon receptors (AhR), a key mechanism that is implicated in several of PAH-induced disease states including lung cancer. The AhR is present in the cytoplasm as a complex with several co-chaperons. When PAHs conjugates to AhR, it leads to the release of other proteins in the complex and initiates translocation of AhR-PAH complex to nucleus and subsequent formation of a hetero dimer of this complex with AhR nuclear translocator protein (ARNT). This heterodimer reacts with DNA through the xenobiotic response element (XRE) located in the promoter region of CYP1A and 1B genes (Shimada et al. 2002).

The Aldo-keto reductases (AKRs) are a superfamily of monomeric NAD(P)H-dependent oxidoreductases. AKRs catalyze the reduction of aldehydes and ketones to yield primary and secondary alcohols on a variety of endogenous substrates and xenobiotics including PAHs (Matsuura et al. 1996; Jin and Penning 2007). Several members of the AKR superfamily are able to oxidize PAH trans-dihydro diols to o-quinones (Smithgall et al. 1986, 1988). The substrate specificity of AKRs covers structurally diverse PAH trans-dihydro diols. PAH o-quinones are electrophilic and highly reactive to endogenous nucleophiles. PAH o-quinones can readily conjugate with cellular thiols to yield L-cysteine, N-acetyl-cysteine (NAC), and GSH leading to their elimination (Murty and Penning 1992a). The oxidative metabolites formed in phase I are enzymatically conjugated to small molecules by enzymes such as sulfo-transferases (SULTs), glutathione transferases (GSTs) and UDP-glucuronyl-transferases (Murty and Penning 1992b; Chou et al. 1998; Bansal

et al. 1981). The oxygenated benzo[a] pyrene derivatives are common substrates of UDP-Glucuronyl transferase (UDPGT) (Bansal et al. 1981). In addition to the liver and kidneys, metabolism of PAHs occur in the adrenal glands, testes, thyroid, lungs, skin, sebaceous glands, and small intestines. The glucuronide and sulfate conjugates of epoxides, dihydro diol derivatives and phenols of these metabolites are excreted in the bile and urine. Glutathione conjugates are further metabolized to mercapturic acids in the kidney and are excreted in the urine. In humans the hydroxylated metabolites of the PAHs are excreted in urine both as free hydroxylated metabolites and as hydroxylated metabolites conjugated to glucuronic acid and sulfate (Bansal et al. 1981).

6.4.2 Carcinogenic Effects of PAHs

The ability of PAHs to cause cancer has been documented as early as 1775 by Sir Percival Pott, who demonstrated a correlation between scrotal cancers in workers exposed to chimney soot. The initial studies with residual oil fly ash, a product of fossil fuel emissions suggested role for transition metals and PAHs as contributors for cancer in occupational boiler workers with the detection of urinary 1-1-hydroxypyrene(OHP) and associated oxidative DNA injury marker, 8-hydroxy-2′-deoxyguanosine(8-OHdg) in these workers (Mukherjee et al. 2004). Reported research in PAH-induced carcinogenesis began with the isolation of BaP from coal tar in 1930 and its ability to induce mouse skin tumors upon repeated painting (Cook et al. 1933). Since then, numerous reports indicate PAHs to cause cancers of several types such as lung, bladder, breast, esophagus, digestive tract and skin. PAHs are carcinogenic in a number of animal models for multiple target organs (Nesnow et al. 1998; Arif et al. 1999; Darwiche et al. 2007; Courter et al. 2008; Wester et al. 2011; Marston et al. 2001; Yu et al. 2006a, b; Mahadevan et al. 2005; Guttenplan et al. 2012). Several laboratory studies have shown dibenzo (a) pyrene as a potent mouse carcinogen (Marston et al. 2001; Yu et al. 2006a, b), as well as capable of crossing trans-placental barriers (Yu et al. 2006a, b; Castro et al. 2008a, b; Shorey et al. 2012).

Atmospheric PAHs concentrations vary from place to place because of variation in emissions; atmospheric transport contributes to the variations by location as well. In addition, differences in respiration rate and genetic susceptibility among individuals can lead to a different risk at the same exposure level. Despite these limitations, PAH exposure from the ambient air had been demonstrated as one of the risk factors for lung cancer (Zhang et al. 2009b). Zhang et al (2009b), had reported increased risk of lung cancer in the urban population of China exposed to PAHs in outdoor ambient air. A more recent study from Brazil, which analyzed the PAH concentrations (B[a] P equivalent) in fraction of particulate matter less than 10 μm (PM10) of the ambient air at multiple sites also observed increased risk for lung cancer (Menezes and Cardeal 2012). Wickramasinghe et al. (2012) observed a significant correlation in lung cancer risk to source PAH exposure only in rural areas of Sri Lanka due to biomass fuel emission.

6.4.3 *Mechanisms of Carcinogenesis*

A comprehensive review of the diverse molecular mechanisms involved in PAH-induced carcinogenesis is beyond the scope of this chapter. The model PAH, B[a]P is one of the most investigated carcinogen and a vast amount of literature on the chemical carcinogenesis mechanisms of this compound is available (Rubin 2001; Siddens et al. 2012). Some of the recent findings on the PAH mediated carcinogenic mechanisms are described below.

Carcinogenic PAH compounds from particulates are taken up by the epithelial lining of tissues such as lung by binding to the cytosolic receptor AhR and its activation initiates a series of downstream events, including inflammation, DNA adduction, cell proliferation, loss of cell to cell adhesion, and eventually tumor formation. AhR is a transcription factor that plays a key role in mediating toxicity of a number of aromatic air pollutants (Denison and Nagy 2003). The nuclear translocation of AhR is dependent on Ary hydrocarbon receptor nuclear translocator (ARNT). AhR is known to interact with multiple signaling pathways: the induction of Cytochrome P450s; cell proliferation; cell-cell contact; and cell cycle regulation. The role of AhR in many types of cancers is well known (Safe et al. 2013). Figures 6.2 and 6.3 are schematic depiction of metabolism of Benzo [a] pyrene and the role of AhR in PAH mediated DNA binding respectively. Studies using AhR deficient mouse

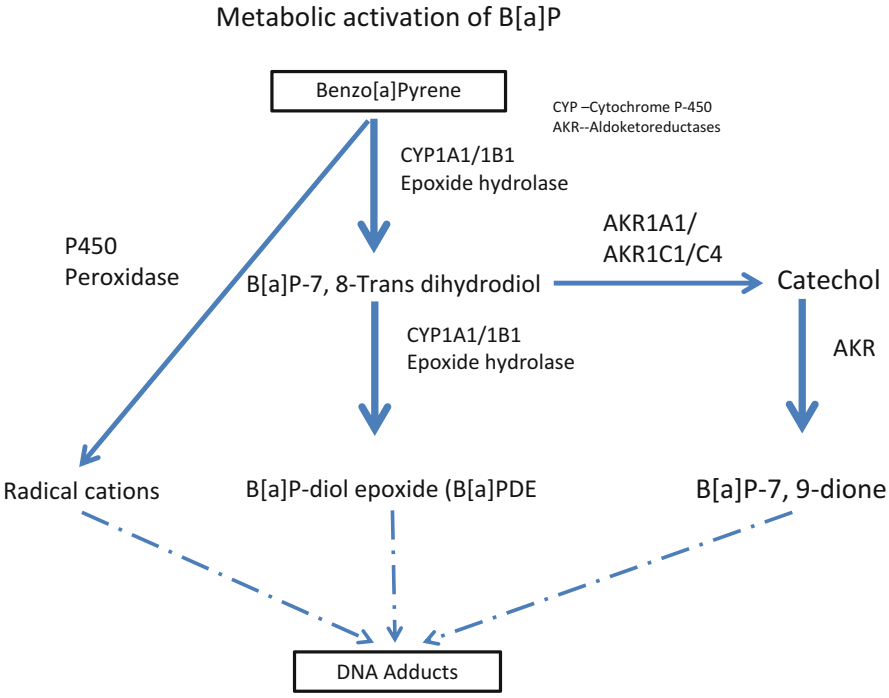

Fig. 6.2 Cytochrome P-450 mediated-benzo (a)-pyrene metabolism

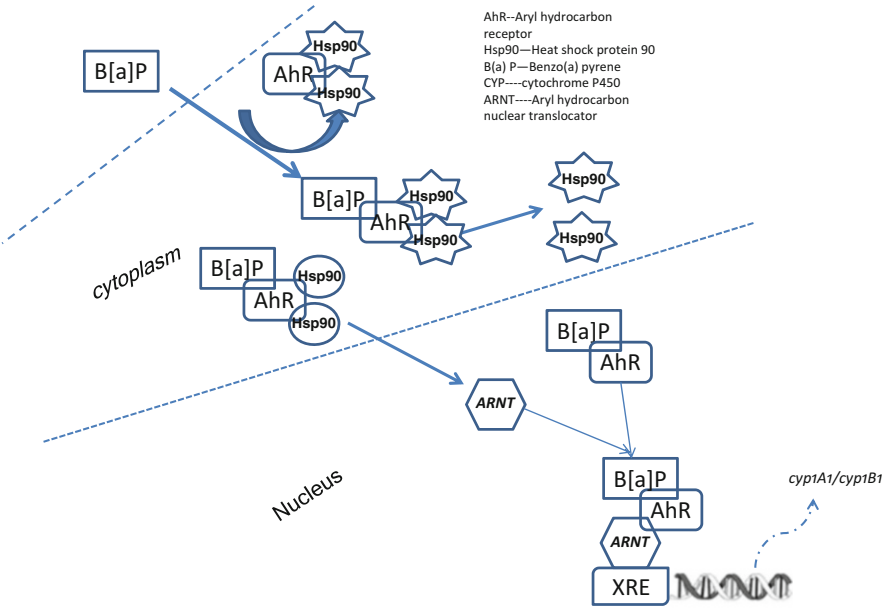

Fig. 6.3 Aryl hydrocarbon receptor-mediated benzo (a) pyrene metabolism and DNA binding

clearly demonstrated the inability of B[a]P to induce CYP1A2 and attenuation of skin carcinogenesis (Shimizu et al. 2000). In mouse strains specifically depleted for ARNT in epidermis, B[a]P failed to induce tumors (Shi et al. 2009; Kuang et al. 2013) suggesting the role or AhR and its nuclear translocation in B[a]P-induced skin tumorigenesis in this animal model.

The activation of PAHs involves metabolism by P450s to generate electrophilic metabolites. The PAH oxy quinones and epoxides generated in this activation step are highly reactive; their phase II detoxification step is elimination of these electrophilic intermediates by conjugation to cellular thiols, which may lead to oxidative stress depending on the load on cellular antioxidant machinery. Alternatively, diverse PAH-metabolites induced free radicals may directly interact with guanine causing DNA damage, resulting in the production of 8-OHdG (Lin et al. 2001).

The enantiomers generated from diol epoxides of PAH metabolism form covalent DNA adducts of varying structures and biological activity. These adducts are normally excised by DNA repair enzymes at different rates for different adducts. The diol epoxides of PAHs bind to guanine and adenine at exocyclic amino groups resulting in the formation of stable adducts with in DNA (Zienolddiny et al. 2006; Schoherr 1928). Several lines of work indicate that DNA adducts block polymerase-mediated replication activity contributing to increased damage by reducing DNA repair activity. This may modify adduct numbers and mutagenicity (Schoherr 1928). Binkova et al (2007), have suggested that polymorphisms in several DNA repair genes involved in nucleotide excision repair (NER) may modify PAH-DNA adduct levels and these adducts may be useful biomarkers to identify individual susceptibility to DNA damage resulting from carcinogenic PAH exposure.

6.5 DNA Repair Pathways

Multiple DNA damage repair pathways play a role in repairing DNA damage induced by PAHs. For example, nucleotide excision repair is the major pathway to repair PAH induced bulky lesions of DNA (Skosareva et al. 2013). Additional repair pathways such as BER homologous recombination repair were also reported under different experimental conditions for various PAHs of different structures or various PAH metabolites (Braithwaite et al. 1998; Meschini et al. 2010; Desler et al. 2009).

The HR mechanism is primarily involved in search for homology and DNA strand invasion through the Rad51-ssDNA presynaptic filament by positioning the invading 3′-end on a template duplex DNA to initiate repair synthesis. This system was found to be principally involved in repair induced by 3 PAHs: 4-nitroquinoline 1-oxide, N-acetoxy-2-acetylaminofluorine, and 1-*N*itroso pyrene. When tested for their relative ability to cause intra-chromosomal homologous recombination between two identical genes that were stably integrated into a mouse cell genome, a dose-dependent increase in recombination frequency with differences among compounds was observed (Bhattacharya et al. 1989).

The mismatch repair (MMR) pathway has been found to play a role in PAH-mediated DNA adduct repair. For example, B[a]P mediated enhancement in lymphomagenisis was demonstrated in Msh2–/– mice in comparison to heterozygous (Msh2+/– mice had been demonstrated by Zienolddiny et al. (2006).

6.6 Second Hand Smoke/Environmental Tobacco Smoke

Involuntary exposure to passive smoke, commonly referred to as second hand smoke (SHS) or environmental tobacco smoke (ETS) or side stream smoke occurs frequently from being in close proximity to cigarette smokers or from exposure to smoke and its components released into the environment. The earliest record of harmful effects of SHS was reported in 1928 (Schoherr 1928). The Surgeon General's report in 1964 classified tobacco smoke as a carcinogenic mixture and after critical evaluation of human and animal data, the US EPA in its report in 1992 classified SHS as a Group A human carcinogen (US 1992). Despite these warnings, in the US about 43.4 million adults continue to smoke, exposing approximately 126 million nonsmokers (CDC 2009) to SHS making and it's a major public health concern. Currently, 7 % of people in high-income countries are covered by comprehensive smoke-free legislation at the national level, and an additional 8 % are covered at the regional level. However, there has been almost no implementation of smoke-free legislation at the subnational level in middle- and low-income countries, despite many of these jurisdictions having the legal authority to do so (World Health Organization 2009). About one-third of adults worldwide are exposed to SHS, which accounts for, 33 % of male and 35 % of female and 40 % of children non-smokers who are exposed to SHS (IARC 2010). The exposure was estimated to

have resulted in about 1 % of worldwide mortality due to lung cancer in nonsmokers (Oberg et al. 2011). The SHS has been implicated in about 47 % deaths in women, 28 % in children, and 26 % in men worldwide (Grimmer et al. 1987). While the risk of lung cancers to SHS exposure are unequivocal (Sheldon et al. 1993), associations have also been reported for colorectal and breast cancers and very weak association for pancreatic, ovarian, and several other types of cancers (World Health Organization 2009; Oberg et al. 2011; Vineis et al. 2004). Due to space limitations, this chapter will focus only on the origin, association and molecular mechanisms implicated in lung cancer from SHS.

Tobacco smoke consists of >2,500 constituents several of which are known human carcinogens (NTP 2005). Both mainstream and side stream smoke are comprised of a vapor phase containing volatile agents, including benzene, vinyl chloride, acrolein, etc., a particulate phase containing semi-volatile and non-volatile agents, such as alkaloids, nicotine and its derivatives, aromatic amines and PAHs. The chemical composition of side stream smoke and mainstream smoke are qualitatively similar, due to difference in the burning temperatures, ageing of smoke and dilution in ambient air; the quantities differ in vapor and particulate phases. However, certain carcinogens, such as aromatic amines have been found to be richer in SHS. Along with these carcinogens, both mainstream and side stream smoke contain a high concentration of reactive organic radicals (RORs) that form on the top of the cigarette at high temperatures. SHS contains not only the gas phase of exhaled smoke, but also the products of combustion of a cigarette. In people, SHS exposure results in up to 50× higher concentration of some of the toxicants than in smokers themselves (A report of the Surgeon General 2006). SHS contains a majority of the IARC group 1 carcinogens benzene, cadmium, 2-aminonaphthalene, nickel, chromium, arsenic and 4-aminobiphenyl; IARC group 2A carcinogens formaldehyde, 1, 3-butadiene and benzo(a) pyrene; the IARC group 2B carcinogens acetaldehyde, isoprene, catechol, acrylonitrile, styrene, NNK, NNN, Lead; and many other carcinogens and genotoxicants (IARC 2004). It has also been observed that the particulate phase of the SHS contains tenfold higher amounts of PAHs than mainstream smoke (Grimmer et al. 1987; Lodovici et al. 2004; Kalaitzoglou and Samara 2006) and is the major source of PAHs in indoor ambient air (Sheldon et al. 1993; Georgiadis et al. 2001; Garfinkel 1981).

One of the first major epidemiologic studies carried out in 1980s indicated a positive association for second hand smoke exposure to a higher risk of developing lung cancer in nonsmoking women married to smokers compared to women married to nonsmokers (Hirayama 1981; Trichopoulos et al. 1981; Apelberg et al. 2013). These three initial studies were followed by numerous investigations that were specifically conducted to evaluate SHS exposure and the risk of lung cancer among nonsmokers. As many as 40 case-control studies and a total of 25 epidemiological studies carried out across the globe confirmed the positive association for workplace secondhand smoke exposure and the risk of lung cancer among lifetime nonsmokers, despite geographic differences in exposure prevalence (A report of the Surgeon General 2006). These epidemiological studies used diverse measurements such as airborne respirable particles and nicotine content to associate the relationship

between exposure to SHS and cancer outcomes (Klepeis et al. 2007). The airborne concentration depends on diverse physical factors, such as the volume of the environment measured and ventilation. It has generally been observed that SHS levels remained relatively stable and decayed over time until ventilation patterns changed indoors, where as in outdoor environments SHS levels dropped immediately to background levels once the source was extinguished (Weaver et al. 1998). In the environments with low SHS exposure, airborne nicotine was non-detectable in the absence of tobacco smoke, while background levels of PM-2.5 from other sources are always present, suggesting that assessment of SHS outdoors cannot be based solely on PM-2.5 measurements, given the limited correlation observed, while both measures can be used indoors when other sources of combustion are absent (Weaver et al. 1998).

Studies relating to urinary trans-, trans-muconoic acid (a marker of benzene exposure) and uptake from SHS has given mixed results (Hecht 2004; Anderson et al. 2001). The urinary metabolites of PAHs, pyrene and phenanthrenone (1-hydroxy pyrene, and hydroxy phenanthranone) respectively were not found to be increased in persons exposed to SHS. The urinary metabolites of tobacco specific markers such as 4 (methyl-nitrasoamine)-1-(3-pyridal)-1-butanone (NNK), which is normally low in smokers is often detected and found elevated in persons exposed to SHS. One study reported about six times higher levels of NNK in women who lived with smokers than who lived with nonsmokers (Anderson et al. 2001; Yamamoto et al. 2013). NNAL-Gluc (4-(methylnitrosoamino)-1-(3-pyridyl)-1-(o-beta-D-glucopyranuronosyl) butane Plus NNAL (4-(methylnitrosamino)-1-3-Pyridyl-1 butanol), is more directly related to cancer risk in SHS than cotinine (Report of the Surgeon general 2006).

Typically, animal studies of second hand smoke often involve exposure to side stream smoke produced by smoking machines. Though these experimental exposures do not fully simulate human exposures, the animals develop tumors. Nevertheless, the animal data provide valuable insights regarding the carcinogenic potential of secondhand tobacco smoke (Coggins 2007; Witschi 2003; Hecht 2005; Toh et al. 2006). Each of the animal model studies with different routes of smoke exposure Viz. nose only or whole body, have their own limitations. These include, difficulty in the accuracy of the dose delivered due to pain and discomfort accompanied with former route and dermal and gastro intestinal absorption of smoke material with the latter. Another inherent issue is that the rodents are obligatory nose breathers with complex nasal turbinates, which filter smoke efficiently and forced smoke exposure in animals leads to a shallow breathing pattern, resulting in scant deposition in lower airways making the rodent a less than ideal model for human exposure (Hecht 2005). In spite of these limitations, animal studies have provided potential biological plausibility for the carcinogenicity of second hand/side stream smoke. These experimental studies in laboratory animals have observed that the exposure to SHS causes biological effects that include: (i) increases or decreases in the activity of phase I enzymes involved in carcinogen metabolism; (ii) increased expression of nitric oxide synthase, xanthine oxidase and various protein kinases; (iii) the formation of smoke-related DNA adducts in several tissues, and (iv) the

presence of urinary biomarkers of exposure to tobacco smoke (Coggins 2007; Witschi 2003; Hecht 2005). Studies using condensates of side stream or mainstream cigarette smoke have produced a spectrum of benign and malignant skin tumors in mice following topical application, and the side stream condensate exhibited higher carcinogenic activity. Side stream smoke condensate was shown to produce a dose-dependent increase in lung tumors in rats following implantation into the lungs (Hecht 2005). In mice, SHS induced tumors primarily occur in peripheral lung as hyperplasia, growing into adenomas and eventually becoming adenocarcinomas. This observation is consistent with the development of non-small cell lung cancer, and particularly of adenocarcinoma in never smokers (Coggins 2007; Witschi 2003; Hecht 2005). Smoke exposure to Hamsters almost exclusively produces laryngeal tumors but not tumors in the deep airways (Witschi 2003; Hecht 2005).

6.6.1 Mechanisms of SHS-Induced Carcinogenesis

It's well known that SHS qualitatively contains essentially the same toxicants and carcinogens in primary tobacco smoke and many of these carcinogens are known to be genotoxicants and induce cancer through genotoxic mechanisms. However, the major difference is that carcinogenic dose resulting from SHS tends to be generally low compared to primary tobacco smoke inhalation. Though small in quantity, the carcinogens in SHS such as NNK undergo detoxification. This results in electrophilic intermediaries that can bind to nucleophilic macromolecules resulting in DNA adducts, subsequently this leads to gene mutations due to DNA mis repair. A significant amount of urinary metabolites of NNK [4-(methyl nitrosamino)-1-(3-Pyridyl)-1-butanol];(NNAL) and its glucuronide conjugate [(NNAL-Gluc)] have been found in the urine of SHS exposed subjects (Hecht 1999; Toh et al. 2006; Wakelee et al. 2007; Meger et al. 2002) suggesting detoxification of NNK, but there is no measureable data on their potential to form adducts with macromolecules. Similarly there is no clear experimental data to support a role for PAHs in the SHS in the mutagenic or carcinogenic process.

Primary tobacco smoke induced lung carcinogenesis has been known to be mediated through mutations in p53 tumor suppressor gene and k-ras oncogenes (Husgafvel-Pursiainen et al. 2000). Epidemiological studies that explored the potential role for these genes in SHS-induced lung tumors failed to find any association due to small number of cases. But, these studies observed that G: C to A: T transitions are the most common mutations among lifetime nonsmokers (Kim et al. 2012) and it was also observed that exposure to SHS doubled the risk of TP53 mutation in never-smokers. In animal models, Transgenic Big Blue mice exposed to side stream smoke for duration of 2–4 months, demonstrated that SHS elicits significant mutagenic response in the lung, trachea and bladder (Kim et al. 2012; Tao et al. 2010). Additional comparative DNA sequencing analysis of lung cellular DNA from SHS exposed Big Blue mouse with lung cancer DNA from known nonsmokers revealed an identical mutation spectrum for TP53 gene and this was significantly

different from lung cancer samples of smokers. Also, the ratio of G: C to A: T transitions and G: C to T: A transversions in the SHS exposed mouse lung and lung cancer from nonsmokers were significantly elevated compared to lung cancer from smokers (Kim et al. 2012). The high incidence of mutations in the bladder tissue of Big Blue mice also provide some experimental support to increased bladder cancer risk observed in lifelong-nonsmokers exposed to SHS (Tao et al. 2010). Additional experimental analysis that investigated overall hypermethylation pattern of tumor suppressor genes on 216 lung cancer patients that includes both smokers and never smokers (Wu et al. 2008) revealed interesting specific patterns of gene expression. The authors indicated increased hypermethylation of these genes including DNA repair gene, O6-methylguanine–DNA methyl transferase (MGMT). This coincides with the fact that the inactivation of MGMT in non-small cell lung cancer, the most frequent cancer in never smokers has been associated with increased occurrence of TP53 mutation, especially the G:C to A:T transition (Wu et al. 2008). The hypermethylation pattern and increased TP53 observed in never smokers due to SHS exposure indirectly suggest that similar mechanisms may be operating in SHS induced lung cancer. Thus far, the accumulated experimental evidence from studies carried out in laboratory animals (usually at a high dose exposure to SHS rather than in human exposure scenarios) indicate multiple pathways to be implicated in the carcinogenesis process and suggests the need to develop better experimental models and design for future studies to gain realistic and relevant knowledge to understand this process in a more cognizant manner.

6.7 Summary

Epidemiological studies point to associations between exposure to air pollution (that contains many well recognized carcinogens) and increase in the incidence of various cancers in population studies. The evidence is stronger in the case of occupational exposure to individual constituents of air pollutants suggesting a potential relationship for carcinogenic effects of air pollution mixtures. The available experimental evidence from laboratory studies for selective air pollutants (viz. formaldehyde, benzene, PAHs and second hand smoke reviewed here), suggest involvement of multiple, but possible common biological pathways. These carcinogenic compounds in the air may enter into humans by inhalation, ingestion or dermal routes. Once these compounds have entered into different organs/portals of entry, they are metabolized/activated to radical/electrophilic moieties by phase I xenobiotic system or chemical specific metabolizing enzymes and conjugated and eliminated by phase II enzymes in liver, lung and other organ systems. The electrophilic/oxidative metabolites generated in this process are capable of initiating carcinogenic process by either genotoxic/mutagenic or non-genotoxic pathways. The former pathways include generation of DNA adducts and DNA-protein adducts. The DNA damage leads to activation of DNA repair machinery to fix damaged DNA. Misrepair of

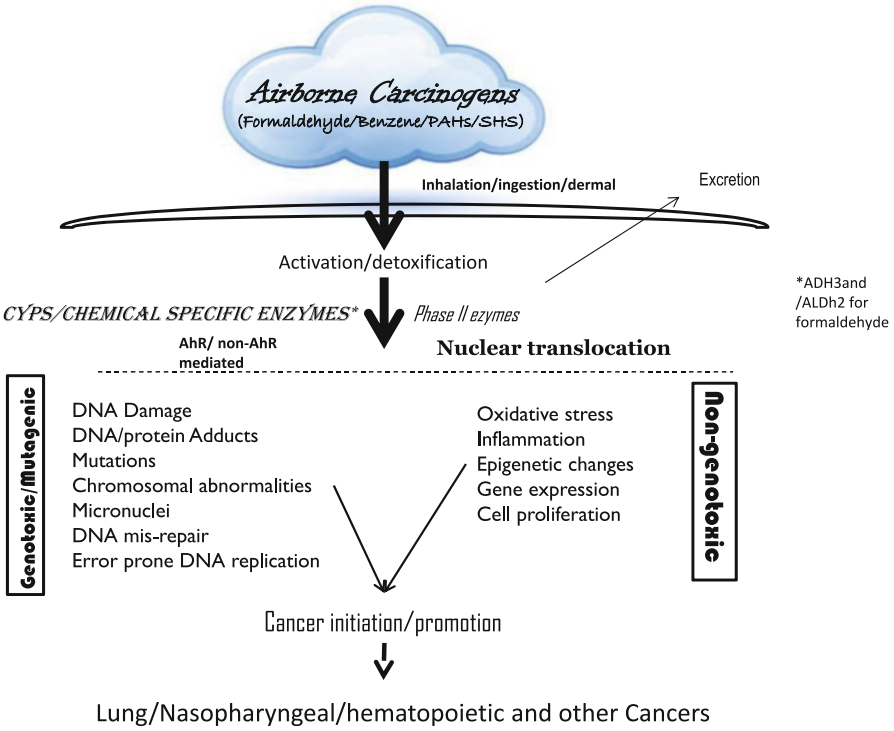

Fig. 6.4 Summary of mechanisms of carcinogenesis by airborne carcinogens

DNA and subsequent error-prone replication may potentially lead to mutagenesis. In addition, these airborne carcinogens may also perturb the cellular homeostasis processes resulting in: (a) oxidative stress; (b) inflammatory responses; and (c) epigenetic and gene expression alterations leading to enhanced cell proliferation. These pathways either independently; or together are implicated in the initiation and or promotion of carcinogenic process and contribute to diverse cancers observed in the population studies. The potential role of multiple pathways implicated in the carcinogenic process mediated by these chemicals is depicted in Fig. 6.4. These diverse pathways may act alone or in different combinations resulting in cancer. Similarly, how specific genotoxic/mutagenic or carcinogenic compounds in the air act alone or together in a synergic or additive way to induce one or more mechanistic pathways referred above, leading to cancer is still largely unknown. Future research efforts to elucidate molecular pathways involved in air- pollution induced carcinogenesis studies may have to develop more integrated approaches that include a combination of high throughput systems biology and to identify specific drivers of cancer that may aid in providing scientific basis for their mode of action to aid in hazard identification and or risk assessment.

Acknowledgements The author expresses sincere appreciation to Drs. George Woodall, Sury Vulimiri, Jason Fitz, Kathleen Newhouse, Yu-Sheng Lin and Bob Sonawane of NCEA, EPA for their critical review and helpful suggestions during the preparation of the manuscript. Sincere appreciation is also for NCEA management for allowing me to take up this task and helping me throughout the agency clearance process.

Disclaimer The views expressed in this Chapter are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

References

A report of the Surgeon General (2006) The health consequences of involuntary exposure to tobacco smoke. pp 1–23, 423–485

Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological profile for formaldehyde (1999) Public Health Service, U.S. Department of Health and Human Services, Atlanta. http://www.atsdr.cdc.gov/toxprofiles/tp111.html

Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological profile for polycyclic aromatic hydrocarbons (1995) Public Health Service, U.S. Department of Health and Human Services, Atlanta. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=122&tid=25

Agency for Toxicological Substances Disease Registry (ATSDR) (2007) Toxicological profile for Benzene. www.atsdr.cdc.gov/toxprofiles/tp3.pdf

Alexander DD, Wagner ME (2010) Benzene exposure and non-Hodgkin lymphoma: a meta-analysis of epidemiologic studies. J Occup Environ Med 52(2):169–189

Anderson KE, Steven G, Carmella C et al (2001) Metabolites of a tobacco-specific lung carcinogen in nonsmoking women exposed to environmental tobacco smoke. J Natl Cancer Inst 93(5):378–381

Andreoli R, Protano C, Manini P et al (2012) Association between environmental exposure to benzene and oxidative damage to nucleic acids in children. Med Lav 103(5):324–337

Apelberg BJ, Hepp LM, Avila-Tang E et al (2013) Environmental monitoring of secondhand smoke exposure. Tob Control 22(3):147–155

Arif JM, Smith WA, Gupta RC (1999) DNA adduct formation and persistence in rat tissues following exposure to the mammary carcinogen dibenzo [a, l]pyrene. Carcinogenesis 20(6):1147–1150

Bansal SK, Zaleski J, Gessner T (1981) Glucuronidation of oxygenated benzo (a) pyrene derivatives by UDP-glucuronyl transferase of nuclear envelope. Biochem Biophys Res Commun 98(1):131–139

Beane Freeman LE, Blair A et al (2009) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute Cohort. J Natl Cancer Inst 101(10):751–761

Bechtold WE, Henderson RF (1993) Biomarkers of human exposure to benzene. J Toxicol Environ Health 40(2–3):377–386

Bechtold WE, Willis JK, Sun JD et al (1992a) Biological markers of exposure to benzene: S-phenylcysteine in albumin. Carcinogenesis 13(7):1217–1220

Bechtold WE, Sun JD, Birnbaum LS et al (1992b) S-phenylcysteine formation in hemoglobin as a biological exposure index to benzene. Arch Toxicol 66(5):303–309

Beland FA, Fullerton NF, Heflich RH (1984) Rapid isolation, hydrolysis and chromatography of formaldehyde-modified DNA. J Chromatogr 308:121–131

Bermudez E, Allen PF (1984) The assessment of DNA damage and repair in rat nasal epithelial cells. Carcinogenesis 5(11):453–458

Bermudez E, Smith-Oliver T, Delehanty LL (1989) The induction of DNA-strand breaks and unscheduled DNA synthesis in F-344 rat hepatocytes following in vivo administration of caprolactam or benzoin. Mutat Res 224(3):361–364

Bhattacharya NP, Maher VC, McCormic JJ (1989) Ability of structurally related polycyclic aromatic carcinogens to induce homologous recombination between duplicated chromosomal sequences in mouse L cells. Mutat Res 211(2):205–214

Bi Y, Li Y, Kong M, Xiao X et al (2010) Gene expression in benzene-exposed workers by microarray analysis of peripheral mononuclear blood cells: induction and silencing of CYP4F3A and regulation of DNA-dependent protein kinase catalytic subunit in DNA double strand break repair. Chem Biol Interact 184(1–2):207–211

Binkova B, Chvatalova I, Lnenickova Z et al (2007) PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms. Mutat Res 620(1–2):49–61

Boström CE, Gerde P, Hanberg A et al (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect 110(Suppl 3):451–488

Braithwaite E, Wu X, Wang Z (1998) Repair of DNA lesions induced by polycyclic aromatic hydrocarbons in human cell-free extracts: involvement of two excision repair mechanisms in vitro. Carcinogenesis 19(7):1239–1246

Buss J, Kuschinsky K, Kewitz H et al (1964) Enteric resorption of formaldehyde. Naunyn Schmiedebergs Arch Pharmacol 247:380–381

Casanova M, Heck H'A (1987) Further studies of the metabolic incorporation and covalent binding of inhaled [3H]- and [14C]formaldehyde in Fischer-344 rats: effects of glutathione depletion. Toxicol Appl Pharmacol 89(1):105–121

Cassee FR, Feron VJ (1994) Biochemical and histopathological changes in nasal epithelium of rats after 3-day intermittent exposure to formaldehyde and ozone alone or in combination. Toxicol Lett 72(1–3):257–268

Castro DJ, Löhr CV, Fischer KA et al (2008a) Lymphoma and lung cancer in offspring born to pregnant mice dosed with dibenzo[a, l]pyrene: the importance of in utero vs. lactational exposure. Toxicol Appl Pharmacol 233(3):454–458

Castro DJ, Baird WM, Pereira CB (2008b) Fetal mouse Cyp1b1 and transplacental carcinogenesis from maternal exposure todibenzo(a, l)pyrene. Cancer Prev Res (Phila) 1(2):128–134

CDC (2009) Tobacco use targeting the nation's leading killer at a glance. Available at http://www.cdc.gov/chronicdisease/resources/publications/aag/pdf/tobacco.pdf

Cheng G, Shi Y, Sturla SJ et al (2003) Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem Res Toxicol 16(2):145–152

Chou HC, Ozawa S, Fu PP et al (1998) Metabolic activation of methyl-hydroxylated derivatives of 7,12-dimethylbenz[a]anthracene by human liver dehydroepiandrosterone-steroid sulfotransferase. Carcinogenesis 19(6):1071–1076

Claxton LD, Woodall GMJR (2007) A review of the mutagenicity and rodent carcinogenicity of ambient air. Mutat Res 636(1–3):36–94

Claxton LD, Mathew PP, Warren SH (2004) The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity. Mutat Res 567(2–3):347–349

Coggins CR (2007) An updated review of inhalation studies with cigarette smoke in laboratory animals. Int J Toxicol 26(4):331–338

Cook JW, Hewett CL, Hieger I (1933) The isolation of a cancer-producing hydrocarbon from coal tar. Parts I, II, and III. J Chem Soc 0:395–405

Cook R, Strum M, Touma JS et al (2007) Inhalation exposure and risk from mobile source air toxics in future years. J Expo Sci Environ Epidemiol 17:95–105

Courter LA, Luch A, Musafia-Jeknic T et al (2008) The influence of diesel exhaust on polycyclic aromatic hydrocarbon-induced DNA damage, gene expression, and tumor initiation in Sencar mice in vivo. Cancer Lett 265(1):135–147

Cronkite EP, Bullis J, Inoue T et al (1984) Benzene inhalation produces leukemia in mice. Toxicol Appl Pharmacol 75(2):358–361

Darwiche N, Ryscavage A, Perez-Lorenzo R, Wright L et al (2007) Expression profile of skin papillomas with high cancer risk displays a unique genetic signature that clusters with squamous cell carcinomas and predicts risk for malignant conversion. Oncogene 26(48):6885–6895

Deltour L, Foglio MH, Duester G (1999) Metabolic deficiency in alcohol dehydrogenase Adh1, Adh3 and Adh4 null mutant mice: Overlapping roles in Adh1 and Adh4 in ethanol clearance and metabolism of retinol to retinoic acid. J Biol Chem 274(24):16796–16801

Demetriou CA, Raascou-Nielsen O, Loft S et al (2012) Biomarkers of ambient air pollution and lung cancer: a systematic review. Occup Environ Med 69(9):619–627

Denison MS, Nagy SR (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 43:309–334

Desler C, Johannessen C, Rasmussen LJ et al (2009) Repair of DNA damage induced by anthanthrene, a polycyclic aromatic hydrocarbon (PAH) without bay or fjord regions. Chem Biol Interact 177(3):212–217

Dhammapala R, Claiborn C, Jimenez J et al (2007) Emission factors of PAHs, methoxyphenols, levoglucosan, elemental carbon and organic carbon from simulated wheat and Kentucky bluegrass stubble burns. Atmos Environ 41(12):2660–2669

Dillon D, Combes R, Zeiger E (1998) The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. Mutagenesis 13(1):19–26

Doolittle DJ, Furlong JW, Butterworth BE (1985) Assessment of chemically induced DNA repair in primary cultures of human bronchial epithelial cells. Toxicol Appl Pharmacol 79(1):28–38

Eastmond DA, Schuler M, Frantz C et al (2001) Characterization and mechanisms of chromosomal alterations induced by benzene in mice and humans. Res Rep Health Eff Inst 103:1–68; discussion 69–80

Egle JL Jr (1972) Retention of inhaled formaldehyde, propionaldehyde, and acrolein in the dog. Arch Environ Health 25:119–124

Emri G, Schaefer D, Held B et al (2004) Low concentrations of formaldehyde induce DNA damage and delay DNA repair after UV irradiation in human skin cells. Exp Dermatol 13(5):305–315

Health and Environment Canada (2012) Report on formaldehyde: indoor air quality. Available at: http://cfpub.epa.gov/ncea/iris_drafts/recorddisplay.cfm?deid=223614

Esposito S, Tenconi R, Lelii M et al (2014) Possible molecular mechanisms linking air pollution and asthma in children. BMC Pulm Med 14(31):1471–1474

Farmer PB, Kaur B, Roach J (2005) The use of S-phenylmercapturic acid as a biomarker in molecular epidemiology studies of benzene. Chem Biol Interact 153–154:97–102

Farris GM, Everitt JI, Irons RD et al (1993) Carcinogenicity of inhaled benzene in CBA mice. Fundam Appl Toxicol 20(4):503–507

Ferkol T, Schraufnagel D (2014) The global burden of respiratory disease. Ann Am Thorac soc 11(3):404–406

Fox CH, Johnson FB, Whiting J et al (1985) Formaldehyde fixation. J Histochem Cytochem 33(8):845–853

Fustinoni S, Consonni D, Campo L et al (2005) Monitoring low benzene exposure: comparative evaluation of urinary biomarkers, influence of cigarette smoking, and genetic polymorphisms. Cancer Epidemiol Biomarkers Prev 14(9):2237–2244

Galbraith D, Gross SA, Paustenbach D (2010) Benzene and human health: a historical review and appraisal of associations with various diseases. Crit Rev Toxicol 40(Suppl 2):1–46

Garfinkel L (1981) Time trends in lung cancer mortality among nonsmokers and a note on passive smoking. J Natl Cancer Inst 66(6):1061–1066

Georgiadis P, Topinka J, Stoikidou M et al (2001) Biomarkers of genotoxicity of air pollution (the AULIS project): bulky DNA adducts in subjects with moderate to low exposures to airborne polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke and other parameters. Carcinogenesis 22(9):1447–1457

Gowans ID, Lorimore SA, McIlrath JM et al (2005) Genotype-dependent induction of transmissible chromosomal instability by gamma-radiation and the benzene metabolite hydroquinone. Cancer Res 65(9):3527–3530

Grafstrom RC, Fornace A Jr, Harris CC (1984) Repair of DNA damage caused by formaldehyde in human cells. Cancer Res 44(10):4323–4327

Grimmer G, Brune H, Dettbarn G et al (1987) Contribution of polycyclic aromatic compounds to the carcinogenicity of sidestream smoke of cigarettes evaluated by implantation into the lungs of rats. Cancer Lett 43(3):173–177

Guttenplan JB, Kosinska W, Zhao ZL et al (2012) Mutagenesis and carcinogenesis induced by dibenzo[a, l]pyrene in the mouse oral cavity: a potential new model for oral cancer. Int J Cancer 130(12):2783–2790

Hamaguchi F, Tsutsui T (2000) Assessment of genotoxicity of dental antiseptics: ability of phenol, guaiacol, p-phenolsulfonic acid, sodium hypochlorite, p-chlorophenol, m-cresol or formaldehyde to induce unscheduled DNA synthesis in cultured Syrian hamster embryo cells. Jpn J Pharmacol 83(3):273–276

Hamilton A (1931) Benzene (Benzol) poisoning: general review. Arch Pathol 11:601–637

Hauptmann M, Stewart PA, Lubin JH et al (2009) Mortality from Lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. J Natl Cancer Inst 101(24):1696–1708

Hecht SS (1999) Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 91(14):1194–1210

Hecht SS (2004) Carcinogen derived biomarkers: applications in studies of human exposure to secondhand tobacco smoke. Tob Control 13:i48–i56

Hecht SS (2005) Carcinogenicity studies of inhaled cigarette smoke in laboratory animals: old and new. Carcinogenesis 26(9):1488–1492

Heck HD, Chin TY, Schmitz MC (1983) Distribution of [14C] formaldehyde in rats after inhalation exposure. In: Gibson JE (ed) Formaldehyde toxicity. Hemisphere Publishing Corporation, Washington, DC, pp 26–37

Hedberg JJ, Griffiths WJ, Nilsson SJ et al (2003) Reduction of s-nitroso glutathione by human alcohol deghydrogenase 3 is an irreversible reaction as analyzed by electrospray mass spectrometry. Eur J Biochem 270(6):1249–1256

Hikiba H, Watanabe E, Barrett JC et al (2005) Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. J Pharmacol Sci 97(1):46–52

Hirayama T (1981) Non-smoking wives of heavy smokers have a higher risk of lung cancer: a study from Japan. Bull World Health Org 78(7):940–942

Husgafvel-Pursiainen K, Boffetta P, Kannio A et al (2000) p53 mutations and exposure to environmental tobacco smoke in a multicenter study on lung cancer. Cancer Res 60(11):2906–2911

IARC (2004) Monograph on evaluating carcinogen risks. Tobacco smoke and involuntary smoking. Lyon. 83:1–1438

IARC (2010) Table 1.5. Monograph on second hand tobacco smoke, Lyon, France 100E:213–255. http://monographs.iarc.fr/ENG/Monographs/vol100E/mono100E-7.pdf. Referenced to Öberg M, Jaakkola MS, Prüss-Üstün A, Schweizer C, Woodward A (2010) Second-hand smoke: assessing the environmental burden of disease at national and local levels. World Health Organization, Geneva (WHO Environmental Burden of Disease Series, No. 18). http://www.who.int/quantifying_ehimpacts/publications/SHS.pdf

IARC (2014) http://www.iarc.fr/en/publications/books/sp161/index.php. In: Kurt Straif, Aaron Cohen, Jonathan Samet (eds) Air pollution and cancer. IARC Scientific Publication No. 161

IARC (2010) monographs on the evaluation of carcinogenic risks to humans vol 92. Some non-heterocyclic polycyclicaromatic hydrocarbons and some related exposures. Available at monographs.ionc.fr/EVG/monographs/vol92/mono92.pdf

Inoue O, Seiji K, Nakatsuka H et al (1989) Urinary t, t-muconic acid as an indicator of exposure to benzene. Br J Ind Med 46(2):122–127

Inoue O, Kanno E, Kakizaki M et al (2000) Urinary phenylmercapturic acid as a marker of occupational exposure to benzene. Ind Health 38(2):195–204

International Agency for Cancer Research (IARC) (2010) Benzene – IARC monographs on the evaluation of carcinogenicity of Benzene. 100F:257–262. monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-24.pdf

International Agency for Research on Cancer (IARC) Monographs (2009) Vol 100: A review and update on occupational carcinogens – a review of human carcinogens – part C: metals, arsenic, dusts and fibres. Lyon, pp 87–89. http://monographs.iarc.fr/ENG/Publications/techrep42/TR42-11.pdf

International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risks to humans (1995), Wood dust and formaldehyde, vol 62. Lyon. http://www.inchem.org/documents/iarc/vol62/wood.html and http://www.inchem.org/documents/iarc/vol62/formal.html

International Agency for Research on Cancer (IARC) Monographs on the evaluation of carcinogenic risks to humans (2006) Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxy-2-propanol, vol 88. IARC, Lyon. http://monographs.iarc.fr/ENG/Monographs/vol88/volume88.pdf

Ishihama M, Toyooka T, Ibuki Y (2008) Generation of phosphorylated histone H2AX by benzene metabolites. Toxicol In Vitro 22(8):1861–1868

Jenkins BM, Jones AD, Turn QT et al (1996a) emission factors for polycyclic aromatic hydrocarbons from biomass burning. Environ Sci Technol 30(8):2462–2469

Jenkins BM, Jones AD, Turn SQ et al (1996b) Particle concentrations, gas-particle partitioning, and species inter correlations for polycyclic aromatic hydrocarbons (PAH) emitted during biomass burning. Atmos Environ 30(22):3825–3835

Jin Y, Penning TM (2007) Aldo-keto reductases and bioactivation/detoxication. Annu Rec Pharmacol Toxicol 47:263–292

Kalaitzoglou M, Samara C (2006) Gas/particle partitioning and yield levels of polycyclic aromatic hydrocarbons and n-alkanes in the mainstream cigarette smoke of commercial cigarette brands. Food Chem Toxicol 44(8):1432–1442

Kamata E, Nakadate M, Uchida O et al (1997) Results of a 28 month – chronic inhalation toxicity study of formaldehyde in male Fisher 344 rats. J Toxicol Sci 22(3):239–254

Kennedy G, Slaich PK, Golding BT et al (1996) Structure and mechanism of formation of a new adduct from formaldehyde and guanosine. Biol Interact 102(2):93–100

Kim SI, Yoon JI, Tommasi S et al (2012) New experimental data linking secondhand smoke exposure to lung cancer in nonsmokers. FASEB J 26(5):1845–1854

Kimbell JS, Overton JH, Subramaniam RP et al (2001) Dosimetry modeling of inhaled formaldehyde: binning nasal flux predictions for quantitative risk assessment. Toxicol Sci 64:111–121

Kleeman MJ, Schauer JJ, Cass GR (1999) Size and composition distribution of fine particulate matter emitted from wood burning, meat charbroiling, and cigarettes. Environ Sci Tech 33:3516–3523

Klepeis NE, Ott WR, Switzer P (2007) Real-time measurement of outdoor tobacco smoke particles. J Air Waste Manage Assoc 57(5):522–534

Kolachana P, Subrahmanyam VV, Meyer K et al (1993) Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells in vitro and in the bone marrow in vivo. Cancer Res 53(5):1023–1026

Kuang D, Zhang W, Deng Q, Zhang X et al (2013) Dose–response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. Environ Sci Technol 47(13):7446–7456

Lan Q, Zhang L, Shen M (2009) Large-scale evaluation of candidate genes identifies associations between DNA repair and genomic maintenance and development of benzene hematotoxicity. Carcinogenesis 30(1):50–58

Li SM, Anlauf KG, Wiebe HA et al (1994) Estimating primary and secondary production of HCHO in eastern North America based on gas-phase measurements and principal component analysis. Geophys Res Lett 21:669–672

Li CT, Lin YC, Lee WJ (2003) Emission of polycyclic aromatic hydrocarbons and their carcinogenic potencies from cooking sources to the urban atmosphere. Environ Health Perspect 111(4):483–487

Li R, Lu ZS, Qiao Y et al (2004) Study on the formaldehyde-induced DNA damage with comet assay. Shi Yan Sheng Wu Xue Bao 37(4):262–268

Liber HL, Benforado K, Crosby RM, Simpson D et al (1989) Formaldehyde-induced spontaneous alterations in human hprt DNA sequence and m-RNA expression. Mutat Res 226(1):31–37
Lin CH, Huang X, Kolbanovskii A, Hingerty BE et al (2001) Molecular topology of polycyclic aromatic carcinogens determines DNA adduct conformation: a link to tumorigenic activity. J Mol Biol 306(5):1059–1080
Liteplo RG, Meek ME (2003) Inhaled formaldehyde: exposure estimation, hazard characterization, and exposure-response analysis. J Toxicol Environ Health B Crit Rev 6(1):85–114
Lodovici M, Akpan V, Evangelisti C et al (2004) Side stream tobacco smoke as the main predictor of exposure to polycyclic aromatic hydrocarbons. J Appl Toxicol 24(4):277–281
Luo L, Jiang L, Geng C et al (2008) Hydroquinone-induced genotoxicity and oxidative DNA damage in HepG2 cells. Chem Biol Interact 173(1):1–8
Magana-Schwencke N, Ekert B (1978) Biochemical analysis of damage induced in yeast by formaldehyde. II. Induction of cross-links between DNA and protein. Mutat Res 51(1):11–19
Mahadevan B, Luch A, Bravo CF et al (2005) Dibenzo [a, l]pyrene induced DNA adduct formation in lung tissue in vivo. Cancer Lett 227(1):25–32
Mallory TB, Gall EA, Brickley WJ et al (1939) Chronic exposure to benzene (benzol). III. The pathologic results. J Ind Hyg Toxicol 21:355–377
Maltoni C, Cotti G, Valgimigli L et al (1982) Hepatocarcinomas in Sprague–Dawley rats, following exposure to benzene by inhalation: First experimental demonstration. Med Lav 73(4):446–450
Maltoni C, Conti B, Cotti G (1983) Benzene: a multipotential carcinogen. Results of long-term bioassays performed at the Bologna Institute of Oncology. Am J Ind Med 4(5):589–630
Maltoni C, Conti B, Cotti G et al (1985) Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: current results and ongoing research. Am J Ind Med 7(5–6):415–446
Maltoni C, Ciliberti A, Cotti G et al (1989) Benzene, an experimental multipotential carcinogen: results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Perspect 82:109–124
Marston CP, Pereira C, Ferguson J et al (2001) Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumor initiation, PAH-DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. Carcinogenesis 22(7):1077–1086
Martin CN, McDermid AC, Garner RC (1978) Testing of known carcinogens and non-carcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. Cancer Res 38(8):2621–2627
Matsuura K, Deyashiki Y, Bunai Y et al (1996) Aldose reductase is a major reductase for isocaproaldehyde, a product of side-chain cleavage of cholesterol, in human and animal adrenal glands. Arch Biochem Biophys 328(2):265–271
Mc Hale CM et al (2012) Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis 33(2):240–252
McQueen CA, Rosado RR, Williams GM (1989) Effect of nalidixic acid on DNA repair in rat hepatocytes. Cell Biol Toxicol 5(2):201–206
Meger M, Meger-Kossien I, Schuler-Metz A et al (2002) Simultaneous determination of nicotine and eight nicotine metabolites in urine of smokers using liquid chromatography – tandem mass spectrometry. J Chromatogr B 778:251–261
Menezes HC, Cardeal ZL (2012) Study of polycyclic aromatic hydrocarbons in atmospheric particulate matter of an urban area with iron and steel mills. Environ Toxicol Chem 31(7):1470–1471
Meschini R, Berni A, Marotta E et al (2010) DNA repair mechanisms involved in the removal of DBPDE-induced lesions leading to chromosomal alterations in CHO cells. Cytogenet Genome Res 128(1–3):124–130
Metz B, Kersten GF, Hoogerhout P et al (2004) Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J Biol Chem 279:6235–6243

Miyachi T, Tsutsui T (2005) Ability of 13 chemical agents used in dental practice to induce sister-chromatid exchanges in Syrian hamster embryo cells. Odontology 93(1):24–29
Monticello TM, Morgan KT, Hurtt ME (1990) Unit length as the denominator for quantitation of cell proliferation in nasal epithelia. Toxicol Pathol 18(1):24–31
Monticello TM, Renne R, Morgan KT (1991) Chemically induced cell proliferation in upper respiratory tract carcinogenesis. Prog Clin Biol Res 369:323–335
Monticello TM, Swenberg JA, Gross EA et al (1996) Correlation of regional and non-linear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res 56(5):1012–1022
Morgan KT, Gross EA, Patterson DL (1986) Distribution, progression and recovery of acute formaldehyde – induced inhibition of nasal mucociliary function in F-344 rats. Toxicol Appl Pharmacol 86(3):448–456
Mukherjee S, Palmer LJ, Kim JY et al (2004) Smoking status and occupational exposure affects oxidative DNA injury in boilermakers exposed to metal fume and residual oil fly ash. Cancer Epidemiol Biomarkers Prev 13(3):454–460
Mullin AH, Rando R, Esmundo F et al (1995) Inhalation of benzene leads to an increase in the mutant frequencies of a lacI transgene in lung and spleen tissues of mice. Mutat Res 327:121–129
Murty VS, Penning TM (1992a) Polycyclic aromatic hydrocarbon (PAH) ortho-quinone conjugate chemistry: kinetics of thiol addition to PAH ortho-quinones and structures of thioether adducts of naphthalene-1, 2- dione. Chem Biol Interact 84(2):169–188
Murty VS, Penning TM (1992b) Characterization of mercapturic acid and glutathionyl conjugates of benzo[a]pyrene-7, 8-dione by two-dimensional NMR. Bioconjug Chem 3(3):218–224
Narot TS, Perez L, Kunzli N et al (2011) Public health importance of triggers of myocardial infarction: a comparative risk assessment. Lancet 377:732–740
National Toxicology program (NTP) Formaldehyde: Report on carcinogens, 12th edn (2011) National Institute of Environmental Health Sciences, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park. http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/formaldehyde.pdf
National Toxicology Program (NTP) Report on carcinogens (2002) 10th edn. National Institute of Environmental Health Sciences, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park. http://ehp.niehs.nih.gov/roc/tenth/profiles/s089form.pdf
National Toxicology Program (NTP) Report on carcinogens (2009) Back ground document for formaldehyde. http://ntp.niehs.nih.gov/ntp/roc/twelfth/2009/…/formaldehyde_bd_final.pdf
National Toxicology Program (NTP) toxicology and carcinogenesis studies of benzene (1989) CAS No. 71-43-2 in F344/N Rats and B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser 289:1–277
Nelson N, Levine RJ, Albert RE et al (1986) Contribution of formaldehyde to respiratory cancer. Environ Health Perspect 70:23–35
Nesnow S, Mass MJ, Ross JA et al (1998) Lung tumorigenic interactions in strain A/J mice of five environmental polycyclic aromatichydrocarbons. Environ Health Perspect 106(Suppl 6):1337–1346
Nolte CG, Schauer JJ, Cass GR (1999) Highly polar organic compounds present in meat smoke. Environ Sci Technol 33(19):3313–3316
NTP (2005) 12th Report on carcinogens. U.S. Department of Health and Human Services, Public Health Service. http://ntp.niehs.nih.gov/go/roc12
Oberg M, Jaakkola MS, Woodward A et al (2011) Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. Lancet 377(9760):139–146
Ohba Y, Morimitsu Y, Watarai A (1979) Reaction of formaldehyde with calf-thymus nucleohistone. Eur J Biochem 100(1):285–293
Oshima J (2000) Comparative aspects of the Werner syndrome gene. In Vivo 14(1):165–172
Pope CA 3rd, Burnett RT, Thun MJ et al (2002) Lung cancer, cardiopulmonary mortality, and long term exposure to fine particulate air pollution. JAMA 287(9):1132–1141

Qu Q, Shore R, Li G et al (2005) Biomarkers of benzene: urinary metabolites in relation to individual genotype and personal exposure. Chem Biol Interact 153–154:85–89
Rappaport SM, Kim S, Lan Q et al (2009) Evidence that humans metabolize benzene via two pathways. Environ Health Perspect 117(6):946–952
Ren X, Lim S, Smith MT et al (2009) Werner syndrome protein, WRN, protects cells from DNA damage induced by the benzene metabolite hydroquinone. Toxicol Sci 107(2):367–375
Reuzel PG, Wilmer JW, Woutersen RA et al (1990) Interactive effects of ozone and formaldehyde on the nasal respiratory lining epithelium in rats. J Toxicol Environ Health 29(3):279–292
Rogge WF, Hildemann LM, Mazurek MA et al (1993) source of fine organic aerosol. 3. Road dust, tire debris and organo metallic break lining dust: Roads as sources and sinks. Environ Sci Technol 27:1892–1904
Rothman N, Haas R, Hayes RB et al (1995) Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. Proc Natl Acad Sci U S A 92(9):4069–4073
Rubin H (2001) Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates. Carcinogenesis 22(12):1903–1930
Ruppert T, Scherer G, Tricker AR et al (1997) trans, trans-muconic acid as a biomarker of non-occupational environmental exposure to benzene. Int Arch Occup Environ Health 69(4):247–251
Safe S, Lee SO, Jin UH (2013) Role of the aryl hydrocarbon receptor in carcinogenesis and potential as a drug target. Toxicol Sci 135(1):1–16
Salthammer T, Mentese S, MarutZky R (2010) Formaldehyde in the indoor environment. Chem Rev 110(4):2536–2572
Schluger NW, Koppaka R (2014) Lung disease in a global context a call for public health action. Ann Am Thorac Soc 11(3):407–416
Schmid O, Speit G (2007) Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis 22(1):69–74
Schoherr E (1928) Second hand smoke contribution to the statistical and clinical features of lung tumors (in German). Z Krebsforsch 27:436–450
Sellakumar AR, Snyder CA, Solomon JJ et al (1985) Carcinogenicity of formaldehyde and hydrogen chloride in rats. Toxicol Appl Pharmacol 81(3):401–406
Shah ASV, Langrish JP, Nair H et al (2013) Global association of air pollution and heart failure: a systematic review and meta analysis. Lancet. doi: http://dx.doi.org/10.1016/S0140-6736(13)60898-3
Shaham J, Bomstein Y, Gurvich R et al (2003) DNA- protein cross links and P^{53} protein expression In relation to occupational exposure to formaldehyde. Occup Environ Med 60(6):403–409
Sheldon L, Clayton A, Keever J et al (1993) Indoor concentrations of polycyclic aromatic hydrocarbons in California residences. California Air Resources Board, Report A033-132. http://www.arb.ca.gov/research/apr/reports/l3041.pdf
Sheltzer JM, Blank HM, Pfau SJ et al (2011) Aneuploidy drives genomic instability in yeast. Science 333(6045):1026–1030
Shen M, Lan Q, Zhang L et al (2006) Polymorphisms in genes involved in DNA double-strand break repair pathway and susceptibility to benzene-induced hematotoxicity. Carcinogenesis 27:2083–2089
Shi S, Yoon DY, Hodge-bellK C et al (2009) The aryl hydro carbon receptor nuclear translocator(ARNT)is required for tumor initiation by Benzo(a) pyrene. Carcinogenesis 30(11):1956–1961
Shimada T, Inoue K, Suzuki Y et al (2002) Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6 J mice. Carcinogenesis 23(7):1199–1207
Shimizu Y, Nakatsura Y, Takahasi Y et al (2000) Benzo(a) pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc Natl Acad Sci 97(2):779–782

Shorey LE, Castro DJ, Baird WM et al (2012) Transplacental carcinogenesis with dibenzo[def, p] chrysene (DBC): timing of maternal exposures determines target tissue response in offspring. Cancer Lett 317(1):49–55

Siddens LK, Larkin A, Krueger SK et al (2012) Polycyclic aromatic hydrocarbons as skin carcinogens: comparison of benzo[a] pyrene, dibenzo [defp] Chrysene and three environmental mixtures in the FVB/N mouse. Toxicol Appl Pharmacol 264(3):377–386

Skosareva LV, Lebedeva NA, Lavrik OI et al (2013) Repair of bulky DNA lesions deriving from polycyclic aromatic hydrocarbons. Mol Biol 47(5):634–644

Skupińska K, Misiewicz I, Kasprzycka-Guttman T (2004) Polycyclic aromatic hydrocarbons: physicochemical properties, environmental appearance and impact on living organisms. Acta Pol Pharm 61(3):233

Smith MT, Rothman N (2000) Biomarkers in the molecular epidemiology of benzene-exposed workers. J Toxicol Environ Health A 61(5–6):439–445

Smith MT, Yager JW, Steinmetz KL, Eastmond DA (1989) Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ Health Perspect 82:23–29

Smithgall TE, Harvey RG, Penning TM (1986) Regio- and stereospecificity of homogeneous 3 alpha-hydroxysteroid-dihydrodiol dehydrogenase for trans-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. J Biol Chem 261(14):6184–6691

Smithgall TE, Harvey RG, Penning TM (1988) Oxidation of the trans-3,4-dihydrodiol metabolites of the potent carcinogen 7,12-dimethylbenz(a)anthracene and other benz(a)anthracene derivatives by 3 alpha-hydroxysteroid-dihydrodiol dehydrogenase: effects of methyl substitution on velocity and stereochemical course of trans-dihydrodiol oxidation. Cancer Res 48(5):1227–1232

Snyder R (2012) Leukemia and benzene. Int J Environ Res Public Health 9(8):2875–2893

Snyder RD, Van Houten B (1986) Genotoxicity of formaldehyde and an evaluation of its effects on the DNA repair process in human diploid fibroblasts. Mutat Res 165(1):21–30

Snyder CA, Goldstein BD, Sellakumar AR et al (1980) The inhalation toxicology of benzene: incidence of hematopoietic neoplasms and hematotoxicity in ARK/J and C57BL/6 J mice. Toxicol Appl Pharmacol 54(2):323–331

Snyder CA, Sellakumar AR, James DJ et al (1988) The carcinogenicity of discontinuous inhaled benzene exposures in CD-1 and C57Bl/6 mice. Arch Toxicol 62(5):331–335

Soffritti M, Maltoni C, Maffei F et al (1989) Formaldehyde: an experimental multipotential carcinogen. Toxicol Ind Health 5(5):699–730

Soffritti M, Belpoggi F, Lambertin L et al (2002) Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann N Y Acad Sci 982:87–105

Solomon DA, Kim T, Diaz-Martinez LA et al (2011) Mutational inactivation of STAG2 causes aneuploidy in human cancer. Science 333:1039–1043

Sorahan T (2011) Occupational benzene exposure and lymphoma risk. Environ Health Perspect 119(11):A468–A469

Speit G, Merk O (2002) Evaluation of mutagenic effects of formaldehyde in vitro: detection of cross links and mutations in mouse lymphoma cells. Mutagenesis 17(3):183–187

Speit G, Schütz P, Merk O (2000) Induction and repair of formaldehyde-induced DNA cross links in repair deficient human cell lines. Mutagenesis 15(1):85–90

Table II (exposure media and potential for Children's exposure) of USEPA BaP summary (2007) Available at http://www.epa.gov/teach/chem_summ/B[a]P_summary.pdf (compilation of Information reflecting the statement in the text described in this table was derived from several resources including studies listed in the TEACH Database (http://www.epa.gov/teach)

Tao L, Xiang YB, Wang R (2010) Environmental tobacco smoke in relation to bladder cancer risk–the Shanghai bladder cancer study [corrected]. Cancer Epidemiol Biomarkers Prev 19(12):3087–3095

Thompson CM, Sonawane B, Grafström RC (2009) The ontogeny, distribution, and regulation of alcohol dehydrogenase 3: implications for pulmonary physiology. Drug Metab Dispos 37(8):1565–1571

Toh CK, Gao F, Lim WT et al (2006) Never-smokers with lung cancer: epidemiologic evidence of a distinct disease entity. J Clin Oncol 24(15):2245–2251
Trichopoulos D, Kalandidi A, Sparros L et al (1981) Lung cancer and passive smoking. Int J Cancer 27(1):1–4
Tyihák E, Bocsi J, Timár F et al (2001) Formaldehyde promotes and inhibits the proliferation of cultured tumour and endothelial cells. Cell Prolif 34(3):135–141
Ura H, Nowak P, Litwin S et al (1989) Effects of formaldehyde on normal xenotransplanted human tracheobronchial epithelium. Am J Pathol 134(1):99–106
US EPA (1992) Respiratory health effects of passive smoking: lung cancer and other disorders. EPA, Washington, DC. oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=36793
US EPA (2009) List of priority chemicals in united states. Available at http://www.epa.gov/oSW/hazard/wastemin/priority.htm
Vineis P, Alavanja M, Garte S et al (2004) Dose–response relationship in tobacco-related cancers of bladder and lung: a biochemical interpretation. Int J Cancer 108(1):2–7
Waidyanatha S, Rothman N, Fustinoni S et al (2001) Urinary benzene as a biomarker of exposure among occupationally exposed and unexposed subjects. Carcinogenesis 22(2):279–286
Wakelee HA, Chang ET, Gomez SL et al (2007) Lung cancer incidence in never smokers. J Clin Oncol 25(5):472–478
Wallace L (1996) Environmental exposure to benzene: an update. Environ Health Perspect 104(Suppl 6):1129–1136
Weaver VM, Buckley TJ, Groopman JD et al (1998) Approaches to environmental exposure assessment in children. Environ Health Perspect 106(Suppl 3):827–832
Wester PW, Muller JJ, Slob W et al (2011) Carcinogenic activity of benzo[a]pyrene in a 2 year oral study in Wistar rats. Food Chem Toxicol 50(3–4):927–935
Wickramasinghe AP, Karunaratne DG, Sivakanesan R (2012) M(10)-bound polycyclic aromatic hydrocarbons: biological indicators, lung cancerrisk of realistic receptors and 'source-exposure-effect relationship' under different source scenarios. Chemosphere 87(11):1381–1387
Wilkins RJ, Macleod HD (1976) Formaldehyde induced DNA-protein crosslinks in Escherichia coli. Mutat Res 36(1):11–16
Williams PR, Panko JM, Unice K et al (2008) Occupational exposures associated with petroleum-derived products containing trace levels of benzene. J Occup Environ Hyg 5(9):565–574
Win LM (2003) Homologous recombination initiated by benzene metabolites: potential role of oxidative stress. Toxicol Sci 72:143–149
Witschi H (2003) Induction of lung cancer by passive smoking in an animal model system. Methods Mol Med 74:441–455
World Health Organization (2009) Report on the global tobacco epidemic, implementing smoke-free environments. http://whqlibdoc.who.int/publications/2009/9789241563918_eng_full.pdf
World Health Organization (WHO) News release (2011) Tackling the global clean air challenge. http://www.who.int/mediacentre/news/releases/2011/air_pollution_20110926/en/index.html
Wu JY, Wang J, Lai JC et al (2008) Association of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation with p53 mutation occurrence in non-small cell lung cancer with different histology, gender, and smoking status. Ann Surg Oncol 11:3272–3277
Xing C, Wang QF, Li B, Tian H et al (2010) Methylation and expression analysis of tumor suppressor genes p15 and p16 in benzene poisoning. Chem Biol Interact 184(1–2):306–309
Yamamoto ML, Chapman AM, Schiest RH (2013) Effects of side stream tobacco smoke and smoke extract on glutathione- and oxidative DNA damage repair-deficient mice and blood cells. Mutat Res 749(1–2):58–65
Yeowell-O'Connell K, Rothman N, Smith MT et al (1998) Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene. Carcinogenesis 19(9):1565–1571
Yeowell-O'Connell K, Rothman N, Waidyanatha S et al (2001) Protein adducts of 1,4-benzoquinone and benzene oxide among smokers and nonsmokers exposed to benzene in China. Cancer Epidemiol Biomarkers Prev 10(8):831–838

Yu Z, Loehr CV, Fischer KA et al (2006a) In utero exposure of mice to dibenzo[a, l]pyrene produces lymphoma in the offspring: role of the aryl hydrocarbon receptor. Cancer Res 66(2):755–762

Yu Z, Mahadevan B, Löhr CV et al (2006b) Indole-3-carbinol in the maternal diet provides chemoprotection for the fetus against transplacental carcinogenesis by the polycyclic aromatic hydrocarbon dibenzo[a, l]pyrene. Carcinogenesis 27(10):2116–2123

Yu K, Shi YF, Yang KY et al (2011) Decreased topoisomerase IIα expression and altered histone and regulatory factors of topoisomerase IIα promoter in patients with chronic benzene poisoning. Toxicol Lett 203(2):111–117

Zar HL, Ferkol TW (2014) The global burden of respiratory disease-impact on child health. Pediatr Pulmonol 49(5):430–434

Zhang L, Rothman N, Wang Y et al (1998) Increased aneusomy and long arm deletion of Chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene. Carcinogenesis 19(11):1955–1961

Zhang L, Lan Q, Guo W et al (2005) Use of octochrome fluorescence in situ hybridization to detect specific aneuploidy among all 24 chromosomes in benzene-exposed workers. Chem Biol Interact 153–154:117–122

Zhang L, Steinmaus C, Eastmond DA et al (2009a) Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. Mutat Res 681:150–168

Zhang Y, Tao S, Shen H et al (2009b) Inhalation exposure to ambient polycyclic aromatic hydrocarbons and lung cancer risk of Chinese population. Proc Natl Acad Sci U S A 106(50):21063–21067

Zhang L, Tang X, Rothman N et al (2010) Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidemiol Biomarkers Prev 19:80–88

Zhang L, Lan Q, Guo W et al (2011) Chromosome-wide aneuploidy study (CWAS) in workers exposed to an established leukemogen benzene. Carcinogenesis 32(4):605–612

Zhong W, Que Hee SS (2004) Formaldehyde-induced DNA adducts as biomarkers of in vitro human nasal epithelial cell exposure to formaldehyde. Mutat Res 563(1):13–24

Zienolddiny S, Ryberg D, Svendsrud DH et al (2006) Msh2 deficiency increases susceptibility to benzo[a]pyrene-induced lymphomagenesis. Int J Cancer 118(11):2899–2902

Chapter 7
Molecular Epidemiology Focused on Airborne Carcinogens

Pavel Rossner Jr., Blanka Binkova, Andrea Rossnerova, and Radim J. Sram

7.1 Introduction

7.1.1 Air Pollutants and Their Impact on Human Health

Humans are constantly exposed to thousands of xenobiotics, that are present in the ambient air, soil, water, as well as in food and various products of human activity. Routes of exposure include inhalation, ingestion and/or dermal contact. Ambient air pollution is considered the most serious in terms of its effect on human health, because it is ubiquitous in both industrialized and developing countries and thus a vast majority of human population suffers from its negative impact. Combustion of fossil fuels due to traffic, local heating and/or industrial production represent a predominant source of air pollution. It has been shown that air pollution has both acute and chronic effects on human health affecting different organs and systems, particularly the respiratory, cardiovascular and nervous systems (Kampa and Castanas 2008). Even though air pollutants are a diverse group of xenobiotics, they can be classified into four categories: gaseous pollutants [SO_2, NOx, CO, ozone and volatile organic compounds (VOCs)], persistent organic pollutants (POPs; e.g. dioxins), metals, and particulate matter (Kampa and Castanas 2008). Biological effects of these compounds may be exerted either through the interaction of the chemicals with biomolecules (nucleic acids, lipids and proteins) thus hampering their function or, in case of nucleic acids, inducing mutations, or by generation of reactive oxygen species (ROS) that cause oxidative damage. In the following text, we will discuss health effects of some of the most important air pollutants.

P. Rossner Jr., Ph.D. • B. Binkova, Ph.D. • A. Rossnerova, Ph.D. • R.J. Sram, M.D., D.Sc. (✉)
Department of Genetic Ecotoxicology, Institute of Experimental Medicine, AS CR,
Videnska 1083, 142 20 Prague 4, Czech Republic
e-mail: sram@biomed.cas.cz

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_7

Volatile organic compounds (VOCs) include a class of organic compounds generated mostly as by-products of fuel combustion in road transportation. Most studies concerning VOCs focused on benzene which is a known human carcinogen responsible for hematological malignancies. The harmful effects of benzene on human health are linked to the formation of ROS that induce oxidative stress, and thus damage DNA and other macromolecules (Barreto et al. 2009). Readers are referred to Chap. 6 for more detailed understanding on the mechanisms involved in benzene-induced carcinogenesis.

Dioxins, polychlorinated biphenyls (PCBs) and pesticides are among the class of persistent organic compounds. These chemicals are characterized by their stability in the environment and by the ability to increase their effect as they move through food chain. Dioxins are produced while burning chlorine-containing material; PCBs were widely used in many industrial products including e.g. coolants, plasticizers, flame retardants or lubricating oils. Many of the compounds are carcinogenic/suspected carcinogens to humans. Dioxins and some of the PCBs interact with aryl hydrocarbon receptor and may thus affect expression of various genes.

Many metals are natural components of the Earth's crust but may be released into the air during combustion or industrial processes. Some of the metals (Ca, Zn, Mg, Fe) at low doses are important cellular components forming specific protein domains (e.g. Zn fingers, hemoglobin molecules). However, at higher concentrations metals may induce ROS formation or interfere with enzyme functions by replacing naturally-occurring ions (e.g. Zn) in protein domains. In this way, carcinogenic properties of some heavy metals may be manifested.

Particulate matter (PM) is a broad class of air pollutants that encompasses particles of various sizes (coarse particles of aerodynamic diameter $\leq$10 μm, fine particles of aerodynamic diameter $\leq$2.5 μm, ultrafine particles of aerodynamic diameter $\leq$100 nm) and chemical composition. PM is mostly emitted during activities associated with burning organic material (local heating, industrial production, power plants, road traffic), but may also arise from natural sources including windblown dust. After inhalation, PM is deposited in upper airways, but smaller particles penetrate to the lungs, some of them reaching alveoli. Ultrafine particles may even enter the bloodstream and may thus be carried to distant parts of the body. Depending on the source of PM, particles may contain metals, reactive gases, material of biological origin or various organic compounds including polycyclic aromatic hydrocarbons (PAHs). PAHs have been identified to be responsible for most of the genotoxic activity of PM (Binkova et al. 1999) causing damage to DNA and proteins by inducing DNA and protein adducts. Inhalation of fine and ultrafine PM also leads to inflammation and subsequent production of reactive oxygen species (Mazzoli-Rocha et al. 2010). The production of ROS, that include e.g. the hydroxyl radical, superoxide anion, or hydrogen peroxide, is caused by both the physical effects of PM (PM is phagocyted by macrophages that consequently produce ROS), and the presence of various chemicals on the surface of PM (e.g. metals, PAHs) with prooxidant properties. It has been repeatedly shown that exposure to PM correlates with increased mortality caused by lung cancer and cardiovascular diseases (Dockery et al. 1993; Pope et al. 1995; Sarnat et al. 2001). Pope et al. suggested that a long term increase in PM2.5 of 10 μg/m^3 is associated with an 8 % increase in lung

cancer mortality in adult men (Pope et al. 2002). Despite the fact that other factors related to cancer incidence, such as smoking habits or inappropriate diet, are probably stronger influences, the absolute number of cancer cases related to air pollution is high due to the high prevalence of exposure (Beaglehole et al. 1993).

After entering the organism, some xenobiotics are metabolized and form active compounds that may interact with cellular macromolecules. Other chemicals do not require metabolic activation and act as direct mutagens/carcinogens.

Many polycyclic aromatic hydrocarbons, products of incomplete combustion of organic material, are typical examples of compounds requiring metabolic activation. Three principal pathways of PAHs metabolism have been proposed (Xue and Warshawsky 2005). **The Bay region dihydrodiol epoxides pathway** involves three enzymatic reactions: oxidation of a double bond catalyzed by cytochrome P450 enzymes to unstable arene oxides, their hydrolysis by microsomal epoxide hydrolases to trans-dihydrodiols and cytochrome P450-catalyzed oxidation to diol-epoxides that can bind to DNA. **The radical cation pathway** includes one electron oxidation catalyzed by P450 peroxidase. In this pathway PAHs are oxidized independently of molecular oxygen; organic or lipid hydroperoxides are used as the oxidant source instead. Radical cations are electrophilic and capable of interacting with nucleophilic centers in cellular macromolecules including DNA. Both pathways lead to formation of reactive intermediates that bind to macromolecules and form adducts. Adducts negatively impact the function of macromolecules and in case of DNA may result in induction of mutations and thus increase the risk of cancer. **Activation through PAH-*o*-quinones** is the third major pathway of PAH metabolism. In this pathway dihydrodiol dehydrogenases catalyze the oxidation of trans-dihydrodiols to PAH *o*-quinones. PAH *o*-quinones are electrophilic metabolites that enter redox cycles and generate ROS thus leading to oxidative damage of DNA and other macromolecules.

Apart from this reaction ROS may be generated by other metabolic processes or by inflammation. These processes are among the endogenous sources of ROS. Exogenous sources include environmental factors such as smoking, diet (Loft et al. 1992; Klaunig and Kamendulis 2004), ultraviolet radiation, ionizing radiation or exposure to environmental pollution (Wu et al. 2004). ROS can attack lipids, proteins and nucleic acids (Cooke et al. 2003). The modification of DNA molecules represents the most serious form of impact of ROS on the organism because it may lead to base changes, mutations, and/or DNA breaks. If ROS attack both DNA strands, double-strand DNA breaks may appear. These breaks may lead either to unstable chromosomal aberrations, or, if homologous recombination or non-homologous end-joining repair seal the breaks, to stable chromosomal translocations. The attack of ROS on lipids that leads to lipid peroxidation may have also potentially serious consequences, as it may damage cellular membranes and inactivate membrane-bound receptors or enzymes. In addition, secondary products of lipid peroxidation, such as aldehydes, are highly reactive and may propagate oxidative stress by reacting with other cellular molecules including proteins (Slade et al. 2010). Oxidation of proteins generates carbonyl groups mostly on side chains of protein molecules. These modifications affect the function of proteins and interfere with enzymatic activity and structural properties of proteins (Dalle-Donne et al. 2006).

7.2 Molecular Epidemiology and Biomarkers

From the health of human population point of view, it is very important to estimate damage to the organism caused by xenobiotics in early stages before exposure-related diseases are manifested. This requirement can be fulfilled by the implementation of human biomonitoring into healthcare practice. In general, human biomonitoring may either concentrate on measurement of xenobiotic levels in body fluids or on analyses of changes of biomolecules. While the former approach is analytically less demanding, it does not address the question of the biological effect of xenobiotics on human organism. This is why the latter approach is preferable. Its application in the recent decades has developed into the field of molecular epidemiology.

Molecular epidemiology aims to merge sophisticated and highly sensitive laboratory methods with analytical epidemiological methods. It bridges from basic research in molecular biology to studies of human cancer causation by combining laboratory measurement of internal dose, biologically effective dose, biological effects and the influence of individual susceptibility with epidemiologic methodologies (Perera and Whyatt 1994). The most common view is that this approach represents a natural convergence of molecular biology and epidemiology (Perera et al. 1998).

Molecular epidemiology focuses on analyses of biomarkers as parameters that allow for quantitative differentiation of subjects exposed to harmful compounds/factors from a normal population. Thus, the biomarkers rather than the disease are used to assess the risk of environmental exposure (Albertini et al. 1996; Albertini 1998). The number of biomarkers available for evaluating genetic and cancer risk in humans is quite large and they may be broadly classified into three groups: biomarkers of exposure, biomarkers of effect and biomarkers of susceptibility (Fig. 7.1). Their utility for human biomonitoring is based on the paradigm of environmentally induced cancer (Committee on Biological Markers of the National Research Council 1987). The biomarkers encompass processes of interaction of xenobiotics with the organism starting with exposure and absorption, followed by their metabolism, distribution, critical target interaction (i.e. damage to macromolecules and repair), and finally resulting in genetic changes and disease. In relation to the recent technical development and emergence of omics technologies new biomarkers, also called omics biomarkers (Bonassi et al. 2013), have appeared (Fig. 7.1). These biomarkers, that can be classified as intermediate omics biomarkers of effect (Vineis et al. 2013), include e.g. analyses of mRNA expression (transcriptomics), DNA methylation (epigenomics) and microRNA (miRNA) expression (micromics). Analyses of expression of selected individual genes have been expanded to gene expression profiling of the whole genome. The biomarkers that strive to address mechanisms of regulation of gene expression include methylation profiles of the genome and miRNA analyses. Although the studies that apply the new biomarkers in the biomonitoring are still relatively scarce, the results are promising and indicate that new avenues have opened in biomarkers research. However, an ultimate answer to the

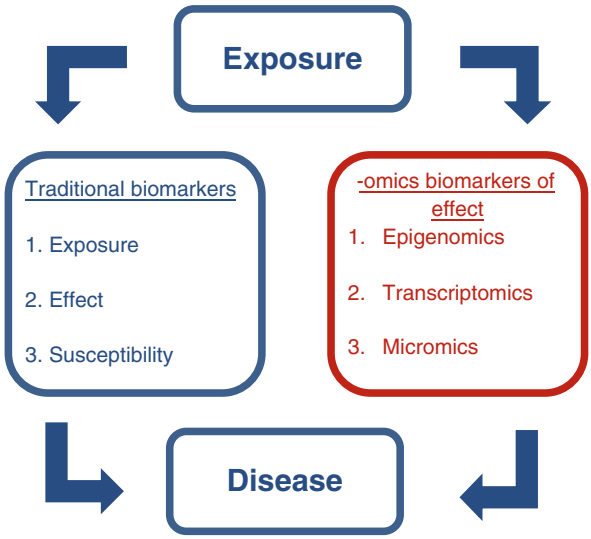

Fig. 7.1 Overview of traditional and omics biomarkers applied in molecular epidemiology and their incorporation into the exposure-disease link

question how xenobiotics impact human health should be provided on the protein level. This answer may be solved in the future when the emerging field of proteomics becomes more advanced and available for researches worldwide.

An ideal biomarker should meet certain criteria. It should be: (1) sensitive enough to be detectable even at low levels of exposure; (2) specific so that it reflects exposure to compounds of interest; (3) standardized and validated so that its analysis is reproducible in both intra- and interlaboratory settings; (4) its analysis should be inexpensive and technically relatively easy to perform; (5) collection of samples for the biomarker analysis should be non-invasive; and (6) its detection method should be high-throughput so that analyses of larger sample sets can be easily performed.

In the following text, we will discuss individual groups of biomarkers, give examples of some of the most commonly used biomarkers and report the results of studies in which the biomarkers have been analyzed.

7.2.1 Biomarkers of Exposure

The concentration of xenobiotics, their metabolites, or levels of modified macromolecules formed as a result of interactions between xenobiotics and target tissue/cell/molecule are included among the biomarkers of exposure. The concentrations of xenobiotics and their metabolites may be measured in body fluids (urine, blood). These biomarkers typically include detection of metals in urine or blood plasma, analyses of metabolites of PAHs, PCBs, pesticides and other xenobiotics in urine and/or blood plasma. However, as mentioned above, these parameters do not reflect the actual effect of the compounds on human organism; they simply serve as

information on the amount of xenobiotics that entered/left the organism. For this reason, biomarkers of biologically effective dose that includes levels of modified cellular macromolecules (proteins, lipids and DNA) are a parameter of choice for molecular-epidemiological studies. DNA or protein adducts have been of particular interest in many studies.

DNA adducts quantify the biologically effective dose of genotoxic compounds that were bound to DNA as a target molecule of carcinogenesis (Binkova et al. 1995, 1996, 1998, 2007; Phillips and Castegnaro 1999). Typical examples of genotoxic compounds include carcinogenic PAHs (c-PAHs) that form bulky DNA adducts or reactive oxygen species (ROS) that induce formation of e.g. 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) as a result of interaction with DNA. If DNA adducts are not effectively repaired, they might be fixed as mutations during replication. Thus, DNA adduct levels have a direct relation to mutagenesis and carcinogenesis. Data are accumulating about the relation of DNA adducts induced by environmental exposure to complex mixture components such as carcinogenic polycyclic aromatic hydrocarbons (Georgiadis et al. 2001; Lewtas 2007) and incidence of malignant tumors and other degenerative diseases (Migliore and Coppede 2002; Binkova et al. 2002).

7.2.1.1 Bulky DNA Adducts

Bulky DNA adducts are markers of exposure to genotoxic aromatic compounds and the ability of an individual to metabolically activate carcinogens and repair DNA damage (Phillips 2005). The use of DNA adducts as a measure of exposure can identify individuals at higher probability of subsequently developing cancer several years prior to the onset (or clinical manifestation) of the disease (Phillips 2005). Bulky DNA adducts determined by the standardized ^{32}P-postlabeling method (Fig. 7.2) are also sensitive biomarkers of environmental exposure to c-PAHs, if the study simultaneously includes personal and stationary monitoring, information on the life style, determination of cotinine, vitamin and lipid levels, as well as genetic

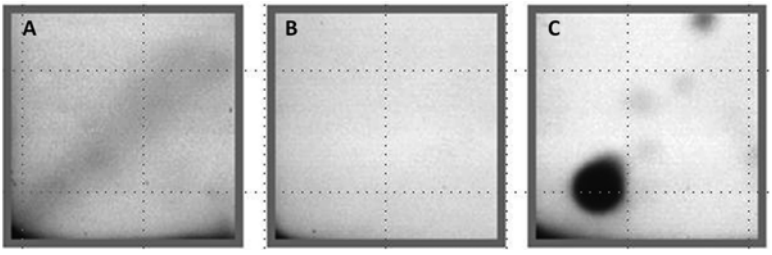

Fig. 7.2 A typical result of bulky DNA adducts analysis by ^{32}P-postlabeling in human peripheral blood lymphocytes. (**a**) DNA isolated from peripheral blood lymphocytes of a human subjects exposed to air pollution; (**b**) A negative control (water blank); (**c**) A positive control (DNA isolated from the lungs of a rat treated with B[a]P)

polymorphisms of metabolic and DNA repair genes (Phillips and Castegnaro 1999; Palli et al. 2001, 2003; Godschalk et al. 2001; Binkova et al. 2007; Georgiadis et al. 2001; Kyrtopoulos et al. 2001; Peluso et al. 1998; Autrup et al. 1999).

^{32}P-postlabeling was widely used in the Czech Republic simultaneously with the personal monitoring of exposure to c-PAHs. The studies included subjects exposed to high levels of air pollutants in Northern Bohemia (B[a]P concentrations up to 7.5 ± 3.6 ng/m^3) (Binkova et al. 1995, 1996), capital city of Prague (groups of city policemen and bus drivers of a total of 950 subjects) (Sram et al. 2011) and in highly polluted Ostrava region (Rossner et al. 2013b). The data obtained for biomarkers of exposure and effect from these studies were used for the pooled analysis. Using multivariate logistic regression, the relationship between personal exposure to B[a]P and DNA adducts measured by ^{32}P-postlabeling was calculated (DNA adducts $= 1.042 + \text{B[a]P} \times 0.077$, $p < 0.001$) (Sram et al. 2011). These results indicate that c-PAH exposure plays a crucial role in DNA adduct formation in lymphocytes.

A pooled analysis of bulky DNA adducts in white blood cells of 3,600 subjects from several European countries was published in 2010 (Ricceri et al. 2010). Lowest DNA adduct levels were observed in spring, followed by summer, autumn and winter. Bulky DNA adduct levels were significantly lower in Northern Europe than in Southern Europe. Authors observed weak associations between bulky DNA adducts and exposure variables. The effect was more pronounced, if DNA adducts were determined in peripheral lymphocytes. In a review of 18 studies that analyzed traffic-associated bulky DNA adducts, including exposure assessment, differences between exposed and control subjects were observed; in nine studies an association between DNA adducts and exposure was detected (DeMarini 2013).

However, the relationship between the exposure to PAHs (B[a]P) and bulky DNA adduct levels is not linear. As Lewtas et al. pointed out, in case of higher occupational exposure to PAHs, as in coke oven workers, the exposure-DNA adduct relationship does not follow dose-response curve. This superlinear response is consistent with saturation of enzyme activity, as would be expected at high doses for carcinogens that require metabolic activation (Lewtas et al. 1997).

7.2.1.2 Oxidative Damage Markers

As mentioned above, all cellular macromolecules may be a target for ROS attack. Although oxidative DNA damage is the most serious because it may lead to mutations (e.g. the presence of 8-oxodG in DNA may result in GC to TA transversions), lipid peroxidation yields highly reactive intermediates that cause secondary damage to cellular structures. Unlike DNA, no repair systems exist for oxidized proteins; they can be either recognized by the proteolytic system and degraded by proteasomes or accumulate in the organism as dysfunctional molecules (Dunlop et al. 2009).

8-oxodG is the most often studied product of DNA oxidation and its presence in urine or lymphocyte DNA was used as a marker of disease or environmental exposure in numerous studies (reviewed e.g. in Loft et al. 2008; Rossner and Sram

2012). Urine is particularly suitable matrix for the analysis of 8-oxodG levels: the samples can be obtained non-invasively, in sufficient quantity, 8-oxodG in urine is very stable and there is no risk of artifactual DNA oxidation during the sample handling. Many studies have shown the effect of traffic-related exposures on 8-oxodG excretion in urine. Traffic exhaust contains a complex mixture of chemicals with the ability to induce ROS production and subsequently oxidative DNA damage. In agreement with this fact, urinary 8-oxodG levels were significantly elevated in taxi drivers (Chuang et al. 2003), highway toll station workers (Lai et al. 2005), city and long-distance bus drivers (Rossner et al. 2007, 2008a; Han et al. 2010) and diesel exhaust emission inspectors (Lee et al. 2010). Interestingly, in another study that followed city policemen in the winter and spring season which differed in levels of air pollutants no effect of seasonal variability was observed (Rossner et al. 2011a).

Air pollution not directly related to traffic resulted in elevated urinary 8-oxodG levels in firefighters (Hong et al. 2000), coke-oven workers (Wu et al. 2003) and boilermakers (Kim et al. 2004). Svecova et al. analyzed the effect of PM10, PM2.5, c-PAHs and B[a]P, on urinary levels of 8-oxodG in 894 children aged 6–10 years living in the Czech Republic. All analyzed pollutants increased oxidative damage within one week of exposure (Svecova et al. 2009). On the other hand, no effect of environmental air pollution on 8-oxodG excretion was observed in a group of office workers and city policemen living in heavily polluted region of the Czech Republic (Rossner et al. 2013a).

Products of lipid peroxidation may be formed by three different mechanisms: free-radical mediated, nonradical-nonenzymatic and enzymatic (Niki 2009). These reactions give rise to a number of products that in low concentrations are important redox signaling mediators. However, at higher concentrations they cause damage to the organism and have been implicated in pathogenesis of various diseases. From the molecular epidemiology point of view, several lipid peroxidation products (LPO) are commonly analyzed: conjugated diens, lipid hydroperoxides, malondialdehyde (MDA)/thiobarbituric acid-reactive substances (TBARS) and F_2-isoprostanes (Moller and Loft 2010). The levels of 15-F_{2t}-isoprostane (15-F2t-IsoP), a commonly used biomarker of lipid peroxidation that is formed from arachidonic acid by a free radical-mediated peroxidation of arachidonic acid independent of cyclooxygenase (Morrow et al. 1990), have been consistently shown to be elevated after exposure to air pollutants including cigarette smoke (Kato et al. 2006), ozone (Chen et al. 2007), c-PAHs and PM (Rossner et al. 2007, 2008b, 2011a, 2013a; Barregard et al. 2006; Nuernberg et al. 2008). On the other hand, levels of lipid hydroperoxides, that are formed as a product of reaction between oxygen and carbon radical in lipids, did not differ between traffic officers and controls sampled in Catania, Italy (Bonina et al. 2008). TBARS levels, that are usually considered a non-specific marker of lipid peroxidation, were positively associated with exposure to $PM_{2.5}$ in a group of senior subjects (Liu et al. 2009). This marker was also affected in subjects who moved to a highly polluted location (Mexico City). Interestingly, the levels of TBARS dropped to normal levels after a 16 weeks stay in the city (Medina-Navarro et al. 1997).

Protein carbonyl groups, used as a marker of protein oxidation, are relatively difficult to induce and thus they probably reflect more severe cases of oxidative stress associated with protein dysfunction (Dalle-Donne et al. 2003). The use of this

marker in molecular-epidemiological studies is not very common and the results are conflicting (Bagryantseva et al. 2010; Ceylan et al. 2006; Rossner et al. 2007, 2008b, 2011a, 2013a). The usefulness of this marker in biomonitoring of the effect of air pollutants on human organism remains to be clarified.

7.2.1.3 Comet Assay

The comet assay (single cell gel electrophoresis, SCGE) is widely used in human biomonitoring to measure DNA damage as a marker of exposure to genotoxic agents or to investigate genoprotective effects (Collins et al. 2014). The comet assay allows the detection of both single and double strand breaks (DSB) depending in assay conditions; DSB represent the principal lesion leading to the formation of chromosomal aberrations. The majority of chemical mutagens induce DSB indirectly via the generation of other DNA lesions such as single strand DNA breaks or oxidative damage that may be converted to DSB during DNA replication or repair (Obe et al. 2002). When combined with specific bacterial repair enzymes, it identifies a broad spectrum of additional lesions including oxidized purines and pyrimidines (Collins 2004). The comet assay is characterized by relative simplicity, low requirements on the number of analyzed cells as well as ability to detect DNA damage independently of the cell cycle.

DeMarini reviewed the use of the method to detect DNA damage induced by traffic in seven exposure groups. In all groups, the higher level of DNA damage was observed in the exposed versus the control populations; the association between exposure levels and DNA damage was observed in all, but one study (DeMarini 2013). Collins et al. further reviewed studies focusing on the effect of air pollution, especially PAHs, on DNA damage. In all ten studies, comet assay detected higher DNA damage in exposed groups (Collins 2004).

Novotna et al. used the comet assay to analyze genetic damage in 54 city policemen (exposed) and 11 controls (working indoors); the sampling was performed in two seasons (January and September). The exposed group displayed significantly higher levels of unspecified DNA damage than controls during both seasons, oxidative DNA damage was significantly higher in the exposed group in January only. The correlation analysis revealed a strong association in the exposed group between the level of oxidative DNA damage and personal exposure to c-PAHs in January (Novotna et al. 2007).

All these studies strongly suggest that the data obtained from the comet assay may serve as an important biomarker of exposure to air pollution.

7.2.2 Biomarkers of Effect

These biomarkers are characterized as measurable biochemical, or physiological alterations within the organism that are known to negatively affect health or are associated with progression of a disease. They include parameters that characterize chromosomal changes or DNA breaks.

7.2.2.1 Chromosomal Aberrations

Chromosomal aberrations in human peripheral blood lymphocytes are recognized as a valuable biomarker of effect in molecular epidemiology. Three basic cytogenetic techniques have been used over time for evaluation of genetic damage – conventional cytogenetic analysis (CCA), analysis of micronuclei (MN) and fluorescent in situ hybridization (FISH).

CCA, as a method focused mainly on unstable aberrations such as chromosomal and chromatid breaks (Fig. 7.3a), has been accepted as a technique suitable for the biological monitoring of genetic damage in somatic cells since the early 1970s. This method was frequently used in various studies to investigate the levels of damage in people exposed to clastogenic agents in the workplace (Natarajan and Obe 1980; Sram et al. 2004). Pooled European data (22,358 subjects) proved that chromosomal aberrations are a valuable standardized and validated biomarker of effect

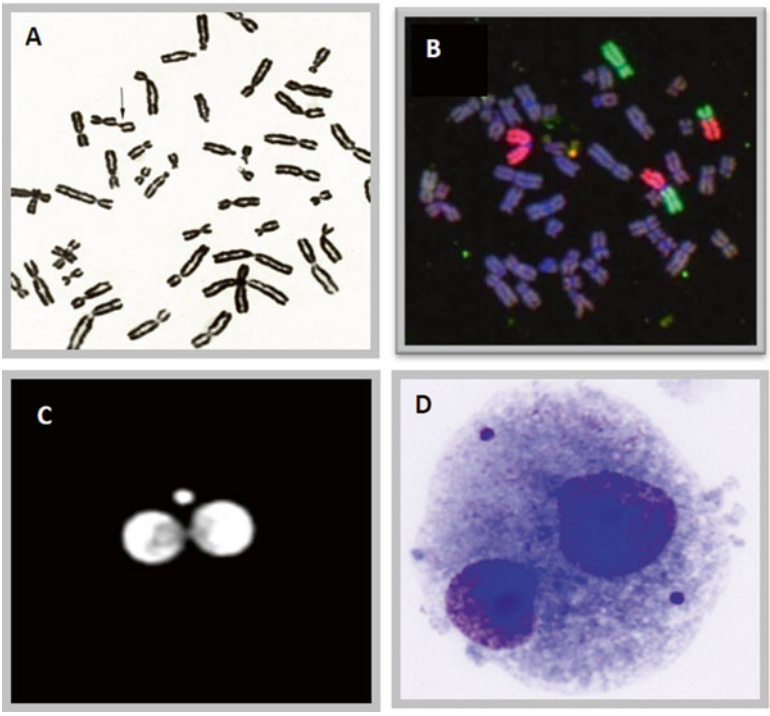

Fig. 7.3 Examples of cytogenetic findings in human peripheral blood lymphocytes, detected by various methodological approaches used in genetic toxicology: (**a**) Chromatid break identified by conventional cytogenetic analysis in metaphases chromosomes; (**b**) Reciprocal translocation between chromosomes #1 (painted *red*) and #4 (painted *green*) identified by fluorescent *in situ* hybridization in metaphases chromosomes; (**c**) Cytochalasin-B-blocked binucleated cell with one micronuclei stained by DAPI, identified by automated image analysis; (**d**) Cytochalasin-B-blocked binucleated cell with two micronuclei stained by Giemsa, identified by visual technique

(Hagmar et al. 2004; Rossner et al. 2005; Bonassi et al. 2008). Low cost of the analysis of Giemsa stained slides is an important advantage of the method; the disadvantage is its laboriousness. This method usually involves evaluation of 100 or 200 well-spread metaphases per subject depending on the size of exposed and/or control groups. There were some efforts for automation of this method, but current state of the art allows scanning metaphases and evaluation of dicentric chromosomes only; both chromosomal and chromatid breaks are not recognized by any automation system yet.

The analysis of **MN** in human peripheral blood lymphocytes that has been used since 1976 (Countryman and Heddle 1976) is the most frequently applied cytogenetic method in molecular epidemiology (Fig. 7.3c, d). Current assay procedure focused on evaluation of MN in binucleated cells (BNC) dates its origin to 1985, when cytochalasin-B was first used to inhibit cytokinesis (Fenech and Morley 1985). MN, represent a measure of both chromosome breakage and chromosome loss. Therefore, an increased frequency of micronucleated cells, used as a biomarker of genotoxic effects, can reflect exposure to agents with clastogenic or aneugenic modes of action (Fenech and Morley 1985). Currently, the MN assay is one of the preferred methods for assessing chromosomal damage as a result of environmental mutagen exposure as well as a tool for genotoxicity testing (Kirsch-Volders et al. 2014).

The HUman MicroNucleus international collaborative project (HUMN), established in 1997, pooled data from more than 6,700 subjects and confirmed that an elevated MN frequency is predictive of an increased cancer risk (Bonassi et al. 2007). Another analysis in this project confirmed that MN frequency is not elevated in moderate smokers and only heavy smokers showed a significant increase in genotoxic damage as measured by the micronucleus assay (Bonassi et al. 2003). Though the visual scoring of MN is relatively easy for a trained person, the scoring of thousands of cells is very time-consuming and tiring work, moreover affected by interpersonal and interlaboratory variability (Fenech et al. 2003). Unlike CCA, there are some validated options for automation and image analysis of MN based on scanning and scoring of MN on Giemsa or DAPI stained slides (Fenech et al. 2013). One of the current biomonitoring studies, the first one that used automated image analysis, showed the impact of season variability of air pollution (concentration of B[a]P) on the frequency of MN in BNC in moderately polluted area (Rossnerova et al. 2009). Using multivariate logistic regression, the relationship between personal exposure to B[a]P and micronuclei expressed as MN/1,000 cells was calculated ($MN = 5.18 + B[a]P \times 1.11$, $p = 0.002$) for this location. Another study performed in highly polluted Ostrava region in the Czech Republic generally failed to show biomarker changes. These results were explained by differences in gene expression between locations and a possibility of adaptive response for population living in highly polluted area was suggested (Rossner et al. 2013a, b, 2014b). This results opened new course for future research.

FISH technique is another method having been used in genetic toxicology since the late 1980s. It is focused mainly on identification of stable chromosomal aberrations like non-reciprocal translocations, reciprocal translocations or insertions,

which are not easily recognized by conventional method (Fig. 7.3b). This method also allows identification of unstable chromosomal aberrations represented e.g. by acentric fragments, but the results are generally limited to the painted chromosomes.

Due to the fact that different laboratories paint different chromosomes by the whole chromosome painting, two important tools were suggested for comparison of results between studies: (1) aberrant cells are classified according to the Protocol for Aberration Identification and Nomenclature (PAINT) (Tucker et al. 1995); (2) the genomic frequencies of translocations (F_G) are calculated by formula suggested by Lucas et al. (1992) where exchange frequencies obtained from each chromosome are calculated for the whole genome by dividing the observed frequencies by the factor of 2.05 f_p (1 − f_p), where f_p is the fraction of painted DNA converted by individual chromosomes.

The background translocation frequency by age, gender, race and smoking status were assessed in pooled data from healthy humans (Sigurdson et al. 2008). FISH technique was successfully used in various human studies for evaluation of the effect of exposure e.g. carcinogenic polycyclic aromatic hydrocarbons (c-PAHs), metals or radiation (Beskid et al. 2007; Palus et al. 2003; Edwards et al. 2004).

Surprising results were observed in the group of city policemen who were examined in two seasons with different concentrations of air pollutants (January and March 2004): the genomic frequency of translocations decreased similarly as did the subjects' exposure to c-PAHs. This suggests that chromosomal aberrations are not so stable in time as originally expected (Sram et al. 2007b). Using multivariate logistic regression the relationship between personal exposure to B[a]P and the genomic frequency of translocations measured by FISH was calculated ($F_G/100 = 1.255 + B[a]P \times 0.082$, $p < 0.05$). When Binkova et al. studied the relationship between chromosomal aberrations and bulky DNA adduct levels in the same subjects, multiple regression analysis indicated that B[a]P-like DNA adducts are a significant predictor of the genomic frequency of translocations (Binkova et al. 2007).

Whole chromosome painting using the FISH technique is more sensitive than the conventional cytogenetic method, which was not affected by the studied concentrations of c-PAHs. Nevertheless this cytogenetic method is generally based on the fact, that the sensitivity of each chromosome to DNA damage is the same. However, some studies discussed different sensitivity of individual chromosomes (Orjuela et al. 2010; Rossner et al. 2014a).

7.2.2.2 Sperm DNA Fragmentation

Sperm DNA fragmentation can be attributed to various pathological conditions including cancer, fever, age, or infection. Many environmental conditions, such as chemotherapy, radiation, air pollution, smoking, as well as ROS can also affect DNA fragmentation in sperm. It is now recognized that elevated sperm DNA fragmentation has a significant effect on reproductive outcome (Evenson et al. 2002;

Larson-Cook et al. 2003). As illustrated below, sperm DNA fragmentation was identified as a sensitive biomarker of air pollution.

Sperm DNA fragmentation is determined by the sperm chromatin structure assay (SCSA). The sperm sample is stained with acridine orange, which is a metachromatic DNA dye that fluoresces green when intercalated into native DNA and shifts to a red fluorescence when associated with collapsed single-stranded DNA. These stained samples are measured by flow cytometry (Evenson et al. 2007; Evenson 2013).

Using the SCSA, Rubes et al. studied the impact of air pollution to sperm DNA damage repeatedly in the same donors living in the polluted Northern Bohemian region (Rubes et al. 2005). DNA fragmentation index (DFI), defined as a percentage of mature sperm with abnormal chromatin/fragmented DNA, was significantly affected by the exposure. Other parameters (sperm concentration, semen volume, sperm morphology and sperm motility) were not associated with air pollution. It was the first study reporting association between exposure to ambient air pollution and DNA fragmentation in human sperm. These results were further confirmed by another study (Rubes et al. 2010), in which DNA fragmentation was observed in mature spermatozoa in subjects exposed to concentration of 1 ng B[a]P/m^3.

7.2.3 Biomarkers of Susceptibility

Biomarkers of susceptibility mostly take into account the role of genetic makeup of the organism in the response to the exposure to xenobiotics. These biomarkers are represented by single nucleotide polymorphisms (SNPs), studied particularly in genes proven to be critical e.g. for metabolic activation of xenobiotics (oxygenases of cytochromes P450), their detoxification (glutathione-S-transferases), or repair pathways (e.g. *XRCC1*, *XPD*, *hOGG1*) (Tuimala et al. 2002; Thacker and Zdzienicka 2003; Kelada et al. 2003). The saturation of the organism by vitamins (e.g. A, C, E, folic acid) is also regarded as a factor affecting susceptibility to the genotoxic and carcinogenic effects of xenobiotics. Vitamins are known to play a significant role as free radical scavengers and antioxidant agents; they also affect the synthesis of DNA repair enzymes (Zijno et al. 2003; Ames 2001; Fenech 2001; Fenech and Ferguson 2001).

It has been shown that levels of biomarkers of exposure and effect are modulated by genetic polymorphisms in relevant genes. Palli et al. demonstrated the effect of polymorphisms in *XPD*, a DNA repair gene, on bulky DNA adduct levels of traffic workers and general population exposed to high levels of genotoxic agents related to vehicle emissions (Palli et al. 2001). The study of Godschalk et al. provided the evidence for combined effects of genetic polymorphisms in *GSTM1*, *GSTT1*, *NAT1* and *NAT2*, genes encoding proteins responsible for detoxification of xenobiotics, on bulky DNA adduct formation in smoking individuals and indicated that simultaneous assessment of multiple genotypes may identify individuals at higher cancer risk (Godschalk et al. 2001). Another study of Palli et al. confirmed that biomarkers of

dietary intake of antioxidants as well as genetic susceptibility markers (*GSTM1*) modulate bulky DNA adduct levels in healthy adults (Palli et al. 2003). Binkova et al. demonstrated that smoking, vitamin C and polymorphisms in *XPD*, *XRCC1* and *GSTM1* are significant predictors for total bulky DNA adduct levels (Binkova et al. 2007). Rubes et al. showed for the first time that men who are homozygous null for *GSTM1* exhibit increased susceptibility to sperm DNA damage associated with exposure to air pollutants (Rubes et al. 2007). In another study, DNA fragmentation index in mature spermatozoa increased after B[a]P exposure and was modulated by a polymorphism in metabolic (*CYP1A1MspI*, *GSTM1*) and DNA repair genes (*XRCC1*, *XPD6*, *XPD23*) (Rubes et al. 2010). In a study by Novotna et al. that used the comet assay to analyze genetic damage in city policemen and controls, regression analysis revealed the influence of genetic polymorphism in *CYP1A1*, *MTHFR*, *MS* and *p53* genes on the level of oxidative and unspecified DNA damage (Novotna et al. 2007). The frequency of stable chromosome aberrations analyzed by the FISH technique was modified by genetic polymorphisms in *CYP1A1*2C*, *GSTP1*, *EPHX1*, *p53* and *MTHFR* genes (Sram et al. 2007a).

In the last couple of years genome-wide association studies (GWAS) showed that many common genetic variants of small, additive effect (McHale et al. 2010) located both in genes and regulatory elements probably play a decisive role in the overall susceptibility of the organism to negative effects of xenobiotics. Thus, nowadays, studies focusing on a small number of SNPs in pre-selected genes are not regarded as sufficient to address the role of genetic susceptibility to e.g. exposure to xenobiotics or to a certain disease; the research in this field has shifted towards large studies that analyze SNPs in thousands of samples using genome-wide approaches (Evangelou and Ioannidis 2013).

7.2.4 Omics Biomarkers

The central dogma of molecular biology that states: “DNA makes RNA makes protein” describes the principle of gene expression. It was formulated by Nobel Prize winner Francis Crick in 1970. The central dogma says “how?” the genetic information flows, but does not answer the question “how many?”, i.e. how many RNAs and proteins are produced during gene expression. For this reason it is important to study the regulatory elements that control intensity of transcription by DNA methylation and intensity of translation by microRNAs binding to the target messenger RNA.

Technical progress and new technologies that became available in the last couple of years made sophisticated genomic methods accessible for a large number of laboratories. As a result, many analyses that would not been possible in the past became a regular part of laboratory routine. Therefore, in the following paragraphs we will focus on new, omics biomarkers: mRNA expression, DNA methylation and miRNA expression. It should be noted that other omics biomarkers exist (e.g. metabolomics and proteomics markers) but they will not be discussed in this text.

7.2.4.1 mRNA Expression

Although the effect of air pollutants on humans may be monitored by the analysis of mRNA expression of individual selected genes (Rossner et al. 2011b), the current trend is to use transcriptomics as a tool for studying genome-wide responses of the organism to environmental exposures (Wild et al. 2013). It has been concluded that transcriptome is a dynamic entity that is highly responsive to environmental exposures (Wild et al. 2013).

Most of the studies analyzing transcriptome changes in exposed subjects use peripheral blood cells. During the last ten years a number of authors reported the effect of air pollutants on global mRNA expression, but a vast majority of them focused on occupational exposures or tobacco smoking (reviewed in Wild et al. 2013). Exposure to benzene (Forrest et al. 2005; McHale et al. 2009), metal fumes (Wang et al. 2005) and diesel exhaust (Peretz et al. 2007) resulted in differential expression of a large number of genes. Studies on the impact of tobacco smoking showed that it is possible to distinguish between subjects exposed and unexposed to tobacco smoke on the basis of transcriptome (Lampe et al. 2004; van Leeuwen et al. 2008a; Wright et al. 2012).

However, studies of the effects of environmental pollutants on gene expression profiles are scarce (van Leeuwen et al. 2006, 2008b; De Coster et al. 2013). In two such studies, higher exposure to air pollutants, which included c-PAHs, was associated with an increased number of deregulated genes (van Leeuwen et al. 2006, 2008b). In the study by De Coster et al. a significant correlation between gene expression modulation and excretion of 1-hydroxypyrene, a marker of PAH exposure, was found (De Coster et al. 2013). In none of these studies detail information from personal monitoring on exposure to environmental pollutants was provided. In addition, these studies were small and included a maximum of 71 subjects from both genders (van Leeuwen et al. 2008a).

Recently, global gene expression analysis in a group of total 312 exposed subjects and 154 controls was conducted with the aim to characterize molecular response of the organism exposed to heavy air pollution (Rossner et al. 2014b). To control for the seasonal variability the samples were collected repeatedly in three different seasons. The exposed group originated from the Ostrava region, a location in the Northeastern part of the Czech Republic that is affected by very high concentrations of air pollutants, particularly c-PAHs. The Ostrava region is one of the most polluted parts of the European Union. A combination of geographical and meteorological conditions (a valley affected by frequent atmospheric inversions), heavy industry and the fact that industrial production exists in the region continually for almost three centuries creates a specific situation suitable for research on environmental air pollution and human health. Given these characteristics a higher number of differentially expressed genes was expected to be found in subjects living in the polluted region. The rationale behind this hypothesis was that the protection of the organism against deleterious effects of air pollution would require greater changes in the transcriptome than in the control subjects. Unexpectedly, despite lower concentrations of air pollutants a higher number of dysregulated genes and

affected KEGG pathways was found in subjects from the control region. In both locations differences between seasons were observed. The quantitative real-time PCR (qRT-PCR) analysis showed a significant decrease in expression of *APEX*, *ATM*, *FAS*, *GSTM1*, *IL1B* and *RAD21* in subjects from Ostrava, in a comparison of winter and summer seasons. In the control subjects, an increase in gene expression was observed for *GADD45A* and *PTGS2*. The authors conclude that high concentrations of pollutants in Ostrava do not increase the number of deregulated genes. This may be explained by adaption of humans to chronic exposure to air pollution. To further explain this phenomenon analyses focused on regulation of mRNA expression are necessary.

7.2.4.2 DNA Methylation

Methylation of cytosine ring at position 5 in CpG sites of DNA leading to formation of 5-methyl-cytosine (5-mC) is an important event in epigenetic changes of cells linked to the control of gene function (Hayatsu 2008). Studies on nuclear DNA methylation changes in white blood cells are rapidly emerging, and thus methylation profiles can serve as a useful biomarker. Molecular epidemiological studies have reported associations between global methylation and several different cancers as well as selected factors including age, gender, race, various environmental exposures or life style factors (Terry et al. 2011).

The level of DNA methylation and changes of methylation profiles can be identified by various methods (Fraga and Esteller 2002; Laird 2010), some of which provide quantitative information about global DNA methylation, while others render qualitative data about gene-specific DNA methylation. Global DNA methylation is most commonly quantified by analyses of highly repetitive sequences like long interspersed nucleotide elements (LINE, e.g. LINE-1), short interspersed nucleotide elements (SINE, e.g. Alu), and pericentromeric satellites (Sat2). For gene-specific methylation, e.g., array methodologies, including the Illumina Infinium Human Methylation 450 K BeadChips interrogating <485,000 CpG sites at single-nucleotide resolution, may be used (Sandoval et al. 2011). The most advanced technology, Whole-Genome Bisulfite Sequencing by Next Generation Sequencing that uses bisulfite treatment combined with high-throughput sequencing is today a top of methodology approaches which allow obtaining both global and gene specific picture of DNA methylome, but due to the price, this method is not used routinely yet. Generally, obtained data can vary by assay types according to their focus on various CpG sites in the genome (Wu et al. 2012; Flom et al. 2011). Moreover, the type of tissue, even different blood cell types can affect global methylation profile, which underlines the functional significance of methylation (Wu et al. 2011; De Bustos et al. 2009).

Currently, there is evidence that DNA methylation in both adults and children is influenced by exposure to environmental pollutants (Terry et al. 2011; Baccarelli and Bollati 2009; Bollati and Baccarelli 2010; De Prins et al. 2013). Several studies suggested that exposure to metals can affect the epigenome (Cheng et al. 2012).

Other study found an inverse correlation between global DNA methylation of Alu, but not of LINE-1 repeated elements, and plasma levels of persistent organic pollutants (POPs) (Rusiecki et al. 2008). Furthermore, a study focused on the changes in DNA methylation patterns in subjects exposed to low doses of benzene showed an association with decreased methylation of LINE-1 and Alu sequences (Bollati et al. 2007). Long-term exposure to PM10 was inversely correlated with methylation in above mentioned repeated elements and demethylation within the promoter of inducible nitric oxide synthase gene (*iNOS*) (Tarantini et al. 2009). *iNOS* methylation was also decreased after acute exposure to PM2.5 (Madrigano et al. 2012). PM10 and PM2.5 exposure have recently been associated with hypomethylation of selected tandem repeats in Beijing, China study groups (Guo et al. 2014). Differences in methylation pattern in children from two regions with various levels of air pollution have recently been analyzed by using the Human Methylation 27 K BeadChips (precursor of 450 K BeadChips) (Rossnerova et al. 2013).

Furthermore, there is a evidence that altered DNA methylation is an important epigenetic mechanism in prenatal programming and that developmental periods are sensitive to environmental stressors. A recent study showed a lower degree of placental global DNA methylation in association with exposure to particulate air pollution in early pregnancy (Janssen et al. 2013). Results of another study that followed non-smoking women during pregnancy suggested that prenatal air polycyclic aromatic hydrocarbons (PAH) exposure was associated with lower global methylation in umbilical cord blood cells and confirmed that global methylation levels were positively associated with the presence of detectable DNA adducts in cord blood (Herbstman et al. 2012). Moreover, a set of genes, *AHRR* (aryl hydrocarbon receptor repressor), *CYP1A1* (cytochrome P450 1A1), and *GFI1* (growth factor independent 1 transcription repressor), with methylation differences present at birth in children whose mothers smoked during pregnancy were each identified by Infinium Illumina Methylation 450 K arrays (Joubert et al. 2012).

7.2.4.3 microRNA Expression

microRNAs are RNA molecules that have been intensively studied in the last few years. The first miRNA, named lin-4, was discovered by Victor Ambros in *Caenorhabditis elegans* in 1993 (Lee et al. 1993). The latest miRBase database [http://www.mirbase.org/, release (v20, June 2013)] contains 24,521 miRNAs identified in 206 various species processed to produce 30,424 mature miRNA products. miRNAs are a class of small (19–25 nucleotides) non-coding RNAs with important role in regulation of gene expression by binding to a target mRNA (Ambros 2004). Various analytical methods like qRT-PCR, Northern blot, microarray or sequencing are used for validation of miRNAs and identification of most altered of them. Mice, rats, and human tissues as well as a various human cell lines are prevalently used in research. Commonly used sources of human samples for this type of analysis are mainly cancer tissues, bronchial tissue, placental cells or peripheral blood lymphocytes. Alternatively, urine or plasma are used for miRNAs profiling, due to the fact, that

cells-derived microvesicules containing miRNAs are released into the plasma and transfer miRNAs between tissues (Bollati et al. 2015). Since significantly different miRNA profiles can be assigned to various types of tumors, miRNAs became important diagnostic, prognostics and therapeutics markers of various types of cancer (Berger and Reiser 2013). Moreover, specific miRNAs are associated with various diseases including pulmonary diseases, such as asthma (Sessa and Hata 2013). The changes of miRNA expression became an established mechanism by which chemical carcinogens induce alterations in target cells (Izzotti and Pulliero 2014).

The important evidence that miRNAs expression is altered by exposure to carcinogens in healthy organisms was obtained in rodents exposed to cigarette smoke (Izzotti et al. 2009a). In another study, a 1 month exposure of mice to cigarette smoke was followed by physiological miRNA expression after 1 week of smoking cessation in comparison with mice that were exposed for 4 months, where alteration of miRNA persisted and resulted in the irreversible loss-of-function of miRNA-base suppression of the expression of oncogenes (Izzotti et al. 2009b; Izzotti et al. 2011). An in vitro study indicated that exposure to maternal cigarette smoke during pregnancy is associated with downregulation of miR-16, –21 and –146a (Maccani et al. 2010). Interestingly, miRNAs were 5.67-fold more sensitive than DNA to the formation of adducts induced by exposure to cigarette smoke (Izzotti and Pulliero 2014). Another study shows association between specific miRNAs (miR-1, –9, –21, –126, –135a, –146a, –155, and –222) and exposure to ambient particulate matter (PM) in a group of elderly males (Fossati et al. 2014). The analysis of the miRNA expression profiles in benzo[a]pyrene (B[a]P)-treated mice revealed the downregulation of miR-122, –142-5p and –150 and the upregulation of miR-29b, –34b-5p and 34c expression (Halappanavar et al. 2011). Other researchers reported associations between overexpression of miR-638 in connection with B[a]P-induced DNA damage (Li et al. 2012). Also association between other airborne carcinogens like diesel exhaust particles, volatile organic compounds, black carbon dust, dimethylbenz[a]anthracene, asbestos or radon and miRNAs were published (Izzotti and Pulliero 2014).

7.3 Conclusions

All discussed studies indicate that bulky DNA adduct levels, the comet assay and analyses of DNA fragmentation in the sperm may be considered sensitive biomarkers of exposure to c-PAHs in polluted air. Stable chromosomal aberrations and unstable chromosomal aberrations measured as frequencies of micronuclei, as well as markers of oxidative damage to DNA and lipid peroxidation can be recommended as reliable biomarkers of effect. However, under specific circumstances, the exposure to environmental pollution may not be reflected on the level of biomarkers. These circumstances are not fully understood yet, but it seems that chronic exposure to intermediate levels of air pollutants and subsequent adaptation of the organism to environmental pollution may play a role. Moreover, to fully understand the

environment-organism relationship it is important to simultaneously identify the gene susceptibility, especially the genetic polymorphisms of metabolic genes and genes encoding DNA repair enzymes. It should be also taken into account that DNA damage may be further affected by life style factors as smoking, environmental tobacco smoke exposure, dietary intake of vitamins (e.g. A, C, E, folic acid), or oxidative damage associated with lipid metabolism (triglycerides, cholesterol, HDL, LDL). It is therefore pertinent to analyze all these endpoints in the biological material in the course of molecular epidemiology studies.

Studies in the Czech Republic suggest that exposure to air pollution exceeding B[a]P concentrations of 1 ng/m^3 represent a risk for DNA damage as indicated by the increase in levels of bulky DNA adducts, the increase of the frequency of stable translocations and micronuclei as well as the increase of DNA fragmentation in the mature sperm. It should be noted, though, that when using biomarkers of exposure and effect, the dose-response effect is detectable only in a certain range of concentrations of xenobiotics; for B[a]P the limit is probably around 10 ng B[a]P/m^3.

New perspectives may be seen in using the omics techniques, e.g. studying mRNA expression as well as regulatory processes, including DNA methylation and miRNA profiles. The ultimate direction in biomarker research should be the application of proteomics.

Summing up, molecular epidemiology studies on environmental exposures to c-PAHs and other airborne carcinogens should be planned as very complex exercises: they should include determination of personal exposure, analyses of damage to DNA and other macromolecules, assessment of gene susceptibility and life style factors. If planned in this way they have a potential to bring new results, which may specify new information important for proper evaluation of human health risk associated with c-PAHs and other airborne carcinogens exposure.

Acknowledgement We would like to acknowledge the great help and support of our friends from National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA, especially Drs. Joellen Lewtas, Lawrence W. Reiter, and Sally Perault Darney. Thanks to their support we were able to establish molecular epidemiology methods in the Czech Republic.

References

Albertini RJ (1998) The use and interpretation of biomarkers of environmental genotoxicity in humans. Biotherapy 11(2–3):155–167

Albertini RJ, Nicklas JA, O'Neill JP (1996) Future research directions for evaluating human genetic and cancer risk from environmental exposures. Environ Health Perspect 104(Suppl 3):503–510

Ambros V (2004) The functions of animal microRNAs. Nature 431(7006):350–355. doi:10.1038/nature02871

Ames BN (2001) DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 475(1–2):7–20

Autrup H, Daneshvar B, Dragsted LO, Gamborg M, Hansen M, Loft S et al (1999) Biomarkers for exposure to ambient air pollution–comparison of carcinogen-DNA adduct levels with other exposure markers and markers for oxidative stress. Environ Health Perspect 107(3):233–238

Baccarelli A, Bollati V (2009) Epigenetics and environmental chemicals. Curr Opin Pediatr 21(2):243–251

Bagryantseva Y, Novotna B, Rossner P Jr, Chvatalova I, Milcova A, Svecova V et al (2010) Oxidative damage to biological macromolecules in Prague bus drivers and garagemen: Impact of air pollution and genetic polymorphisms. Toxicol Lett 199:60–68. doi:10.1016/j.toxlet.2010.08.007, S0378-4274(10)01629-2 [pii]

Barregard L, Sallsten G, Gustafson P, Andersson L, Johansson L, Basu S et al (2006) Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal Toxicol 18(11):845–853

Barreto G, Madureira D, Capani F, Aon-Bertolino L, Saraceno E, Alvarez-Giraldez LD (2009) The role of catechols and free radicals in benzene toxicity: an oxidative DNA damage pathway. Environ Mol Mutagen 50(9):771–780. doi:10.1002/em.20500

Beaglehole R, Bonita R, Kjellstrom T (1993) Basic epidemiology. WHO, Geneva

Berger F, Reiser MF (2013) Micro-RNAs as potential new molecular biomarkers in oncology: have they reached relevance for the clinical imaging sciences? Theranostics 3(12):943–952. doi:10.7150/thno.7445

Beskid O, Binkova B, Dusek Z, Rossner P, Solansky I, Kalina I et al (2007) Chromosomal aberrations by fluorescence in situ hybridization (FISH) – biomarker of exposure to carcinogenic PAHs. Mutat Res 620(1–2):62–70

Binkova B, Lewtas J, Miskova I, Lenicek J, Sram R (1995) DNA adducts and personal air monitoring of carcinogenic polycyclic aromatic hydrocarbons in an environmentally exposed population. Carcinogenesis 16(5):1037–1046

Binkova B, Lewtas J, Miskova I, Rossner P, Cerna M, Mrackova G et al (1996) Biomarker studies in northern Bohemia. Environ Health Perspect 104(Suppl 3):591–597

Binkova B, Topinka J, Mrackova G, Gajdosova D, Vidova P, Stavkova Z et al (1998) Coke oven workers study: the effect of exposure and GSTM1 and NAT2 genotypes on DNA adduct levels in white blood cells and lymphocytes as determined by 32P-postlabelling. Mutat Res 416(1–2):67–84

Binkova B, Vesely D, Vesela D, Jelinek R, Sram RJ (1999) Genotoxicity and embryotoxicity of urban air particulate matter collected during winter and summer period in two different districts of the Czech Republic. Mutat Res 440(1):45–58

Binkova B, Smerhovsky Z, Strejc P, Boubelik O, Stavkova Z, Chvatalova I et al (2002) DNA-adducts and atherosclerosis: a study of accidental and sudden death males in the Czech Republic. Mutat Res 501(1–2):115–128. doi:S0027510702000192 [pii]

Binkova B, Chvatalova I, Lnenickova Z, Milcova A, Tulupova E, Farmer PB et al (2007) PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms. Mutat Res 620(1–2):49–61. doi:10.1016/j.mrfmmm.2007.02.022, S0027-5107(07)00103-0 [pii]

Bollati V, Baccarelli A (2010) Environmental epigenetics. Heredity 105(1):105–112. doi:10.1038/hdy.2010.2

Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D et al (2007) Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res 67(3):876–880. doi:10.1158/0008-5472.CAN-06-2995

Bollati V, Angelici L, Rizzo G, Pergoli L, Rota F, Hoxha M et al (2015) Microvesicle-associated microRNA expression is altered upon particulate matter exposure in healthy workers and in A549 cells. J Appl Toxicol 35(1):59–67

Bonassi S, Neri M, Lando C, Ceppi M, Lin YP, Chang WP et al (2003) Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. Mutat Res 543(2):155–166

Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N et al (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis 28(3):625–631. doi:10.1093/carcin/bgl177

Bonassi S, Norppa H, Ceppi M, Stromberg U, Vermeulen R, Znaor A et al (2008) Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis 29(6):1178–1183. doi:10.1093/carcin/bgn075, bgn075 [pii]

Bonassi S, Taioli E, Vermeulen R (2013) Omics in population studies: a molecular epidemiology perspective. Environ Mol Mutagen 54(7):455–460. doi:10.1002/em.21805

Bonina FP, Puglia C, Frasca G, Cimino F, Trombetta D, Tringali G et al (2008) Protective effects of a standardised red orange extract on air pollution-induced oxidative damage in traffic police officers. Nat Prod Res 22(17):1544–1551. doi:10.1080/14786410701740401

Ceylan E, Kocyigit A, Gencer M, Aksoy N, Selek S (2006) Increased DNA damage in patients with chronic obstructive pulmonary disease who had once smoked or been exposed to biomass. Respir Med 100(7):1270–1276

Chen C, Arjomandi M, Balmes J, Tager I, Holland N (2007) Effects of chronic and acute ozone exposure on lipid peroxidation and antioxidant capacity in healthy young adults. Environ Health Perspect 115(12):1732–1737

Cheng TF, Choudhuri S, Muldoon-Jacobs K (2012) Epigenetic targets of some toxicologically relevant metals: a review of the literature. J Appl Toxicol 32(9):643–653. doi:10.1002/jat.2717

Chuang CY, Lee CC, Chang YK, Sung FC (2003) Oxidative DNA damage estimated by urinary 8-hydroxydeoxyguanosine: influence of taxi driving, smoking and areca chewing. Chemosphere 52(7):1163–1171

Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26(3):249–261. doi:10.1385/MB:26:3:249

Collins A, Koppen G, Valdiglesias V, Dusinska M, Kruszewski M, Moller P et al (2014) The comet assay as a tool for human biomonitoring studies: the ComNet project. Mutat Res 759:27–39. doi:10.1016/j.mrrev.2013.10.001

Committee on Biological Markers of the National Research Council (1987) Biological markers in environmental health research. Environ Health Perspect 74:3–9

Cooke MS, Evans MD, Dizdaroglu M, Lunec J (2003) Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 17(10):1195–1214

Countryman PI, Heddle JA (1976) The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. Mutat Res 41(2–3):321–332

Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R (2003) Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 329(1–2):23–38

Dalle-Donne I, Aldini G, Carini M, Colombo R, Rossi R, Milzani A (2006) Protein carbonylation, cellular dysfunction, and disease progression. J Cell Mol Med 10(2):389–406

De Bustos C, Ramos E, Young JM, Tran RK, Menzel U, Langford CF et al (2009) Tissue-specific variation in DNA methylation levels along human chromosome 1. Epigenetics Chromatin 2(1):7. doi:10.1186/1756-8935-2-7

De Coster S, van Leeuwen DM, Jennen DG, Koppen G, Den Hond E, Nelen V et al (2013) Gender-specific transcriptomic response to environmental exposure in Flemish adults. Environ Mol Mutagen 54(7):574–588. doi:10.1002/em.21774

De Prins S, Koppen G, Jacobs G, Dons E, Van de Mieroop E, Nelen V et al (2013) Influence of ambient air pollution on global DNA methylation in healthy adults: a seasonal follow-up. Environ Int 59:418–424. doi:10.1016/j.envint.2013.07.007

DeMarini DM (2013) Genotoxicity biomarkers associated with exposure to traffic and near-road atmospheres: a review. Mutagenesis 28(5):485–505. doi:10.1093/mutage/get042

Dockery DW, Pope CA 3rd, Xu X, Spengler JD, Ware JH, Fay ME et al (1993) An association between air pollution and mortality in six U.S. cities. N Engl J Med 329(24):1753–1759. doi:10.1056/NEJM199312093292401

Dunlop RA, Brunk UT, Rodgers KJ (2009) Oxidized proteins: mechanisms of removal and consequences of accumulation. IUBMB Life 61(5):522–527. doi:10.1002/iub.189

Edwards A, Voisin P, Sorokine-Durm I, Maznik N, Vinnikov V, Mikhalevich L et al (2004) Biological estimates of dose to inhabitants of Belarus and Ukraine following the Chernobyl accident. Radiat Prot Dosimetry 111(2):211–219. doi:10.1093/rpd/nch039

Evangelou E, Ioannidis JP (2013) Meta-analysis methods for genome-wide association studies and beyond. Nat Rev Genet 14(6):379–389. doi:10.1038/nrg3472

Evenson DP (2013) Sperm chromatin structure assay (SCSA(R)). Methods Mol Biol 927:147–164. doi:10.1007/978-1-62703-038-0_14

Evenson DP, Larson KL, Jost LK (2002) Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23(1):25–43

Evenson DP, Kasperson K, Wixon RL (2007) Analysis of sperm DNA fragmentation using flow cytometry and other techniques. Soc Reprod Fertil Suppl 65:93–113

Fenech M (2001) The role of folic acid and vitamin B12 in genomic stability of human cells. Mutat Res 475(1–2):57–67

Fenech M, Ferguson LR (2001) Vitamins/minerals and genomic stability in humans. Mutat Res 475(1–2):1–6

Fenech M, Morley AA (1985) Measurement of micronuclei in lymphocytes. Mutat Res 147(1–2):29–36

Fenech M, Bonassi S, Turner J, Lando C, Ceppi M, Chang WP et al (2003) Intra- and inter-laboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes. Results of an international slide-scoring exercise by the HUMN project. Mutat Res 534(1–2):45–64

Fenech M, Kirsch-Volders M, Rossnerova A, Sram R, Romm H, Bolognesi C et al (2013) HUMN project initiative and review of validation, quality control and prospects for further development of automated micronucleus assays using image cytometry systems. Int J Hyg Environ Health 216(5):541–552. doi:10.1016/j.ijheh.2013.01.008

Flom JD, Ferris JS, Liao Y, Tehranifar P, Richards CB, Cho YH et al (2011) Prenatal smoke exposure and genomic DNA methylation in a multiethnic birth cohort. Cancer Epidemiol Biomarkers Prev 20(12):2518–2523. doi:10.1158/1055-9965.EPI-11-0553

Forrest MS, Lan Q, Hubbard AE, Zhang L, Vermeulen R, Zhao X et al (2005) Discovery of novel biomarkers by microarray analysis of peripheral blood mononuclear cell gene expression in benzene-exposed workers. Environ Health Perspect 113(6):801–807

Fossati S, Baccarelli A, Zanobetti A, Hoxha M, Vokonas PS, Wright RO et al (2014) Ambient particulate air pollution and microRNAs in elderly men. Epidemiology 25(1):68–78. doi:10.1097/EDE.0000000000000026

Fraga MF, Esteller M (2002) DNA methylation: a profile of methods and applications. Biotechniques 33(3):632, 4, 6–49

Georgiadis P, Topinka J, Stoikidou M, Kaila S, Gioka M, Katsouyanni K et al (2001) Biomarkers of genotoxicity of air pollution (the AULIS project): bulky DNA adducts in subjects with moderate to low exposures to airborne polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke and other parameters. Carcinogenesis 22(9):1447–1457

Godschalk RW, Dallinga JW, Wikman H, Risch A, Kleinjans JC, Bartsch H et al (2001) Modulation of DNA and protein adducts in smokers by genetic polymorphisms in GSTM1, GSTT1, NAT1 and NAT2. Pharmacogenetics 11(5):389–398

Guo L, Byun HM, Zhong J, Motta V, Barupal J, Zheng Y et al (2014) Effects of short-term exposure to inhalable particulate matter on DNA methylation of tandem repeats. Environ Mol Mutagen 55(4):322–335. doi:10.1002/em.21838

Hagmar L, Stromberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C et al (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res 64(6):2258–2263

Halappanavar S, Wu D, Williams A, Kuo B, Godschalk RW, Van Schooten FJ et al (2011) Pulmonary gene and microRNA expression changes in mice exposed to benzo(a)pyrene by oral gavage. Toxicology 285(3):133–141. doi:10.1016/j.tox.2011.04.011

Han YY, Donovan M, Sung FC (2010) Increased urinary 8-hydroxy-2′-deoxyguanosine excretion in long-distance bus drivers in Taiwan. Chemosphere 79(9):942–948. doi:10.1016/j.chemosphere.2010.02.057, S0045-6535(10)00238-9 [pii]

Hayatsu H (2008) The bisulfite genomic sequencing used in the analysis of epigenetic states, a technique in the emerging environmental genotoxicology research. Mutat Res 659(1–2):77–82. doi:10.1016/j.mrrev.2008.04.003

Herbstman JB, Tang D, Zhu D, Qu L, Sjodin A, Li Z et al (2012) Prenatal exposure to polycyclic aromatic hydrocarbons, benzo[a]pyrene-DNA adducts and genomic DNA methylation in cord blood. Environ Health Perspect. doi:10.1289/ehp.1104056

Hong YC, Park HS, Ha EH (2000) Influence of genetic susceptibility on the urinary excretion of 8-hydroxydeoxyguanosine of firefighters. Occup Environ Med 57(6):370–375

Izzotti A, Pulliero A (2014) The effects of environmental chemical carcinogens on the microRNA machinery. Int J Hyg Environ Health 217(6):601–627

Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S (2009a) Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. FASEB J 23(3):806–812. doi:10.1096/fj.08-121384

Izzotti A, Calin GA, Steele VE, Croce CM, De Flora S (2009b) Relationships of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J 23(9):3243–3250. doi:10.1096/fj.09-135251

Izzotti A, Larghero P, Longobardi M, Cartiglia C, Camoirano A, Steele VE et al (2011) Dose-responsiveness and persistence of microRNA expression alterations induced by cigarette smoke in mouse lung. Mutat Res 717(1–2):9–16. doi:10.1016/j.mrfmmm.2010.12.008

Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A et al (2013) Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 10(1):22. doi:10.1186/1743-8977-10-22

Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK et al (2012) 450 K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ Health Perspect 120(10):1425–1431. doi:10.1289/ehp.1205412

Kampa M, Castanas E (2008) Human health effects of air pollution. Environ Pollut 151(2):362–367. doi:10.1016/j.envpol.2007.06.012, S0269-7491(07)00284-9 [pii]

Kato T, Inoue T, Morooka T, Yoshimoto N, Node K (2006) Short-term passive smoking causes endothelial dysfunction via oxidative stress in nonsmokers. Can J Physiol Pharmacol 84(5):523–529

Kelada SN, Eaton DL, Wang SS, Rothman NR, Khoury MJ (2003) The role of genetic polymorphisms in environmental health. Environ Health Perspect 111(8):1055–1064

Kim JY, Mukherjee S, Ngo LC, Christiani DC (2004) Urinary 8-hydroxy-2′-deoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to fine particulates. Environ Health Perspect 112(6):666–671

Kirsch-Volders M, Bonassi S, Knasmueller S, Holland N, Bolognesi C, Fenech MF (2014) Commentary: critical questions, misconceptions and a road map for improving the use of the lymphocyte cytokinesis-block micronucleus assay for in vivo biomonitoring of human exposure to genotoxic chemicals-a HUMN project perspective. Mutat Res 759:49–58. doi:10.1016/j.mrrev.2013.12.001

Klaunig JE, Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 44:239–267

Kyrtopoulos SA, Georgiadis P, Autrup H, Demopoulos NA, Farmer P, Haugen A et al (2001) Biomarkers of genotoxicity of urban air pollution. Overview and descriptive data from a molecular epidemiology study on populations exposed to moderate-to-low levels of polycyclic aromatic hydrocarbons: the AULIS project. Mutat Res 496(1–2):207–228

Lai CH, Liou SH, Lin HC, Shih TS, Tsai PJ, Chen JS et al (2005) Exposure to traffic exhausts and oxidative DNA damage. Occup Environ Med 62(4):216–222. doi:10.1136/oem.2004.015107, 62/4/216 [pii]

Laird PW (2010) Principles and challenges of genomewide DNA methylation analysis. Nat Rev Genet 11(3):191–203. doi:10.1038/nrg2732

Lampe JW, Stepaniants SB, Mao M, Radich JP, Dai H, Linsley PS et al (2004) Signatures of environmental exposures using peripheral leukocyte gene expression: tobacco smoke. Cancer Epidemiol Biomarkers Prev 13(3):445–453

Larson-Cook KL, Brannian JD, Hansen KA, Kasperson KM, Aamold ET, Evenson DP (2003) Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril 80(4):895–902

Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854

Lee MW, Chen ML, Lung SC, Tsai CJ, Yin XJ, Mao IF (2010) Exposure assessment of PM2.5 and urinary 8-OHdG for diesel exhaust emission inspector. Sci Total Environ 408(3):505–510. doi:10.1016/j.scitotenv.2009.10.012, S0048-9697(09)00955-3 [pii]

Lewtas J (2007) Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. Mutat Res 636(1–3):95–133

Lewtas J, Walsh D, Williams R, Dobias L (1997) Air pollution exposure-DNA adduct dosimetry in humans and rodents: evidence for non-linearity at high doses. Mutat Res 378(1–2):51–63

Li D, Wang Q, Liu C, Duan H, Zeng X, Zhang B et al (2012) Aberrant expression of miR-638 contributes to benzo(a)pyrene-induced human cell transformation. Toxicol Sci 125(2):382–391. doi:10.1093/toxsci/kfr299

Liu L, Ruddy T, Dalipaj M, Poon R, Szyszkowicz M, You H et al (2009) Effects of indoor, outdoor, and personal exposure to particulate air pollution on cardiovascular physiology and systemic mediators in seniors. J Occup Environ Med 51(9):1088–1098. doi:10.1097/JOM.0b013e3181b35144

Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE (1992) Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 13(12):2241–2247

Loft S, Hogh Danielsen P, Mikkelsen L, Risom L, Forchhammer L, Moller P (2008) Biomarkers of oxidative damage to DNA and repair. Biochem Soc Trans 36(Pt 5):1071–1076. doi:10.1042/BST0361071, BST0361071 [pii]

Lucas JN, Awa A, Straume T, Poggensee M, Kodama Y, Nakano M et al (1992) Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int J Radiat Biol 62(1):53–63

Maccani MA, Avissar-Whiting M, Banister CE, McGonnigal B, Padbury JF, Marsit CJ (2010) Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21, and miR-146a in the placenta. Epigenetics 5(7):583–589

Madrigano J, Baccarelli A, Mittleman MA, Sparrow D, Spiro A 3rd, Vokonas PS et al (2012) Air pollution and DNA methylation: interaction by psychological factors in the VA Normative Aging Study. Am J Epidemiol 176(3):224–232. doi:10.1093/aje/kwr523

Mazzoli-Rocha F, Fernandes S, Einicker-Lamas M, Zin WA (2010) Roles of oxidative stress in signaling and inflammation induced by particulate matter. Cell Biol Toxicol 26(5):481–498. doi:10.1007/s10565-010-9158-2

McHale CM, Zhang L, Lan Q, Li G, Hubbard AE, Forrest MS et al (2009) Changes in the peripheral blood transcriptome associated with occupational benzene exposure identified by cross-comparison on two microarray platforms. Genomics 93(4):343–349. doi:10.1016/j.ygeno.2008.12.006

McHale CM, Zhang L, Hubbard AE, Smith MT (2010) Toxicogenomic profiling of chemically exposed humans in risk assessment. Mutat Res 705(3):172–183. doi:10.1016/j.mrrev.2010.04.001

Medina-Navarro R, Lifshitz A, Wacher N, Hicks JJ (1997) Changes in human serum antioxidant capacity and peroxidation after four months of exposure to air pollutants. Arch Med Res 28(2):205–208

Migliore L, Coppede F (2002) Genetic and environmental factors in cancer and neurodegenerative diseases. Mutat Res 512(2–3):135–153

Moller P, Loft S (2010) Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. Environ Health Perspect 118(8):1126–1136. doi:10.1289/ehp.0901725
Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ (1990) A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A 87(23):9383–9387
Natarajan AT, Obe G (1980) Screening of human populations for mutations induced by environmental pollutants: use of human lymphocyte system. Ecotoxicol Environ Saf 4(4):468–481
Niki E (2009) Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med 47(5):469–484. doi:10.1016/j.freeradbiomed.2009.05.032, S0891-5849(09)00330-X [pii]
Novotna B, Topinka J, Solansky I, Chvatalova I, Lnenickova Z, Sram RJ (2007) Impact of air pollution and genotype variability on DNA damage in Prague policemen. Toxicol Lett 172(1–2):37–47. doi:10.1016/j.toxlet.2007.05.013
Nuernberg AM, Boyce PD, Cavallari JM, Fang SC, Eisen EA, Christiani DC (2008) Urinary 8-isoprostane and 8-OHdG concentrations in boilermakers with welding exposure. J Occup Environ Med 50(2):182–189. doi:10.1097/JOM.0b013e31815cf6cc
Obe G, Pfeiffer P, Savage JR, Johannes C, Goedecke W, Jeppesen P et al (2002) Chromosomal aberrations: formation, identification and distribution. Mutat Res 504(1–2):17–36
Orjuela MA, Liu X, Warburton D, Siebert AL, Cujar C, Tang D et al (2010) Prenatal PAH exposure is associated with chromosome-specific aberrations in cord blood. Mutat Res 703(2):108–114. doi:10.1016/j.mrgentox.2010.08.004
Palli D, Russo A, Masala G, Saieva C, Guarrera S, Carturan S et al (2001) DNA adduct levels and DNA repair polymorphisms in traffic-exposed workers and a general population sample. Int J Cancer 94(1):121–127
Palli D, Masala G, Vineis P, Garte S, Saieva C, Krogh V et al (2003) Biomarkers of dietary intake of micronutrients modulate DNA adduct levels in healthy adults. Carcinogenesis 24(4):739–746
Palus J, Rydzynski K, Dziubaltowska E, Wyszynska K, Natarajan AT, Nilsson R (2003) Genotoxic effects of occupational exposure to lead and cadmium. Mutat Res 540(1):19–28
Peluso M, Merlo F, Munnia A, Valerio F, Perrotta A, Puntoni R et al (1998) 32P-postlabeling detection of aromatic adducts in the white blood cell DNA of nonsmoking police officers. Cancer Epidemiol Biomarkers Prev 7(1):3–11
Perera FP, Whyatt RM (1994) Biomarkers and molecular epidemiology in mutation/cancer research. Mutat Res 313:117–129
Perera FP, Whyatt RM, Jedrychowski W, Rauh V, Manchester D, Santella RM et al (1998) Recent developments in molecular epidemiology: a study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. Am J Epidemiol 147(3):309–314
Peretz A, Peck EC, Bammler TK, Beyer RP, Sullivan JH, Trenga CA et al (2007) Diesel exhaust inhalation and assessment of peripheral blood mononuclear cell gene transcription effects: an exploratory study of healthy human volunteers. Inhal Toxicol 19(14):1107–1119. doi:10.1080/08958370701665384
Phillips DH (2005) DNA adducts as markers of exposure and risk. Mutat Res 577(1–2):284–292. doi:10.1016/j.mrfmmm.2005.03.008
Phillips DH, Castegnaro M (1999) Standardization and validation of DNA adduct postlabelling methods: report of interlaboratory trials and production of recommended protocols. Mutagenesis 14(3):301–315
Pope CA 3rd, Thun MJ, Namboodiri MM, Dockery DW, Evans JS, Speizer FE et al (1995) Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. Am J Respir Crit Care Med 151(3 Pt 1):669–674. doi:10.1164/ajrccm/151.3_Pt_1.669
Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K et al (2002) Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287(9):1132–1141
Ricceri F, Godschalk RW, Peluso M, Phillips DH, Agudo A, Georgiadis P et al (2010) Bulky DNA adducts in white blood cells: a pooled analysis of 3,600 subjects. Cancer Epidemiol Biomarkers Prev 19(12):3174–3181. doi:10.1158/1055-9965.EPI-10-0314

Rossner P Jr, Sram RJ (2012) Immunochemical detection of oxidatively damaged DNA. Free Radic Res 46(4):492–522. doi:10.3109/10715762.2011.632415

Rossner P, Boffetta P, Ceppi M, Bonassi S, Smerhovsky Z, Landa K et al (2005) Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environ Health Perspect 113(5):517–520

Rossner P Jr, Svecova V, Milcova A, Lnenickova Z, Solansky I, Santella RM et al (2007) Oxidative and nitrosative stress markers in bus drivers. Mutat Res 617:23–32

Rossner P Jr, Svecova V, Milcova A, Lnenickova Z, Solansky I, Sram RJ (2008a) Seasonal variability of oxidative stress markers in city bus drivers – part I: oxidative damage to DNA. Mutat Res 642:14–20

Rossner P Jr, Svecova V, Milcova A, Lnenickova Z, Solansky I, Sram RJ (2008b) Seasonal variability of oxidative stress markers in city bus drivers – part II: oxidative damage to lipids and proteins. Mutat Res 642:21–27

Rossner P Jr, Rossnerova A, Sram RJ (2011a) Oxidative stress and chromosomal aberrations in an environmentally exposed population. Mutat Res 707(1–2):34–41. doi:10.1016/j.mrfmmm.2010.12.005, S0027-5107(10)00312-X [pii]

Rossner P Jr, Uhlirova K, Beskid O, Rossnerova A, Svecova V, Sram RJ (2011b) Expression of XRCC5 in peripheral blood lymphocytes is upregulated in subjects from a heavily polluted region in the Czech Republic. Mutat Res 713:76–82. doi:10.1016/j.mrfmmm.2011.06.001

Rossner P Jr, Rossnerova A, Spatova M, Beskid O, Uhlirova K, Libalova H et al (2013a) Analysis of biomarkers in a Czech population exposed to heavy air pollution. Part II: chromosomal aberrations and oxidative stress. Mutagenesis 28(1):97–106. doi:10.1093/mutage/ges058

Rossner P Jr, Svecova V, Schmuczerova J, Milcova A, Tabashidze N, Topinka J et al (2013b) Analysis of biomarkers in a Czech population exposed to heavy air pollution. Part I: bulky DNA adducts. Mutagenesis 28(1):89–95. doi:10.1093/mutage/ges057

Rossner P Jr, Rossnerova A, Beskid O, Tabashidze N, Libalova H, Uhlirova K et al (2014a) Nonhomologous DNA end joining and chromosome aberrations in human embryonic lung fibroblasts treated with environmental pollutants. Mutat Res 763-764C:28–38. doi:10.1016/j.mrfmmm.2014.03.006

Rossner P Jr, Tulupova E, Rossnerova A, Libalova H, Gmuender H, Svecova V et al (2014b) Gene expression profiling in populations exposed to different levels of respirable air particles. Mutagenesis, Mutation Research (submitted)

Rossnerova A, Spatova M, Rossner P, Solansky I, Sram RJ (2009) The impact of air pollution on the levels of micronuclei measured by automated image analysis. Mutat Res 669(1–2):42–47. doi:10.1016/j.mrfmmm.2009.04.008, S0027-5107(09)00146-8 [pii]

Rossnerova A, Tulupova E, Tabashidze N, Schmuczerova J, Dostal M, Rossner P Jr et al (2013) Factors affecting the 27 K DNA methylation pattern in asthmatic and healthy children from locations with various environments. Mutat Res 741–742:18–26

Rubes J, Selevan SG, Evenson DP, Zudova D, Vozdova M, Zudova Z et al (2005) Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. Hum Reprod 20(10):2776–2783. doi:10.1093/humrep/dei122

Rubes J, Selevan SG, Sram RJ, Evenson DP, Perreault SD (2007) GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res 625(1–2):20–28. doi:10.1016/j.mrfmmm.2007.05.012

Rubes J, Rybar R, Prinosilova P, Veznik Z, Chvatalova I, Solansky I et al (2010) Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res 683(1–2):9–15. doi:10.1016/j.mrfmmm.2009.09.010, S0027-5107(09)00290-5 [pii]

Rusiecki JA, Baccarelli A, Bollati V, Tarantini L, Moore LE, Bonefeld-Jorgensen EC (2008) Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit. Environ Health Perspect 116(11):1547–1552. doi:10.1289/ehp.11338

Sandoval J, Heyn H, Moran S, Serra-Musach J, Pujana MA, Bibikova M et al (2011) Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome. Epigenetics 6(6):692–702

Sarnat JA, Schwartz J, Suh HH (2001) Fine particulate air pollution and mortality in 20 U.S. cities. N Engl J Med 344(16):1253–1254. doi:10.1056/NEJM200104193441614

Sessa R, Hata A (2013) Role of microRNAs in lung development and pulmonary diseases. Pulm Circ 3(2):315–328. doi:10.4103/2045-8932.114758

Sigurdson AJ, Ha M, Hauptmann M, Bhatti P, Sram RJ, Beskid O et al (2008) International study of factors affecting human chromosome translocations. Mutat Res 652(2):112–121. doi:10.1016/j.mrgentox.2008.01.005, S1383-5718(08)00019-3 [pii]

Slade PG, Williams MV, Brahmbhatt V, Dash A, Wishnok JS, Tannenbaum SR (2010) Proteins modified by the lipid peroxidation aldehyde 9,12-dioxo-10(E)-dodecenoic acid in MCF7 breast cancer cells. Chem Res Toxicol 23(3):557–567. doi:10.1021/tx9002808

Sram RJ, Rossner P, Smerhovsky Z (2004) Cytogenetic analysis and occupational health in the Czech Republic. Mutat Res 566(1):21–48. doi:S1383574203000346 [pii]

Sram RJ, Beskid O, Binkova B, Chvatalova I, Lnenickova Z, Milcova A et al (2007a) Chromosomal aberrations in environmentally exposed population in relation to metabolic and DNA repair genes polymorphisms. Mutat Res 620(1–2):22–33. doi:10.1016/j.mrfmmm.2007.02.019, S0027-5107(07)00100-5 [pii]

Sram RJ, Beskid O, Rossnerova A, Rossner P, Lnenickova Z, Milcova A et al (2007b) Environmental exposure to carcinogenic polycyclic aromatic hydrocarbons – the interpretation of cytogenetic analysis by FISH. Toxicol Lett 172(1–2):12–20

Sram RJ, Binkova B, Beskid O, Milcova A, Rossner P, Rossner P Jr et al (2011) Biomarkers of exposure and effect—interpretation in human risk assessment. Air Qual Atmos Health 4(3–4):161–167

Svecova V, Rossner P Jr, Dostal M, Topinka J, Solansky I, Sram RJ (2009) Urinary 8-oxodeoxyguanosine levels in children exposed to air pollutants. Mutat Res 662(1–2):37–43. doi:10.1016/j.mrfmmm.2008.12.003, S0027-5107(08)00317-5 [pii]

Tarantini L, Bonzini M, Apostoli P, Pegoraro V, Bollati V, Marinelli B et al (2009) Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. Environ Health Perspect 117(2):217–222. doi:10.1289/ehp.11898

Terry MB, Delgado-Cruzata L, Vin-Raviv N, Wu HC, Santella RM (2011) DNA methylation in white blood cells: association with risk factors in epidemiologic studies. Epigenetics 6(7):828–837

Thacker J, Zdzienicka MZ (2003) The mammalian XRCC genes: their roles in DNA repair and genetic stability. DNA Repair (Amst) 2(6):655–672

Tucker JD, Morgan WF, Awa AA, Bauchinger M, Blakey D, Cornforth MN et al (1995) A proposed system for scoring structural aberrations detected by chromosome painting. Cytogenet Cell Genet 68(3–4):211–221

Tuimala J, Szekely G, Gundy S, Hirvonen A, Norppa H (2002) Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity. Carcinogenesis 23(6):1003–1008

van Leeuwen DM, van Herwijnen MH, Pedersen M, Knudsen LE, Kirsch-Volders M, Sram RJ et al (2006) Genome-wide differential gene expression in children exposed to air pollution in the Czech Republic. Mutat Res 600(1–2):12–22. doi:10.1016/j.mrfmmm.2006.05.032

van Leeuwen DM, Gottschalk RW, Schoeters G, van Larebeke NA, Nelen V, Baeyens WF et al (2008a) Transcriptome analysis in peripheral blood of humans exposed to environmental carcinogens: a promising new biomarker in environmental health studies. Environ Health Perspect 116(11):1519–1525. doi:10.1289/ehp.11401

van Leeuwen DM, Pedersen M, Hendriksen PJ, Boorsma A, van Herwijnen MH, Gottschalk RW et al (2008b) Genomic analysis suggests higher susceptibility of children to air pollution. Carcinogenesis 29(5):977–983. doi:10.1093/carcin/bgn065

Vineis P, van Veldhoven K, Chadeau-Hyam M, Athersuch TJ (2013) Advancing the application of omics-based biomarkers in environmental epidemiology. Environ Mol Mutagen 54(7):461–467. doi:10.1002/em.21764

Wang Z, Neuburg D, Li C, Su L, Kim JY, Chen JC et al (2005) Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. Environ Health Perspect 113(2):233–241

Wild CP, Scalbert A, Herceg Z (2013) Measuring the exposome: a powerful basis for evaluating environmental exposures and cancer risk. Environ Mol Mutagen 54(7):480–499. doi:10.1002/em.21777

Wright WR, Parzych K, Crawford D, Mein C, Mitchell JA, Paul-Clark MJ (2012) Inflammatory transcriptome profiling of human monocytes exposed acutely to cigarette smoke. PLoS One 7(2):e30120. doi:10.1371/journal.pone.0030120

Wu MT, Pan CH, Huang YL, Tsai PJ, Chen CJ, Wu TN (2003) Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. Environ Mol Mutagen 42(2):98–105. doi:10.1002/em.10176

Wu LL, Chiou CC, Chang PY, Wu JT (2004) Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics [Review] [54 refs]. Clin Chim Acta 339(1–2):1–9

Wu HC, Delgado-Cruzata L, Flom JD, Kappil M, Ferris JS, Liao Y et al (2011) Global methylation profiles in DNA from different blood cell types. Epigenetics 6(1):76–85. doi:10.4161/epi.6.1.13391

Wu HC, Wang Q, Delgado-Cruzata L, Santella RM, Terry MB (2012) Genomic methylation changes over time in peripheral blood mononuclear cell DNA: differences by assay type and baseline values. Cancer Epidemiol Biomarkers Prev 21(8):1314–1318. doi:10.1158/1055-9965.EPI-12-0300

Xue W, Warshawsky D (2005) Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol 206(1):73–93. doi:10.1016/j.taap.2004.11.006, S0041-008X(04)00514-9 [pii]

Zijno A, Andreoli C, Leopardi P, Marcon F, Rossi S, Caiola S et al (2003) Folate status, metabolic genotype, and biomarkers of genotoxicity in healthy subjects. Carcinogenesis 24(6):1097–1103. doi:10.1093/carcin/bgg064, bgg064 [pii]

Chapter 8
Diabetes and Metabolic Syndrome

Xiaoquan Rao*, Cuiqing Liu*, and Sanjay Rajagopalan

8.1 Introduction

The International Diabetes Federation estimates that in the year of 2013, at least 371 million people have suffered from diabetes mellitus (DM), a number that is expected to reach 566 million by the year 2030. Over four million people died in 2012 as a consequence of suffering from diabetes and $471 billion were spent due to diabetes in 2012 alone. Thus, diabetes represents an uncontrolled global epidemic and is a leading cause of global morbidity and mortality (http://www.idf.org/fact-sheets/diabetes-cvd). Scientific efforts over the last several decades have been focused primarily on factors such as inactivity and diet. Although genetic factors are thought to play an important role, at least on the basis of genome-wide association studies, few candidate genes have emerged that would help explain the relatively high population prevalence of this condition today (Murea et al. 2012). Based on these findings one may conclude that either, non-genetic (predominantly environmental) factors may be important or the interaction of common genetic variants with pervasive environmental factors may ultimately help explain predisposition (Sears and Genuis 2012).

Air pollution due to outdoor and household sources represent major risk factors for chronic disease due to continued need for fossil fuels. The recent global burden

*Author contributed equally with all other contributors.

X. Rao, M.D., Ph.D. • S. Rajagopalan, M.D. (✉)
Division of Cardiovascular Medicine, Department of Medicine, University of Maryland School of Medicine, 110 S. Paca St., 7th floor, Room 7-N-100, Baltimore 21201, MD, USA
e-mail: srajagopalan@medicine.umaryland.edu

C. Liu, Ph.D.
Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH, USA

Department of Physiology, Medical College, Hangzhou Normal University, Hangzhou, Zhejiang, China

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_8

of disease (GBD) document provides evidence to support an important impact of air-pollution in global morbidity and mortality (Rajagopalan and Brook 2012). While this work represents the best estimate of the impact of risk factors such as air-pollution on human health, there is evidence that the cumulative burden and impact of air pollution may be much more profound (Rajagopalan and Brook 2012). One of the reasons for this is that air-pollution may also predispose to other risk factors such as hypertension and diabetes, which in turn may have direct effects of morbidity and mortality. Thus, through both direct effects on human health as well as through their impact on these risk factors, air pollution may have a much larger impact than previously recognized. This chapter will mainly discuss: (1) Epidemiology of air pollution-associated diabetes/metabolic syndromes; (2) Pathological studies on organ systems that provide insights into air pollution-mediated diabetes/metabolic syndromes; (3) Potential signaling molecules/receptors involved in the pathogenesis of air-pollution mediated diabetes/metabolic syndromes; and (4) Summary and future directions. In this study the majority of references are to type 2 diabetes (Type 2 DM) unless otherwise specified.

8.2 Epidemiology of Outdoor Air Pollution-Associated Diabetes/Metabolics Syndrome

8.2.1 Studies Linking Air-Pollution with Prevalence of Diabetes

An interesting question is whether the growing increase in prevalence of diabetes in low and middle income countries (4/5 individuals with diabetes now resides in these countries) represents the influence of pervasive levels of air-pollutants in these countries. Based on a World Health Organization (WHO) database that reviewed outdoor air pollution data in >1,100 cities in 91 countries, all of the top countries for particulate matter (PM) air pollution in the world belong to low and middle income areas with rapidly urbanizing populations. The mean annual average value for PM <2.5 μg mass ($PM_{2.5}$) in the top 10 countries was roughly fivefold higher than the U.S. National Ambient Air Quality Standard of 15 μg/m^3 and the WHO standard of 10 μg/m^3. Thus, even small associations between air-pollution and diabetes may translate into larger numbers given the pervasive nature of air pollutants globally (Brook et al. 2010). Conversely, even modest reductions in air-pollution may translate into substantial reductions in the prevalence of DM and consequent morbidity and mortality secondary to diabetes. An association between particulate matter and/or traffic-related air pollutants and type 2 DM has been demonstrated in several epidemiological studies (Table 8.1). One of the first studies to document an association was a study that utilized the Ontario health insurance plan database, to explore a potential association between DM prevalence and air pollution (Brook et al. 2008). The exposure assessment in this study was based on field measurements and land use regression models capable of predicting fine scale variation of NO_2 levels, as a

Table 8.1 Epidemiological associations between air pollutants, diabetes, and insulin resistance

Location	Subject number	Main pollutants	Principal findings	Publish year
Studies related to diabetes prevalence or incidence				
Ontario, Canada	7,634 subjects	NO_2	OR for DM prevalence (1.04; 95 % CI 1.00–1.08) increased the in women per 1 ppb NO_2. No significant association in men	2008 (Brook et al. 2008)
Ruhr, Germany	1,775 nondiabetic women	NO_2; PM_{10} (mean: 47 μg/m^3)	Adjusted HR for developing DM over mean 16 years ranged from 1.15 to 1.42 per IQR increase in PM_{10} or in relation to traffic-exposures or NO_2	2010 (Kramer et al. 2010)
United States	Unknown, estimated by county level	$PM_{2.5}$	Adjusted DM prevalence associated with $PM_{2.5}$ at county-level (1 % increase per 10 μg/m^3) and persistently associated with $PM_{2.5}$ at counties below current annual standards ($PM_{2.5}$ < 15 μg/m^3)	2010 (Pearson et al. 2010)
United States	NHS: 74,412 subjects	$PM_{2.5}$, PM_{10}, $PM_{10-2.5}$	Most pollutants not significantly associated with increased HR for developing DM in adjusted model	2011 (Puett et al. 2011)
	HPFS: 15,048 subjects	Mean $PM_{2.5}$: 17.5–18.3 μg/m^3		
	Non-diabetes subjects			
Los Angeles, USA	3,992 women	NO_2 and $PM_{2.5}$	NO_2 exposure associated with 10 year incidence of DM (adjusted HR 1.25; 95 % CI 1.07–1.46). $PM_{2.5}$ not related	2012 (Coogan et al. 2012)
Denmark	51,818 nondiabetic participants	NO_2 level (IQR: 4.9 μg/m^3)	NO_2 not related to all new DM cases over mean 9.7 years. Positive associations with confirmed DM (1.04; 95 % CI 1.00–1.08) with larger effects in nonsmokers and active people	2012 (Andersen et al. 2012)
Ontario, Canada	62,012 nondiabetic adults	$PM_{2.5}$	The adjusted hazard ratio for a 10 μg/m^3 increase in $PM_{2.5}$ was 1.11 (95%CI: 1.02, 1.21)	2013 (Chen et al. 2013)
Studies related to markers of insulin sensitivity				
Iran	374 children	Mean PM_{10}: 150 μg/m^3	HOMA-IR increased by 1.1 adjusted for other health parameters in relation to PM_{10} levels	2009 (Kelishadi et al. 2009)

(continued)

Table 8.1 (continued)

Location	Subject number	Main pollutants	Principal findings	Publish year
Taiwan	1,023 elderly adults	Mean PM_{10}: 35 μg/m^3	Significant associations with increases in fasting glucose and HbA1c level with PM_{10}	2011 (Chuang et al. 2011)
Michigan, USA	25 subjects	Mean $PM_{2.5}$: 11.5 μg/m^3	Increased HOMA-IR (0.7, 95 % CI 0.1–1.3) with a 10 μg/m^3 $PM_{2.5}$ exposure	2013 (Brook et al. 2013a)
German	397 children	Mean PM_{10}: 21.2 μg/m^3; Mean $PM_{2.5}$: 14 μg/m^3; Mean NO_2: 21.7 μg/m^3	HOMA-IR increased 17 % in related to NO_2; 18.7 % in PM_{10} levels. HOMA-IR also increased in related to $PM_{2.5}$ without statistical significance	2013 (Thiering et al. 2013)
Beijing, China	50 subjects	BC (mean: 7.2 μg/m^3) and $PM_{2.5}$ (mean: 150.6 μg/m^3)	Minimum average detectable effect of HOMA-IR is 2.81 with 10 μg/m^3 PM and 1 μg/m^3 black carbon	2013 (Sun et al. 2013)
Studies related to diabetes-associated mortality				
Canada	2.1 million adults	Mean $PM_{2.5} \cong 8.7$ μg/m^3	The adjusted HR of diabetes-associated mortality for a 10 μg/m^3 $PM_{2.5}$ was 1.49 (5 % CI: 1.37, 1.62)	2013 (Brook et al. 2013b)
Danish	52,061 subjects	NO_2 (mean 16.9 μg/m^3)	Mean levels of NO_2 at the residence were significantly associated with mortality from diabetes	2013 (Raaschou-Nielsen et al. 2013)
Montreal, Canada	158,350 elderly adults	$PM_{2.5}$ (mean 8.74 μg/m^3), NO_2 (mean 37.90 μg/m^3), O_3, CO and SO_2	Positive associations were found between daily non-accidental mortality and all air pollutants but O_3	2013 (Goldberg et al. 2013)

CI confidence interval, *DM* diabetes mellitus, *HOMA-IR* homeostasis model assessment of insulin resistance, *HPFS* health professionals follow-up study, *HR* hazard ratio, *IQR* interquartile range, *LUR* land use regression, *NHS* nurses' health study, NO_2 nitrogen dioxide, *OR* odds ratio, PM_{10} particulate matter with diameter < 10 μm, $PM_{2.5}$ particulate matter with diameter < 2.5 μm, $PM_{10-2.5}$ particulate matter with diameter between 2.5 and 10 μm

surrogate for traffic related air pollution. After adjusting for age, BMI and income, a positive relationship between NO_2 exposure and DM prevalence (OR 1.04; 95 % CI: 1.00–1.08) was seen in women but not in men. Another cohort study conducted by Chen et al suggested that long-term $PM_{2.5}$ might contribute to the development of diabetes (Chen et al. 2013). After adjusting for covariates such as smoking, body mass index, physical activity and neighborhood-level household income in Cox proportional hazards models, the adjusted hazard ratio for a 10-ug/m3 increase in $PM_{2.5}$ was 1.11 (95 % CI: 1.02, 1.21) (Chen et al. 2013). Pearson et al. conducted a cross-sectional ecological study using data obtained from the centers of disease control (CDC), in which the relationship between $PM_{2.5}$ levels and diabetes prevalence in the US was assessed by multivariate regression models (Pearson et al. 2010). A significant association between $PM_{2.5}$ levels and diabetes prevalence was revealed after adjustment for multiple co-variates including exclusion of minority populations that have a high prevalence of DM. One important aspect of the analysis was that the findings remained significant even when restricted to counties with $PM_{2.5} < 15$ μg/m^3 (annual US EPA limit). This finding suggests that the relationship seems to extend to levels considered admissible at least from the perspective of regulatory standards in effect in the US. In the Danish Diet Cancer and Health Cohort, of 57,053 subjects of whom 51,818 were eligible and followed-up for 9.7 years, NO_2 levels (a surrogate for traffic related air-pollution) were associated with DM on multivariate models adjusted for co-variates (Andersen et al. 2012). In another cohort study in Europe, comprising of 1,775 women aged from 54 to 55 years without diabetes, the hazard for diabetes was increased by 15–42 % per interquartile range of PM_{10} or NO_2 levels (used as a proxy of traffic-related exposure) over 16 years. The associations remained robust after adjusting for age, body mass index, socioeconomic status, and exposure to several non-traffic-related sources of air pollution and persisted when different spatial scales were used to assess exposure (Kramer et al. 2010). Sensitivity analyses indicated that women with high blood C3c levels (a complement fragment) were more susceptible for PM-related risk of diabetes than those with low C3c levels. Interesting results from African American women living within the greater Los Angeles area, have also shown an association between new onset DM and long-term exposure to NO_2 (IRR 1.25; 95%CI 1.07–1.46), a metric of traffic-related pollution (Coogan et al. 2012). On the other hand, the pooled analyses of the Nurses' Health Study and the Health Professionals' follow-Up showed less compelling findings at least for traffic related air-pollution (Puett et al. 2011). DM incidence was only modestly associated with proximity of residence location to roadways while multiple other exposure metrics were not significant.

8.2.2 Studies Linking Air-Pollution with Glycemic Control and Insulin Resistance

There are a growing number of prospective studies that have demonstrated a link between insulin resistance and air-pollution. In a study in Iranian children living in cities exposed to extremely high PM_{10} levels (averaging rough 150 μg/m^3), after adjustment for age, gender, body mass index, waist circumference, healthy eating index and physical activity, prior 7-day exposure to particulate air pollution was associated with insulin resistance and plasma markers of inflammation (Kelishadi et al. 2009). In another study conducted in Taiwan, long-term exposure to fine particles (mean level ≅ 35 μg/m^3) was associated with elevations in HbA1c among 1,023 elderly individuals (Chuang et al. 2011). Recently, a prospective cohort study in Beijing also revealed a positive relation between air pollution and insulin resistance measures. The mean level of $PM_{2.5}$ in Beijing is 150.6 μg/m^3 and black carbon is 7.2 μg/m^3. An increase of 10 μg/m^3 PM and 1 μg/m^3 black carbon is sufficient to result in a detectable effect on Homeostasis Model Assessment of insulin resistance (HOMA-IR) (Sun et al. 2013). Importantly, the association remained positive in studies with relatively normal or even low levels of air pollution (PM_{10} ≅ 21.2 μg/m^3; $PM_{2.5}$ ≅ 14 μg/m^3). Thiering et al. collected fasting blood samples from 397 children in two prospective German birth cohort studies. The investigators, demonstrated an association between air-pollution measures and insulin resistance with a per 2SD increase in traffic-related air pollutant being associated with increases in HOMA-IR [increase of 17.0 % with NO_2 (95 % CI 5.0, 30.3); increase of 18.7 % with PM_{10} (95 % CI 2.9, 36.9)]. Effect estimates for $PM_{2.5}$ (22.5 %, 95 % CI 0.9, 51.5) were elevated, but the difference did not reach statistical significance ($p = 0.062$) (Thiering et al. 2013). Brook et al. conducted a sub-acute ambient-level exposure prospective cohort study on 25 healthy adults for five consecutive days of daily 4–5-h long ambient air pollution exposure. HOMA-IR was measured at three time points: 7 days prior to exposure, on the last exposure-day and 7 days after completion. A 10 μg/m^3 increase in sub-acute $PM_{2.5}$ exposure was associated with increased HOMA-IR [$\beta = +0.7$, 95 % confidence interval (CI) 0.1–1.3; $p = 0.023$], suggesting that even low levels of $PM_{2.5}$ (mean level ≅ 11.5 μg/m^3) may reduce metabolic insulin sensitivity (Brook et al. 2013a).

8.2.3 Studies Linking Air Pollution and DM Morbidity/Mortality

There are relatively few studied that have examined the relationship between air pollution and diabetes-associated mortality. In a study in the Boston area, residents participating in multiple pharmaceutical studies that were pooled together, 6-day moving averages of four particle metrics ($PM_{2.5}$, particle number, black carbon, and sulfates) were associated with decreased vascular reactivity among patients

with diabetes but not those without diabetes (O'Neill et al. 2005). Interquartile range increases in sulfate were associated with decreased flow-mediated dilation and nitroglycerin-mediated vascular reactivity among those with diabetes. Black carbon increases were associated with decreased flow-mediated vascular reactivity, and $PM_{2.5}$ was associated with reduced nitroglycerin-mediated reactivity. The effects of air-pollutants were stronger in type 2 DM than type I diabetes (O'Neill et al. 2005). Brook et al. found long term-exposure to $PM_{2.5}$, even at low levels, is related to an increased risk of mortality attributable to diabetes. These authors evaluated 2.1 million adults from the 1991 Canadian census mortality follow-up study. An increase in risk for diabetes-related mortality (HR, 1.49; 95 % CI, 1.37–1.62) was associated with a 10 $\mu g/m^3$ elevation in $PM_{2.5}$ exposure in fully adjusted Cox proportional hazards survival models (Brook et al. 2013b). Raaschou-Nielsen et al. followed up 52,061 participants in the Danish Diet, Cancer and Health cohort for diabetes-related mortality and traced their residential addresses since 1971. Mean levels of NO_2, an indicator of traffic-related air pollution, were significantly associated with mortality of diabetes. Exposure to NO_2 above 19.4 $\mu g/m^3$ was associated with a mortality-rate ratio of 2.15 (95 % CI 1.21, 3.83) per 10 $\mu g/m^3$ NO_2 after adjustment for potential confounders (Raaschou-Nielsen et al. 2013). A study conducted in Montreal, Quebec, among persons 65 years of age and older between 1990 and 2003 to assess daily mortality change associated with air pollution found a positive association among people having diabetes and total mortality (mean percent change in daily mortality = 3.45 with air pollution) (Goldberg et al. 2013).

In summary, these studies demonstrate a link between air-pollution exposure and susceptibility to Type II DM as well as diabetes-associated mortality. The varying associations noted between studies may be related to a range of factors that may differ across and within studies. These include differences in population characteristics, risk factors, individual susceptibilities, robustness of the cohort data and the absolute prevalence/incidence rates of DM, technical aspects of the exposure assessment methodologies, pollution types/sources, and the degree and duration of air-pollution exposures. The sex-specific differences in some of these studies may relate to differences in biologic susceptibility.

8.3 Insights from Studies on Organ Systems in Air Pollution-Mediated Diabetes/Metabolic Syndrome

In addition to epidemiologic evidence, a variety of animal studies also confirmed the role of air-pollution in insulin resistance and diabetes (Table 8.2). The prevailing view of DM as a consequence of inadequate synthesis of the peptide insulin has now been replaced by a highly complex view, where there are defects in a multitude of organ systems that are all concerned with the regulation of glucose and in the interpretation of insulin action. Glucose homeostasis is achieved through a balance of input (e.g., dietary, gluconeogenesis) and tissue uptake/utilization coordinated by

Table 8.2 Associations between air pollutants and insulin resistance in mice in last 5 years

Duration	Models	Main pollutants	Principal findings	Publish year
24 weeks	C57BL/6 mice on HFD	*$PM_{2.5}$ (72.7 μg/m^3)	$PM_{2.5}$ exposed mice have exaggerated systemic inflammation, enlarged adipose tissue and increased whole-body insulin resistance	2009 (Sun et al. 2009)
10 weeks	C57BL/6 mice	*$PM_{2.5}$ (74.6 μg/m^3)	$PM_{2.5}$ exposure induces both oxidative stress and ER stress in mouse lung and liver tissues through PERK/eIF2α/ATF6/ IRE1α-mediated UPR pathway	2010 (Laing et al. 2010)
10 weeks	C57BL/6 & p47 (phox−/−) mice on HFD or NCD	*$PM_{2.5}$ (111.0 μg/m^3)	$PM_{2.5}$ increases fat content, macrophage infiltration in adipose tissue and vascular function in young mice. P47 (phox) deficiency improves insulin resistance in response to $PM_{2.5.}$	2010 (Xu et al. 2010)
10 months	C57BL/6 mice	*$PM_{2.5}$ (94.4 μg/m^3)	$PM_{2.5}$ induces IR in adipose tissue, liver and skeletal muscle possibly through increased cytokine, mitochondrial alteration, oxidation and decreased Akt phosphorylation	2011 (Xu et al. 2011a)
2 months	ApoE−/− mice	*$PM_{2.5}$ (96.89 μg/m3)	$PM_{2.5}$ resulted in an increase of superoxide production and decreases of mitochondrial number and UCP1 expression in WAT and BAT. WAT-specific genes and BAT-specific genes are also abnormal	2011 (Xu et al. 2011b)
3 or 10 weeks	C57BL/6 mice	*$PM_{2.5}$ (74.6 μg/m^3)	$PM_{2.5}$ induces a NASH-like phenotype and impairs hepatic glucose metabolism after 10 weeks exposure	2013 (Zheng et al. 2013)
17 weeks	C57BL/6 & CCR2−/− mice on HFD	*$PM_{2.5}$ (116.9 μg/m^3)	$PM_{2.5}$ exposure increase whole body IR and hepatic lip accumulation. CCR2−/− partially rescued the effect of $PM_{2.5.}$	2013 (Liu et al. 2013b)
10 months	C57BL/6 mice	*$PM_{2.5}$ (94.4 μg/m^3)	$PM_{2.5}$ induces macrophage infiltration, unfolded protein response, and lipid deposition in white adipose tissue	2013 (Mendez et al. 2013)

*$PM_{2.5}$ concentrated particulate matter compared to ambient air with diameter < 2.5 μm, *HFD* high-fat diet, *NCD* normal chow diet, *CCR2* C-C chemokine receptor type 2, *NASH* non-alcoholic steatohepatitis, *WAT* white adipose tissue, *BAT* brown adipose tissue, *HOMA-IR* homeostasis model assessment of insulin resistance, *ER* endoplasmic reticulum, *UPR* unfolded protein response, *IR* insulin resistance

the production of insulin by the beta cell (Saltiel and Kahn 2001; Taniguchi et al. 2006; Qatanani and Lazar 2007). Three target tissues are of primal importance with regards to the effects of insulin: fat, liver, and skeletal muscle (Saltiel and Kahn 2001). These organs represent the ability of the body to store, produce, and dispose glucose respectively with the interaction of these sites synergistically required for glucose homeostasis. As the major sites where insulin executes its metabolic action, these organs are also the primary determinants of insulin resistance (Qatanani and Lazar 2007; Kahn and Flier 2000; Shulman 2000). However, insulin resistance in each organ manifests itself clinically in very different ways, although they share the base effect of insulin action. For example, hepatic insulin resistance is responsible for elevated hyperglycemia, due to the inability of insulin to suppress hepatic gluconeogenesis while lipid biosynthesis remains intact (Brown and Goldstein 2008). In contrast, insulin resistance in adipose tissue and skeletal muscle manifests as elevated lipolysis and glucose intolerance, respectively, resulting in hyperlipidemia, hyperglycemia, and compensatory hyperinsulinemia (Kahn and Flier 2000; Shulman 2000). With increasing insulin resistance in the liver and skeletal muscle, blood glucose concentrations progressively rise, which, in turn, leads to compensatory and progressive hyperinsulinemia. This eventually leads to β-cell exhaustion, an inability to synthesize insulin and florid type 2 DM (Kahn and Flier 2000; Shulman 2000). Consistent with this temporal progression of type 2 DM, the onset of frank DM is preceded by years or even decades when glucose control is more or less maintained with HbA1c within normal limits. During this phase however, abnormalities in glucose in response to a meal (impaired glucose tolerance, IGT) or fasting abnormalities in glucose may be evident (impaired fasting glucose, IFG). Considerable effort has been expended over the last decade to define the mechanisms and origins of IR. These determinants can be classified broadly into intrinsic and extrinsic factors. Generally speaking, cell-intrinsic factors include ER stress, oxidative stress, mitochondrial dysfunction, intracellular lipid deposition/imbalance, and anabolic demand. In contrast, extrinsic factors modulating peripheral insulin signaling comprise alterations in circulating inflammatory cytokines, adipokines and serum fatty acid composition (Qatanani and Lazar 2007). Despite their biological diversity, a striking majority of these determinants converge on the common pathway of inflammation. For instance, inflammatory cytokines, saturated fatty acids, hypoxia, and ER stress converge on inhibitor of nuclear factor-κB (NF-κB), kinase-β (IKKβ), and Jun kinase (JNK). Those pathways directly inhibit insulin action via serine phosphorylation of insulin receptor substrate 1 and 2 (IRS-1 and IRS-2). Moreover, activation of NF-κB and AP-1 transcription factors by these kinases further induces transcription of inflammatory cytokines and establishes an autocrine/paracrine feed-forward loop of inflammation. Thus metabolic inflammation functions as a common pathway that drives insulin resistance. The emerging view of air-pollution is that it functions as a risk factor that promotes inflammation and in this sense may function similar to other risk factors such as high-calorie diets, physical inactivity etc. In the following paragraphs we will review the evidence linking inflammation in response to exposure as a potential causal mechanism linking air-pollution exposure with T2DM.

8.3.1 *Visceral Adipose Tissue (VAT) Inflammation and Oxidative Stress*

Macrophages function as sentinels of the innate immune system, and are responsible for sensing, integrating, and responding to a multitude of stimuli in their external environment. Macrophage exhibit two distinct response patterns designated as classical (M1) and alternative (M2) activation (Gordon 2003). Classically activated M1 macrophages are short-lived, highly inflammatory and have potent bactericidal potential, whereas alternatively activated M2 cells are associated with enduring and regulatory/reparative responses (Martinez et al. 2009; Odegaard and Chawla 2011). M1 cells secrete large amounts of pro-inflammatory cytokines (e.g., IL-1β, IL-6, IL-12, TNFα), express high level of costimulatory molecules that are important in T-cell activation (e.g., MHC, CD40, CD86), and produce bactericidal mediators, such as nitric oxide, via iNOS. Conversely, M2 macrophages have a distinct secretory phenotype (e.g., IL-10, TGFβ,), express numerous pattern recognition receptors (e.g., mannose receptor, dectin, CD301), and metabolize arginine to produce biosynthetic precursors (e.g., polyamines, proline) via arginase 1. Type 2 DM in humans and animal models is associated with increased levels of recruitment and/or activation of innate immune cells in visceral adipose depots. In an important study by Sun et al, exposure to concentrated airborne particulates (CAP, primarily $PM_{2.5}$) using a versatile aerosol concentration enrichment system (VACES) in conjunction with high-fat diet (HFD) was evaluated in C57Bl/6 mice for duration of 128 days. The mice were pre-fed with HFD for 10 weeks prior to exposure. Exposure to CAP resulted in an increase in adipose tissue macrophages with a shift to a pro-inflammatory phenotype characterized by an increase of total macrophages in the visceral adipose and a pro-inflammatory "M1 phenotype" typified by TNFα, IL-6 and a decrease in IL-10, MgI1 gene expression (Sun et al. 2009). In mechanistic experiments in the same study, conducted to understand whether, the increase in adipose tissue macrophages, represents a consequence of increased recruitment, the effects of intra-tracheally delivered $PM_{2.5}$ was studied in a transgenic model of yellow-fluorescent protein expression restricted to monocytes (*c-fmsYFP*). These mice were fed high-fat diet to induce insulin resistance and under these circumstances, $PM_{2.5}$ exposure resulted in a doubling in the number of endothelial adherent YFP^+ cells in mesenteric fat with a sixfold increase in monocytes within adipose (Sun et al. 2009). Thus $PM_{2.5}$ facilitated migration and adhesion of YFP^+ cells into fat depots. In subsequent experiments Xu et al investigated the effects of early exposure to $PM_{2.5}$ exposure (at age of 3 weeks) on development of insulin resistance and compared and contrasted the effects of both normal diet (ND) and high-fat diet (HFD) administered in conjunction with $PM_{2.5}$ exposure. $PM_{2.5}$ exposure resulted in significant increase in glucose levels in mice on ND by an intraperitoneal glucose tolerance test (Xu et al. 2011a). $PM_{2.5}$ exposure in ND-fed mice led to elevations in homeostasis model assessment index-insulin resistance (HOMA-IR) and TNF-α compared with the FA-exposed mice (Xu et al. 2011a). HFD (regardless of FA or $PM_{2.5}$ exposure) significantly increased HOMA-IR and TNF-α. Adipocyte size was

increased in the $PM_{2.5}$-exposed mice fed an ND in both visceral fat (FA, $2{,}137 \pm 45\ \mu m^2$; $PM_{2.5}$, $2{,}698 \pm 80\ \mu m^2$; $P < 0.01$) and subcutaneous fat (FA, $1{,}039 \pm 27\ \mu m^2$; $PM_{2.5}$, $1{,}355 \pm 30\ \mu m^2$; $P < 0.05$) with corresponding increases in visceral fat content with $PM_{2.5}$ exposure in the ND group. The increase in adipocyte size was extreme in the HFD group alone, not permitting assessment of further increase due to $PM_{2.5}$ exposure. In light of the importance of oxidative stress mechanisms in driving inflammation and IR in visceral fat, we tested the concept that one of the major sources of reactive oxygen species, the nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) system may play a role in the pathogenesis of IR in response to $PM_{2.5}$ exposure. Age-matched male, wild-type C57BL/6 mice and mice deficient in a critical cytosolic sub-unit (p47phox) of NADPH oxidase ($p47phox^{-/-}$) were exposed to $PM_{2.5}$ or FA. The HOMA-IR index from $p47phox^{-/-}$ mice exposed to $PM_{2.5}$ was significantly attenuated and comparable to those of the mice exposed to FA. Plasma inflammatory biomarkers in the $p47phox^{-/-}$ mice and the wild-type C57BL/6 mice were similar. Notably, the previously noted difference in TNF-α in wild-type C57BL/6 exposed to $PM_{2.5}$ compared to FA, was blunted by the absence of a functional NADPH oxidase (Xu et al. 2010). In both visceral and subcutaneous fat of $p47phox^{-/-}$ mice fed an ND, adipocyte sizes were similar when the mice were exposed to $PM_{2.5}$ or FA. $PM_{2.5}$ exposure alone (normal chow diet) resulted in a heightened chemotactic ability of adipose tissue in in-vitro studies (Xu et al. 2010). A recent study by Mendez also suggested chronic $PM_{2.5}$ exposure induced macrophage infiltration and lipid deposition into white adipose tissue consistent with these earlier findings (Mendez et al. 2013).

8.3.2 The Role of Endoplasmic Reticulum (ER) and Oxidative Stress in Liver, Lung, and Adipose Tissue

Endoplasmic reticulums (ER) stress represents an evolutionarily conserved pathway and is designed to alleviate protein misfolding in response to diverse cellular stressors. This pathway is also referred to as the unfolded protein response (UPR) (Walter and Ron 2011). ER stress has been demonstrated to represent an important pathway that may contribute to the pathogenesis of IR in liver and white adipose tissue and may represent a pathophysiological mechanism linking PM exposure with insulin resistance. The UPR is known to intersect with a variety of inflammatory and stress-signaling systems such as the NF-κB and c-Jun N-terminal kinase (JNK) pathways as well oxidative stress responses, all of which may influence lipid and glucose metabolism. Further, insights on how ER stress is regulated by $PM_{2.5}$ may potentially provide insights into how an inhaled stressor could mediate systemic effects via activation of the immune system. ER stress in the lung for instance has been demonstrated with several inhaled environmental triggers, like cigarette smoke, diesel exhaust, or allergens, which cause a dysregulation in ER homeostasis (Osorio et al. 2013). However, there appears to be endogenous mechanisms that prevent

activation of this response from inducing deleterious end-organ effects such as fibrosis. For instance, recent studies have highlighted the importance of ER stress in combination with additional cellular stressors in activation of the innate immune response in the lung. Mice expressing a mutated form of surfactant C develop ER stress but required an additional trigger to induce fibrosis in the lung suggesting a facilitative role of dysfunctional surfactant in induction of ER stress as well as the need for additional triggers for activation of inflammatory response and fibrosis (Lawson et al. 2011). Laing et al demonstrated that UPR-associated proteins ATF4 (activating transcription factor 4), heat shock protein (Hsp70), Hsp90, and binding immunoglobulin protein (BiP) significantly increase in cultured human bronchial epithelial cells exposed to $PM_{2.5}$ (Laing et al. 2010). GRP94 (glucose regulatory peptide 94) and BiP were increased in lungs, liver and white adipose tissue of mice (but not aorta and spleen) exposed to concentrated $PM_{2.5}$ (Laing et al. 2010), indicating activation of the ATF6 (activating transcription factor 6), a key sensor and regulator of ER stress in these organs (Laing et al. 2010). The other 2 key proximate sensors of ER stress include IRE1a (Inositol Requiring 1a), PERK (double-stranded RNA-activated protein kinase-like ER kinase). Phosphorylation of PERK and eIF2a was increased in the liver along with induction of C/EBP homologous transcription factor CHOP/GADD153 (Laing et al. 2010). The latter is associated with apoptosis in the lung and liver. Indeed Laing et al demonstrated evidence of increased apoptosis (Laing et al. 2010). Mendez et al recently reported that IRE1α is activated in the white adipose tissue during chronic exposure to $PM_{2.5}$ (Mendez et al. 2013). The activation of ER stress in adipose tissue may represent a molecular mechanism by which air pollutants induce adipocyte hypertrophy and macrophage infiltration in adipose tissue (Mendez et al. 2013). The studies by Laing et al also demonstrate a critical proximate role for NADPH oxidase in activation of the ER stress response (Laing et al. 2010). In these studies, cultured cells (RAW264.7) overexpressing Mn-SOD or dominant negative N17Rac1 (a cytosolic sub-unit of the macrophage NAPDH oxidase) were exposed to in-vitro $PM_{2.5}$. $PM_{2.5}$ exposure increased levels of phosphorylated eIF2α, CHOP, and GADD34 in the control RAW264.7 cells, while these levels were suppressed with cells expressing Mn-SOD or dominant negative N17Rac1 indicating that ROS, produced through mitochondrial electron transport and/or NADPH oxidase pathways, is critical for the activation of $PM_{2.5}$-induced UPR pathway. The factors involved in the ER stress are summarized as Fig. 8.1.

In subsequent experiments, Zheng et al demonstrated that $PM_{2.5}$ exposure resulted in a non-alcoholic steatohepatitis (NASH)-like phenotype characterized by hepatic steatosis, lobular and portal inflammation, depletion/redistribution of glycogen levels of and perisinusoidal fibrosis in the liver of mice exposed to $PM_{2.5}$ for 10 weeks. Down-regulation of the IRS1-mediated signaling and peroxisome proliferator-activated receptor (PPARγ2) expression in the liver was observed with these changes being associated with increases in HOMA-IR and elevated glucose following IP-GTT challenge (Zheng et al. 2013). $PM_{2.5}$ exposure led to activation of the JNK pathway with elevated levels of phosphorylated JNK in the liver of mice exposed to $PM_{2.5}$. In in-vitro studies, $PM_{2.5}$ activated AP-1, the downstream transactivator regulating the expression of pro-inflammatory cytokines under JNK control.

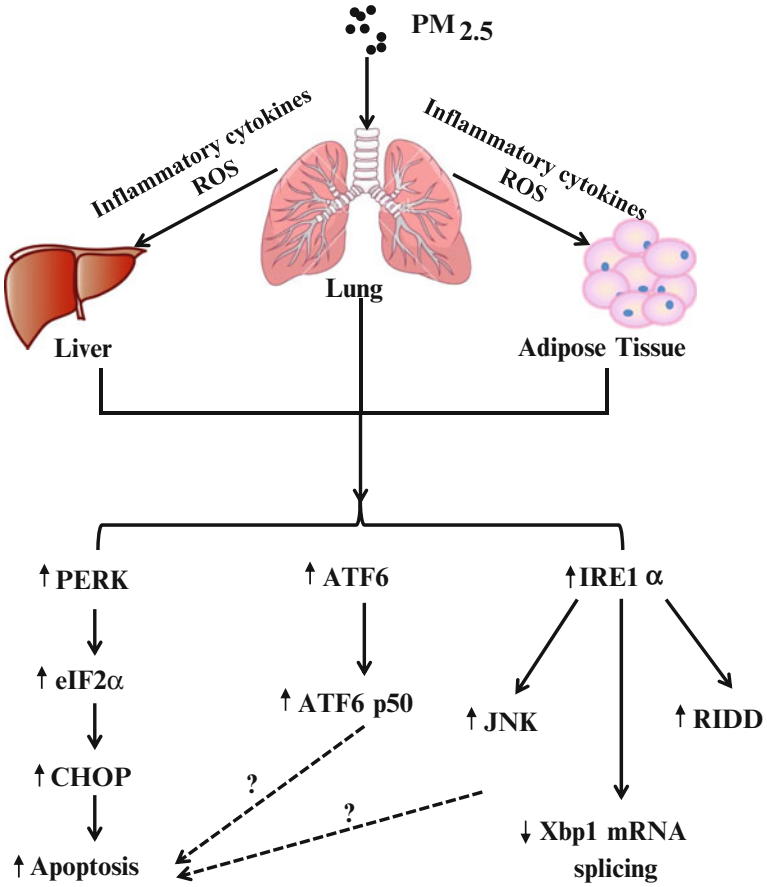

Fig. 8.1 ER stress signal pathways involved in air pollution. Inhaled $PM_{2.5}$ results in the activation of PERK, ATF6, and IRE1α. These three key ER stress pathways subsequently induce the adverse effects of air pollution

Interestingly in this study, inhibition of JNK activation with a chemical JNK inhibitor was not able to overcome enhanced IL-6 expression in response to $PM_{2.5}$ suggesting that other inflammatory triggers may be involved. Indeed TLR2, TLR4 expression in the liver was also enhanced concomitant with elevated levels of phosphorylated JNK in the liver of $PM_{2.5}$-exposed mice (Zheng et al. 2013). While the tissue distributions of the pattern recognition receptors were not discerned in this work, it is tempting to speculate that activated monocytes may be the source of heightened levels of TLR4 and TLR2. Collectively these studies suggest activation of multiple inflammatory cascades at least in the liver that may contribute to insulin resistance. The mechanisms through which inflammatory recruitment to the liver and adipose tissue will be discussed in the section on signaling pathways of importance.

8.3.3 Brown Adipose Tissue (BAT) Dysfunction

Mitochondrial dysfunction is a key abnormality in Type 2 DM. Defective fatty acid metabolism through β-oxidation in mitochondria results in the accumulation of intracellular metabolites such as fatty-acyl CoA diacylglycerol and ceramide in both skeletal muscle and liver (Lowell and Shulman 2005). Multiple abnormalities in mitochondrial rich BAT have been noted in C57BL/6 mice in response to long durations (10 months) of $PM_{2.5}$ exposure and in $ApoE^{-/-}$ mice over shorter durations (2 months) (Xu et al. 2011a, b). Visible decreases in BAT mass and decreased mitochondrial size in BAT depots were observed in long term $PM_{2.5}$ exposure compared to FA (Xu et al. 2011b). These changes were accompanied by increased oxidative and nitrosative stress in BAT, along with Phase II antioxidant gene induction including NF-E2-related factor 2 (Nrf2), NAD(P)H quinone oxidoreductase 1 and glutamate-cysteine ligase modifier subunit. Long-term $PM_{2.5}$ exposure significantly decreased the mRNA levels of the brown adipocyte-specific genes Ucp1, Prdm16, Pgc-1α, and Pparγ2 in the WAT, although no significant differences in C/EBPβ, Cidea, Dio2, or Elovl3 between the PM2.5 and FA were noted. $PM_{2.5}$ exposure significantly reduced mRNA levels of Ucp1 and Pgc-1α in BAT, although no significant changes in the other brown adipocyte-specific genes between the two groups (Xu et al. 2011b). Consistent with the decrease in Ucp1 gene expression, Ucp1 protein was also reduced in the BAT of $PM_{2.5}$ exposed animals (Xu et al. 2011b). In subsequent studies, our group also noted that $PM_{2.5}$ exposure decreased O_2 consumption and heat production in a genetic KKay diabetic model (unpublished observations). Taken together, these data demonstrate that alterations in BAT may account for key alterations metabolism in response to $PM_{2.5}$ exposure.

8.3.4 Central Nervous System as an Integrator of Metabolic Effects with PM

Inflammation in key regions of the hypothalamus as a mediator of peripheral abnormalities in glucose homeostasis and energy imbalance has been reported by a number of groups (Thaler et al. 2013; De Souza et al. 2005; Zhang et al. 2008). Thaler et al have reported hypothalamic inflammatory signaling as evidenced by up regulation of IL-6 and nuclear factor κB (NF-κB) very early on (within days) prior to substantial weight gain in rodent models of HFD feeding. Furthermore, both reactive gliosis and inflammatory markers suggested neuronal injury in the hypothalamic arcuate nucleus as early as the first week of high-fat feeding (Thaler et al. 2012). In additional studies with rodent models of diet-induced obesity, increased inflammatory signaling in the mediobasal hypothalamus has been demonstrated and appears to play a role in the genesis of peripheral inflammation and altered energy homeostasis (Thaler et al. 2012; Posey et al. 2009; Ryan et al. 2012). Enhanced expression and activation of hypothalamic IKKβ/NF-κB in obesity have been

reported in both leptin-deficient ob/ob mice and high fat diet-induced obese animals with inhibition of IKKb ameliorating insulin resistance and obesity (Zhang et al. 2008). Purkayastha et al have demonstrated an important role for ER stress in the hypothalamus in the induction of peripheral inflammation and glucose abnormalities. Obesity-associated metabolic and blood pressure disorders were partially reversed by interruption of ER stress with tauroursodeoxycholic acid (TUDCA) (Purkayastha et al. 2011). These findings may provide potential cues regarding central pathways that may regulate peripheral metabolic dysfunction in response to $PM_{2.5}$. However an important initial question pertains to how a trigger like PM may permeate the CNS to induce peripheral abnormalities such as inflammation?

Previous experiments from Block et al found that air pollution may cause neuroinflammation, oxidative stress and pathological alterations such as reactive gliosis (Block and Calderon-Garciduenas 2009). Nakane et al found that particles can be directly translocated along the olfactory nerve into the olfactory bulb (Nakane 2012). Olofsson et al found that $PM_{2.5}$ and/or ozone exposure may directly affect vagal afferents that may play an important role in regulation of peripheral inflammatory responses (Olofsson et al. 2012). Fonken et al demonstrated that long term $PM_{2.5}$ exposure (over 10 months) resulted in hippocampal inflammatory cytokine expression and impairments in spatial learning memory and behavior (Fonken et al. 2011). Given the importance of the hypothalamus in energy balance with multiple environmental and internal signals serving as cues to trigger the requisite behavioral and physiological response to maintain energy homeostasis, it may be reasoned that inflammatory triggers such as $PM_{2.5}$ inhalation may result in hypothalamic inflammation and impact peripheral glycemic control, metabolism, and inflammation. Recent experiments from our group in KKay mice, a genetically susceptible mouse model of Type II DM, revealed that exposure even over a few weeks is sufficient to induce increases in TNFα and IL-6 and reactive gliosis in the medial basal hypothalamus, suggesting an important role of air pollution in mediating hypothalamic inflammation (unpublished observations). Therefore, the hypothalamus may be particularly vulnerable to environmental signals such as $PM_{2.5}$ as the blood brain barrier is relatively permeable in this part of the brain.

In light of the importance of hypothalamic TNFα in response to high fat diet, we additionally hypothesized that this cytokine may play a role in $PM_{2.5}$-mediated insulin resistance and its inhibition may prevent progression of IR in the KKay model. However, TNFα blockade did not show any effects on $PM_{2.5}$-induced changes in glucose metabolism and energy homeostasis (unpublished observations). Inhibition of IKKβ, the enzyme regulating cytokines production, however completely corrected $PM_{2.5}$-induced alterations (Fig. 8.2). These results suggest that hypothalamic IKKβ rather than TNFα is critical for $PM_{2.5}$-mediated peripheral effects and suggest TNFα-independent pathways as downstream of IKKβ in $PM_{2.5}$ mediated peripheral effects. These findings suggest that peripheral inflammation in response to PM2.5 may represent a result of inflammatory changes in the CNS and mechanistically contribute to the development of metabolic insulin resistance in response to air-pollution.

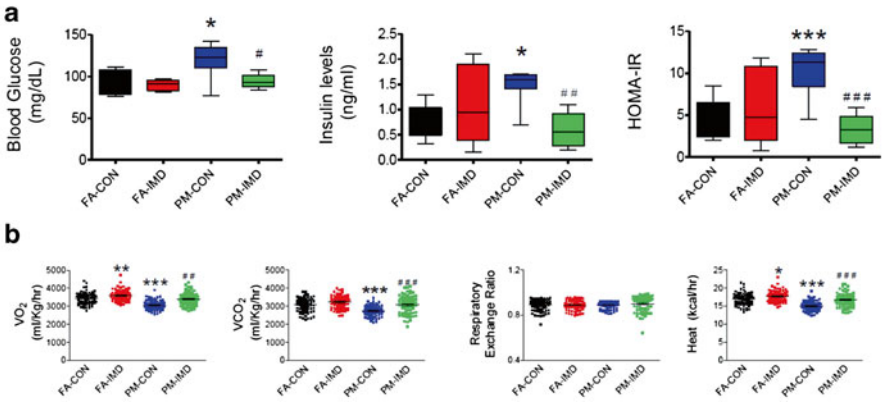

Fig. 8.2 Metabolic cage assessment following $PM_{2.5}$ exposure and in response to Intracerebroventricular (ICV) infusion of IMD-0354 in KKay mice exposed to $PM_{2.5}$. (**a**) After ICV infusion of IMD-0354 or DMSO vehicle, the fasting blood glucose and insulin levels were determined and HOMA-IR calculated accordingly. (**b**) Energy homeostasis response (O_2 consumption, CO_2 production, respiratory exchanging ratio and heat production) to ICV infusion of IMD-0354 in KKay mice exposed to $PM_{2.5}$. $^{*}P<0.05$, $^{***}P<0.001$ when compared PM-CON group with FA-CON group; $\#P<0.05$, $\#\#P<0.01$, $\#\#\#P<0.001$ when compared PM-IMD group with PM-CON group. n = 7–8 per group (Part of the data were reproduced from Environ Health Perspect; DOI:10.1289/ehp.1306841)

8.4 Signaling Pathways of Relevance with Air-Pollution Exposure

8.4.1 Toll-Like Receptors (TLRs) and Nucleotide Oligomerization Domain Receptors (NLRs) as Particulate Matter Sensors

The mechanisms by which PM is sensed initially by the lung and how these signals are transduced systemically remain a continuing challenge in the field. While direct translocation of particles has been raised as a simple mechanism that may contribute to systemic effects, the evidence supporting the direct translocation of $PM_{2.5}$ or even ultrafine particles is slim (Brook et al. 2004). On the other hand leachable materials present on the surface of $PM_{2.5}$ or UFPs such as soluble metals and organics may represent pathways whereby inhaled particles may engineer a systemic response. A broad encompassing mechanism by which particles may be sensed and could result in an inflammatory response is via pattern recognition receptors (PRRs) (Brook et al. 2004). The innate immune system recognizes various environmental threats such as pathogens and invading antigens through a limited amount of cell surface or intracellular receptors that recognize conserved molecular structural motifs or patterns unique to microorganisms and other environment threats. Each PRR can bind to a large amount of pathogen specific molecules sharing a certain structural

motif. Therefore, a limited number of PRRs can recognize different kinds of pathogens and initiate immune response immediately after encountering pathogens. Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), as the most important and best-characterized PRRs, play an essential role in pathogen-induced immune response (Lamkanfi and Kanneganti 2010; Vandanmagsar et al. 2011). As the principal initial cellular sensors to inhaled particles, both alveolar macrophage and bronchial epithelial cells have been shown to express PRRs such as TLRs and NLRs. TLRs can be directly activated by biologic components intrinsic to PM such as LPS and peptides and gaseous co-pollutants such as ozone (Deiuliis et al. 2011; Takeda and Akira 2007). Indeed earlier studies have suggested a link between TLR2 and TLR4 polymorphisms and susceptibility to air pollution related childhood asthma (Kerkhof et al. 2010). Components such as LPS as part of coarse PM may play a dominant role in urbanized environments in Asia where LPS-rich sources may contribute to air pollutant levels. In a recent prospective study in an urban population in Beijing, Sun et al reported that LPS binding protein (LBP) is an important predictor for the development of Type 2 DM on multivariate analysis after adjustment of most risk factors including CRP (Sun et al. 2010). Emerging studies suggested that LBP from activation of the innate immune system may serve as a surrogate for LPS in inflammatory disorders (Takeda and Akira 2007; Lepper et al. 2007). Shoenfelt et al have suggested that different ambient air particles might use different receptors according to the composition of particles. Exposure of macrophages to $PM_{2.5}$ that had high levels of redox active metals and low levels of endotoxin induced cytokine secretion in a TLR2-dependent mechanism. In contrast, exposure to PM_{10} that had high levels of endotoxin activates macrophage in a TLR4-dependent mechanism. However, fine and coarse air pollution particles-elicited inflammatory response shared the same downstream signaling pathways, MyD88, despite differential utilization of TLR2 and TLR4 (Sun et al. 2010; Shoenfelt et al. 2009).

Release of endogenous damage associated molecular patterns (DAMPs) in response to PM may represent additional mechanisms for TLR/NLR activation that may exacerbate the overactive pathways in obesity/insulin resistance. In a recent study, Vandanmagsar et al. demonstrated a key role for lipotoxicity associated ceramide accumulation in the pathogenesis of Type 2 DM by activating Nalp3, a NLR (Vandanmagsar et al. 2011). A number of DAMPs including oxidized phospholipid components (Liu et al. 2013a) and hyaluronan fragments (Deiuliis et al. 2011; Kampfrath et al. 2011) were shown to be released in response to PM and/or gaseous components. Palmitoyl-arachidonyl phosphocholine (PAPC), an abundant phospholipid in lung lavage fluid, activates TLR4 and is implicated in diverse lung injury (Imai et al. 2008). Release of oxidized PAPC may facilitate chemokine production and activate innate immune response in the lung and subsequently mediate efflux of inflammatory monocytes from the bone marrow to other locations including the lung, visceral adipose tissue or perivascular fat (Kampfrath et al. 2011). Kampfrath et al demonstrated that $PM_{2.5}$ exposure leads to an egress of $CD11b^{+}$, $Ly6C^{hi}$ inflammatory monocytes from the bone marrow to circulation and to tissue niches such as the peri-vascular fat via circulation (Kampfrath et al. 2011).

These monocytes then produce excessive superoxide that is involved in dysregulation of vascular tone characterized by vasoconstriction. Consistent with these findings, deficiency in TLR4 or the NADPH oxidase sub-unit Nox2 ($Nox2^{-/-}$) ameliorated these abnormal vascular responses (Kampfrath et al. 2011). Activation of NADPH oxidase is required for the increase of superoxide production in $PM_{2.5}$ exposure as evidenced by increased phosphorylation of the p47 subunit in aortic homogenates in $PM_{2.5}$ exposed animals which was prevented by TLR4 deficiency suggesting that TLR4 activation was required for NADPH oxidase activation (Kampfrath et al. 2011). In parallel in-vitro experiments, we also demonstrated that NADPH oxidase activation by ox-PAPC in macrophages could be prevented by inhibition of interleukin-1 receptor-associated kinase (IRAK), suggesting that TLR4 mediated IRAK phosphorylation was upstream of NADPH oxidase (Kampfrath et al. 2011). Ozone a gaseous component of air-pollution exposure in animal models may lead to degradation of hyaluronan and activation of TLR4/MyD88 pathways (Li et al. 2010, 2011). These modifications of endogenous proteins and phospholipids may circulated systemically and represent secondary mediators that may elicit systemic effects in response to air-pollution exposure. Consistent with these experimental data, human experiments demonstrate a rather rapid inflammatory response with exposure to both particulates and gaseous pollutants (Balcells et al. 2010). Ozone exposure for instance has recently been shown to result in an elevation of interleukin-8 and a decrease in plasminogen activator inhibitor-1 at the end of 2-h of exposure. There was a 104 % increase in IL-1β and C-reactive protein level 24 h after ozone exposure. The investigators also noted a 51.3 % decrease in the high-frequency component of heart rate variability, and a 1.2 % increase in QT duration compared to pre-exposure levels suggestive of rather rapid autonomic dysfunction (Devlin et al. 2012). Collectively these changes argue for inflammatory changes in response to both particulate and gaseous constituents with findings consistent with possible regulation by neural circuits. Figure 8.3 details our current understanding of inflammatory pathways involved with PM.

8.4.2 CC-Chemokine Receptor 2 (CCR2) as a Relevant Pathway Responsible for Immune Cell Recruitment

CCR2 plays a critical role in the entry of innate immune cells into tissues through direct interaction with its ligands such as CCL2 (also called MCP-1), CCL7, CCL8 and CCL12 (Charo and Ransohoff 2006; Proudfoot 2002). Reduced egress of monocytes from systemic reservoirs resulting in a reduction of these cells in the circulation and into tissue niches such as VAT was noted in $CCR2^{-/-}$ mice (Tsou et al. 2007). Recent studies have shown that the CCR2/MCP-1 system is not only critical to VAT inflammation but also in the recruitment of macrophages to the liver in response to a high-fat diet (Oh et al. 2012). Infiltration of monocytes is enhanced in obesity via local tissue cues with a progressive transformation of these cells to a

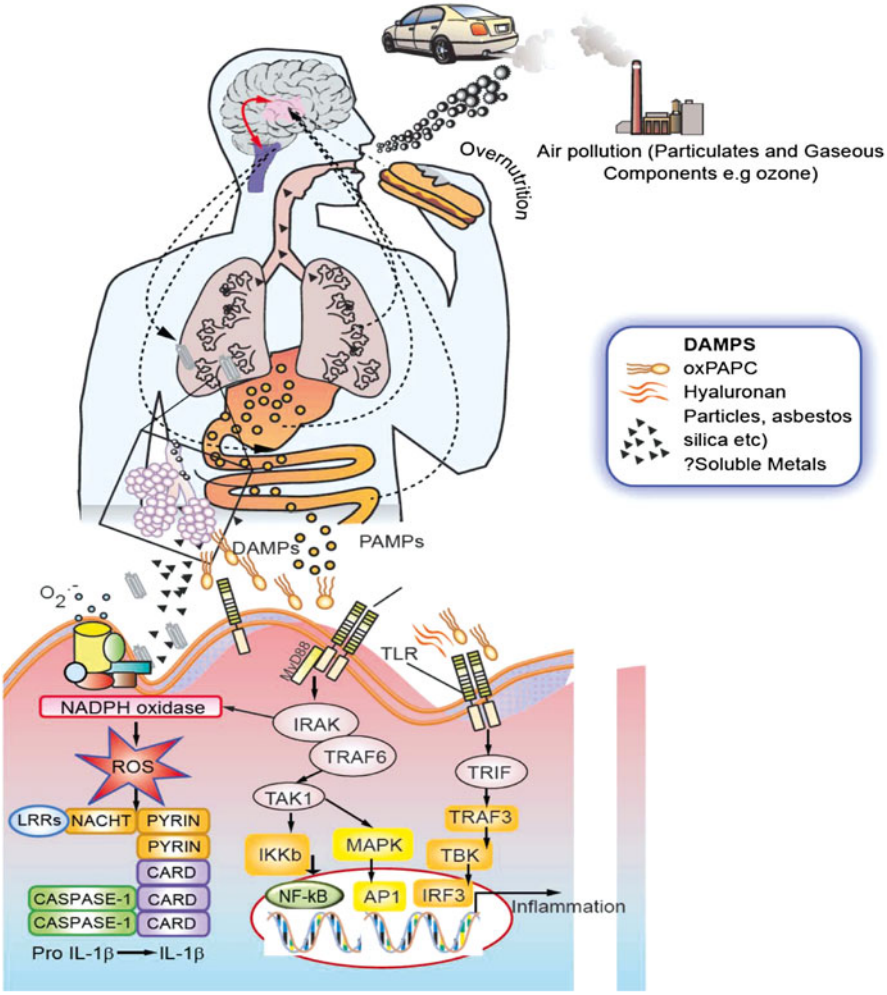

Fig. 8.3 Hypothesized mechanisms of air pollution-mediated cardiovascular disease. Inhalational or nutritional signals either directly or via the generation of signals such as DAMPs may serve to activate innate immune mechanisms such as the TLR and NLR. *DAMP* damage-associated molecular pattern, *AP1* activator protein 1, *CARD* caspase activation and recruitment domain, *IKKb* IκB kinase b, *IRAK* interleukin receptor-associated kinase, *IRF3* interferon regulatory factor 3, *MAPK* mitogen-activated protein kinase, *MyD88* myeloid differentiation primary response gene 88, *NAFLD* nonalcoholic fatty liver disease, *PAMP* pathogen-associated molecular pattern, *PAPC* palmitoyl-arachidonyl phosphocholine, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *TAK* transforming growth factor-β-activated kinase, *TBK* TANK-binding kinase 1, *TRAF* TNF receptor-associated factor, *TRIF* Toll/IL-1 receptor-domain-containing adapter-inducing interferon-β, *UCP-1* uncoupling protein-1, *WAT* white adipose tissue (Note: Copyright 2012 American Diabetes Association; From Diabetes®, Vol. 61, 2012; 3,037–3,045; Reprinted with permission from *The American Diabetes Association*)

CD11c$^+$ status, resulting in a polarization of the local adipose milieu to an M1 state from a predominantly M2 state under normal diet conditions (Oh et al. 2012; Lumeng et al. 2007). Consistent with this concept, CCR2 deficiency attenuates obesity, VAT inflammation and systemic IR with hematopoietic CCR2 deficiency being essential for improvement (Weisberg et al. 2006; Ito et al. 2008). Given the importance of the CCR2/MCP-1 system in regulating monocyte/macrophage chemotaxis and inflammation in obesity-induced IR, ablation of CCR-2 ameliorates glucose homeostasis and inflammation in visceral adipose tissue (Tsou et al. 2007; Weisberg et al. 2006; Ito et al. 2008). In a recent study by Liu et al, CD11b$^+$Gr-1^{low}7/4^{hi} cells, F4/80$^+$/CD11b$^+$ and F4/80$^+$/CD11c$^+$ cells in VAT increased in circulation in response to $PM_{2.5}$ exposure, whereas CCR2 deficiency markedly reduced immune cell numbers in the peripheral circulation and VAT (Liu et al. 2013b). We noted a significantly higher number of CD11c$^+$ cells (absolute numbers) in $PM_{2.5}$ exposure (Fig. 8.4), strongly suggesting that these cells in VAT are a consequence of recruitment rather than polarization of existing cell populations (Liu et al. 2013b). In addition, CCR2 is also involved in $PM_{2.5}$-induced changes in lipid metabolism. SREBP1c the transcription factor involved in regulation of fatty acid synthesis and triglyceride metabolism was upregulated with $PM_{2.5}$ exposure with normalization in response to CCR2 deficiency (Liu et al. 2013b).

Circulating glucose levels reflect a balance between glucose production and utilization. Accounting for ~80 % of insulin-stimulated whole-body glucose disposal, skeletal muscle is the most affected organ with respect to impaired insulin-stimulated glucose disposal in states of IR (DeFronzo et al. 1979). GLUT-4 expression in

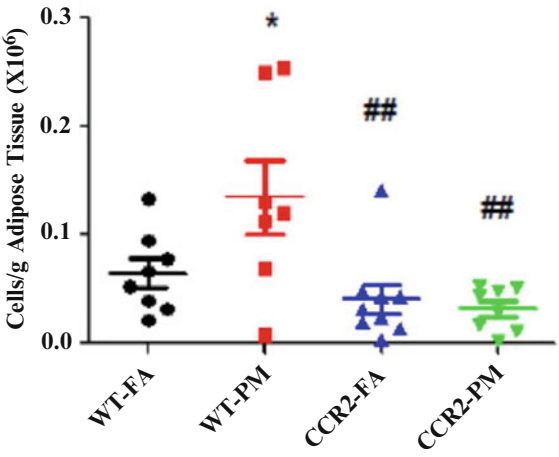

Fig. 8.4 PM enhanced the recruitment of classically activated ATM in a CCR2-dependent manner. Stromal vascular fraction (SVF) isolated from epididymal fat of WT or CCR2−/− mice exposed to FA or PM was used for cytometric detection of M1. The absolute number of M1 in adipose tissue was shown. *, $p<0.05$ compared with WT-FA; ##, $p<0.01$ compared with WT-PM (This data was adapted from Environ Health Perspect; DOI:10.1289/ehp.1306841)

skeletal muscle of C57BL/6 mice, has been shown to be decreased in response to $PM_{2.5}$ exposure, indicating a defect in glucose utilization upon $PM_{2.5}$ exposure. Gluconeogenesis is tightly regulated by insulin signaling (suppressed) with mitigation of this suppression with IR (in the face of continued insulin-mediated PC) is required for this process (Jitrapakdee 2012). Liu et al found reduced expression of G6pase, FBPase and PC mRNA levels with no alteration of PEPCK levels in response to $PM_{2.5}$ exposure, suggesting an adaptive negative feedback regulation of hyperglycemia (Liu et al. 2013b). In addition, Glut-2, a transporter in liver cells that functions to mediate glucose uptake in the liver for glycolysis, was reduced by $PM_{2.5}$ exposure, leading to an attenuated glucose uptake by the liver and hyperglycemia in $PM_{2.5}$ exposure (Liu et al. 2013b). Although CCR2 deficiency showed no improvement in ChRE-binding protein (ChREBP) or L-type pyruvate kinase (L-PK), the normalized GLUT-2 expression and GK overexpression in the CCR2 deficiency phenotype may be expected to alleviate glucose dysregulation induced by $PM_{2.5}$ exposure. Additional experimentation will be required to clarify the mechanism. However, CCR2 deficiency did not correct impaired vascular function (decreased relaxation to both acetylcholine and insulin) induced by $PM_{2.5}$, suggesting that CCR2 does not mediate air pollution-induced abnormalities in vascular endothelium. The study by Liu et al also demonstrated that $PM_{2.5}$ selectively up-regulates p38, which was partially abrogated by CCR2 deficiency (Liu et al. 2013b). In this study no changes in JNK were noted which were at variance with prior studies by Zheng et al which did demonstrate increase in JNK phosphorylation (Zheng et al. 2013). These differences may relate to differences in exposure conditions and/or strains used in the studies. p38 MAPK belongs to a family of evolutionarily conserved serine-threonine MAPKs that link extracellular signals to intracellular machinery regulating a plethora of cellular processes. Together with JNK, they are described as stress-activated protein kinases, which can be activated by environmental or genotoxic stress (Coulthard et al. 2009; Morrison and Davis 2003; Chang and Karin 2001). Taken together these findings seem to suggest a role for p38 and JNK pathways in the pathogenesis of IR and a role for CCR2 plays a pivotal role in the $PM_{2.5}$-mediated susceptibility to IR. Figure 8.5 depicts a schema of CCR2 dependent and independent mechanisms by which PM2.5 mediates IR.

8.5 Summaries and Future Directions

In summary, a variety of biological processes including inflammatory response, ER stress, and oxidative stress are involved in the physiopathologic response to air pollution. Characterizations of those pathologic changes such as inflammatory response or oxidative damages to nucleobases could serve as biomarkers of air pollution. Evidence from epidemiologic studies, limited exposure studies in humans and animal experiments support that inflammatory responses to environmental factors act as a central mechanism that mediates cardiometabolic effects of $PM_{2.5}$. As a non-traditional factor, air pollution is pervasive in the urban environment and

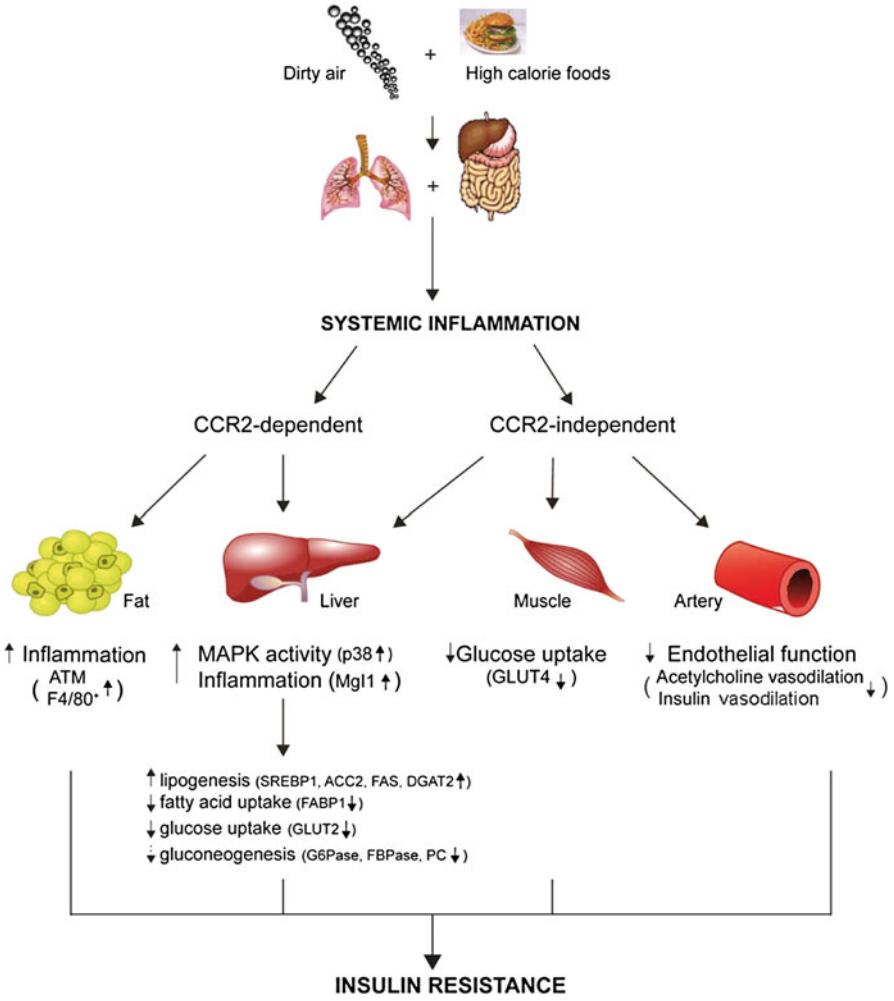

Fig. 8.5 CCR2-dependent and -independent mechanisms involved in $PM_{2.5}$-induced progression of insulin resistance. Exposure to $PM_{2.5}$ synergizes with high fat diet to induce systemic inflammation. $PM_{2.5}$ enhances inflammation and MAPK activity in liver and adipose tissue via a CCR2-dependent pathway. $PM_{2.5}$ also reduces glucose uptake in muscles/liver and aortic endothelial dysfunction through a CCR2-independent pathway. *MAPK* mitogen-activated protein kinase, *GLUT4* glucose transporter type 4, *SREB1* sterol regulatory element-binding protein 1, *ACC2* acetyl-CoA carboxylase 2, *FAS* fatty acid synthase, *DGAT2* diacylglycerol O-acyltransferase 2, *FABP1* fatty acid-binding protein 1, *GLUT2* glucose transporter type 2, *G6Pase* glucose-6-phosphatase, *FBPase* fructose 1,6-bisphosphatase, *PC* pyruvate carboxylase

may provide low level synergism with other dominant factors in accelerating propensity for T2DM. Future studies are warranted to gain greater insights into the molecular mechanisms involved, the responsible pollutants (eg, components, sizes/sources); the role of combined exposures to mixtures (eg, ozone plus PM) and

susceptibility factors (eg, gene-environment interactions, vulnerable populations). Finally an integrated of effects across multiple systems and organs is needed, together with insights on pathways that serve as regulators of systems. The findings of $PM_{2.5}$ induced CNS inflammation while early, may represent at least one potential pathway by which a multitude of metabolic and inflammatory pathways could converge. Work in the next few years that focus on such broad integrative pathways juxtaposed with a system based understanding of $PM_{2.5}$ effects (transcriptome, proteome and metabolome) and intervention of those pathways as a therapeutic strategy is warranted.

Acknowledgement This work was supported by a grant from US EPA (R834797) and grants from NIH (RO1 ES015146, R01ES017290 and R21 DK088522).

References

Andersen ZJ, Raaschou-Nielsen O, Ketzel M, Jensen SS, Hvidberg M, Loft S et al (2012) Diabetes incidence and long-term exposure to air pollution: a cohort study. Diabetes Care 35(1):92–98

Balcells M, Martorell J, Olive C, Santacana M, Chitalia V, Cardoso AA et al (2010) Smooth muscle cells orchestrate the endothelial cell response to flow and injury. Circulation. [In Vitro Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 121(20):2192–2199

Block ML, Calderon-Garciduenas L (2009) Air pollution: mechanisms of neuroinflammation and CNS disease. Trends Neurosci. [Research Support, N.I.H., Extramural Review]. 32(9):506–516

Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M et al (2004) Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation. [Consensus Development Conference Review]. 109(21):2655–2671

Brook RD, Jerrett M, Brook JR, Bard RL, Finkelstein MM (2008) The relationship between diabetes mellitus and traffic-related air pollution. J Occup Environ Med 50(1):32–38

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV et al (2010) Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121(21):2331–2378

Brook RD, Xu X, Bard RL, Dvonch JT, Morishita M, Kaciroti N et al (2013a) Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. Sci Total Environ. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 448:66–71

Brook RD, Cakmak S, Turner MC, Brook JR, Crouse DL, Peters PA et al (2013b) Long-term fine particulate matter exposure and mortality from diabetes in Canada. Diabetes Care 36(10):3313–3320

Brown MS, Goldstein JL (2008) Selective versus total insulin resistance: a pathogenic paradox. Cell Metab. [Comment Review]. 7(2):95–96

Chang L, Karin M (2001) Mammalian MAP kinase signalling cascades. Nature. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 410(6824):37–40

Charo IF, Ransohoff RM (2006) The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med. [Review]. 354(6):610–621

Chen H, Burnett RT, Kwong JC, Villeneuve PJ, Goldberg MS, Brook RD et al (2013) Risk of incident diabetes in relation to long-term exposure to fine particulate matter in Ontario, Canada. Environ Health Perspect 121(7):804–810

Chuang KJ, Yan YH, Chiu SY, Cheng TJ (2011) Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup Environ Med 68(1):64–68

Coogan PF, White LF, Jerrett M, Brook RD, Su JG, Seto E et al (2012) Air pollution and incidence of hypertension and diabetes in African American women living in Los Angeles. Circulation 125(6):767–772

Coulthard LR, White DE, Jones DL, McDermott MF, Burchill SA (2009) p38(MAPK): stress responses from molecular mechanisms to therapeutics. Trends Mol Med. [Review]. 15(8):369–379

De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC et al (2005) Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinol. [Research Support, Non-U.S. Gov't]. 146(10):4192–4199

DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. [Research Support, U.S. Gov't, P.H.S.]. 237(3):E214–E223

Deiuliis JA, Kampfrath T, Zhong J, Oghumu S, Maiseyeu A, Chen LC et al (2011) Pulmonary T cell activation in response to chronic particulate air pollution. Am J Physiol Lung Cell Mol Physiol 9

Devlin RB, Duncan KE, Jardim M, Schmitt MT, Rappold AG, Diaz-Sanchez D (2012) Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126(1):104–111

Fonken LK, Xu X, Weil ZM, Chen G, Sun Q, Rajagopalan S et al (2011) Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology. Mol Psychiatry. [Research Support, N.I.H., Extramural]. 16(10):987–995, 973

Goldberg MS, Burnett RT, Stieb DM, Brophy JM, Daskalopoulou SS, Valois MF et al (2013) Associations between ambient air pollution and daily mortality among elderly persons in Montreal, Quebec. Sci Total Environ. [Research Support, Non-U.S. Gov't]. 463–464:931–942

Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol. [Research Support, Non-U.S. Gov't Review]. 3(1):23–35

Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, van Loo G et al (2008) Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell 133(2):235–249

Ito A, Suganami T, Yamauchi A, Degawa-Yamauchi M, Tanaka M, Kouyama R et al (2008) Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. J Biol Chem. [Research Support, Non-U.S. Gov't]. 283(51):35715–35723

Jitrapakdee S (2012) Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis. Int J Biochem Cell Biol. [Research Support, Non--U.S. Gov't Review]. 44(1):33–45

Kahn BB, Flier JS (2000) Obesity and insulin resistance. J Clin Invest. [Research Support, Non--U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 106(4):473–481

Kampfrath T, Maiseyeu A, Ying Z, Shah Z, Deiuliis JA, Xu X et al (2011) Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways. Circ Res 108(6):716–726

Kelishadi R, Mirghaffari N, Poursafa P, Gidding SS (2009) Lifestyle and environmental factors associated with inflammation, oxidative stress and insulin resistance in children. Atherosclerosis 203(1):311–319

Kerkhof M, Postma DS, Brunekreef B, Reijmerink NE, Wijga AH, de Jongste JC et al (2010) Toll-like receptor 2 and 4 genes influence susceptibility to adverse effects of traffic-related air pollution on childhood asthma. Thorax. [Multicenter Study Research Support, Non-U.S. Gov't]. 65(8):690–697

Kramer U, Herder C, Sugiri D, Strassburger K, Schikowski T, Ranft U et al (2010) Traffic-related air pollution and incident type 2 diabetes: results from the SALIA cohort study. Environ Health Perspect 118(9):1273–1279

Laing S, Wang G, Briazova T, Zhang C, Wang A, Zheng Z et al (2010) Airborne particulate matter selectively activates endoplasmic reticulum stress response in the lung and liver tissues. Am J Physiol Cell Physiol. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 299(4):C736–C749

Lamkanfi M, Kanneganti TD (2010) Nlrp3: an immune sensor of cellular stress and infection. Int J Biochem Cell Biol. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 42(6):792–795

Lawson WE, Cheng DS, Degryse AL, Tanjore H, Polosukhin VV, Xu XC et al (2011) Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs. Proc Natl Acad Sci U S A 108(26):10562–10567

Lepper PM, Schumann C, Triantafilou K, Rasche FM, Schuster T, Frank H et al (2007) Association of lipopolysaccharide-binding protein and coronary artery disease in men. J Am Coll Cardiol 50(1):25–31

Li Z, Potts EN, Piantadosi CA, Foster WM, Hollingsworth JW (2010) Hyaluronan fragments contribute to the ozone-primed immune response to lipopolysaccharide. J Immunol. [Research Support, N.I.H., Extramural]. 185(11):6891–6898

Li Z, Potts-Kant EN, Garantziotis S, Foster WM, Hollingsworth JW (2011) Hyaluronan signaling during ozone-induced lung injury requires TLR4, MyD88, and TIRAP. PloS One. [Research Support, N.I.H., Extramural]. 6(11):e27137

Liu C, Ying Z, Harkema J, Sun Q, Rajagopalan S (2013a) Epidemiological and experimental links between air pollution and type 2 diabetes. Toxicol Pathol. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 41(2):361–373

Liu C, Xu X, Bai Y, Wang TY, Rao X, Wang A et al (2013b) Air pollution-mediated susceptibility to inflammation and insulin resistance: influence of CCR2 pathways in mice. Environ Health Perspect 22

Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307(5708):384–387

Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117(1):175–184

Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol. [Research Support, Non-U.S. Gov't Review]. 27:451–483

Mendez R, Zheng Z, Fan Z, Rajagopalan S, Sun Q, Zhang K (2013) Exposure to fine airborne particulate matter induces macrophage infiltration, unfolded protein response, and lipid deposition in white adipose tissue. Am J Transl Res 5(2):224–234

Morrison DK, Davis RJ (2003) Regulation of MAP kinase signaling modules by scaffold proteins in mammals. Annu Rev Cell Dev Biol. [Research Support, U.S. Gov't, P.H.S. Review]. 19:91–118

Murea M, Ma L, Freedman BI (2012) Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. Review Diabet Stud: RDS. [Review]. Spring 9(1):6–22

Nakane H (2012) Translocation of particles deposited in the respiratory system: a systematic review and statistical analysis. Environ Health Prev Med 17(4):263–274

Odegaard JI, Chawla A (2011) Alternative macrophage activation and metabolism. Annu Rev Pathol. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 6:275–297

Oh DY, Morinaga H, Talukdar S, Bae EJ, Olefsky JM (2012) Increased macrophage migration into adipose tissue in obese mice. Diabetes. [Research Support, N.I.H., Extramural]. 61(2):346–354

Olofsson PS, Rosas-Ballina M, Levine YA, Tracey KJ (2012) Rethinking inflammation: neural circuits in the regulation of immunity. Immunol Rev 248(1):188–204

O'Neill MS, Veves A, Zanobetti A, Sarnat JA, Gold DR, Economides PA et al (2005) Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. Circulation. [Meta-Analysis Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. 111(22):2913–2920

Osorio F, Lambrecht B, Janssens S (2013) The UPR and lung disease. Semin Immunopathol. [Review]. 35(3):293–306

Pearson JF, Bachireddy C, Shyamprasad S, Goldfine AB, Brownstein JS (2010) Association between fine particulate matter and diabetes prevalence in the U.S. Diabetes Care 33(10):2196–2201

Posey KA, Clegg DJ, Printz RL, Byun J, Morton GJ, Vivekanandan-Giri A et al (2009) Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. Am J Physiol Endocrinol Metab 296(5):E1003–E1012

Proudfoot AE (2002) Chemokine receptors: multifaceted therapeutic targets. Nat Rev Immunol. [Review]. 2(2):106–115

Puett RC, Hart JE, Schwartz J, Hu FB, Liese AD, Laden F (2011) Are particulate matter exposures associated with risk of type 2 diabetes? Environ Health Perspect 119(3):384–389

Purkayastha S, Zhang H, Zhang G, Ahmed Z, Wang Y, Cai D (2011) Neural dysregulation of peripheral insulin action and blood pressure by brain endoplasmic reticulum stress. Proc Natl Acad Sci U S A. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 108(7):2939–2944

Qatanani M, Lazar MA (2007) Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes Dev. [Review]. 21(12):1443–1455

Raaschou-Nielsen O, Sorensen M, Ketzel M, Hertel O, Loft S, Tjonneland A et al (2013) Long-term exposure to traffic-related air pollution and diabetes-associated mortality: a cohort study. Diabetologia. [Research Support, Non-U.S. Gov't]. 56(1):36–46

Rajagopalan S, Brook RD (2012) The indoor-outdoor air-pollution continuum and the burden of cardiovascular disease: an opportunity for improving global health. Glob Heart 7(3):207–213

Ryan KK, Woods SC, Seeley RJ (2012) Central nervous system mechanisms linking the consumption of palatable high-fat diets to the defense of greater adiposity. Cell Metab 15(2):137–149

Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414(6865):799–806

Sears ME, Genuis SJ (2012) Environmental determinants of chronic disease and medical approaches: recognition, avoidance, supportive therapy, and detoxification. J Environ Public Health. [Research Support, Non-U.S. Gov't Review]. 2012:356798

Shoenfelt J, Mitkus RJ, Zeisler R, Spatz RO, Powell J, Fenton MJ et al (2009) Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. J Leukoc Biology. [Research Support, N.I.H., Extramural]. 86(2):303–312

Shulman GI (2000) Cellular mechanisms of insulin resistance. J Clin Invest. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. 106(2):171–176

Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB et al (2009) Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation 119(4):538–546

Sun L, Yu Z, Ye X, Zou S, Li H, Yu D et al (2010) A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. Diabetes Care 33(9):1925–1932

Sun Z, Mukherjee B, Brook RD, Gatts GA, Yang F, Sun Q et al (2013) Air-Pollution and Cardiometabolic Diseases (AIRCMD): a prospective study investigating the impact of air pollution exposure and propensity for type II diabetes. Sci Total Environ. [Research Support, N.I.H., Extramural]. 448:72–78

Takeda K, Akira S (2007) Toll-like receptors. Curr Protoc Immunol/edited by John E Coligan [et al.]. Chapter 14:Unit 14 2

Taniguchi CM, Emanuelli B, Kahn CR (2006) Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 7(2):85–96

Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO et al (2012) Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. 122(1):153–162

Thaler JP, Guyenet SJ, Dorfman MD, Wisse BE, Schwartz MW (2013) Hypothalamic inflammation: marker or mechanism of obesity pathogenesis? Diabetes. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. 62(8):2629–2634

Thiering E, Cyrys J, Kratzsch J, Meisinger C, Hoffmann B, Berdel D et al (2013) Long-term exposure to traffic-related air pollution and insulin resistance in children: results from the GINIplus and LISAplus birth cohorts. Diabetologia. [Research Support, Non-U.S. Gov't]. 56(8):1696–1704

Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP et al (2007) Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. J Clin Invest. [Research Support, N.I.H., Extramural]. 117(4):902–909

Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL et al (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 17(2):179–188

Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334(6059):1081–1086

Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K et al (2006) CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 116(1):115–124

Xu X, Yavar Z, Verdin M, Ying Z, Mihai G, Kampfrath T et al (2010) Effect of early particulate air pollution exposure on obesity in mice: role of p47phox. Arterioscler Thromb Vasc Biol 30(12):2518–2527

Xu X, Liu C, Xu Z, Tzan K, Zhong M, Wang A et al (2011a) Long-term exposure to ambient fine particulate pollution induces insulin resistance and mitochondrial alteration in adipose tissue. Toxicol Sci 124(1):88–98

Xu Z, Xu X, Zhong M, Hotchkiss IP, Lewandowski RP, Wagner JG et al (2011b) Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues. Part Fibre Toxicol. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, Non-P.H.S.]. 8:20

Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D (2008) Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 135(1):61–73

Zheng Z, Xu X, Zhang X, Wang A, Zhang C, Huttemann M et al (2013) Exposure to ambient particulate matter induces a NASH-like phenotype and impairs hepatic glucose metabolism in an animal model. J Hepatol. [Research Support, N.I.H., Extramural Research Support, Non--U.S. Gov't]. 58(1):148–154

Chapter 9
Air Pollution, Lipids and Atherosclerosis

Jesus A. Araujo and Michael E. Rosenfeld

9.1 Introduction

Extensive epidemiological evidence supports the association of air pollution with adverse health effects leading to increased morbidity and mortality of worldwide significance (Brook et al. 2004, 2010; Bhatnagar 2006). Air pollution-related deaths are mostly due to cardiovascular and cerebrovascular diseases (Pope et al. 2004), which together accounted for 80 % of all deaths worldwide (40 % each) attributed to ambient air pollution in 2012 (World Health Organization 2014). This is of large significance as heart disease remained in 2011 as the top leading cause of death in the US (Hoyert and Xu 2012) and in the world (World Health Organization 2013); and cerebrovascular diseases were the fourth cause of death and leading cause of disability in the US in 2011 (Hoyert and Xu 2012; Towfighi and Saver 2011). Exposure to air pollution is now recognized as a relevant cardiovascular risk factor given its potential to affect large numbers of people around the globe. The nature of the cardiovascular effects of air pollution and potential mechanisms involved have been the focus of two Concensus Statements from the American Heart Association in 2004 (Brook et al. 2004) and 2010 (Brook et al. 2010), targeted to the healthcare professionals, which underlines the importance of this topic for the general community.

J.A. Araujo, M.D., Ph.D. (✉)
Division of Cardiology, Department of Medicine, David Geffen School of Medicine at UCLA, 10833 Le Conte Avenue, CHS 43-264, 90095, P.O. Box 951679, Los Angeles, CA, USA
e-mail: JAraujo@mednet.ucla.edu

M.E. Rosenfeld, Ph.D.
Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_9

Ischemic cardiovascular and cerebrovascular diseases share a major pathogenic substrate, the development of atherosclerotic plaques and subsequent thromboembolic events. Plaques develop in the large and medium-sized elastic muscular arteries, as a result of atherosclerosis, an inflammatory process characterized by the accumulation of lipids and fibrous elements in the vascular wall (Lusis 2000). Infiltrating lipids come from circulating low-density lipoprotein (LDL) particles that are retained in the vascular wall. The retention of LDL is favored by its oxidative modification which leads to activation of endothelial cells, monocyte recruitment with internalization into the vasculature, differentiation into macrophages and generation of foam cells by increased lipid uptake. Vascular infiltration of lipids and inflammatory cells further enhances oxidative stress and a vicious cycle of inflammation. While the pathogenic role of vascular oxidative stress has been challenged by the argument that oxidative modifications present in the plaques could be consequential rather than causal to lesion formation (Stocker and Keaney 2004), it does appear that the interplay between pro-oxidant and anti-oxidant factors in the vasculature may determine the degree of reactive oxygen species (ROS) generation in a way that can affect lesion formation (Araujo et al. 2012). Thus, established risk factors such as diabetes and cigarette smoking or novel risk factors such as exposure to air pollution enhance ROS generation in the vasculature and promote atherogenesis (Araujo 2011).

Air pollution is a complex mixture of compounds with gaseous (ozone, carbon monoxide, sulfur and nitrogen oxides) and particle phases, the cardiovascular effects have been mostly ascribed to the particulate matter (PM) components (Bhatnagar 2006; Brook et al. 2010; Araujo and Nel 2009). Indeed, the ambient PM pollution ranked within the top 10 leading risk factors associated with global burden of disease worldwide, resulting in 3.0 % of the global disability-adjusted life-years (Lim et al. 2012). Ambient particles can be classified according to their aerodynamic diameter with size fractions of PM_{10} ("thoracic" particles, <10 μm), $PM_{2.5–10}$ ("coarse" particles, 2.5–10 μm), $PM_{2.5}$ (fine particles, <2.5 μm) and UFP (ultrafine particles, <0.1 μm) that are derived from various sources and by a variety of processes characteristic of each size fraction (U.S.EPA 2004). While the associations of exposure to PM_{10} and $PM_{2.5}$ with cardiovascular endpoints appear to be stronger than with the gaseous pollutants, two recent meta-analyses, one of 34 studies (with 17 time-series and 17 case-crossover designs) and the other of 35 studies, revealed that with the exception of ozone, all the main air pollutants that were studied (PM_{10}, $PM_{2.5}$, carbon monoxide, nitrogen dioxide and sulfur dioxide) were significantly associated with an increased risk for myocardial infarction (MI) (Mustafic et al. 2012) and heart failure hospitalizations (Shah et al. 2013).

Air pollution-mediated cardiovascular actions include a plethora of varied effects on atherosclerosis, platelet aggregability and thrombosis, vasoreactivity, arrhythmias and possibly cardiac systolic function that are likely responsible for the association with increased risk for myocardial infarction (Mustafic et al. 2012), cerebrovascular events (Miller et al. 2007) and heart failure hospitalizations (Shah et al. 2013). Various mechanisms have been proposed to explain how inhalation of ambient

pollutants could result in systemic cardiovascular effects such as: (1) activation of pulmonary receptors resulting in autonomic nervous system imbalance and the development of dysrhythmias, (2) induction of pulmonary and systemic inflammation, (3) access of particles, gases or their chemical constituents to the systemic circulation (Araujo and Nel 2009). The latter pathways can mediate air pollution-related acute pro-thrombotic as well as chronic pro-atherosclerotic effects, largely responsible for acute coronary syndromes, cerebrovascular events and ischemic heart disease. In this chapter, we will focus our attention on air pollution-mediated effects on atherosclerosis.

9.2 Air Pollutants and Atherosclerosis

9.2.1 Human Studies Reveal Associations Between Exposure to Air Pollutants and Atherosclerosis

Several studies support the association between air pollution and subclinical measures of atherosclerosis in humans (Table 9.1). Kunzli et al. reported in 2005 on a cross-sectional study where the degree of carotid intima-medial thickness (CIMT) in 798 individuals correlated with an increase of 5.9 % for every 10 μg/m^3 rise in $PM_{2.5}$ levels (Kunzli et al. 2005) (Table 9.1). Data from the Multi-Ethnic Study of Atherosclerosis collected at baseline further support the association of PM with atherosclerosis (Diez Roux et al. 2008). Diez Roux et al. reported that PM_{10} exposures assessed over long-term (20-year means and 2001 mean) and 20-year $PM_{2.5}$ exposures were correlated with a 1–3 % increase in CIMT per 21 μg/m^3 increase in PM_{10} or 12.5 μg/m^3 increase in $PM_{2.5}$, respectively (Diez Roux et al. 2008). In addition, Allen et al. reported that $PM_{2.5}$ exposures were correlated with an increased risk for aortic calcification in a related study (Allen et al. 2009) (Table 9.1). The PM and atherosclerosis association was further confirmed by results from the Heinz Nixdorf Recall (HNR) study, a population-based cohort of 4,814 participants where exposure to $PM_{2.5}$, PM_{10} and distance to high traffic was associated with an increase in CIMT (Bauer et al. 2010). In this study, the association of 1-year exposure to $PM_{2.5}$ with CIMT was strong in comparison to all major known risk factors for atherosclerosis and stronger than for larger PM_{10} particles. This is consistent with the greater effects of the $PM_{2.5}$ fraction on long-term cardiovascular mortality (Pope et al. 2002) and the notion that the cardiovascular effects are favored by a smaller particle size (Araujo and Nel 2009). This was an extension of the previous report on the HNR study, subjects living >200 m away from a major road, were compared to subjects living within 101 to 200 m, 51 to 100 or less than 50 m showed an 8 %, 34 % and 63 % increase in the probability of having a high coronary artery calcium (CAC) score, respectively (Table 9.1). The potential influence of PM exposure levels with the progression of atherosclerotic disease was first reported by

Table 9.1 Human studies linking exposure to air pollution with subclinical atherosclerosis

Study	Air pollutant	Evaluation of atherosclerosis	Major findings
Kunzli et al. (2005)	$PM_{2.5}$	CIMT	5.9 % increased in CIMT per every 10 μg $PM_{2.5}$/m^3
	Ozone		
Hoffman et al. (2007)	$PM_{2.5}$	CACS	Increased CAC scores with shorter distances to a major road
	Distance to major road		
Diez Roux et al. (2008)	PM_{10}	CIMT	1–3 % increase in CIMT per every increase in 21 and 12.5 μg/m^3 of PM_{10} and $PM_{2.5}$ respectively
	$PM_{2.5}$	CACS	
		BAI	
Allen et al. (2009)	$PM_{2.5}$	Aortic calcification	6 % increase in the risk of aortic calcification with a 10 μg/m^3 increase in $PM_{2.5}$
	Distance to major road		
Bauer et al. (2010)	$PM_{2.5}$	CIMT	4.3 %, 1.7 % and 1.2 % increases in CIMT per interdecile range increases in $PM_{2.5}$ (4.2 μg/m^3), PM_{10} (6.7 μg/m^3) and distance to traffic (1,939 m), respectively
	PM_{10}		
	Distance to high traffic		
Kunzli et al. (2010)	$PM_{2.5}$	CIMT	Greater annual progression of CIMT among individuals living < 100 m from a highway
	Distance to highway or major road		
Adar et al. (2013)	$PM_{2.5}$	CIMT	2.5 μg/m^3 higher $PM_{2.5}$ levels associated with greater CIMT progression over 5 years (increase of 5.0 μm/year) while greater reductions in $PM_{2.5}$ over a fixed baseline $PM_{2.5}$ associated with slowed CIMT progression

Studies are listed in chronological order based on the year of publication
CIMT carotid intima-media thickness, *CACS* coronary artery calcium score, *BAI* brachial artery index

Kunzli et al. who found that the annual rate of CIMT progression among individuals living within 100 m of a highway was accelerated and more than twice the population mean rate of progression (Kunzli et al. 2010). A recent report from a 5-year follow-up of the MESA study showed that among 5,362 participants with a mean annual progression of 14 μm/year, subjects exposed to 2.5 μg/m^3 exhibited a greater rate of CIMT progression over 5 years (increase of 5.0 μm/year). Importantly, reductions in $PM_{2.5}$ exposure from a fixed baseline were associated with a slower progression in the CIMT (−2.8 μm/year per each 1 μg/m^3 reduction in $PM_{2.5}$) (Adar et al. 2013). Associations of short-term and long-term exposures to $PM_{2.5}$ and retinal vessel diameters, expressed as central retinal arteriolar equivalents and central retinal venular equivalents, were also reported in the MESA study, further supporting the notion that small increases in short-term and long-term air pollution exposures are associated with vascular events (Adar et al. 2010).

9.2.2 *Animal Studies Support a Causality Link*

While the epidemiological studies support an association between exposure to air pollution and atherosclerosis in humans, studies in experimental animals suggest that exposure to air pollution can induce the progression and/or changes in the composition of atherosclerotic lesions. In addition, several of these studies show that exposure to air pollution also results in increased systemic pro-oxidative effects leading to enhanced lipid peroxidation in various tissues. Table 9.2 summarizes several of these studies using various animal models.

Exposures to air pollutants have been performed using intra-tracheal instillations (I.T.) of PM as well as inhalation of polluted air, concentrated ambient particles (CAPs) or motor vehicle emissions such as diesel exhaust (DE), gasoline exhaust (GE) and mixed vehicular emissions (MVE: DE+GE). These various exposure modalities differ with regard to how difficult they are to perform and with regard to how much they model "real life" scenarios. Thus, I.T. administrations are easier experimentally but more limited in physiological relevance. On the other hand, inhalation of CAPs and motor vehicle emissions are more difficult to conduct and have larger variability but better reproduce "real life" scenarios However, both types of approaches are informative in understanding and dissecting relevant pathological events.

I.T. administration of PM_{10} or carbon black have been shown to stimulate atherosclerotic lesion formation in Watanabe hyperlipidemic rabbits (Suwa et al. 2002; Yatera et al. 2008) and low density lipoprotein receptor deficient (LDL-$R^{-/-}$) mice (Niwa et al. 2007), respectively. In those studies, atherosclerosis was assessed both in the coronary arteries (Suwa et al. 2002) and in the aorta (Yatera et al. 2008; Suwa et al. 2002; Niwa et al. 2007). The effects of concentrated $PM_{2.5}$ have primarily been evaluated with long-term inhalation exposures. Six studies have been reported where concentrated $PM_{2.5}$ led to enhanced atherosclerosis in apolipoprotein E null mice (Apo $E^{-/-}$). For example, animals were exposed to fine CAPs in both suburban Sterling Forest, New York (Chen and Nadziejko 2005; Chen et al. 2010; Sun et al. 2005, 2008; Quan et al. 2010) or in urban Manhattan, New York (Ying et al. 2009) and the CAPs from both environments had accelerating effects on atherosclerosis. Chen and Nadziejko first reported that 39–41 week-old $ApoE^{-/-}$ mice fed a chow diet and exposed to 10X ambient concentrations of $PM_{2.5}$ for 6 h per day, 5 days per week for 5 months led to a 57 % increase in the percentage of atherosclerotic plaque area in the aortic root (Gunnison and Chen 2005) (Table 9.2). Sun et al. then showed that younger 6-week-old $ApoE^{-/-}$ mice fed a chow diet and exposed to similar conditions for 6 months displayed an upward trend in the percentage of aortic atherosclerotic plaque area (45 % increase), that was not statistically significant (Sun et al. 2005). The feeding of a high fat diet however, resulted in a statistically significant 58 % increase in aortic root plaque area following exposure to $PM_{2.5}$ together with an impaired vasomotor response (Sun et al. 2005). In another study, Sun et al. reported that $ApoE^{-/-}$ mice fed a high fat diet and exposed to concentrated $PM_{2.5}$ also resulted in enhancement of the plaque area in the aortic arch as assessed by ultrasound bio-microscopy (Sun et al. 2008). The $PM_{2.5}$ exposures also led to

Table 9.2 Animal studies evaluating the effect of air pollution on lipids and atherosclerosis

Study	Air pollutant	Animal model	Diet	Major findings (Induced by the exposure to air pollutant vs. FA controls)
Suwa et al. (2002)	I.T. PM_{10}	Watanabe rabbits	Chow	Increase in % lesional volume in coronary arteries and aorta
	2 days/week × 4 week			Increase in circulating polymorphonuclear leukocytes
				No effects on plasma lipids
Chen and Nadziejko (2005)	Inhaled CAPs($PM_{2.5}$)	$ApoE^{-/-}$, $LDL^{-/-}$ mice & $ApoE^{-/-}$ mice	Chow	Increase in % lesional area in whole aorta in $ApoE^{-/-}$ mice but not in $ApoE^{-/-}$, $LDL^{-/-}$ mice
	6 h/day, 5 days/ week × 5 month		Chow	
Sun et al. (2005)	Inhaled CAPs($PM_{2.5}$)	$ApoE^{-/-}$ mice	Chow or CED	Increase in % lesional area in cross-sections of aorta in CED-fed mice and N.S. increase in chow-fed mice
	6 h/day, 5 days/ week × 6 month			Small increase in total plasma cholesterol in CED-fed mice but small decrease in chow-fed mice
Niwa et al. (2007)	I.T. Carbon black	$LDL\text{-}R^{-/-}$ mice	CED	Increase in % lesional area in whole aorta
	1x/week × 10 week			Increase in C-reactive protein
Sun et al. (2008)	Inhaled CAPs($PM_{2.5}$)	$ApoE^{-/-}$ mice	Chow or CED	Increase in % lesional area in aorta in CED-fed mice and N.S increase in chow-red mice, assessed by ultrasound
	6 h/day, 5 days/ week × 6 m			Plaques had a greater content of tissue factor protein
Yatera et al. (2008)	I.T. PM_{10}	Watanabe rabbits	Chow	Increase in % lesional volume and % lesional area in the aorta
	2 days/week × 4 week			Increase in circulating monocytes 24 h after first I.T treatment
Araujo et al. (2008)	Inhaled CAPs($PM_{2.5}$ & UFP)	$ApoE^{-/-}$ mice	Chow	Increase in lesional area in aortic root of UFP-exposed mice N.S increase in $PM_{2.5\text{-exposed}}$ mice
	3x/week × 5 week			Small increase in plasma total cholesterol in the $PM_{2.5}$ group
				Increase in liver MDA in $PM_{2.5}$ and UFP-exposed mice
				Dysfunctional plasma HDL in mice exposed to $PM_{2.5}$ and UFP, more prominent in UFP (HDL became pro-inflammatory)

Soares et al. (2009)	Inhaled polluted ambient air	$LDLR^{-/-}$ mice	CED	Increase in aortic wall thickness in the CED-fed mice but not in % lesional area
			Chow	
				Increase in LDL susceptibility to oxidation, plasma anti-oxLDL and anti-apoB antibodies, which was more pronounced in CED-fed mice
Ying et al. (2009)	Inhaled CAPs($PM_{2.5}$)	$ApoE^{-/-}$ mice	CED	Increase in % lesional area in cross-sections of aorta
	6 h/day, 5 days/ week×4 month			Increase in iNOS and Nitrotyrosine in the aorta
Lund et al. (2009)	Inhaled GE	$ApoE^{-/-}$ mice	CED	Increase in aortic TBARS and MMP-9, inhibited by Tempol
	6 h/day×1 or 7 days			Increase in aortic ET-1, inhibited by BQ-123 (ET_A receptor antagonist)
	+/– Tempol			
	+/– BQ-123			
Campen et al. (2010)	Inhaled DE	$ApoE^{-/-}$ mice	CED	Increase in macrophage content but not % lesional area in both DE and filtered DE-exposed mice
	Inhaled DEG			Increase in aortic TBARS in both DE and filtered-DE mice
				No effects on plasma oxLDL
Chen et al. (2010)	Inhaled CAPs($PM_{2.5}$)	$ApoE^{-/-}$ mice	Chow	Increase in % lesional area in brachiocephalic and left common arteries by ultrasound. Increase was similar to that induced by second hand smoke with a concentration 3 times higher
	6 h/day, 5 days/ week×6 month			
Quan et al. (2010)	Inhaled CAPs($PM_{2.5}$)	$ApoE^{-/-}$ mice	Chow	Increase in % lesional area in aorta and brachiocephalic artery, largest in CAPs or CAPs+DEG on each location, respectively
	DE, filtered-DE, CAPs+filtered-DE			
	5 h/day, 4 days/ week×3&5 month			No effects on plasma lipids

(continued)

Table 9.2 (continued)

Study	Air pollutant	Animal model	Diet	Major findings (Induced by the exposure to air pollutant vs. FA controls)
Kampfrath et al. (2011)	Inhaled CAPs($PM_{2.5}$)	C57BL/6 mice	Chow	Increase in superoxide production in perivascular fat and aorta, Increase in TNFα and MCP-1 in the lungs and plasma, that was Tlr4-dependent
	6 h/day, 5 days/ week×20 week	$Nox2^{-/-}$ mice		
		Tlr4*Lps-d mice*		Increase in oxPAPC in the BALF
				No effects on plasma lipids
Bai et al. (2011)	Inhaled DE	$ApoE^{-/-}$ mice	Chow	Increase in plaque lipid content, cellularity, foam cell formation and smooth muscle cell content
	6 h/day, 5 days/ week×7 week			Increase in systemic lipid and DNA oxidation (8-isoprostane and 8-OH-dG in urine)
				No effects on plasma lipids
Lund et al. (2011)	Inhaled GE, DE and MVE	$ApoE^{-/-}$ mice	CED	MVE increased aortic TBARS and oxidized plasma lipoprotein, inhibited by LOX-1 receptor Ab
	+/– Ab αLOX-1 receptor			MVE led to upregulation of aortic LOX-1, ET-1 and MMP-9
	6 h/day×7 days			
Yin et al. (2013)	Inhaled DE	$ApoE^{-/-}$ mice	Chow	Development of pro-oxidative and pro-inflammatory HDL. No effects on cholesterol efflux capacity
	6 h/day, 5 days/ week×2 week			Increase in oxidized lipids in the blood (8-isoprostanes, 12-HETE, 13-HODE), BALF (12- and 15-HETEs, 13-HODE) and liver (5-HETEs)
				Decreased plasma PON-1 activity
				Activation of 5-lipooxygenase in the liver

Li et al. (2013)	Inhaled reaerosolized UFP +/− D-4 F	LDL-R$^{-/-}$ mice	CED	Increase in lesional area in the aortic root
	5 h/day, 3 days/ week × 10 week			Degree of HDL anti-oxidant dysfunction was associated with aortic atherosclerosis and improved by D-4F
				Increase in plasma oxidized lipids (5-,12- and 15-HETEs, 9-&13-HODEs)
				Increase plasma serum amyloid protein and TNF-α
				No effects on plasma levels of total cholesterol, decrease in HDL and increase in triglycerides

Studies are shown in chronological order based on the year of publication
I.T. intratraqueal, *CAPs* concentrated ambient particles, *DE* diesel emissions, *DEG* DE gases achieved by filtering of whole DE, *GE* gasoline exhaust, *MVE* mixed motor vehicle emissions (DE+GE), *FA* filtered air, *CED* cholesterol enriched diet, *N.S.* not significant

atherosclerotic plaques that were enriched with tissue factor, a pro-thrombotic factor that may have played a causative role or may have simply been an indicator of greater atherosclerotic plaque burden. In addition, a recent study showed that $PM_{2.5}$ exposures led to pro-oxidative effects that were NADPH oxidase and Toll Like Receptor-4-dependent (TLR-4) (Kampfrath et al. 2011). Concentrated $PM_{2.5}$ appears to exert a greater promotion of plaque formation when compared to inhaled side-stream tobacco smoke and assessed by ultrasound bio-microscopy (Chen et al. 2010).

Of interest, while the majority of studies with $PM_{2.5}$ exposures have reported increased atherosclerotic burden (Chen and Nadziejko 2005; Sun et al. 2005, 2008; Quan et al. 2010; Ying et al. 2009), comparable studies of the effects of inhalation of diesel exhaust report only changes in plaque composition (Campen et al. 2010; Bai et al. 2011). Only a single study to date has reported that a 5-month exposure to whole diesel exhaust leads to enhanced size of atherosclerotic plaques in the brachiochephalic artery (Quan et al. 2010).

Various mechanisms have been proposed to mediate PM-induced atherosclerosis as illustrated in Fig. 9.1. The differences in the degree to which aortic atherosclerosis was enhanced by $PM_{2.5}$ in the various CAPs studies are likely due to several factors. These include differences in the length of the exposures, the type of experimental diet, the PM composition and the gender and age of the mice, and thus the extent of atherosclerosis already existing at the time of exposure. For example, while 4–6 month-exposures to concentrated $PM_{2.5}$ in New York (Chen and Nadziejko 2005; Chen et al. 2010; Sun et al. 2005, 2008; Ying et al. 2009) yielded positive results, 5-week exposures to concentrated $PM_{2.5}$ in Los Angeles only resulted in a non-statistically significant trend for increased atherosclerotic lesions (Araujo et al. 2008). Further, while the study from Ying et al. showed that 4-month inhalation exposures to concentrated $PM_{2.5}$ from the Upper East side of Manhattan (6 h/day, 5 days/week) of cholesterol enriched diet (CED)-fed 6-week-old $ApoE^{-/-}$ mice resulted in ~89 % increase in aortic atherosclerotic plaques over controls (Ying et al. 2009), CED-fed mice that were exposed for 5–6 months to CAPs from Sterling Forest, New York resulted in a 58–68 % increase in atherosclerotic lesions over controls (Sun et al. 2005, 2008). As noted by the authors, the larger effects with the Manhattan $PM_{2.5}$ could have been due to higher $PM_{2.5}$ levels and/or a higher relative content of organic carbon (OC) and elemental carbon (EC) in the Manhattan $PM_{2.5}$ (Ying et al. 2009). Data from the National Particle Components Toxicity (NPACT) Initiative sponsored by the Health Effects Institute suggests that PM chemical composition is likely to influence the degree to which it enhances atherogenesis (Vedal et al. 2013; Lippmann et al. 2013). However, the determination of the specific constituents mainly responsible for these toxic effects has remained elusive.

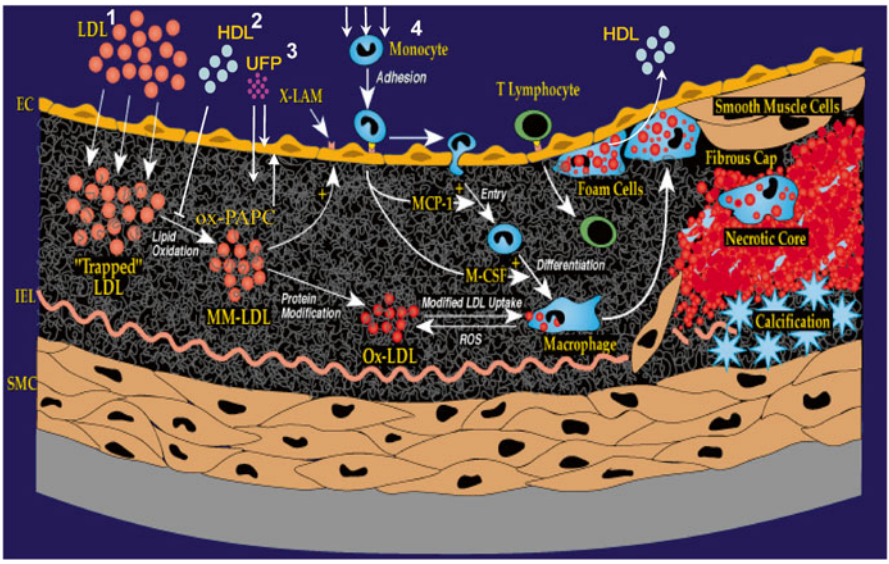

Fig. 9.1 Potential mechanisms how air pollutants promote atherogenesis. The artery wall is infiltrated by lipids originating from circulating LDL, followed by oxidative modification in the subendothelial space, monocyte chemotaxis, differentiation into macrophages and foam cell formation in the early stages of atherosclerotic lesion formation. Release of inflammatory mediators and a vicious cycle of inflammation ensue. More advanced stages of the disease include smooth muscle cell proliferation, formation of fibrous caps, necrotic cores, calcification, rupture, hemorrhage and thrombosis. Possible mechanisms for how PM enhances atherosclerosis include: (*1*) Increased susceptibility for LDL oxidation, (*2*) Development of dysfunctional HDL with loss of HDL anti-oxidant and anti-inflammatory properties, (*3*) Systemically translocated UFP or their chemical constituents may synergize with ox-PAPC generated within ox-LDL in the activation of proatherogenic molecular pathways in endothelial cells, (*4*) Inflammatory mediators released from tissues at the point of entry (e.g. lungs or gastrointestinal tract) may promote monocyte chemotaxis into the vessels (Modified from Araujo and Nel 2009)

9.3 Modulating Factors

9.3.1 Diet

There have been discrepancies between various studies about the effects of cholesterol-enriched diets on the degree of PM-induced atherogenesis. Two studies have reported a significant enhancement of atherosclerosis when ApoE$^{-/-}$ mice were fed a CED but only displayed suggestive trends when mice were fed a chow diet (Sun et al. 2005, 2008). At the same time, one study showed that the $PM_{2.5}$ pro-atherogenic effects were not detected under the extreme hyperlipidemia developed by ApoE×LDL-R double knockout mice (Chen and Nadziejko 2005). As previously noted, it is possible that under certain conditions, the extent of atherosclerotic lesions induced by the diet or genetic cross prior to or during exposure (“noise”)

could mask the effects induced by PM ("signal") so that the signal-to-noise ratio would be too low to be detected. Thus, a more advanced age or a longer length of feeding of a CED resulting in higher levels of hyperlipidemia, could favor a greater development of background atherosclerotic plaques.

It has also been debated whether PM-induced atherogenesis is a function of the very high concentrations of CAPs that animals are exposed to with inhalations or I.T regimes. Soares et al. addressed this issue by exposing LDL-$R^{-/-}$ mice to 1X ambient air (non-filtered) vs. filtered air at a location 20 m away from the roadside in downtown Sao Paulo, Brazil. Although mice exposed to non-filtered air developed atherosclerotic plaques that were comparable in lipid content to those developed by controls, the aortic walls were thicker, suggesting a PM concentration dependent contribution of other components (either cellular or non-cellular) (Soares et al. 2009). However, in this study, additional lipid endpoints were magnified by the administration of a high cholesterol diet as is discussed below.

9.3.2 Particle Size and Composition

There is still the question of whether particle size has any role in PM-mediated pro-atherogenic effects. This has been addressed by a single study by Araujo et al. that tested the hypothesis that the smaller size and greater redox potential of UFP would result in greater pro-atherogenic effects than $PM_{2.5.}$ $ApoE^{-/-}$ mice were fed a chow diet and subjected to concentrated $PM_{2.5}$, UFP or filtered air for 5 h/day, 3 days per week for 5 weeks and effects were assessed by measuring the mean atherosclerotic lesion area in the aortic root. UFP-exposed mice developed 25 % and 55 % greater aortic plaque area as compared to $PM_{2.5}$ or FA-exposed mice respectively, despite an exposure mass that was ~ 4 times less than the $PM_{2.5}$. (Araujo et al. 2008), Given that the concentrator technology employed in this study (VACES: Versatile Aerosol Concentration Enrichment System) generated overlapping CAPs aerosols, it was difficult to estimate the true relative pro-atherogenic strength of UFP vs. $PM_{2.5}$. While it is possible that the UFP fraction could concentrate the PM pro-atherogenic effects, a clear demonstration of this will require the straight comparison of UFP to the accumulation mode particles in the 0.1–2.5 μm range (Araujo and Nel 2009). Interestingly, in this study, the UFP contained a greater relative content of polyaromatic hydrocarbons (PAHs), a group of redox-active compounds that can facilitate free radical reactions (Li et al. 2003; Ntziachristos et al. 2007; Ayres et al. 2008). Additional studies are required to compare $PM_{2.5}$ and UFP exposures in other locations in the world where the effects of different particle size and composition on atherosclerosis can be assessed.

9.3.3 *Gaseous Pollutants*

There has been a continuous debate about whether air pollution-mediated pro-atherogenic effects are all due to the particulate components or are also due to the gaseous constituents, especially since the epidemiological studies have consistently identified associations with the PM. While one meta-analysis has found positive associations of gaseous pollutants such as CO, and sulfur and nitrogen dioxides with MI (Mustafic et al. 2012), the studies included in that meta-analysis can not be extrapolated to imply a role for the gases in atherogenesis since they may induce MI by non-atherosclerotic effects. There is however, experimental data that support a potential role for gaseous pollutants in promoting atherosclerosis. Lewis et al. showed that exposures of CED-fed C57BL6 mice to carbon disulfide (CS_2), a known volatile organic compound, at 500 and 800 ppm CS_2 for 6 h/day, 5 days/week for up to 20 weeks, markedly enhanced aortic atherosclerosis (Lewis et al. 1999). In addition, Chuang et al. reported that $ApoE^{-/-}$ mice exposed to 0.5 ppm O_3 for 8 h/day, 5 days/week for 8 weeks exhibited more than double the degree of aortic atherosclerosis than the filtered air-exposed controls (Chuang et al. 2009) (Table 9.2). Furthermore, Campen et al. examined the effects of whole vs. particle-filtered diesel exhaust emissions on atherosclerotic lesion formation in CED-fed 10-week-old male $ApoE^{-/-}$ mice over 50 days. Although they did not find significant differences in the extent of aortic plaque formation between the various groups, the mice exposed to whole diesel exhaust developed atherosclerotic lesions with a greater macrophage content (Campen et al. 2010) (Table 9.2). Interestingly, exposure to whole diesel exhaust led to upregulation of MMP-9 in the aorta, consistent with similar effects reported for gasoline exhaust (Lund et al. 2007). While particle-filtering tended to diminish the effects of the diesel emissions on plaque macrophage content, it didn't alter the degree of aortic MMP-9 upregulation (Campen et al. 2010), suggesting that both gaseous and particulate components could be affecting different pathways. It is possible that gaseous and particulate pollutants could exert different but cooperative effects, which will need to be explored in more detail in the future. The contribution of particulate and gaseous components in motor vehicle emissions has also been addressed in the NPACT studies, which are soon to be reported.

9.4 Pathogenic Mechanisms

9.4.1 *Role of ROS and Inflammation*

PM-enhanced atherosclerosis is most likely the result of systemic pro-oxidant and pro-inflammatory effects (Araujo 2011). Long-term exposure to $PM_{2.5}$ activates NADPH oxidase via upregulation of the NADPH oxidase subunits p47phox and Rac1 (Ying et al. 2009), resulting in increased production of superoxide in monocytes, aortic tissue and perivascular fat (Kampfrath et al. 2011). Increased

generation of ROS leads to oxidative stress in systemic tissues. This is supported by data showing that exposure to CAPs in the $PM_{2.5}$ and UFP size ranges led to increased hepatic lipid peroxidation that was accompanied by an upregulation of Nrf2-regulated anti-oxidant genes in the UFP-exposed mouse livers (Araujo et al. 2008; Gong et al. 2007), while $PM_{2.5}$ exposure led to enhanced formation of 3-nitrotyrosine residues (Sun et al. 2005; Ying et al. 2009). Chronic exposure to urban air pollution enhanced the susceptibility of LDL to oxidation in hyperlipemic LDL-$R^{-/-}$ mice fed a high fat diet (Soares et al. 2009). Inhalation of diesel exhaust also enhanced lipid peroxidation in the liver (Yin et al. 2013). Plasma HETEs and HODEs, additional markers of increased lipid peroxidation, were induced by exposure to diesel exhaust and re-aerosolized ultrafine particles in $ApoE^{-/-}$ and LDL-$R^{-/-}$ mice, respectively (Yin et al. 2013; Li et al. 2013).

PM exposure has also been associated with increased pro-inflammatory mediators in the systemic circulation in animals (Tamagawa et al. 2008; Mutlu et al. 2007). I.T. administration of residual oil fly ash (ROFA) to rats (Nurkiewicz et al. 2004, 2006) led to greater vascular ROS generation, as assessed by the Tetranitro Blue Tetrazolium (TNBT) reduction method (Nurkiewicz et al. 2006), and resulted in a dose-dependent impairment of systemic endothelium-dependent arterial dilation, increased leukocyte rolling and adhesion, as well as deposition of myeloperoxidase in the spinotrapezius muscle microcirculation (Nurkiewicz et al. 2004, 2006). In New Zealand White Rabbits and mice, I.T. PM_{10} administration resulted in significantly increased serum IL-6 and TNF-α levels (Tamagawa et al. 2008; Mutlu et al. 2007). Interestingly, lack of IL-6 ameliorated the PM_{10}-mediated systemic pro-inflammatory response as well as a PM_{10} induced pro-coagulant state in mice (Mutlu et al. 2007), suggesting an important role for IL-6 in the mediation of PM induced systemic inflammatory effects. Likewise, inhalation of fine CAPs led to an elevation in circulating IL-6 and TNF-α (Sun et al. 2009) together with increases in circulating adipokines, such as resistin. There was also a change in the balance of macrophages in adipose tissue towards the pro-inflammatory M1 phenotype, suggestive of a systemic pro-inflammatory state (Sun et al. 2009). Furthermore, it has been shown that PM induces increased monocyte migration to atherosclerotic vessels in Watanabe rabbits (Yatera et al. 2008). The simultaneous presence of pro-oxidative and pro-inflammatory effects has led to a linear causal paradigm for the PM-pro-atherogenic effects as shown in Fig. 9.2a.

Several additional *in vitro* studies support the concept that the pro-oxidative effects induced by air pollutants occur directly in cells of the vasculature such as endothelial cells (Bai et al. 2001; Hirano et al. 2003; Li et al. 2006, 2010; Montiel-Davalos et al. 2010), macrophages (Soukup et al. 2000; Hiura et al. 1999; Li et al. 2004; Ohyama et al. 2007; Lee and Kang 2002; Chio et al. 2007; Goldsmith et al. 1998) and possibly smooth muscle cells (Sun et al. 2008). These pro-oxidative effects are strongly linked to the induction of pro-inflammatory responses in the same cell types as previously reviewed by Araujo (2011). These include the activation of the NF-kB (Li et al. 2010; Montiel-Davalos et al. 2010), p38 MAPK (Mo et al. 2009; Sumanasekera et al. 2007) and ERK1/2 pathways (Mo et al. 2009) with subsequent upregulation of pro-inflammatory factors such as TNF-α, IL-8, and monocyte

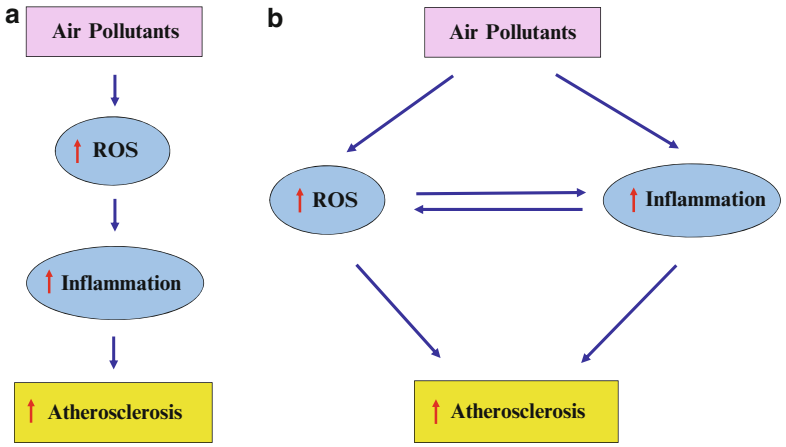

Fig. 9.2 Mechanistic models for the induction of proatherogenic effects induced by air pollutants. (**a**) Linear causal paradigm. (**b**) Alternative non-linear model

chemotactic protein-1 (MCP-1), (Li et al. 2010; Montiel-Davalos et al. 2010), and adhesion molecules such as VCAM (Li et al. 2010), E-selectin, P-selectin (Montiel-Davalos et al. 2007) in endothelial cells. There is also an air pollution induced increase in production of TNF-α, IL-6 (Alfaro-Moreno et al. 2002; Fujii et al. 2002; Osornio-Vargas et al. 2003; Pozzi et al. 2003; Becker et al. 2005a; Amakawa et al. 2003), IL-8 (Becker et al. 2005b; Monn and Becker 1999), IL1-α (Brown et al. 2004), IL1-β (Jimenez et al. 2002), granulocyte macrophage colony-stimulating factor (GM-CSF) (Fujii et al. 2002; Ishii et al. 2005) and macrophage-inflammatory protein-2 (MIP-2) (Imrich et al. 2007) in macrophages.

Controlled exposure studies, as well as panel and cross-sectional studies in humans also support the association of exposure to PM with increased systemic oxidative stress, via the detection of biomarkers of oxidative alteration of proteins, lipids and/or DNA in the circulating blood or in products excreted in the urine, as reviewed by Moller and Loft (2010). For example, Liu et al. reported the association of increases in exposure to black carbon and $PM_{2.5}$ with an elevation in plasma levels of thiobarbituric acid reactive substances (TBARS), a measure of reactive aldehydes derived from the oxidation of fatty acids, in 28 nonsmoking seniors (Liu et al. 2009). This is in agreement with earlier reports of increased serum TBARS in association with $PM_{2.5}$ exposures (Sorensen et al. 2003) or after moving or living in a polluted urban location such as Mexico city (Medina-Navarro et al. 1997; Sanchez-Rodriguez et al. 2005). Measures of oxidation in circulating blood are highly relevant since they may imply the involvement of oxidatively modified plasma lipoproteins such as LDL and/or HDL, key players in the promotion or protection from atherosclerosis, respectively (Araujo and Nel 2009). PM exposures also associated with increased blood levels of GM-CSF, IL-6, IL-1, sTNF-RII, CRP and CD40 ligand (sCD40L) (van Eeden et al. 2001; Delfino et al. 2008; Ruckerl et al. 2007; Chuang et al. 2007; Dubowsky et al. 2006).

Interestingly, the ability of diesel exhaust particulates (DEP) in the ultrafine range generated from the same engine in idling vs. driving modes led to particles with different chemical compositions that had divergent pro-oxidant vs. pro-inflammatory potentials (Li et al. 2010). This indicates that PM can activate various pathways, not all ROS-related. In addition, pro-inflammatory effects have not always been associated with pro-oxidative events which suggests that air pollutants can directly activate inflammatory pathways, leading to a potential redefinition of the ROS and inflammation mechanistic model (Fig. 9.2b).

9.4.2 Role of the Lungs in Mediating Systemic Effects

Systemic pro-oxidative and pro-inflammatory effects could be derived from increased production of ROS and development of inflammation in the lungs that somehow get transduced to the systemic tissues. This may be due to the release of inflammatory mediators into the blood. However, it is also conceivable that particles, gases or their chemical constituents enter into the systemic circulation with direct action at the target vascular sites. There are multiple reports that support the ability of PM to induce local pro-oxidant and pro-inflammatory responses in the lungs (Gunnison and Chen 2005; Wang et al. 2008). Studies with intra-tracheal administration of PM have shown induction of obvious pulmonary inflammation, evidenced by increased total cell counts in the bronchoalveolar lavage fluid (BALF), modification of the BALF cell differential and infiltration of the lungs by inflammatory cells with the development of histologically-defined pulmonary inflammatory foci (Gunnison and Chen 2005; Wang et al. 2008). I.T. administration of PM also leads to up-regulation of inflammatory genes (Ayres et al. 2008; Wang et al. 2008; Wise et al. 2006). Suwa et al. found that I.T. administration of PM_{10} resulted in a systemic response characterized by increased blood counts of polymorphonuclear cells and circulating band cells, together with an increased volume of atherosclerotic lesions in the coronary arteries. In addition, they observed that there was a correlation between the percentage of alveolar macrophages containing particles and the vol/vol of atherosclerotic lesions in the vessels ($r = 0.53$, $p < 0.05$) (Suwa et al. 2002) suggesting that the pulmonary response is associated with the atherosclerotic effects. However, while some studies have shown that inhaled ultrafine carbon particles (Andre et al. 2006) or CAPs (Lei et al. 2004) can also lead to increased inflammatory cell counts in the BALF or increased inflammatory cytokines (Kampfrath et al. 2011), others have shown no effects on either BALF cell counts, histology or lung pro-inflammatory gene expression (Gunnison and Chen 2005; Ito et al. 2008; Heidenfelder et al. 2009). These different responses could be due to the variability in CAPs composition or length of exposures (Saldiva et al. 2002; Morishita et al. 2004). Consistent with the latter, we have observed that DE for 2 weeks (Yin et al. 2013) or CAPs for 5 weeks (Araujo et al. 2008) did not result in the development of obvious pulmonary inflammation (Araujo et al. 2008; Yin et al. 2013), but clearly led to lipid and pro-atherogenic effects, respectively. While

this suggests that CAPs and motor vehicle emissions can bypass the lungs, it is also possible that they lead to activation of inflammatory molecular pathways without histological evidence of overt pulmonary inflammation or local activation of cells leading to pan-systemic activation of the immune system. Thus, both fine CAPs (Kampfrath et al. 2011) and DE (Yin et al. 2013) have been shown to lead to enhanced lipid peroxidation in the lungs resulting in increased levels in the BALF of oxidized phospholipids (Kampfrath et al. 2011) and oxidized fatty acids (Yin et al. 2013), respectively. Lipid peroxidation in the BALF was accompanied by increased levels of TNF-α, MCP-1 and IL-12 in lung homogenates and increased plasma levels of TNF-α and MCP-1 (Kampfrath et al. 2011), effects that were blunted in mice deficient in TLR-4. This suggests that activation of pathways participating in innate immunity was required (Kampfrath et al. 2011). However, other CAPs studies have not found increased plasma levels of pro-inflammatory cytokines, suggesting that this may not be a generalized mechanism for how inhalation of air pollutants promotes atherosclerosis. Therefore, while CAPs and DE promote oxidation events in the BALF, it is still not clear whether and/or how these effects are associated with pro-atherosclerotic actions.

9.5 Effects on Lipids: A Potential Missing Link

Sun et al. observed that ApoE$^{-/-}$ mice exposed to fine CAPs for 6 months exhibited a small decrease in total plasma cholesterol on a chow diet but a small increase in total cholesterol when animals were fed a high-fat diet (Sun et al. 2005), without any effects on the levels of plasma triglycerides. Araujo et al. noted that chow-fed ApoE$^{-/-}$ mice exposed for 5 weeks to fine but not to ultrafine CAPs showed a small increase in total plasma cholesterol without any effects on HDL cholesterol levels (Araujo et al. 2008). Likewise, Li et al. reported that inhalation of re-aerosolized UFP for 10 weeks did not affect plasma levels of total cholesterol but led to decreased HDL cholesterol and higher plasma triglyceride levels (Li et al. 2013) (Table 9.2) in LDL-R$^{-/-}$ mice fed a high fat diet. However, other studies have not observed any effects on the quantitative levels of plasma lipids in either normolipidemic (Kampfrath et al. 2011) or hyperlipidemic animals, either following intra-tracheal administration (Suwa et al. 2002), inhalation of CAPs (Quan et al. 2010) or inhalation of MVE (Bai et al. 2001). Altogether, it appears that these effects on plasma lipids are highly dependent on the genetic background, type of diet and length of exposure. In addition, the effects on total plasma cholesterol have been relatively small (Sun et al. 2005; Araujo et al. 2008) and unlikely to be a major driver of the overall pro-atherosclerotic effects. For instance, while 5-week exposure to fine CAPs did lead to a small increase in total plasma cholesterol levels, this only resulted in a trend towards larger plaques (Araujo et al. 2008). In contrast, exposure to ultrafine CAPs for a similar length of time did not influence total plasma cholesterol levels but significantly promoted atherosclerosis (Araujo et al. 2008).

While quantitative effects on plasma lipids may not account for the observed effects on atherosclerosis, qualitative effects on plasma lipids may be quite important. Campen et al. (2010) and Lund et al. (2009) have shown that exposure of ApoE null mice to DE or GE respectively, led to increased oxidized lipids (TBARS) in the aorta. DE exposure alone did not increase plasma TBARS. However, exposure of $ApoE^{-/-}$ mice to MVE did result in enhanced plasma TBARS (Lund et al. 2011), suggesting a greater toxicity for MVE in comparison to DE alone. We have recently shown that $ApoE^{-/-}$ mice exposed to DE for 2 weeks led to increased plasma levels of 8-isoprostanes, 12-HETEs and 13-HODEs, oxidized products of arachidonic and linoleic acid, respectively (Yin et al. 2013). This is consistent with the findings of Bai et al. who reported that $ApoE^{-/-}$ mice exposed to diesel exhaust for 7 weeks led to increased urinary excretion of 8-isoprostane and 8-OH-dG (Bai et al. 2011), measures indicative of increased systemic oxidation of lipids and DNA, respectively. We also found that diesel exhaust led to increased susceptibility to oxidation by air of a plasma lipoprotein fraction enriched in VLDL and LDL (Yin et al. 2013). This is consistent with the study from Soares et al. which indicated that chronic exposure to ambient levels of urban air pollution led to enhanced susceptibility of LDL to oxidation and increased titers of antibodies against oxLDL and apoB in hyperlipemic $LDL\text{-}R^{-/-}$ mice fed a high cholesterol diet (Soares et al. 2009). Li et al. also showed that increased HETEs and HODEs could be measured in the LDL fraction of $LDL\text{-}R^{-/-}$ mice exposed to inhaled re-aerosolized UFP for 10 weeks (Li et al. 2003). Altogether, this indicates that ambient PM and motor vehicle emissions can increase lipid peroxidation in the plasma, resulting in LDL particles that are either more oxidized or more susceptible to oxidation than filtered air controls.

We have also shown that qualitative effects on plasma lipoproteins appear to alter the functional properties of the HDL particles. There are a number of protective functions of HDL that are negatively correlated with atherosclerosis and CAD. Thus, exposure of $ApoE^{-/-}$ mice to fine and ultrafine CAPs for 5 weeks led to the development of dysfunctional pro-inflammatory HDL (Araujo et al. 2008). The degree of HDL dysfunction was particle size-dependent since UFP exposures led to a greater degree of dysfunction than did $PM_{2.5}$ (Araujo et al. 2008). The anti-inflammatory properties of HDL were measured using an LDL-induced monocyte chemotactic assay in a co-culture of endothelial cells and smooth muscle cells. In comparison to $PM_{2.5,}$ exposure to UFP not only failed to inhibit the LDL-mediated inflammatory effects but promoted more monocyte migration. The degree of HDL dysfunction correlated with the extent of atherosclerosis and a systemic Nrf2-regulated anti-oxidant response. This supports the notion that dysfunctional HDL can either play a role in disease pathogenesis or serve as a marker for the PM-mediated systemic pro-oxidant and/or pro-inflammatory effects. Indeed, pro-inflammatory HDL is predictive of the susceptibility to atherosclerosis in humans (Ansell et al. 2003) and in rabbits (Van Lenten et al. 2007). Furthermore, HDL's reverse cholesterol transport function inversely associates with carotid intima-media thickness and the likelihood of angiographic coronary artery disease (CAD) (Khera et al. 2011) while HDL's anti-oxidant function is significantly impaired in subjects with acute coronary syndrome, as compared with healthy subjects or those with stable CAD (Patel et al.

2011). More recently, we have shown that inhalation of diesel exhaust for as short as 2 weeks not only led to the development of pro-inflammatory HDL but also caused the loss of the anti-oxidant properties of the HDL, turning the HDL particles from anti-oxidant into pro-oxidant lipoproteins. The anti-oxidant dysfunction correlated with markers of lipid peroxidation in the blood suggesting that it may be related to lipid peroxidation within the HDL particles (Yin et al. 2013). Of interest, the kinetics of recovery of the anti-oxidant and anti-inflammatory capacities was different, indicating that they may be affected in a different manner. Thus, exposure to CAPs and diesel exhaust lead to plasma lipids that are functionally pro-atherogenic and characterized by increased oxidation or greater susceptibility to oxidation of the LDL particles and by the development of HDL with dysfunctional anti-oxidant and anti-inflammatory properties. It is possible that these effects on the lipid functional profiles could represent the missing link between air pollutant-induced effects in the lungs and the pro-atherosclerotic effects observed in the blood vessels. Alternatively, enhanced oxidation of plasma lipids could function as a marker of systemic lipid peroxidation induced by the air pollutants and occur parallel to the vascular effects, which will need to be evaluated in the future.

9.6 Novel Biomarkers of Air Pollution-Induced Cardiovascular Effects

Work with experimental animals, discussed in this chapter; support the notion that associations between air pollution, atherosclerosis and ischemic cardiovascular events are causal. Therefore, there is need for the identification of biomarkers aimed at the detection of pro-atherosclerotic effects that could be induced at different times after the exposure to air pollutants. Recent animal work has shown that air pollutant exposures result in oxidative modifications of plasma lipids, resulting in increased plasma levels of TBARS, 8-isoprostanes, HETEs, HODEs and qualitative alterations of various plasma lipoproteins, conducive to oxidized LDL and dysfunctional HDL. While some measures of systemic oxidative stress such as plasma TBARS and MDA have been tested in human panel and cross-sectional studies (Moller and Loft 2010), the results have not always been encouraging. Determining whether novel markers, such as HETEs and HODEs or those oriented to evaluate HDL functionality, identified in experimental animals, translate into human subjects exposed to “real life” levels of air pollutants would be very important as they could provide greater sensitivity and be more informative on the pathological relevance of the cardiovascular effects that are induced.

Different markers can be more or less informative. Some markers offer a greater sensitivity while other markers provide a greater specificity for the detection of lipid oxidation in the plasma. For example, TBARS are known to be a sensitive marker but relatively unspecific, while MDA, (an end product of lipid peroxidation and measured in the TBARS assay), should be more specific. Whether evaluating HETEs and HODEs (more upstream oxidative products than MDA), yields a better

balance of optimal sensitivity and specificity needs to be tested. Markers could be informative of the acuity vs. chronicity of the health effects. Thus, while LDL oxidizability is indicative of acute, and perhaps hyperacute effects, the degree of LDL oxidation and development of anti-oxLDL antibodies are more likely informative of subacute and chronic effects that require a longer time for the establishment of an acquired immune response against oxidized LDL. The combination of various markers may also be informative of the kinetics of the effects. For instance, air pollutant effects on LDL oxidizability may be reversed more rapidly than effects on HDL anti-oxidant capacity, as suggested by our studies with diesel exhaust exposures in ApoE null mice (Yin et al. 2013). The specific type of biomarkers chosen can also be informative of the nature of the cardiovascular responses that are induced and the potential clinical implications. Thus, induction of HDL reverse cholesterol transport dysfunction is probably more indicative of a greater susceptibility for development of atherosclerosis (Khera et al. 2011) while induction of HDL anti-oxidant dysfunction may be more indicative of the propensity to develop acute coronary syndromes in subjects with CAD (Patel et al. 2011). Therefore, there is great potential for translation of these new measures of lipid peroxidation into novel sensitive and specific biomarkers of the type and nature of human cardiovascular disease responses to AP.

9.7 Conclusions

In the last few years, there has been marked progress in our understanding of the cardiovascular effects of air pollution. Based on the cumulative evidence, we can conclude at the current time: (1) Epidemiological studies support a positive association between the exposure to ambient PM and the development and progression of atherosclerosis, (2) Experimental animal work using hypercholesterolemic rabbits, $ApoE^{-/-}$ and $LDLR^{-/-}$ mice suggest that those associations are very likely to be causal, (3) PM-mediated enhancement of atherosclerosis is strongly associated with the development of systemic pro-oxidant and pro-inflammatory responses, (4) PM-induced pro-atherogenic effects are favored by a small particle size as ultrafine ambient particles appear to be more pro-atherogenic than the bigger particles found in the fine size fraction, (5) The degree of PM-enhancement of atherosclerosis can be modulated by the experimental diet, length of exposures and likely to be affected by PM chemical composition, (6) Gaseous pollutants may also play a role in mediating atherogenesis or enhancing the toxicity of PM-induced atherosclerosis, (7) Exposure to $PM_{2.5}$, ultrafine particles and diesel exhaust lead to enhanced systemic lipid peroxidation and pro-atherogenic plasma lipid profiles, characterized by LDL particles that are oxidatively modified or more susceptible to oxidation and dysfunctional HDL particles that lose their anti-oxidant and anti-inflammatory properties and even promote further oxidation and inflammation.

While PM-induction of systemic pro-oxidant and pro-inflammatory responses could be important in the enhancement of atherosclerosis, the mechanisms for the

development of those effects are still unknown as well as the precise role for the lungs in their systemic transduction. There is need of epidemiological and exposure studies with UFP to test the hypothesis in humans that UFP carry a larger toxicity than bigger particles. PM effects on circulating lipoproteins need to be characterized in detail and confirmed in humans, since they may yield a better understanding of PM-mediated pathogenesis and could be a promising biomarker of cardiovascular health effects.

Acknowledgments Writing of this article was supported by the National Institute of Environmental Health Sciences, National Institutes of Health (ONES RO1 Award ES016959 to Jesus A. Araujo).

References

Adar SD, Klein R, Klein BE, Szpiro AA, Cotch MF, Wong TY, O'Neill MS et al (2010) Air pollution and the microvasculature: a cross-sectional assessment of in vivo retinal images in the population-based multi-ethnic study of atherosclerosis (MESA). PLoS Med 7:e1000372

Adar SD, Sheppard L, Vedal S, Polak JF, Sampson PD, Diez Roux AV, Budoff M, Jacobs DR Jr, Barr RG, Watson K, Kaufman JD (2013) Fine particulate air pollution and the progression of carotid intima-medial thickness: a prospective cohort study from the multi-ethnic study of atherosclerosis and air pollution. PLoS Med 10(4):e1001430

Alfaro-Moreno E, Martinez L, Garcia-Cuellar C, Bonner JC, Murray JC, Rosas I, Rosales SP et al (2002) Biologic effects induced in vitro by PM10 from three different zones of Mexico City. Environ Health Perspect 110:715–720

Allen RW, Criqui MH, Diez Roux AV, Allison M, Shea S, Detrano R, Sheppard L, Wong ND, Stukovsky KH, Kaufman JD (2009) Fine particulate matter air pollution, proximity to traffic, and aortic atherosclerosis. Epidemiology 20(2):254–264

Amakawa K, Terashima T, Matsuzaki T, Matsumaru A, Sagai M, Yamaguchi K (2003) Suppressive effects of diesel exhaust particles on cytokine release from human and murine alveolar macrophages. Exp Lung Res 29:149–164

Andre E, Stoeger T, Takenaka S, Bahnweg M, Ritter B, Karg E, Lentner B et al (2006) Inhalation of ultrafine carbon particles triggers biphasic pro-inflammatory response in the mouse lung. Eur Respir J 28:275–285

Ansell BJ, Navab M, Hama S, Kamranpour N, Fonarow G, Hough G, Rahmani S et al (2003) Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. Circulation 108:2751–2756

Araujo J (2011) Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis. Air Qual Atmos Health 4:79–93

Araujo JA, Nel AE (2009) Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress. Part Fibre Toxicol 6:24

Araujo JA, Barajas B, Kleinman M, Wang X, Bennett BJ, Gong KW, Navab M, Harkema J, Sioutas C, Lusis AJ, Nel AE (2008) Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. Circ Res 102(5):589–596

Araujo JA, Zhang M, Yin F (2012) Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Front Pharmacol 3:119

Ayres JG, Borm P, Cassee FR, Castranova V, Donaldson K, Ghio A, Harrison RM et al (2008) Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential. A workshop report and consensus statement. Inhal Toxicol 20:75–99

Bai Y, Suzuki AK, Sagai M (2001) The cytotoxic effects of diesel exhaust particles on human pulmonary artery endothelial cells in vitro: role of active oxygen species. Free Radic Biol Med 30:555–562

Bai N, Kido T, Suzuki H, Yang G, Kavanagh TJ, Kaufman JD, Rosenfeld ME, van Breemen C, Eeden SF (2011) Changes in atherosclerotic plaques induced by inhalation of diesel exhaust. Atherosclerosis 216(2):299–306

Bauer M, Moebus S, Mohlenkamp S, Dragano N, Nonnemacher M, Fuchsluger M, Kessler C, Jakobs H, Memmesheimer M, Erbel R, Jockel KH, Hoffmann B (2010) Urban particulate matter air pollution is associated with subclinical atherosclerosis: results from the HNR (Heinz Nixdorf Recall) study. J Am Coll Cardiol 56(22):1803–1808

Becker S, Mundandhara S, Devlin RB, Madden M (2005a) Regulation of cytokine production in human alveolar macrophages and airway epithelial cells in response to ambient air pollution particles: further mechanistic studies. Toxicol Appl Pharmacol 207:269–275

Becker S, Dailey L, Soukup JM, Silbajoris R, Devlin RB (2005b) TLR-2 is involved in airway epithelial cell response to air pollution particles. Toxicol Appl Pharmacol 203:45–52

Bhatnagar A (2006) Environmental cardiology: studying mechanistic links between pollution and heart disease. Circ Res 99:692–705

Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, Luepker R et al (2004) Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655–2671

Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F et al (2010) Particulate matter air pollution and cardiovascular disease. An update to the scientific statement from the American Heart Association. Circulation 121:2331–2378

Brown DM, Donaldson K, Stone V (2004) Effects of PM10 in human peripheral blood monocytes and J774 macrophages. Respir Res 5:29

Campen MJ, Lund AK, Knuckles TL, Conklin DJ, Bishop B, Young D, Seilkop S, Seagrave J, Reed MD, McDonald JD (2010) Inhaled diesel emissions alter atherosclerotic plaque composition in ApoE(-/-) mice. Toxicol Appl Pharmacol 242(3):310–317

Chen LC, Nadziejko C (2005) Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. V. CAPs exacerbate aortic plaque development in hyperlipidemic mice. Inhal Toxicol 17(4–5):217–224

Chen LC, Quan C, Hwang JS, Jin X, Li Q, Zhong M, Rajagopalan S, Sun Q (2010) Atherosclerosis lesion progression during inhalation exposure to environmental tobacco smoke: a comparison to concentrated ambient air fine particles exposure. Inhal Toxicol 22(6):449–459

Chio C-P, Chen S-C, Chiang K-C, Chou W-C, Liao C-M (2007) Oxidative stress risk analysis for exposure to diesel exhaust particle-induced reactive oxygen species. Sci Total Environ 387:113–127

Chuang KJ, Chan CC, Su TC, Lee CT, Tang CS (2007) The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am J Respir Crit Care Med 176:370–376

Chuang GC, Yang Z, Westbrook DG, Pompilius M, Ballinger CA, White CR, Krzywanski DM et al (2009) Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol 297:L209–L216

Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Gillen DL, Kleinman MT et al (2008) Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease. Environ Health Perspect 116:898–906

Diez Roux AV, Auchincloss AH, Franklin TG, Raghunathan T, Barr RG, Kaufman J, Astor B, Keeler J (2008) Long-term exposure to ambient particulate matter and prevalence of subclinical atherosclerosis in the Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol 167(6):667–675

Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR (2006) Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. Environ Health Perspect 114:992–998

Fujii T, Hayashi S, Hogg JC, Mukae H, Suwa T, Goto Y, Vincent R et al (2002) Interaction of alveolar macrophages and airway epithelial cells following exposure to particulate matter produces mediators that stimulate the bone marrow. Am J Respir Cell Mol Biol 27:34–41

Goldsmith CA, Imrich A, Danaee H, Ning YY, Kobzik L (1998) Analysis of air pollution particulate-mediated oxidant stress in alveolar macrophages. J Toxicol Environ Health A 54:529–545

Gong KW, Zhao W, Li N, Barajas B, Kleinman M, Sioutas C, Horvath S et al (2007) Air-pollutant chemicals and oxidized lipids exhibit genome-wide synergistic effects on endothelial cells. Genome Biol 8:R149

Gunnison A, Chen LC (2005) Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. VI. Gene expression in heart and lung tissue. Inhal Toxicol 17:225–233

Heidenfelder BL, Reif DM, Harkema JR, Cohen Hubal EA, Hudgens EE, Bramble LA, Wagner JG et al (2009) Comparative microarray analysis and pulmonary changes in Brown Norway rats exposed to ovalbumin and concentrated air particulates. Toxicol Sci 108:207–221

Hirano S, Furuyama A, Koike E, Kobayashi T (2003) Oxidative-stress potency of organic extracts of diesel exhaust and urban fine particles in rat heart microvessel endothelial cells. Toxicology 187:161–170

Hiura TS, Kaszubowski MP, Li N, Nel AE (1999) Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J Immunol 163:5582–5591

Hoffmann B, Moebus S, Mohlenkamp S, Stang A, Lehmann N, Dragano N, Schmermund A, Memmesheimer M, Mann K, Erbel R, Jockel KH (2007) Residential exposure to traffic is associated with coronary atherosclerosis. Circulation 116(5):489–496

Hoyert DL, Xu J (2012) Deaths: preliminary data for 2011. Natl Vital Stat Rep 61:1–52

Imrich A, Ning Y, Lawrence J, Coull B, Gitin E, Knutson M, Kobzik L (2007) Alveolar macrophage cytokine response to air pollution particles: oxidant mechanisms. Toxicol Appl Pharmacol 218:256–264

Ishii H, Hayashi S, Hogg JC, Fujii T, Goto Y, Sakamoto N, Mukae H et al (2005) Alveolar macrophage-epithelial cell interaction following exposure to atmospheric particles induces the release of mediators involved in monocyte mobilization and recruitment. Respir Res 6:87

Ito T, Suzuki T, Tamura K, Nezu T, Honda K, Kobayashi T (2008) Examination of mRNA expression in rat hearts and lungs for analysis of effects of exposure to concentrated ambient particles on cardiovascular function. Toxicology 243:271–283

Jimenez LA, Drost EM, Gilmour PS, Rahman I, Antonicelli F, Ritchie H, MacNee W et al (2002) PM(10)-exposed macrophages stimulate a proinflammatory response in lung epithelial cells via TNF-alpha. Am J Physiol Lung Cell Mol Physiol 282:L237–L248

Kampfrath T, Maiseyeu A, Ying Z, Shah Z, Deiuliis JA, Xu X, Kherada N et al (2011) Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways. Circ Res 108(6):716–726

Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC et al (2011) Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 364:127–135

Kunzli N, Jerrett M, Mack WJ, Beckerman B, LaBree L, Gilliland F, Thomas D, Peters J, Hodis HN (2005) Ambient air pollution and atherosclerosis in Los Angeles. Environ Health Perspect 113(2):201–206

Kunzli N, Jerrett M, Garcia-Esteban R, Basagana X, Beckermann B, Gilliland F, Medina M, Peters J, Hodis HN, Mack WJ (2010) Ambient air pollution and the progression of atherosclerosis in adults. PLoS ONE 5(2):e9096

Lee CC, Kang JJ (2002) Extract of motorcycle exhaust particles induced macrophages apoptosis by calcium-dependent manner. Chem Res Toxicol 15:1534–1542

Lei YC, Chen MC, Chan CC, Wang PY, Lee CT, Cheng TJ (2004) Effects of concentrated ambient particles on airway responsiveness and pulmonary inflammation in pulmonary hypertensive rats. Inhal Toxicol 16:785–792

Lewis JG, Graham DG, Valentine WM, Morris RW, Morgan DL, Sills RC (1999) Exposure of C57BL/6 mice to carbon disulfide induces early lesions of atherosclerosis and enhances arterial fatty deposits induced by a high fat diet. Toxicol Sci 49:124–132

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M et al (2003) Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111:455–460

Li N, Alam J, Venkatesan MI, Eiguren-Fernandez A, Schmitz D, Di Stefano E, Slaughter N et al (2004) Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. J Immunol 173:3467–3481

Li Z, Hyseni X, Carter JD, Soukup JM, Dailey LA, Huang YC (2006) Pollutant particles enhanced H_2O_2 production from NAD(P)H oxidase and mitochondria in human pulmonary artery endothelial cells. Am J Physiol Cell Physiol 291:C357–C365

Li R, Ning Z, Majumdar R, Cui J, Takabe W, Jen N, Sioutas C et al (2010) Ultrafine particles from diesel vehicle emissions at different driving cycles induce differential vascular pro-inflammatory responses: implication of chemical components and NF-kappaB signaling. Part Fibre Toxicol 7:6

Li R, Navab M, Pakbin P, Ning Z, Navab K, Hough G, Morgan TE, Finch CE, Araujo JA, Fogelman AM, Sioutas C, Hsiai T (2013) Ambient ultrafine particles alter lipid metabolism and HDL anti-oxidant capacity in LDLR-null mice. J Lipid Res 54(6):1608–1615

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, AlMazroa MA et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224–2260

Lippmann M, Chen LC, Gordon T, Ito K, Thurston GD (2013) National Particle Component Toxicity (NPACT) Initiative: integrated epidemiologic and toxicologic studies of the health effects of particulate matter components. Res Rep Health Eff Inst 177:5–13

Liu L, Ruddy T, Dalipaj M, Poon R, Szyszkowicz M, You H, Dales RE et al (2009) Effects of indoor, outdoor, and personal exposure to particulate air pollution on cardiovascular physiology and systemic mediators in seniors. J Occup Environ Med 51:1088–1098

Lund AK, Knuckles TL, Obot Akata C, Shohet R, McDonald JD, Gigliotti A, Seagrave JC et al (2007) Gasoline exhaust emissions induce vascular remodeling pathways involved in atherosclerosis. Toxicol Sci 95:485–494

Lund AK, Lucero J, Lucas S, Madden MC, McDonald JD, Seagrave JC, Knuckles TL, Campen MJ (2009) Vehicular emissions induce vascular MMP-9 expression and activity associated with endothelin-1-mediated pathways. Arterioscler Thromb Vasc Biol 29(4):511–517

Lund AK, Lucero J, Harman M, Madden MC, McDonald JD, Seagrave JC, Campen MJ (2011) The oxidized low-density lipoprotein receptor mediates vascular effects of inhaled vehicle emissions. Am J Respir Crit Care Med 184(1):82–91

Lusis AJ (2000) Atherosclerosis. Nature 407:233–241

Medina-Navarro R, Lifshitz A, Wacher N, Hicks JJ (1997) Changes in human serum antioxidant capacity and peroxidation after four months of exposure to air pollutants. Arch Med Res 28:205–208

Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, Kaufman JD (2007) Long-term exposure to air pollution and incidence of cardiovascular events in women. N Engl J Med 356:447–458

Mo Y, Wan R, Chien S, Tollerud DJ, Zhang Q (2009) Activation of endothelial cells after exposure to ambient ultrafine particles: the role of NADPH oxidase. Toxicol Appl Pharmacol 236:183–193

Moller P, Loft S (2010) Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. Environ Health Perspect 118(8):1126–1136

Monn C, Becker S (1999) Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (PM2.5) and coarse particles (PM10-2.5) in outdoor and indoor air. Toxicol Appl Pharmacol 155:245–252

Montiel-Davalos A, Alfaro-Moreno E, Lopez-Marure R (2007) PM2.5 and PM10 induce the expression of adhesion molecules and the adhesion of monocytic cells to human umbilical vein endothelial cells. Inhal Toxicol 19(Suppl 1):91–98

Montiel-Davalos A, Ibarra-Sanchez Mde J, Ventura-Gallegos JL, Alfaro-Moreno E, Lopez-Marure R (2010) Oxidative stress and apoptosis are induced in human endothelial cells exposed to urban particulate matter. Toxicol In Vitro 24:135–141

Morishita M, Keeler G, Wagner J, Marsik F, Timm E, Dvonch J, Harkema J (2004) Pulmonary retention of particulate matter is associated with airway inflammation in allergic rats exposed to air pollution in urban Detroit. Inhal Toxicol 16:663–674

Mustafic H, Jabre P, Caussin C, Murad MH, Escolano S, Tafflet M, Perier MC et al (2012) Main air pollutants and myocardial infarction: a systematic review and meta-analysis. JAMA 307:713–721

Mutlu GM, Green D, Bellmeyer A, Baker CM, Burgess Z, Rajamannan N, Christman JW et al (2007) Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway. J Clin Invest 117:2952–2961

Niwa Y, Hiura Y, Murayama T, Yokode M, Iwai N (2007) Nano-sized carbon black exposure exacerbates atherosclerosis in LDL-receptor knockout mice. Circ J 71(7):1157–1161

Ntziachristos L, Froines J, Cho A, Sioutas C (2007) Relationship between redox activity and chemical speciation of size-fractionated particulate matter. Part Fibre Toxicol 4:5

Nurkiewicz TR, Porter DW, Barger M, Castranova V, Boegehold MA (2004) Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation. Environ Health Perspect 112:1299–1306

Nurkiewicz TR, Porter DW, Barger M, Millecchia L, Rao KM, Marvar PJ, Hubbs AF et al (2006) Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. Environ Health Perspect 114:412–419

Ohyama M, Otake T, Adachi S, Kobayashi T, Morinaga K (2007) A comparison of the production of reactive oxygen species by suspended particulate matter and diesel exhaust particles with macrophages. Inhal Toxicol 19(Suppl 1):157–160

Osornio-Vargas AR, Bonner JC, Alfaro-Moreno E, Martinez L, Garcia-Cuellar C, Ponce-de-Leon Rosales S, Miranda J et al (2003) Proinflammatory and cytotoxic effects of Mexico City air pollution particulate matter in vitro are dependent on particle size and composition. Environ Health Perspect 111:1289–1293

Patel PJ, Khera AV, Jafri K, Wilensky RL, Rader DJ (2011) The anti-oxidative capacity of high-density lipoprotein is reduced in acute coronary syndrome but not in stable coronary artery disease. J Am Coll Cardiol 58:2068–2075

Pope CA 3rd, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD (2002) Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287:1132–1141

Pope CA 3rd, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ (2004) Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation 109:71–77

Pozzi R, De Berardis B, Paoletti L, Guastadisegni C (2003) Inflammatory mediators induced by coarse (PM2.5-10) and fine (PM2.5) urban air particles in RAW 264.7 cells. Toxicology 183:243–254

Quan C, Sun Q, Lippmann M, Chen LC (2010) Comparative effects of inhaled diesel exhaust and ambient fine particles on inflammation, atherosclerosis, and vascular dysfunction. Inhal Toxicol 22(9):738–753

Ruckerl R, Phipps RP, Schneider A, Frampton M, Cyrys J, Oberdorster G, Wichmann HE et al (2007) Ultrafine particles and platelet activation in patients with coronary heart disease – results from a prospective panel study. Part Fibre Toxicol 4:1

Saldiva PH, Clarke RW, Coull BA, Stearns RC, Lawrence J, Murthy GG, Diaz E et al (2002) Lung inflammation induced by concentrated ambient air particles is related to particle composition. Am J Respir Crit Care Med 165:1610–1617

Sanchez-Rodriguez MA, Retana-Ugalde R, Ruiz-Ramos M, Munoz-Sanchez JL, Vargas-Guadarrama LA, Mendoza-Nunez VM (2005) Efficient antioxidant capacity against lipid peroxide levels in healthy elderly of Mexico City. Environ Res 97:322–329

Shah ASV, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K, Newby DE et al (2013) Global association of air pollution and heart failure: a systematic review and meta-analysis. Lancet 382:1039–1048

Soares SR, Carvalho-Oliveira R, Ramos-Sanchez E, Catanozi S, da Silva LF, Mauad T, Gidlund M, Goto H, Garcia ML (2009) Air pollution and antibodies against modified lipoproteins are associated with atherosclerosis and vascular remodeling in hyperlipemic mice. Atherosclerosis 207(2):368–373

Sorensen M, Daneshvar B, Hansen M, Dragsted LO, Hertel O, Knudsen L, Loft S (2003) Personal PM2.5 exposure and markers of oxidative stress in blood. Environ Health Perspect 111:161–166

Soukup JM, Ghio AJ, Becker S (2000) Soluble components of Utah Valley particulate pollution alter alveolar macrophage function in vivo and in vitro. Inhal Toxicol 12:401–414

Stocker R, Keaney JF Jr (2004) Role of oxidative modifications in atherosclerosis. Physiol Rev 84:1381–1478

Sumanasekera WK, Ivanova MM, Johnston BJ, Dougherty SM, Sumanasekera GU, Myers SR, Ali Y et al (2007) Rapid effects of diesel exhaust particulate extracts on intracellular signaling in human endothelial cells. Toxicol Lett 174:61–73

Sun Q, Wang A, Jin X, Natanzon A, Duquaine D, Brook RD, Aguinaldo JG, Fayad ZA, Fuster V, Lippmann M, Chen M, Rajagopalan S (2005) Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294(23):3003–3010

Sun Q, Yue P, Kirk RI, Wang A, Moatti D, Jin X, Lu B, Schecter AD, Lippmann M, Gordon T, Chen LC, Rajagopalan S (2008) Ambient air particulate matter exposure and tissue factor expression in atherosclerosis. Inhal Toxicol 20(2):127–137

Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, Cai Y et al (2009) Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation 119:538–546

Suwa T, Hogg JC, Quinlan KB, Ohgami A, Vincent R, van Eeden SF (2002) Particulate air pollution induces progression of atherosclerosis. J Am Coll Cardiol 39(6):935–942

Tamagawa E, Bai N, Morimoto K, Gray C, Mui T, Yatera K, Zhang X et al (2008) Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction. Am J Physiol Lung Cell Mol Physiol 295:L79–L85

Towfighi A, Saver JL (2011) Stroke declines from third to fourth leading cause of death in the United States: historical perspective and challenges ahead. Stroke 42:2351–2355

U.S.EPA (2004) Air quality criteria for particulate matter, Final report, Oct 2004. U.S. Environmental Protection Agency, Washington, DC, EPA 600/P-99/002aF-bF

van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T, Qui D et al (2001) Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am J Respir Crit Care Med 164:826–830

Van Lenten BJ, Wagner AC, Navab M, Anantharamaiah GM, Hama S, Reddy ST, Fogelman AM (2007) Lipoprotein inflammatory properties and serum amyloid A levels but not cholesterol levels predict lesion area in cholesterol-fed rabbits. J Lipid Res 48:2344–2353

Vedal S, Campen MJ, McDonald JD, Larson TV, Sampson PD, Sheppard L, Simpson CD et al (2013) National Particle Component Toxicity (NPACT) initiative report on cardiovascular effects. Res Rep Health Eff Inst 178:5–8

Wang T, Moreno-Vinasco L, Huang Y, Lang GD, Linares JD, Goonewardena SN, Grabavoy A et al (2008) Murine lung responses to ambient particulate matter: genomic analysis and influence on airway hyperresponsiveness. Environ Health Perspect 116:1500–1508

Wise H, Balharry D, Reynolds LJ, Sexton K, Richards RJ (2006) Conventional and toxicogenomic assessment of the acute pulmonary damage induced by the instillation of Cardiff PM10 into the rat lung. Sci Total Environ 360:60–67

World Health Organization (2013) World health statistics. World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

World Health Organization (2014) Burden of disease from ambient air pollution for 2012. Public Health, Social and Environmental Determinants of Health Department, World Health Organization, Geneva, Switzerland

Yatera K, Hsieh J, Hogg JC, Tranfield E, Suzuki H, Shih CH, Behzad AR, Vincent R, van Eeden SF (2008) Particulate matter air pollution exposure promotes recruitment of monocytes into atherosclerotic plaques. Am J Physiol Heart Circ Physiol 294(2):H944–H953

Yin F, Lawal A, Ricks J, Fox JR, Larson T, Navab M, Fogelman AM, Rosenfeld ME, Araujo JA (2013) Diesel exhaust induces systemic lipid peroxidation and development of dysfunctional pro-oxidant and pro-inflammatory high-density lipoprotein. Arterioscler Thromb Vasc Biol 33(6):1153–1161

Ying Z, Kampfrath T, Thurston G, Farrar B, Lippmann M, Wang A, Sun Q, Chen LC, Rajagopalan S (2009) Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species. Toxicol Sci 111(1):80–88

Chapter 10
Particulate Air Pollution and CNS Health

Alison Elder, Joel Schwartz, and Günter Oberdörster

10.1 Introduction and Background

Results of abundant toxicological and epidemiological studies have confirmed that exposure to particulate matter (PM) at concentrations encountered at numerous sites around the globe results in acute and chronic inflammatory conditions not only in the respiratory tract, but also in the cardiovascular system (Brook et al. 2010; Breysse et al. 2013; Fanning et al. 2009). PM-induced inflammatory events may result in severe chronic disease states, including lung cancer (Pope et al. 2002), myocardial events, and possibly neurodegenerative diseases.

This chapter focuses on toxicological and epidemiological studies that report effects of PM on the central nervous system (CNS) (Table 10.1). The focus of the toxicological investigations is on in vivo studies, primarily those that employ inhalation exposures. Epidemiological studies include cohort, panel, cross-sectional, and controlled human exposure designs.

10.1.1 Particulate Matter Size Categories

Urban airborne particulate matter (PM) typically can be described by four modes (Fig. 10.1) which include the three broad categories, PM_{10}, $PM_{2.5}$ and $PM_{0.1}$. These define size fractions of particulate matter of less than 10 μm and less than 2.5 μm in

A. Elder, Ph.D. (✉) • G. Oberdörster, D.M.V., Ph.D.
Department of Environmental Medicine, University of Rochester, Rochester, NY, USA
e-mail: Alison_Elder@urmc.rochester.edu; Gunter_Oberdorster@urmc.rochester.edu

J. Schwartz, Ph.D.
Departments of Environmental Health and Epidemiology, Harvard School of Public Health, Boston, MA, USA
e-mail: jschwrtz@hsph.harvard.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_10

Table 10.1 Particulate exposure and CNS effects: existing data

Animal/human experimental:	
Calderón-Garcidueñas et al. (1999, 2003, 2004, 2008b)	Mexico City air pollution associated with CNS inflammatory and neurodegenerative changes; increased brain COX-2 and β-amyloid (humans); DNA damage, neuronal tangles (dogs)
Campbell et al. (2005)	Two-week ultrafine/fine PM near-highway exposure in ovalbumin-sensitized mice led to increased brain NF-κB and IL-1α
Crüts et al. (2005)	Diesel exhaust exposure caused an increase in fast-wave EEG activity in humans
Veronesi et al. (2005)	Five-month exposure to ultrafine/fine PM induced loss of dopaminergic neurons in substrantia nigra of ApoE$^{-/-}$ mice
Elder et al. (2006)	Twelve-day exposure to Mn oxide ultrafine particles led to inflammatory cell activation and oxidative stress in olfactory bulb and other brain regions
Kleinman et al. (2008)	Six-week exposure to concentrated near-highway ultrafine PM in ApoE null mice led to activation of inflammatory mediator transcription factors
Gerlofs-Nijland et al. (2010)	Four-week Diesel exhaust exposure induced elevations in rat striatal TNF-α and IL-1α
Suzuki et al. (2010)	Mice exposed in utero to Diesel PM had reduced locomotor activity
Fonken et al. (2011)	Ten-month $PM_{2.5}$ exposure led to oxidative stress and inflammatory changes in mouse hippocampus and to decreased learning and memory
Allen et al. (2013)	Mice exposed as neonates or adults to ultrafine PM have preference for immediate reward upon behavioral testing
Guerra et al. (2013)	Two-month coarse/fine/ultrafine Mexico City ambient air exposures in rats led to region- and PM size-specific increases in oxidative stress, inflammation, and unfolded protein responses
Epidemiological:	
Rauh et al. (2004)	Second-hand tobacco smoke exposure in pre- and post-natal periods associated with decreased cognitive function in children
Calderón-Garcidueñas et al. (2008a, 2011)	Mexico City air pollution associated with cognitive changes in children
Suglia et al. (2008)	Decrease in verbal and non-verbal intelligence and memory in children in association with traffic-related particles
Zeng et al. (2010)	Poor air quality in China associated with poor cognitive function in older adults
Power et al. (2011)	Cognitive function decline in older men in association with black carbon exposure
Volk et al. (2011, 2013)	Proximity to roadways and exposure to traffic-related pollutants or $PM_{2.5}$ in utero or in early life predict higher likelihood of autism
Weuve et al. (2012)	$PM_{2.5}$ and PM_{10} levels associated with cognitive function decline in adult females
Wellenius et al. (2012)	Association between proximity to roadways and cognitive function changes in elderly
Becerra et al. (2013)	$PM_{2.5}$ and ozone exposure during pregnancy increase odds of autistic disorder in children

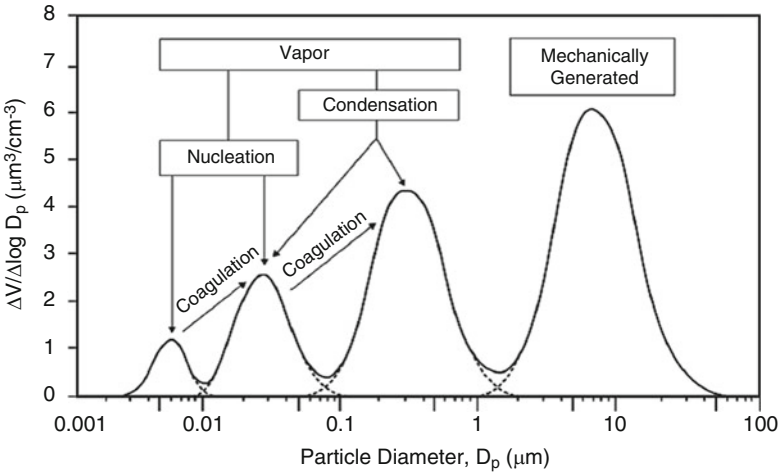

Fig. 10.1 Idealized size distribution of airborne particulate matter (Adapted from EPA 2004)

aerodynamic diameter and less than 0.1 μm in thermodynamic diameter, which includes categories of coarse particles ($PM_{10–2.5}$), fine particles ($\leq PM_{2.5}$), and ultrafine particles (UFP; $\leq PM_{0.1}$). Details about formation processes, composition and atmospheric behavior have been described in Chap. 1 (Hopke et al.). Coarse PM originates from mechanical processes such as grinding, cutting, and wind-blown dust (road dust, tire, brake wear), which settle in a relatively short time (hours) over short distances, whereas fine particles travel over very long distances and stay airborne for a long time after their generation from diverse combustion sources and heating processes as well as various precursor gases. UFP are formed by many natural processes (e.g., gas-to-particle conversions, volcanoes) and by anthropogenic activities (e.g., internal combustion engines, power plants, fumes); intentionally-engineered nanoparticles are defined to be of the same size as UFP. The physicochemical properties of ambient UFP vary widely depending on their origin; they consist of elemental and organic carbon, metals, and salts, all of which have different dissolution or leaching behavior. Shapes can vary from spherical to chain-like structures of differing agglomeration and aggregation states. Due to their high surface area per volume or mass, UFP chemical reactivity is likely to be greater than that of larger particles and their number concentration (particles per cm^3) is very high, whereas their mass concentration is very low (Finlayson-Pitts and Pitts 2000). However, as pointed out by Hinds (1982), an extremely high number concentration does not persist long due to both homogeneous coagulation of UFP and heterogeneous coagulation with larger particles, as indicated in Fig. 10.1.

Numerous epidemiological studies have reported the correlation between particulate air pollution and increased morbidity and mortality of susceptible parts of the population. For example, Schwartz and Marcus (1990) compiled data of the earlier London air pollution episodes of the 1950s, showing an initial steep increase in daily deaths at lower particulate concentrations and a flatter slope at higher

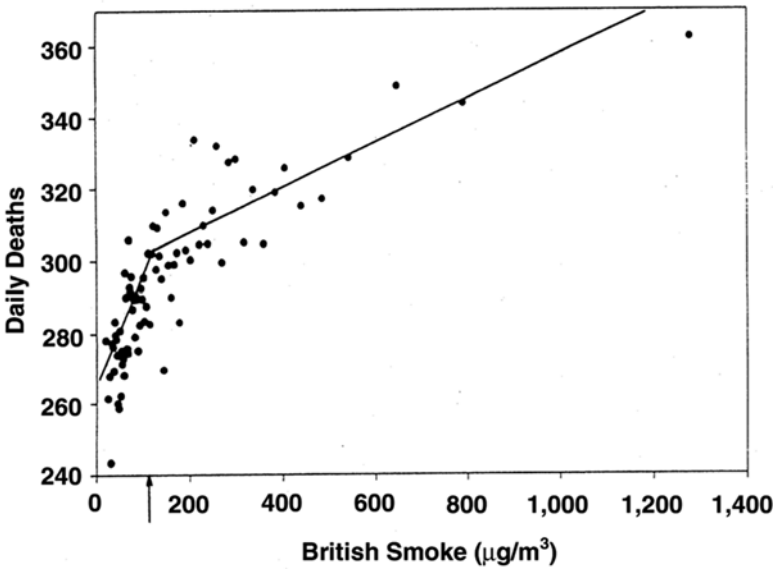

Fig. 10.2 Daily mortality and pollution in London (1958–1972) (Schwartz and Marcus 1990) (Adapted by Oberdörster et al. 2000)

particle concentrations (Fig. 10.2). A regression analysis of this data set (Oberdörster et al. 2000) identified an inflection point at a particle concentration of 128 μg/m^3, which was interpreted as being possibly indicative of the greater toxicity of a high number of ultrafine particles at lower mass concentrations and lower toxicity at higher mass concentrations after heterogeneous coagulation to fine particles.

10.1.2 Particle Deposition

Deposition of inhaled ambient PM in the respiratory tract is governed by breathing mode and activity level, airway geometry, particle size, density, shape and hygroscopicity and ambient humidity. Figure 10.3 depicts deposition efficiency of airborne particles of unit density ranging from about 1 nm to 20 μm in the three regions of the human respiratory tract, assuming nasal breathing with light exercise. The efficient nasal filtering capacity both for very small (<5 nm) and very large PM (>2 μm) is obvious; it is due to very different mechanisms involving high diffusional movement of the smallest UFP and high gravitational settling combined with inertial impaction of the larger particles. These deposition mechanisms are based on the aerodynamic and thermodynamic properties of the particles, the former becoming more effective with increasing particle size and the latter with decreasing particle

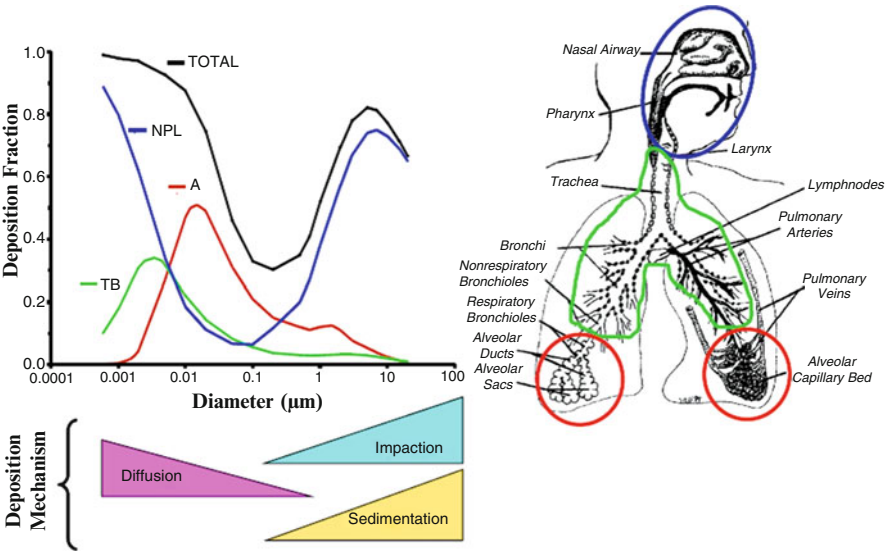

Fig. 10.3 Fractional deposition of inhaled particles in the human respiratory tract (ICRP 1994). Data shown for nose-breathing individual. Area outlined in: *blue*, nasopharyngeal-laryngeal (extrathoracic) region; *green*, tracheobronchial region; *red*, alveolar region (Adapted with permission from reference #51: Oberdörster et al. (2009). Copyright © American Scientific Publishers)

size (Fig. 10.3). As a result, there is a minimum of deposition around particle sizes of 0.2–0.5 μm, indicating the transition between the aerodynamic and thermodynamic domains of particle behavior. Of note is the high deposition efficiency of UFP in all regions of the respiratory tract, with peaks for ~20 nm particles in the alveolar region, ~10–15 nm in the tracheobronchial region, and less than ~5 nm in the nasopharyngeal-laryngeal (extrathoracic) region. This is of significance considering that sensory nerves embedded in the mucosa of the latter two regions can serve as conduits for locally-deposited UFP to reach sensitive sites in the CNS, as well as peripheral ganglia, as will be discussed later.

When viewing the deposition efficiencies in Fig. 10.3, it is important to understand that the particle sizes given on the abscissa refer to singlet airborne particles of a given size. For agglomerated or aggregated particles, the respective size of the agglomerate/aggregate applies. For example, a large agglomerate made up of 50 nm particles may have a size of several hundred nm or even micrometers. Furthermore, a high deposition efficiency in a given region of the respiratory tract does not necessarily mean that this area receives a high dose per unit epithelial surface area. For example, 20 nm particles depositing with a highest 50 % efficiency in the alveolar region translates to a deposited dose per unit alveolar surface area which is lowest compared to the tracheobronchial and extrathoracic airways because of the orders of magnitude higher alveolar surface area.

10.1.3 Particle Disposition

The fate of inhaled PM following deposition in the respiratory tract depends on the physicochemical properties of PM and the site of deposition. Deposited larger particles are mainly cleared by macrophage phagocytosis from the alveolar region towards the mucociliary escalator of the conducting airways (tracheobronchial region) and by mucociliary clearance from the tracheobronchial region and the naso-oro-pharynx via swallowing into the gastrointestinal tract. Agglomerated/aggregated UFP can also be cleared via macrophage phagocytosis and subsequent mucociliary function. A smaller portion of larger particles is also taken up into interstitial lymphatic channels towards local lymph nodes, a recognized pathway for UFP that becomes more important under heavy deposited particle load conditions. Particles may also be cleared via dissolution. In addition to these pathways, UFP can also be cleared via translocation into the blood or lymph and along sensory neurons, as discussed below.

A major difference between UFP and larger particles relates to their biodistribution following deposition in the airways: The translocation of ultrafine (or nano-sized) particles across epithelial barriers, e.g., the alveolar-capillary barrier into the bloodstream, and their translocation along axons and dendrites of neuronal cells are hallmarks of nanoparticle biokinetics to reach secondary target organs. Translocation rates from the respiratory tract are generally very low, on the order of 1–2 % of the deposited dose, as will be discussed later; however, the translocation of UFP from the upper respiratory tract to the olfactory bulb of the brain has been estimated to be higher, ~11 % of the dose deposited on the olfactory mucosa (Elder et al. 2006). A major focus of this article is, therefore, on studies with ultrafine and nanosized particles because of their propensity to translocate from the portal of entry to secondary organs. Such translocation implies that, in contrast to larger particles, a direct effect of UFP on secondary target tissues can be postulated as pointed out later. However, consideration should also be given to soluble constituents of PM of all sizes, which can be absorbed and distributed via blood and lymph circulation to secondary tissues or interact/bind with cellular structures or tissue fluids. Determination of dissolution rates and of the chemical nature of soluble or leachable PM constituents should be part of a detailed physicochemical characterization of ambient PM for all sizes.

Figure 10.4 summarizes distinct translocation pathways for inhaled UFP from the lower and upper respiratory tract to the brain based on experimental findings with engineered nanoparticles. Uptake into and transport within blood and lymph circulation is a potential route for access to the brain. However, the tight junctions of the blood-brain barrier (BBB) form a neuroprotective layer that restricts movement of particles and solutes much more efficiently than tight junctions in other organs of the body. Inflammatory processes, osmotic destruction of the tight junctions of the BBB, or coating of nanoparticles with specific proteins targeting endothelial LDL receptors can result in greater passage of nanoparticles into the brain (Kreuter 2004). In contrast, the aforementioned translocation of nanoparticles

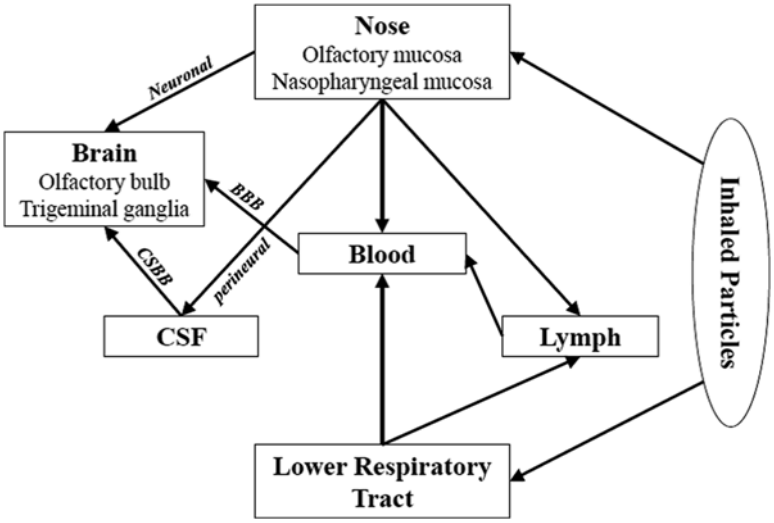

Fig. 10.4 Potential translocation pathways for inhaled ultrafine or nanosized particles from the respiratory tract to the brain (*CSF* cerebrospinal fluid, *BBB* blood-brain barrier, *CSBB* cerebrospinal-brain barrier) (Adapted with permission from reference #51: Oberdörster et al. (2009). Copyright © American Scientific Publishers)

along axons and dendrites of the olfactory and trigeminal sensory nerves originating in the nasal and oropharyngeal areas circumvents the tight BBB, so this conduit functions as a direct pathway for UFP to the olfactory bulb and the trigeminal ganglion of the brain (DeLorenzo 1970; Oberdörster et al. 2004; Elder et al. 2006; Hunter and Dey 1998; Hunter and Undem 1999). Another translocation pathway to CNS structures involves perineural transport of nanoparticles to the cerebrospinal fluid (CSF) along the olfactory nerve as described in studies by Czerniawska (1970) and Zhang et al. (2006). However, although it is still a prevailing view that drugs injected into CSF will also directly enter the brain parenchyma, transport from the CSF space to the brain is restricted by a very tight cerebrospinal fluid brain barrier of the choroid plexus. Thus, nanoparticles that are translocated to the CSF – together with CSF itself – exit rapidly into the general blood circulation, with only very little entering the CSF microcirculation to reach the brain parenchyma (Pardridge 2011).

10.2 Animal Studies

The cardiopulmonary effects of particulate air pollution have been appreciated for some time, following decades of epidemiological, controlled clinical, animal, and in vitro mechanistic studies. Because the respiratory tract is a primary target for airborne PM, the effects in lungs are understandable. However, the targeting of the

cardiovascular system gained acceptance after similar findings in humans and animals converged upon three main mechanistic explanations for the observed effects (Fig. 10.5): the generation of inflammatory mediators that then travel via the circulation to distal target tissues, autonomic nervous system activation, and the direct delivery via translocation of particles or their constituents to target tissues (Brook et al. 2010). Two of these mechanistic pathways will be discussed in this section of the chapter, namely translocation and inflammatory/oxidative stress responses, to explore the potential impact of particulate air pollution on the CNS using evidence that is drawn from animal models.

10.2.1 Particle Translocation

The translocation of particles from sites of deposition in the respiratory tract to other sites is a normal clearance process and has been known for some time, although the conditions under which this occurs and the governing mechanisms are not as well understood. For example, Ferin et al. (1992) showed that fine and ultrafine titanium dioxide particles migrated from the alveolar region of the lung following subchronic inhalation exposure to the hilar lymph nodes, a process that was particle size-dependent, with greater mass accumulation for the UFP and only some lung overload-induced accumulation for fine particles. Several studies of the translocation of inhaled ultrafine or nanosized very poorly soluble particles (iridium, elemental carbon, iridium/carbon) have demonstrated that the majority of the lung deposited dose remains in the lung in the short term (Mills et al. 2006; Wiebert et al. 2006). However, a measurable but small fraction is transported to distal tissues, typically less than 1–2 % of the total deposited doses following a single tracheal inhalation exposure (Kreyling et al. 2002, 2009). Distal target tissues include spleen, liver, heart, bone marrow, kidney, and brain. Two points about these studies are noteworthy. First, the dissolution of the particles used in these studies was very carefully documented to support conclusions regarding the nature of what was transported, either particulate or solute. Secondly, the nasal region of the respiratory tract was bypassed in these studies, which draws attention to the vascular pathways by which particles may get into the CNS (Fig. 10.4). The areas of the BBB with fenestrated endothelia, namely the circumventricular organs, may represent points of entry into the brain parenchyma if the particles are small enough or if components of inhaled particulates can be transported across the barrier (Fry and Ferguson 2007).

Aside from the vasculature, the olfactory mucosa in the nose represents a potential site for particles to access the brain (Figs. 10.4 and 10.5). DeLorenzo (1970) demonstrated in neuronal tracing studies in squirrel monkeys that silver-coated colloidal gold (50 nm) could travel from the olfactory epithelial surface through the cribriform plate into the olfactory bulb, thus providing an explanation for earlier studies showing that polio virus (~30 nm) infected the brain via this pathway. Employing a whole-body inhalation exposure model – whereby particle deposition

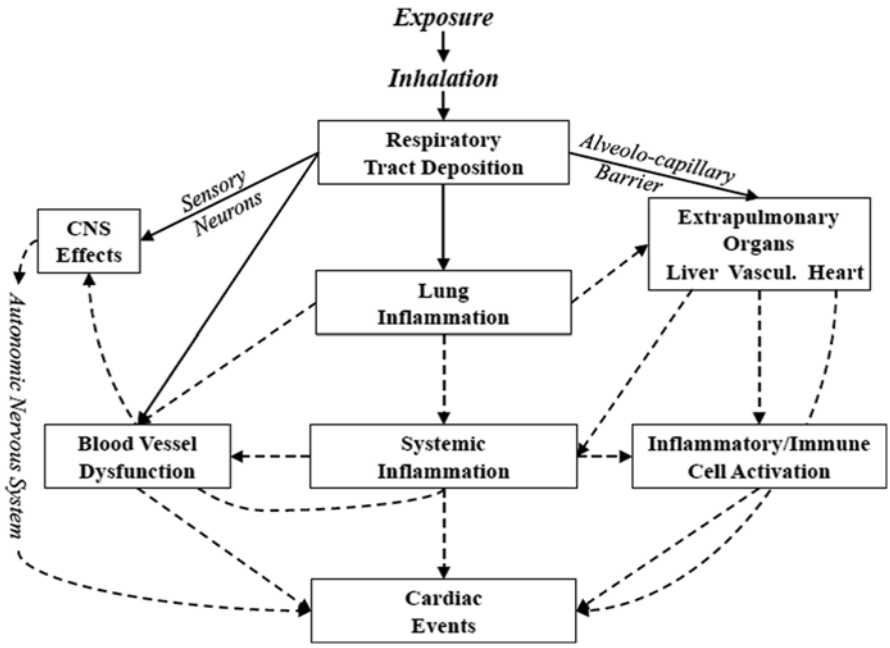

Fig. 10.5 Potential effects following exposure to airborne particulate matter (*solid arrows*, particle translocation; *dotted arrows*, mediators or dissolved PM components) (Adapted from Oberdörster et al. 2005)

in the nose occurs – Oberdörster et al. (2004) demonstrated the time-dependent accumulation of ^{13}C in olfactory bulb, cerebellum, and cerebrum following a 6-h exposure to ^{13}C elemental carbon UFP (35–37 nm; 150–170 μg/m^3). Yu et al. (2007) and Balasubramanian et al. (2013) also demonstrated the time- and particle size-dependent accumulation of gold nanoparticles (40–80 nm) in brain tissues following repeated (5–15 days) whole-body inhalation exposures, in particular the olfactory bulb, septum, entorhinal cortex, frontal cortex, hippocampus, striatum, and cerebellum. The finding of translocated gold nanoparticles in the entorhinal cortex is significant, as it raises the possibility that the particles crossed synapses, a finding that was reported by DeLorenzo (1970), who found gold in pre- and post-synaptic compartments of the olfactory bulb after intranasal administration. Confirmation of entorhinal cortex targeting by particles would also lend support to the hypothesis that inhaled UFP may contribute to neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Olfactory function impairment is common in these diseases, which has led to the hypothesis that xenobiotics can be transported synaptically to the primary olfactory cortex of the brain (Doty 2008, 2012; Morgan and Murphy 2002).

Elder et al. (2006) showed the highest accumulation of manganese in the olfactory bulb and lesser, but still significant, build-up in the striatum and frontal cortex

following 6- or 12-day whole-body inhalation exposures to poorly-soluble manganese oxide UFP (~500 μg/m^3, 18×10^6/cm^3, 30 nm), with evidence for short-term, rapid accumulation via olfactory neuronal transport to the olfactory bulb. Nevertheless, because exposures were repeated and, in the case of manganese oxide, some in vivo solubilization would be expected, vascular transport cannot be ruled out. Work from Dorman et al. (2001, 2004) also showed the importance of in vivo dissolution in the transport of manganese-containing compounds to the brain (olfactory bulb and striatum, in particular) that were delivered via nose-only inhalation exposure, with accumulation being heightened for more soluble materials and smaller particle sizes.

10.2.2 CNS Inflammation

Calderón-Garcidueñas and colleagues reported that exposure to polluted Mexico City air resulted in oxidative DNA damage in the nasal epithelium; oxidative stress and inflammatory mediator production, innate immune cell activation, and accumulation of beta-amyloid – a pathological hallmark of Alzheimer's disease – and α-synuclein in brain tissue from humans and dogs; and deficits in cognition, memory, and executive function and increased prefrontal lesions in children (Calderón-Garcidueñas et al. 1999, 2003, 2004, 2008a, b, 2011). Because of the lack of exposure control or characterization in these studies, however, it is not possible to conclude that particulate air pollution was causally associated with these changes. Guerra et al. (2013) used an inertial separator to expose adult rats to coarse, fine, and ultrafine Mexico City PM for 2 months and reported size fraction- and brain region-dependent upregulation in genes for superoxide dismutase, interleukin-1β, tumor necrosis factor-α, heme oxygenase-1, and markers of the unfolded protein response, as well as elevated activities of Nrf-2 and NF-κB (transcription factors in the pro-inflammatory cascade). These findings more directly implicate PM as being responsible for the observed increases in pro-inflammatory mediators, but it is important to note that gas-phase constituents of the pollutant mixture would still be present.

Using a stationary particle concentrator to expose ovalbumin-sensitized mice to Southern CA traffic-related PM or filtered air for 2 weeks, Campbell et al. (2005) reported that particles smaller than 180 nm in diameter induced increases in NF-κB activation and interleukin-1α in cortical tissue. Using a similar aerosol, Kleinman et al. (2008) showed that a 6-week exposure of ApoE$^{-/-}$ mice led to the activation of several inflammation-related transcription factors and to astrocyte activation in cortical tissue in an exposure concentration-dependent manner. Using the same animal model (ApoE$^{-/-}$ mice), Veronesi et al. (2005) showed a decrease in tyrosine hydroxylase staining – interpreted as a loss of dopaminergic neurons – in the substantia nigra region of the striatum following inhalation exposure for 5 months to concentrated $PM_{2.5}$, which also includes UFP. Exposure of mice to concentrated Columbus, OH, $PM_{2.5}$ for 10 months also resulted in elevated mRNA levels for

tumor necrosis factor-α, interleukin-1β, and heme oxygenase-1 in the hippocampus, which is critical for learning and memory (Fonken et al. 2011). Diesel exhaust exposure (4 weeks) has also been shown to cause significant elevations in striatal tumor necrosis factor-α and interleukin-1α protein levels in rats (Gerlofs-Nijland et al. 2010). The links between CNS inflammatory processes, persistent innate immune cell (microglia) activation, neurodegeneration, and cognitive impairment (Akiyama et al. 2000; Amor et al. 2010; Block et al. 2007; Block and Calderón-Garcidueñas 2009; Rainero et al. 2004) have fueled a growing interest in exploring the relationship between exposure to ambient air pollution and neurodegenerative diseases.

10.2.3 Behavioral Changes

Few studies have examined CNS functional or behavioral outcomes following particulate air pollutant exposures. In utero exposure to diesel PM has been shown in a mouse model to reduce spontaneous locomotor activity and to alter neurotransmitter levels (as well as their metabolites and turnover) in prefrontal cortex of weanlings (Suzuki et al. 2010). In the $PM_{2.5}$ study described in the previous paragraph, Fonken et al. (2011) also evaluated hippocampus-mediated behavioral changes. After documenting that body weight, gross olfactory function, motor function, sensorimotor responses, muscle tone, and serum corticosterone levels were constant, the authors found that the mice exposed to particulates exhibited decreases in spatial learning and memory, as well as despair behavior upon forced swim testing. More recently, Allen et al. (2013) showed that male mice that were exposed to concentrated ambient UFP (~80 nm in diameter) in the immediate postnatal period (8 days over 2 weeks), as adults (4 days), or both demonstrated preference for immediate reward (increased impulsivity) in fixed-ratio waiting-for-reward behavioral testing, particularly in those mice that received postnatal exposures. These changes were not related to hyperactivity or changes in locomotor function.

Taken as a whole, these findings suggest that inhaled particles that deposit in the upper or lower respiratory tract may impact several different brain centers to affect functional changes through mechanisms that are likely to be mediated through local oxidative stress and inflammation/inflammatory cell activation, either centrally or peripherally. The olfactory transport pathway may be able to deliver enough of the deposited dose by circumventing BBB – when particles are small enough – to initiate such responses, especially upon repeated exposures. Transport of particles or their constituents via the blood also needs to be considered for some particles that readily release solutes upon in vivo dissolution that can be transported across the BBB. The relative contribution of these pathways – neuronal versus BBB – to CNS dosimetry requires more investigation, as does a kinetic analysis of particle clearance pathways from the brain. Recent results showing inflammatory changes in the heart via nodose ganglia activation without obvious lung or systemic inflammation highlight the role of sensory neurons in the tracheobronchial region in extrapulmonary effects of inhaled particles (Kan et al. 2012); such response initiation pathways also

need to be considered in regards to effects observed in the brain. In addition, the inflammatory, neurodegenerative, functional, and behavioral outcomes following exposure to air pollution need to be viewed in relation to brain dosimetry in order to develop a more mature understanding of the relative vulnerability of the CNS, much as we now have for the pulmonary and cardiovascular systems. This should include characterization of particle size, chemistry, surface reactivity, and in vivo dissolution rates, all of which are determinants of dose-response relationships in the brain. Lastly, the relative contributions of brain versus systemic inflammatory processes to CNS effects need to be clarified, as distinguishing between these pathways could have implications regarding therapeutic interventions.

While the preceding text has focused largely on PM, air pollution is a complex mixture and other components are delivered to the respiratory tract along with the particulates. Indeed, the composition of air pollution – including of the particles themselves – is spatially and temporally dynamic. Ozone is a ubiquitous gaseous pollutant that is known to perturb the barrier function of the epithelium in all respiratory tract regions, leading to increased permeability (Bhalla et al. 1986; Kleeberger et al. 2001). Such a change in barrier function could affect translocation rates to secondary target tissues. Other inflammatory stimuli, like endotoxin, induce similar permeability changes. Such stimuli also lead to the recruitment of phagocytic cells, which could shift the balance between cell-mediated particle clearance and clearance via translocation. As to inflammatory and oxidative stress-related responses that occur in secondary tissues as a result of air pollutant exposures, little is understood about the components that might initiate responses. Metals, volatile organics, and polycyclic aromatic hydrocarbons have been associated with adverse CNS outcomes (Block et al. 2012) and may also underlie the observed effects with PM if these species are adsorbed to particle surfaces or otherwise carried into the respiratory tract. The uncertainties relating the components of air pollution that are causally related to initiation of response should be addressed in future research.

10.3 Epidemiological Studies

10.3.1 Changes in Cognition and Behavior in Association with Exposure

The last 8 years has seen a rapid development in human studies of the effects of air pollution on cognition. The neural network is still developing rapidly in the first years of a child's life, and cognitive function begins to fall when people reach their 50s. Hence, children and the elderly have been the focus of most of the research. The first clear report from an epidemiological cohort study was from Suglia et al. (2008), who compared cognitive function of children to their lifetime PM exposure measured as black carbon. They used a geographic exposure model, calibrated to 82 different monitoring locations, to estimate the geographic variation of traffic

particles in Boston. From this model, they estimated chronic exposure to traffic particles for school-aged children living in Boston. Higher PM exposure was associated with decrements in cognitive function of the order of two IQ points. A subsequent study of 671 older men living in the Boston area used the same black carbon exposure model and observed that higher levels of exposure over the previous one to 11 years was associated with worse cognitive function (Power et al. 2011). Black carbon in urban areas is predominantly from traffic, like UFP. However, it is more stable, and black carbon that is transported from upwind cities can be an important source of exposure. Hence, it reflects both fresh and aged traffic particles.

The two studies above took place in the Boston metropolitan area and focused on traffic particles because those types of particles are the ones that show substantial variability across addresses within a city. Other studies have demonstrated effects more broadly with air pollution. In a study of 15,973 older adults in China, residents of areas with poorer air quality over the previous 7–10 years, measured by an index of ambient particulate and gas concentrations, were more likely to have poor cognitive function (Zeng et al. 2010). More recently, an even larger study of 19,409 U.S. nurses used land use regression exposure models that predicted long term $PM_{2.5}$ and PM_{10} concentrations at the addresses of each nurse. Nurses with higher baseline exposure (that is, before the first cognitive exam) had faster rates of decline in cognitive function over time (Weuve et al. 2012).

Another study of elderly subjects, the Project Mobilize study, reported an association with residential proximity to major roadways, but not with black carbon, and impaired cognitive function in elderly inhabitants of Boston (Wellenius et al. 2012). In another study of older subjects, Ranft and coworkers (2009) found associations between long term particle exposure and cognitive function in elderly women, and Chen and Schwartz (2009) reported associations with ozone and, more weakly, with PM_{10} in the NHANES III study.

More studies of children have also appeared. In 2009, Freire and coworkers reported an association of traffic related air pollution and cognitive function in a large, well controlled cohort study in Spain. Simultaneously, Wang et al. (2009) published a study comparing cognitive function in schoolchildren in a high- and low-traffic neighborhood in Quanzhou, China. There was no difference in PM_{10} between the two locations, only in traffic pollution. The children from the higher traffic school had lower cognitive performance. More recently, Gatto et al. (2013) and van Kempen et al. (2012) have also reported associations, particularly with traffic pollutants, with cognitive performance in children.

Second hand tobacco smoke particles have broad similarities with ambient particles and, consistent with this, Rauh and colleagues measured prenatal and post-natal exposure to second-hand tobacco smoke and Bayley Scales of Infant Development in 226 urban children from pregnancy to several years of age (Rauh et al. 2004). Prenatal second hand smoke exposure (dichotomized as yes/no) predicted a five point decrement in the Bayley Mental Developmental Index scores ($p = 0.02$). Secondhand smoke is enriched in combustion particles compared to primary tobacco smoke, which has more gases.

In addition to these studies of cognition in elderly and young subjects, there is also a growing epidemiological literature about associations between traffic-related air pollutant exposures and developmental disorders such as autism. A small case-control study (309 cases, 249 controls) conducted in the Los Angeles, CA, area showed that living within close proximity (<309 yards) to a major freeway is associated with a higher odds ratio (OR) for being diagnosed with autism (OR, 1.86; 95 % confidence interval (CI), 1.04–3.45). Estimate adjustment for exposure throughout pregnancy resulted in a doubling of the odds ratio (Volk et al. 2011). A follow-up study (279 cases, 245 controls) examined associations between estimates of traffic-related pollutant exposures or regional criteria pollutant levels and risk of autism (Volk et al. 2013). Children who had the highest exposures to traffic-related pollution in the first year of life had a 3.10 times higher likelihood (95 % CI, 1.76–5.57) of having autism. Similarly, an increase of 8.7 $\mu g/m^3$ $PM_{2.5}$ during the first year of life was associated with a 2.12 times higher odds of having autism (95 % CI, 1.45–3.10). Another case-control study in the same region of the US, but with larger case (7603) and control (76,030) groups, also examined associations between autism diagnoses and criteria air pollutant exposures that were estimated using land-use regression modeling (Becerra et al. 2013). The authors reported an estimated 5–15 % increase in odds of autistic disorder per interquartile range increase over the entire pregnancy in $PM_{2.5}$ (4.68 $\mu g/m^3$) and a 6–12 % increase in odds per interquartile range increase in ozone (11.54 ppb). Odds ratios were further increased when these two pollutants were included in the same model. In addition, children with autism diagnoses had lower gestational ages at birth and lower birth weights than the controls, as has been previously reported. It is interesting to note that Miranda et al. (2013) and Pereira et al. (2014) showed increased incidence of preterm birth in association with increases in traffic-related pollution (proximity to roadways) or $PM_{2.5}$, respectively.

In general, cognitive impairment and dementia have been associated with oxidative stress and inflammation, which, as noted elsewhere, is clearly generated by PM. This makes the findings described above quite plausible. Crüts and colleagues (2005) exposed young healthy human volunteers using a cross-over study design to diesel exhaust or filtered air for 1 h and found increased β2 electroencephalogram wave activity in frontal cortex in diesel-exposed individuals. The authors concluded from these findings that cortical stress could lead to functional changes, which, if confirmed, provides another mechanism by which cognition and behavior could be altered. Another major cause of cognitive impairment in the elderly is reduced blood supply to the brain as a consequence of atherosclerosis. Again, PM has been demonstrated in human and in experimental studies to accelerate atherosclerosis. The cognitive decrements associated with air pollution have substantial impacts on the effected individuals. A meta-analysis of studies relating cognitive function measured in childhood or young adults to subsequent lifetime earnings found that a one IQ point decrease in ability was associated with a one percent reduction in lifetime earnings, lower probability of attending college, and more time spent unemployed (Schwartz 1994). Hence the reduced cognitive ability due to air pollution exposure is an important consequence. Decreased cognitive function in the elderly is

associated with faster onset of dementia, including Alzheimer's disease. To see the importance of this, one paper has forecasted that a broadly applied intervention that delays the onset of Alzheimer's disease by 2 years, could reduce the number of prevalent cases in the U.S. by about two million over a 40-year interval (Brookmeyer et al. 1998), which could result in substantial decreases in cost of care.

10.4 Conclusions

In considering the impact of particulate air pollution on CNS health, there are several key points to keep in mind. The first of these relate to the disposition of particulates in the air and upon deposition in the respiratory tract. The four modes of typical traffic-related ambient PM include coarse (2.5–10 μm), fine (≤2.5 μm) and ultrafine (≤0.1 μm) PM (ultrafine PM consists of two modes; see Fig. 10.1). Inhalation exposure targets all regions of the respiratory tract, with major deposition of UFP (included in fine particle fraction) in nasopharyngeal (<5 nm), tracheobronchial (~10–15 μm), and alveolar (~20 nm) regions; coarse particles (>2.5 μm) deposit to a high degree in the nasopharyngeal region. Secondly, translocation across the alveolo-capillary barrier of inhaled PM after deposition in the respiratory tract occurs at low dose rates for UFP only and, for larger PM, only at much higher deposited doses. Higher rates of translocation along sensory nerves of the upper respiratory tract, thus circumventing the tight BBB, have been demonstrated only for nano-sized particles (UFP). Thirdly, PM of all sizes can induce oxidative stress/inflammation at the portal of entry, the respiratory tract, and in secondary organs, depending on dose, preexisting disease, genetic factors, and age. Effects in secondary organs, including the CNS, can be due to direct translocation (for UFP) or to locally-released inflammatory mediators that distribute to the systemic circulation (all PM sizes) or via neurogenic stimulation of airway receptors (all PM sizes). CNS inflammatory changes, cognitive impairment, and behavioral changes have been demonstrated following inhalation exposure to PM and to engineered nanoparticles in toxicological studies in rodents. Epidemiological studies show cognitive impairment and behavioral changes in association with PM exposure in children and adults. Lastly, UFP appear to have a greater potential to induce adverse CNS effects due to their ubiquitous nature and their propensity to reach this target site directly. Thus, exposure to inhaled PM – in particular UFP – may be causally related to the initiation or progression of neurodegenerative diseases.

Several areas for future research can be identified from the preceding text. First, little is known about the impact of epithelial barrier function on UFP delivery to secondary target tissues, like the brain. Other pollutants could perturb the barrier, which might have a role in the dose that gets delivered to the tissues and the translocation kinetics. The physicochemical properties of the PM that are associated with outcome severity are also largely unknown. This knowledge will contribute to an understanding of whether there is a set of common response mechanisms that act in an independent manner with respect to exposure or, alternatively, if the origins of

response are dependent on the physicochemical characteristics of the air pollution mixture. Similarly, by exploring the incidence and severity of CNS responses in different locations, information will be gained both about the universality of the findings and about the components of PM and the pollutant mixtures that are causally associated with the observed outcomes. Lastly, controlled studies should focus as much as possible on realistic mixtures and exposure concentration ranges. Effort should also be devoted towards understanding differences in response when high doses are delivered over an acute exposure period – which can overwhelm innate defenses and activate response pathways that would not normally be operative – versus those that result from chronic, low-level exposures.

References

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM et al (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21(3):383–421

Allen JL, Conrad K, Oberdörster G, Johnston CJ, Sleezer B, Cory-Slechta DA (2013) Developmental exposure to concentrated ambient particles and preference for immediate reward in mice. Environ Health Perspect 121:32–38

Amor S, Puentes F, Baker D, van der Valk P (2010) Inflammation in neurodegenerative diseases. Immunology 129(2):154–169

Balasubramanian SK, Poh KW, Ong CN, Kreyling WG, Ong WY, Yu LE (2013) The effect of primary particle size on biodistribution of inhaled gold nano-agglomerates. Biomaterials 34:5439–5452

Becerra TA, Wilhelm M, Olesen J, Cockburn M, Ritz B (2013) Ambient air pollution and autism in Los Angeles County, California. Environ Health Perspect 121(3):380–386

Bhalla DK, Mannix RC, Kleinman MT, Crocker TT (1986) Relative permeability of nasal, tracheal, and bronchoalveolar mucosa to macromolecules in rats exposed to ozone. J Toxicol Environ Health 17(2–3):269–283

Block ML, Calderón-Garcidueñas L (2009) Air pollution: mechanisms of neuroinflammation and CNS disease. Trends Neurosci 32(9):506–516

Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57–69

Block ML, Elder A, Auten RL, Bilbo SD, Chen H, Chen JC et al (2012) The outdoor air pollution and brain health workshop. Neurotoxicology 33(5):972–984

Breysse PN, Delfino RJ, Dominici F, Elder ACP, Frampton MF, Froines JR et al (2013) US EPA particulate matter research centers: summary or research results for 2005–2011. Air Qual Atmos Health 23:333–355. doi:10.1007/s11869-012-0181-8

Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux AV et al (2010) Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121(21):2331–2378

Brookmeyer R, Gray S, Kawas C (1998) Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health 88:1337–1342

Calderón-Garcidueñas L, Wen-Wang L, Zhang Y, Rodriguez-Alcaraz A, Osnaya N, Villarreal-Calderón A et al (1999) 8-Hydroxy-2′-deoxyguanosine, a major mutagenic oxidative DNA lesion, and DNA strand breaks in nasal respiratory epithelium of children exposed to urban pollution. Environ Health Perspect 107:469–474

Calderón-Garcidueñas L, Maronpot RR, Torres-Jardón R, Henriquez-Roldán C, Schoonhoven R, Acuña-Ayala H et al (2003) DNA damage in nasal and brain tissues of canines exposed to air

pollutants is associated with evidence of chronic brain inflammation and neurodegeneration. Toxicol Pathol 31(5):524–538

Calderón-Garcidueñas L, Reed W, Maronpot RR, Henriquez-Roldán C, Delgado-Chavez R, Calderón-Garcidueñas A et al (2004) Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. Toxicol Pathol 32(6):650–658

Calderón-Garcidueñas A, Mora-Tiscareño A, Ontiveros E, Gómez-Garza G, Barragán-Mejia G, Broadway J et al (2008a) Air pollution, cognitive deficits and brain abnormalities: a pilot study with children and dogs. Brain Cogn 68(2):117–127

Calderón-Garcidueñas L, Solt AC, Henriquez-Roldán C, Torres-Jardón R, Nuse B, Herritt L et al (2008b) Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particle deposition, and accumulation of amyloid β-42 and α-synuclein in children and young adults. Toxicol Pathol 36:289–310

Calderón-Garcidueñas L, Engle R, Mora-Tiscareño A, Styner M, Gómez-Garza G, Zhu H et al (2011) Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. Brain Cogn 77(3):345–355

Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C et al (2005) Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. Neurotoxicology 26:133–140

Chen J, Schwartz J (2009) Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology 30(2):231–239

Crüts B, van Etten L, Törnqvist H, Blomberg A, Sandström T, Mills NL et al (2008) Exposure to diesel exhaust induces changes in EEG in human volunteers. Part Fibre Toxicol 5(4):6

Czerniawska A (1970) Experimental investigations on the penetration of ^{198}Au from nasal mucous membrane into cerebrospinal fluid. Acta Laryngol 70:58–61

DeLorenzo AJD (1970) The olfactory neuron and the blood-brain barrier. In: Wolstenholme GF, Knight J (eds) Taste and smell in vertebrates, CIBA Foundation symposium series. J&A Churchill, London, pp 151–176

Dorman DC, Struve MF, James AR, Marshall MW, Parkinson CU, Wong BA (2001) Influence of particle solubility on the delivery of inhaled manganese to the rat brain: manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14-day) exposure. Toxicol Appl Pharmacol 170(2):79–87

Dorman DC, McManus BE, Parkinson CU, Manuel CA, McElveen AM, Everitt JI (2004) Nasal toxicity of manganese sulfate and manganese phosphate in young male rats following subchronic (13-week) inhalation exposure. Inhal Toxicol 16(6–7):481–488

Doty RL (2008) The olfactory vector hypothesis of neurodegenerative disease: is it viable? Ann Neurol 63:7–15

Doty RL (2012) Olfactory dysfunction in Parkinson disease. Nat Rev Neurol 8(6):329–339

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J et al (2006) Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114(8):1172–1178

Fanning EW, Froines JR, Utell MJ, Lippmann M, Oberdörster G, Frampton M et al (2009) Particulate matter (PM) research centers (1999–2005) and the role of interdisciplinary center-based research. Environ Health Perspect 117(2):167–174

Ferin J, Oberdörster G, Penney DP (1992) Pulmonary retention of ultrafine and fine particles in rats. Am J Respir Cell Mol Biol 6(5):535–542

Finlayson-Pitts BJ, Pitts JN (2000) Particles in the troposphere. In: Finlayson-Pitts BJ, Pitts JN (eds) Chemistry of the upper and lower atmosphere: theory, experiments, and applications. Academic, San Diego, pp 349–439

Fonken LK, Xu X, Weil ZM, Chen G, Sun Q, Rajagopalan R et al (2011) Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology. Mol Psychiatry 16(10):987–995

Freire C, Ramos R, Puertas R, Lopez-Espinosa M, Julvez J, Aguilera I et al (2010) Association of traffic-related air pollution with cognitive development in children. J Epidemiol Community Health 64:223–228

Fry M, Ferguson AV (2007) The sensory circumventricular organs: brain targets for circulating signals controlling ingestive behavior. Physiol Behav 91:413–423

Gatto NM, Henderson VW, Hodis HN, St. John JA, Lurmann F, Chen JC et al (2013) Components of air pollution and cognitive function in middle-aged and older adults in Los Angeles. Neurotoxicology 40C:1–7. doi:10.1016/j.neuro.2013.09.004

Gerlofs-Nijland ME, van Berlo D, Cassee FR, Schins RP, Wang K, Campbell A (2010) Effect of prolonged exposure to diesel engine exhaust on proinflammatory markers in different regions of the rat brain. Part Fibre Toxicol 7:12

Guerra R, Vera-Aguilar E, Uribe-Ramirez M, Gookin G, Camacho J, Osornio-Vargas AR et al (2013) Exposure to inhaled particulate matter activates early markers of oxidative stress, inflammation and unfolded protein response in rat striatum. Toxicol Lett 222:146–154

Hinds WC (1982) Time for number concentration to halve and particle size to double by simple monodisperse coagulation. In: Aerosol technology: Properties, behavior, and measurements of airborne particles. Wiley, New York, pp 235–239

Hunter DD, Dey RD (1998) Identification and neuropeptide content of trigeminal neurons innervating the rat nasal epithelium. Neuroscience 83(2):591–599

Hunter DD, Undem BJ (1999) Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea. Am J Respir Crit Care Med 159(6):1943–1948

International Committee on Radiological Protection (1994) Human respiratory tract model for radiological protection. A report of Committee 2 of the ICRP. Pergamon Press, Oxford

Kan H, Wu Z, Young SH, Chen TH, Cumpston JL, Chen F et al (2012) Pulmonary exposure of rats to ultrafine titanium dioxide enhances cardiac protein phosphorylation and substance P synthesis in nodose ganglia. Nanotoxicology 6(7):736–745

Kleeberger SR, Reddy SP, Zhang LY, Cho HY, Jedlicka AE (2001) Toll-like receptor 4 mediates ozone-induced murine lung hyperpermeability via inducible nitric oxide synthase. Am J Physiol 280(2):L326–L333

Kleinman MT, Araujo JA, Nel A, Sioutas C, Campbell A, Cong PQ et al (2008) Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways. Toxicol Lett 178(2):127–130

Kreuter J (2004) Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. J Nanosci Nanotechnol 4(5):484–488

Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health Part A 65:1513–1530

Kreyling WG, Semmler-Behnke M, Seitz J, Scymczak W, Wenk A, Mayer P et al (2009) Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. Inhal Toxicol 21(Suppl. 1):55–60

Mills NL, Amin N, Robinson SD, Anand A, Davies J, Patel D et al (2006) Do inhaled carbon particles translocate directly into the circulation in humans? Am J Respir Crit Care Med 173(4):426–431

Miranda ML, Edwards SE, Chang HH, Auten RL (2013) Proximity to roadways and pregnancy outcomes. J Expo Sci Environ Epidemiol 23(1):32–38

Morgan CD, Murphy C (2002) Olfactory event-related potentials in Alzheimer's disease. J Int Neuropsychol Soc 8(6):753–763

Oberdörster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R et al (2000) Acute pulmonary effects of ultrafine particles in rats and mice. Health Effects Institute, Cambridge, MA

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W et al (2004) Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 16(6–7):437–445

Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–829

Oberdörster G, Elder A, Rinderknecht A (2009) Nanoparticles and the brain: cause for concern? J Nanosci Nanotechnol 9:4996–5007
Pardridge WM (2011) Drug transport in brain via the cerebrospinal fluid. Fluid Barrier CNS 8(1):4
Pereira G, Belanger K, Ebisu K, Bell ML (2014) Fine particulate matter and risk of preterm birth in Connecticut in 2000–2006: a longitudinal study. Am J Epidemiol 179(1):67–74
Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K et al (2002) Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287(9):1132–1141
Power MS, Weisskopf MG, Alexeeff SE, Coull BA, Spiro A III, Schwartz J (2011) Traffic-related air pollution and cognitive function in a cohort of older men. Environ Health Perspect 119(5):682–687
Rainero I, Bo M, Ferrero M, Valfrè W, Vaula G, Pinessi L (2004) Association between the interleukin-1alpha gene and Alzheimer's disease: a meta-analysis. Neurobiol Aging 25(10):1293–1298
Ranft U, Schikowski T, Sugiri D, Krutmann J, Krämer U (2009) Long-term exposure to traffic-related particulate matter impairs cognitive function in the elderly. Environ Res 109(8):1004–1011
Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A et al (2004) Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. Neurotoxicol Teratol 26(3):373–385
Schwartz J (1994) Societal benefits of reducing lead exposure. Environ Res 66:105–124
Schwartz J, Marcus A (1990) Mortality and air pollution in London: a time series analysis. Am J Epidemiol 131(1):185–194
Suglia SF, Gryparis A, Wright RO, Schwartz J, Wright RJ (2008) Association of black carbon with cognition among children in a prospective birth cohort study. Am J Epidemiol 167(3):280–286
Suzuki T, Oshio S, Iwata M, Saburi H, Odagiri T, Udagawa T et al (2010) In utero exposure to a low concentration of diesel exhaust affects spontaneous locomotor activity and monoaminergic system in male mice. Part Fibre Toxicol 3:7
US EPA (2004) Air quality criteria for particulate matter. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC
van Kempen E, Fischer P, Janssen N, Houthuijs D, van Kamp I, Stansfeld S et al (2012) Neurobehavioral effects of exposure to traffic-related air pollution and transportation noise in primary schoolchildren. Environ Res 115:18–25
Veronesi B, Makwana O, Pooler M, Chen LC (2005) Effects of subchronic exposure to concentrated ambient particles: VII. Degeneration of dopaminergic neurons in ApoE$^{-/-}$ mice. Inhal Toxicol 17:235–241
Volk HE, Hertz-Picciotto I, Delwiche L, Lurmann F, McConnell R (2011) Residential proximity to freeways and autism in the CHARGE study. Environ Health Perspect 119:873–877
Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R (2013) Traffic-related air pollution, particulate matter, and autism. JAMA Psychiatry 70(1):71–77
Wang S, Zhang J, Zeng X, Zeng Y, Wang S, Chen S (2009) Association of traffic-related air pollution with children's neurobehavioral functions in Quanzhou, China. Environ Health Perspect 117(10):1612–1618
Wellenius GA, Boyle LD, Coull BA, Liberg WP, Gryparis A, Schwartz J et al (2012) Residential proximity to major roadway and cognitive function in community-dwelling seniors: results from the MOBILIZE Boston study. Arch Intern Med 60(11):2075–2080
Weuve J, Puett RC, Schwartz J, Yanosky JD, Laden F, Grodstein F (2012) Exposure to particulate air pollution and cognitive decline in older women. Arch Intern Med 172(3):219–227
Wiebert P, Sanchez-Crespo A, Seitz J, Falk R, Philipson K, Kreyling WG et al (2006) Negligible clearance of ultrafine particles retained in healthy and affected human lungs. Eur Respir J 28(2):286–290

Yu LE, Yung LL, Ong CN, Tan YL, Balasubramanian KS, Hartono D et al (2007) Translocation and effects of gold nanoparticles after inhalation exposure in rats. Nanotoxicology 1(3):235–242

Zeng Y, Gu D, Purser J, Hoenig H, Christakis N (2010) Associations of environmental factors with elderly health and mortality in China. Am J Public Health 100(2):298–305

Zhang QZ, Zha LS, Zhang Y, Jiang WM, Lu W, Shi ZQ et al (2006) The brain targeting efficiency following nasally applied MPEG-PLA nanoparticles in rats. J Drug Target 14(5):281–290

Chapter 11
Air Pollution and Immune Function

Robert M. Tighe, Jennifer Wheeler, and John W. Hollingsworth

11.1 Introduction

The lung is continuously exposed to the external environment. Appropriate responses to inhaled foreign material and toxicants are essential to homeostatic maintenance of the lung and survival of the host. This requires tight regulation of pulmonary immunity to direct appropriate microbial pathogen defense responses while limiting secondary lung injury. A growing body of research provides insight into how inhaled environmental exposures can alter normal immune system responses. These altered responses can either dampen or exacerbate the intensity of immune function and thereby contributing to the pathogenesis of numerous common human diseases. In this chapter, we provide an overview of recent advances in our understanding of the impact of air pollution on host immune function.

The immune system is broadly divided into innate and adaptive immunity. The innate immune system is a highly evolutionary conserved system designed for mediating the initial responses to pathogens or irritants. Innate response incorporates a variety of cells and cellular responses which direct not only clearance of pathogens and/or irritants but also initial inflammatory cascades. These initial responses are dependent on germline encoded pattern recognition of pathogens or

R.M. Tighe, M.D. • J. Wheeler, Ph.D.
Department of Internal Medicine, Duke University Medical Center, Durham, NC, USA

J.W. Hollingsworth, M.D. (✉)
Department of Internal Medicine, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Ohio State University, Columbus, OH, USA
e-mail: john.hollingsworth@osumc.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_11

endogenous motifs. These motifs direct cell specific responses which allow the host to respond to injury or irritants without the requirement of prior exposure. Alternatively, the adaptive immune system, or acquired immune system, allows the host to recognize prior exposures; and then augment subsequent responses to repeat exposures. This system, therefore, provides immunologic memory. This immunologic memory allows the host to adapt and respond to recurrent challenges that are present in the environment. When the immune system works appropriately, there is an innate response which deals with the initial host-pathogen reaction. This then can contribute to the development of immunologic memory through the adaptive immune system. The adaptive immune system then allows the host to have a more vigorous and specific immune response in the setting of subsequent re-exposures to the pathogen or antigen. In addition, as was initially proposed by the late Charles Janeway (1989), there is communication or cross-talk between the innate and adaptive immune system, which provides the foundation for our current understanding of immunity. We now recognize that common environmental exposures can result in perturbations of either the innate or adaptive immune system and contribute to diseases pathogenesis.

In this chapter, we will focus on three major environmental pollutants: (1) particulate matter; (2) vehicle exhaust/diesel exhaust; and (3) ambient ozone. As summarized in Figs. 11.1 and 11.2, we will define the mechanisms that these environmental pollutants can modify innate and adaptive immune responses. In gaining insight into

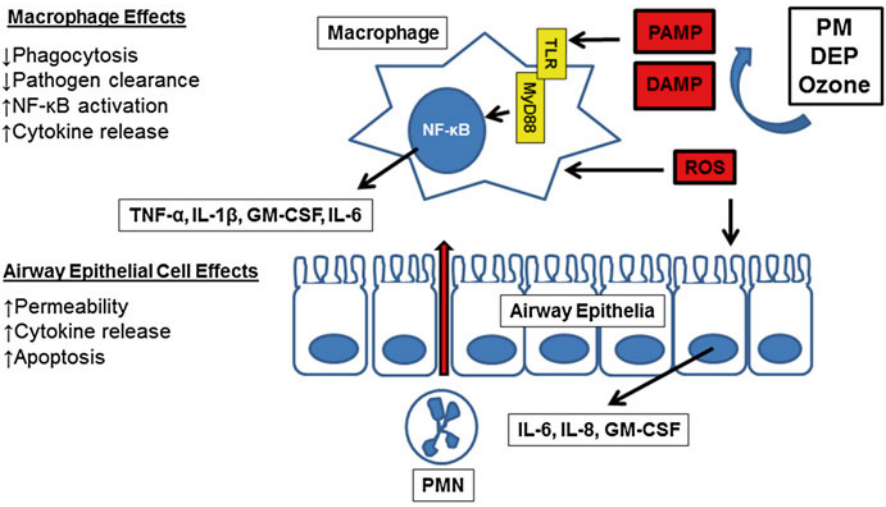

Fig. 11.1 Innate immune response to air pollutants. Inhalation of environmental air pollutants results in exposure to pathogen associated molecular patterns (PAMP), release of damage associated molecular patterns (DAMP), and generation of reactive oxygen species (ROS). These conserved intermediates can result in activation of lung macrophages and airway epithelia. Activation of innate immunity results in alteration of epithelial barrier function, release of cytokines and growth factors, and the recruitment and maturation of inflammatory cells

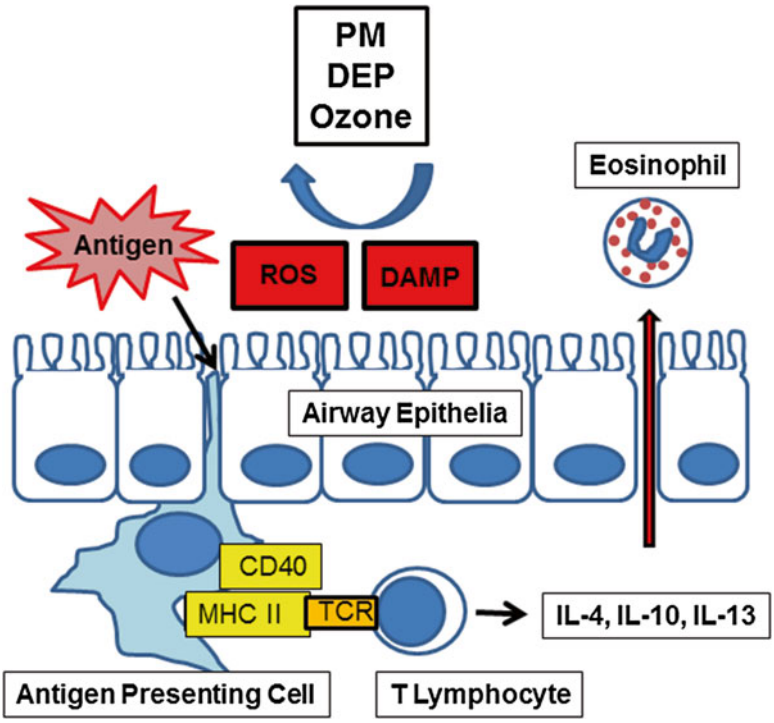

Fig. 11.2 Adaptive immune response to air pollutants. Inhalation of environmental air pollutants can facilitate activation of the adaptive immune system that can modify memory response to either pathogens or allergic antigens. Factors released after exposure to air pollution can facilitate adjuvancy resulting in host sensitization to otherwise inert antigens and thereby generating an immunological memory response. For example, exposure to air pollution can enhance sensitization to allergen by both enhancing antigen presentation and the polarization of T helper lymphocytes. This effect can promote allergic disease and exacerbate existing disease through enhanced influx of effector cells including eosinophil and mast cells. In allergic disease, these effects can enhance production of IgG, IgE, Th2 cytokines, and augment airway hyperresponsiveness (AHR)

these basic mechanisms, we appreciate the broad and lasting impact of environmental exposures on human health and gain insight into potential means to mitigate adverse health consequences related to common urban air pollutants.

11.2 Ambient Particulate Matter

Particulate matter (PM) refers to the composition of particles which are present in ambient air and represent a major environmental pollutant. Indoor and outdoor PM exposures are estimated to be the third and ninth respective leading cause of modifiable risk factor-related death world-wide (Lim et al. 2012). Current evidence

supports that exposure to PM can alter both local and systemic immune response. In the respiratory system, these alterations in immune responses are associated with both increased admissions for respiratory ailments and respiratory related deaths (Atkinson et al. 2001; Schwartz 1995). PM-induced alterations in systemic immune responses are associated with both increased cardiovascular events and higher mortality rates (Pope et al. 2009; Eftim et al. 2008; Brook et al. 2010). These negative health effects occur with both short- and long-term PM exposures and can be mitigated by reducing PM exposure. However, because PM will never be completely excluded from the environment, better control of these health effects will require a comprehensive understanding of the mechanisms of innate and adaptive immune responses.

11.2.1 PM and the Innate Immune System

11.2.1.1 Barrier Function/Epithelial Cells

One of the primary functions of the lung is to provide a site for exchange of gasses, principally oxygen and carbon dioxide. As a consequence of this function in gas exchange, the lung is exposed on a continual basis to the external environment. Maintaining separation between the external environment and the host while preserving the ability for gas exchange requires a thin, but intact barrier. This barrier is principally composed of epithelial cells. Epithelia along with alveolar macrophages, in the setting of a constant barrage of irritants, microbes and pollutants, principally function to maintain an intact barrier. The integrity of this barrier requires direct connections between epithelial cells which are mediated by epithelial cell barrier proteins. *In vitro* exposures of cultured human or rat epithelial cells to PM induce alveolar epithelial barrier dysfunction through oxidant mediated alteration in the barrier proteins: occludin and ZO-1 (Caraballo et al. 2011). In addition to alterations in barrier proteins, *in vitro* PM exposure causes epithelial cell apoptosis which can also enhance epithelial permeability (Urich et al. 2009; Soberanes et al. 2006). Though these *in vitro* findings suggest a link between epithelial permeability and PM exposure, this link has been more difficult to demonstrate *in vivo*. For an example, an *in vivo* exposure of PM_{10} to healthy non-smokers demonstrated no evidence of enhanced permeability or alteration in lung function (Brauner et al. 2009). This discrepancy between *in vivo* and *in vitro* exposures likely reflects challenges with present experimental models of PM exposure. One such concern is that PM-related health effects appear principally to exacerbate existing chronic cardiopulmonary conditions. Therefore, exposure to healthy humans in studies may not adequately reflect the biological response in subjects with existing disease. It is also likely that *in vitro* models do not adequately replicate the normal complex lung biology that includes multiple cell types and crosstalk between these cells. For example, macrophages are critical for the phagocytosis of PM components. PM-induced phagocytosis by macrophages can have broad direct and indirect effects on epithelial cell

function. One hypothesis is that PM phagocytosis can generate macrophage-derived inflammatory and oxidant stress responses which then activate and modify epithelial cells and barrier function. Another hypothesis is that the phagocytic capacity of macrophages becomes overwhelmed thereby allowing PM to come into contact with epithelial cells and exert direct detrimental effects to barrier function. Finally, in the setting of maximal macrophage PM phagocytosis, it appears that macrophage-derived anti-oxidant pathways become overwhelmed leading to oxidants-derived modification of barrier function (Caraballo et al. 2011).

In addition to their important role in barrier function, epithelial cells are also a critical source of cytokines and growth factors in response to particulate matter. PM_{10} exposure of primary human bronchial epithelial cells resulted in a dose-dependent increase in cytokine production (GM-CSF, IL-1β and IL-8) (Fujii et al. 2001). This cytokine release was most robust with PM_{10} as compared to the fine and ultrafine particle components (Becker et al. 2005a). Additionally, the epithelial cell cytokine response to PM appeared to be dependent on toll-like receptor 2 (TLR2) (Becker et al. 2005b). Interestingly, in this study, toll-like receptor 4 (TLR4) signaling was not required for epithelial cell responses to PM. Evidence of intracellular signaling in epithelial cells by PM is supported by NF-κB activation, which is downstream of TLRs and appeared to be a contributing to cytokine release by epithelial cells (Churg et al. 2005; Dagher et al. 2007; Kennedy et al. 1998; Quay et al. 1998).

11.2.1.2 Macrophages

Macrophages are central to the pulmonary response to inhaled particulates via their roles as scavenger cells and as central mediators of pulmonary immune responses. The two principal pulmonary macrophages, alveolar and interstitial macrophages, are critical components of the innate immune system and have been extensively studied in the setting of exposure to PM. PM exposure alters both macrophages phagocytic capacity and their ability to initiate immune responses. This effect on macrophage-derived immune responses occurs as a result of PM induced up-regulation of pattern recognition, and scavenger receptors and by activation of intracellular pathways including NF-κB and MAPK. The combined effect results in activation of pro-inflammatory cascades, which directs further local and systemic effects.

One of the principle functions of macrophages is phagocytosis. This function is critical in PM exposures. PM distributes throughout the tracheobronchial tree depending on size. Multiple mechanisms exist for clearance of particles in the lung including: chemical clearance, mucociliary clearance, lymphatic drainage, and phagocytosis (Oberdorster et al. 2005). Though phagocytosis occurs in other innate immune cells such as epithelial cells, macrophages are clearly the most efficient cell type for this function. Macrophage-directed phagocytosis is therefore critical to protecting of the lung from the adverse effects of PM.

In the setting of heavy PM exposure, it is evident that macrophages can be saturated by particles and can become overloaded. Work by several investigators highlighted that macrophages can become functionally overloaded by particles leading to adverse consequences (Miyata and van Eeden 2011; Oberdorster et al. 1992; Bellmann et al. 1992). One of the primary outcomes has been the observation that macrophages exposed to particles have reduced ability to clear a secondary exposure to pathogens. This observation is based primarily on *in vitro* studies where macrophages were exposed to PM of varying composition and then subsequently exposed to fluorescently labeled yeast to monitor clearance (Becker and Soukup 1998, 2003; Becker et al. 2003). These studies demonstrated that there is not only a defect in internalization, but also in opsonization of pathogens after PM exposure. Though less well studied, PM exposure also appears to impair phagocytic function *in vivo* (Renwick et al. 2004). The specific mechanisms by which PM exposure causes impaired phagocytosis remains poorly defined. One potential explanation for the PM-induced impairment in phagocytosis is from the effect PM components including metals and/or LPS. Zhou and colleagues demonstrated that reactive oxidant species, derived from soluble metals in $PM_{2.5}$, appeared to responsible for impaired internalization, clearance and killing of *Streptococcus pneumonia* (Zhou and Kobzik 2007). Additionally, LPS, contained in PM_{10}, also has the ability to reduce the phagocytic capacity of macrophages (Becker et al. 2003). PM alters receptor expression on the surface of macrophages and effects phagocytosis (Becker and Soukup 1998). Exposure to PM_{10} resulted in lower expression of CD11b and CD29 in both alveolar macrophages and blood monocytes. Furthermore, CD14 was down-regulated in blood monocytes and CD11c was similarly down-regulated in alveolar macrophages. The expression of CD11b is critical for the proper binding of opsonized pathogens, which can then be phagocytized. CD14 is a co-stimulatory molecule for TLR4 signaling and is important for phagocytosis of gram negative bacteria (Grunwald et al. 1996).

One of the prime mediators of innate immune responses is that of pattern recognition receptors (PRR). These receptors recognize specific molecular motifs called pathogen-associated molecular patterns. These PRRs can be further divided into PRRs which are critical to signaling and PRRs which are critical for opsonin-independent endocytosis. The TLRs are the critical signaling PRRs while the scavenger receptors are the primary endocytic PRRs. Both receptor families are critical to macrophage-derived innate immune responses to PM.

One of the principle functions of the TLRs is to initiate immune responses to those pathogens by identifying pathogen-derived microbial materials. Since many of these microbial components are found also in PM, it has long been felt that microbial components found in PM are responsible for PM-derived TLR signaling (Miyata and van Eeden 2011). Much of the work on PM-derived TLR signaling has focused on TLR2 and TLR4. Several lines of investigation have clearly demonstrated that PM-induced cytokine production is dependent on both TLR2 and TLR4 (Shoenfelt et al. 2009; Becker et al. 2002). Additionally, these TLR2 and TLR4-dependent cytokine responses were specific to certain size fractions of PM. It

appeared that PM_{10} responses required TLR4 signaling while $PM_{2.5}$ required TLR2 (Shoenfelt et al. 2009). This may have to do with the fact that LPS, the canonical TLR4 agonist, can be a component of PM_{10} (Soukup and Becker 2001). Supporting this finding, the use of an LPS inactivator, polymyxin B, reduced the majority of the PM_{10}-induced TLR4 signaling (Shoenfelt et al. 2009). Both the TLR2 and TLR4 PM-induced effects require their common downstream adaptor protein MyD88. This activation of the TLRs then mediated a downstream cascade leading to cytokine production. This downstream cytokine production is thought to be mediated primarily through the nuclear factor κ light chain enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) (Miyata and van Eeden 2011).

Phagocytosis of un-opsonized particles by macrophages is critical to the host ability to respond to inhalation of ambient particulate matter. Since PM is composed of several non-biologic components, which are not recognized by classic opsonin-dependent phagocytosis, un-opsonized phagocytosis is particularly critical to PM responses (Hiraiwa and van Eeden 2013). This type of phagocytosis is dependent on the scavenger-type receptors (Kobzik 1995). Previous work has defined two specific scavenger receptors: class A scavenger receptors (SR-A) and macrophage receptor with collagenous structure (MARCO) as being the predominant receptors for alveolar macrophage-dependent non-opsonized particle phagocytosis (Arredouani et al. 2006; Palecanda et al. 1999). The loss of these receptor functions led to a defect in particle internalization and clearance. Despite the understanding of how scavenger receptors are important for the internalization of particles, less is understood about their effects on cellular responses. Furthermore, we are unaware of any studies, to date, of mixed exposures. Much of the evidence supporting the cellular effects has focused on single agent exposures such as silica, titanium dioxide, or latex beads. Interestingly, utilizing silica and titanium dioxide instillation models, it was demonstrated that SR-A and MARCO expression is important for reducing TNF-α and neutrophil influx (Arredouani et al. 2006; Beamer and Holian 2005; Thakur et al. 2009). It is also known that TLR4 activation via a TLR4-MyD88-IRAK4 pathway causes an increase in SR-A expression (Chen et al. 2010; Xiang et al. 2009). SR-A activation may therefore be a mechanism by which PM host-responses are down-regulated, specifically TLR4 mediated activation (Mukhopadhyay et al. 2011). The specific intracellular mechanisms of this process and the direct connection to ambient PM *in vivo* remain to be elucidated, but scavenger receptors seem to contribute a critical role in the innate response to PM.

11.2.2 PM and the Adaptive Immune System

Exposure to inhaled PM appears to modify adaptive immunity. However, the mechanisms are less well understood. The adaptive immune system is particularly important for the development of allergic airway diseases such as asthma. Additionally, asthma prevalence is clearly increased in areas of elevated PM exposure (Brunekreef

and Forsberg 2005). This association between increased asthma prevalence and regions of high PM exposure suggests that PM may have a role modulating adaptive immune responses such as the development of allergic airways disease.

Development of allergy requires sensitization to antigen in context of an adjuvant and subsequent challenge to antigen. Exposure to PM could impact either sensitization or challenge to antigen. Evidence exists that PM can augment the development of allergic airways disease (Lippmann 2007; Bernstein et al. 2004; D'Amato et al. 2005). PM can function as an adjuvant (Kleinman et al. 2007; Steerenberg et al. 2003a, b), which could impact the prevalence of allergic disease through increasing sensitization to otherwise inert antigens. The ability to act as an adjuvant is greatest in the UFP component of PM. This fraction can direct allergic inflammation, allergic sensitization and mucus production in mice (Li et al. 2009). These effects appear to be in part dependent on UFP-derived oxidant potential.

In addition to functioning as an adjuvant, PM can also direct the development of allergic airways disease by enhancing antigen presentation and T cell activation by macrophages and dendritic cells. Both pulmonary macrophages and dendritic cells have the ability to present antigen and generate adaptive immune responses. The presentation of antigen occurs through interaction of major histocompatibility complex (MHC) with T-cell receptor (TCR) on antigen presenting cells (APCs). In normal human subjects, APCs appear to up-regulate MHC II expression with *in vivo* PM exposure (Alexis et al. 2006). In addition to antigen presentation, T cells require a second signal to become fully activated, called a co-stimulatory signal. Co-stimulatory signals CD80/86 and CD40L are up-regulated in dendritic cells and monocytes after PM exposure (Becker and Soukup 2003; Williams et al. 2007a). This PM-induced increase in co-stimulatory signals resulted in enhanced proliferation and cytokine production from alloreactive CD4+ T cells (Williams et al. 2007a). This response appears to depend on the specific type of particle exposure. Ambient particulate matter, when compared to diesel exhaust particles or carbon black particles, generated a more robust allergic responses including eosinophilic infiltration; Th2 cytokine release including IL-4, IL-5 and IL-13; and cell surface expression of co-stimulatory markers (Bezemer et al. 2011).

In addition to its effect on supporting the development of allergic airways disease, PM also appears to exacerbate pre-existing disease. A growing epidemiologic literature supports that PM causes increased admissions for asthma exacerbations and increased use of bronchodilator medications (Atkinson et al. 2001; Rabinovitch et al. 2006; Spira-Cohen et al. 2011; von Klot et al. 2002). These human epidemiologic findings were also observed in an animal model where PM exposure exacerbated pre-existing experimental allergic airways disease (Wang et al. 2008). This PM induced exacerbation was associated with increased airway resistance, neutrophil and eosinophil influx, and enhanced IL-4, eotaxin and IL-5 production. The specific mechanism of this effect remains undetermined. The cumulative data highlights the ability of ambient PM to elaborate allergic airway disease via augmentation of the adaptive immune system.

11.3 Vehicular Traffic/Diesel Exhaust

A major source of environmental air pollution is from vehicular traffic. Epidemiological investigations have found associations between incidence and exacerbation of various airways diseases and proximity to major roadways. Due to the health implications of roadway exposures, research has focused on investigating how vehicular traffic pollution impacts human health through modulation of innate and adaptive immune responses (Laumbach and Kipen 2012; Saxon and Diaz-Sanchez 2005; Riedl 2008). Traffic-derived air pollution consists of a complex mixture of noxious gases, such as sulfur dioxide and nitrogen dioxide, and carbonaceous particulate matter (PM) coated with organic chemicals, termed diesel exhaust particles (DEP) or diesel exhaust emissions (DEE, DE). DEP can range in size from nanoparticles to coarse (approximately 0.2 μm in size) and upon inhalation are deposited throughout the respiratory tree dependent on particle size (Oberdorster 1996). Further, the chemicals adsorbed on the surface of DEP include polycyclic aromatic hydrocarbons (PAH) and their derivatives, aliphatic hydrocarbons and aldehydes (Totlandsdal et al. 2012). It is important to note that there is not a universally accepted experimental DEP standard. Instead, a variety of sources, conditions and collection techniques have been employed to generate DEP for experimental use and therefore effects of DEP can vary considerably depending on these differences (McDonald et al. 2011; Stevens et al. 2009; Aust et al. 2002). Therefore, differences in experimental outcomes in similar models must be further evaluated for differences in specific DEP composition and source. Given that immune responses are not simply governed by the action of one singular lineage, but rather involve complex cooperation between various cell types over time, the impact of DEP exposure on a variety of immune and non-immune cell populations have been investigated both in naïve and antigen challenge models.

11.3.1 DEP and the Innate Immune System

11.3.1.1 Lung Epithelial Cells

Airway epithelial cells act as a physical barrier to inhaled pathogens and pollutants and often are the first line of defense against foreign molecules and pathogens. As such, innate immune responses are routinely initiated following epithelial cell exposure to foreign agents. Many studies have investigated *in vitro* epithelial exposure to DEP and have found varying mechanism through which DEP could impact immune response activation. One of the most consistent findings is that increased cytokine and chemokine levels are observed upon epithelial cell exposure to DEP. Specifically, dose-dependent increases in both GM-CSF and IL-8 were found following DEP exposure of primary human airway epithelial cells (nasal and bronchial) as well as

immortalized BEAS-2B cells (Ohtoshi et al. 1998; Bleck et al. 2006). In a separate study, increased message levels of IL-6 and IL-8 were observed in human BEAS-2B cells exposed to DEP (Totlandsdal et al. 2010). These studies support that DEP exposure may affect immune cell recruitment or maturation through altered cytokine production. Indeed, co-culture of DEP-exposed, primary human bronchial epithelial cells with immature dendritic cells (DC) induced dendritic cell maturation via GM-CSF (Bleck et al. 2006). These mature DC were then shown to elicit T cell differentiation, whereas DC cultured with unexposed epithelial cells or DC directly exposed to DEP had no effect on T cell differentiation (Bleck et al. 2006). This finding suggests an important role of DEP interaction with epithelia in DC maturation. Additionally, the frequency of monocytes, conventional DC's (cDC) and inflammatory DC's was elevated following DEP exposure in mice compared to saline controls (Provoost et al. 2012). Further, this DEP mediated recruitment was shown to be regulated by CCR2, a critical receptor for CC cytokines such as CCL2 which is produced by respiratory epithelial cells. Another study has linked CCR7 to regulating DC recruitment to draining lymph nodes following DEP treatment in mice (Provoost et al. 2010). Collectively, these findings support a major role of epithelial cells following DEP exposure in producing factors which regulate DC maturation and migration.

11.3.1.2 Myeloid Immune Cells

Like epithelial cells, innate immune cells such as macrophages, monocytes, neutrophils, and DCs, are critical sentinels of the immune system. DCs are of particular importance due to their role in initiating adaptive immune responses to pathogens and foreign stimuli. In addition to affecting DC function indirectly through impact on epithelial cells, DEP exposure has been shown to directly impact human DC phenotype and function. DCs exposed to DEP have been shown to increase expression of markers of activation and maturation CD40, CD80 and CD86 (Porter et al. 2007). In fact, when compared to treatment with CD40L, a known inducer of DC maturation, similar patterns of surface protein expression are observed following DEP exposure to DCs (Porter et al. 2007). Interestingly, while mature DC have been shown to have reduced uptake of extracellular antigens compared to immature DC, DEP exposed DC have elevated fluorescein isothiocyanate (FITC) dextran uptake than non-exposed (Porter et al. 2007). These findings suggest that DEP induces a heightened activated state in DC, while still allowing endocytosis. Similar to results in epithelial cells, DEP also directly alters cytokine production in naïve DCs (Porter et al. 2007). Increased production of TNFα, IL-12, IL-6 and IL-18 were observed following DEP treatment of DCs (Porter et al. 2007; Braun et al. 2010). Intriguingly, some studies have found that direct DC exposure to DEP does not affect some or any aspects of DC phenotype, cytokine production or function (Provoost et al. 2010; Braun et al. 2010). In these studies, bone marrow derived DCs (BMDC) were exposed to DEP, as opposed to Porter et al., which investigated the impact of DEP exposure on human-derived blood DC populations. These findings suggest that there could be species-specific sensitivities to DEP.

Macrophages and monocytes have also been shown to be functionally altered by DEP exposure. Like DC's, macrophages are known to phagocytose DEP (Hiura et al. 1999). Organic extracts of DEP induced the production of reactive oxygen species (ROS) and apoptosis of macrophages (Hiura et al. 1999, 2000). Interestingly, human monocyte derived macrophages (MDM) cultured in the presence of DEP also had increased cell death compared to control (Chaudhuri et al. 2012). Furthermore, these MDM had reduced expression of CD14, CD11b, HLA-DR, and CD86 (Chaudhuri et al. 2012). DEP exposure also affects the physical appearance of macrophages. In a chronic model of DEP exposure in rats, 2 years of daily DEP exposure caused smoothing of the surface of alveolar macrophages, whereas filtered air control macrophages had a ruffled exterior. However, the functional significance of this change in morphology was not evaluated (Castranova et al. 2001). Interestingly, it appears that naïve NK cells are also sensitive to DEP exposure, showing altered cytokine release and cell-killing proteins granzyme B and perforin (Muller et al. 2013a). Collectively, these studies suggest that DEP may differentially affect immune cell populations in a cell type specific manner.

11.3.2 Effects of DEP on Pathogen Immunity and Asthma

In addition to affecting aspects of innate immunity, DEP exposure has also been shown in several models to modulate immune responses to a variety of secondary challenges. Specifically, bacterial and viral infection models as well as sensitization to allergens have been shown to be affected following DEP exposure in both rodents and humans. While the mechanisms underlying these effects have not been well established, certain pathogen response pathways have been documented to be sensitive to DEP-mediated immunomodulation.

11.3.2.1 Bacteria and Bacterial Products

Lipopolysaccharide (LPS) is a major component of the outer membrane of gram negative bacteria and often used as a powerful inducer of immune responses. The frequency of human peripheral blood monocytes producing IFNγ, TNF-α, IL-6 IL-10 and IL-1β is reduced following co-exposure of DEP and LPS *in vitro* (Sarkar et al. 2012). Further, both DEP and the organic extracts of DEP inhibited the LPS-mediated production of both IL-1 and TNF-α following *in vitro* exposure to rat alveolar macrophages. Importantly, this study demonstrated that exposure to DEP either *in vivo* or *ex vivo* prior to LPS stimulation modulated cytokine production by macrophages (Castranova et al. 2001).

Mycobacterium tuberculosis infection has been studied following both chronic and acute DEP exposure in mice and human cells. Although modest changes in cytokine gene expression were observed in whole lung tissue, at 6 months pathogen load in the lungs were elevated in mice chronically exposed to DEP (Hiramatsu et al. 2005). Human peripheral blood monocytes (PBMC) have been shown to

uptake both DEP and *M. tuberculosis* bacteria in the same region of the cell, which could indicate that DEP may interact directly with the mycobacterium (Sarkar et al. 2012). Further, co-exposure of PBMC with DEP and *M. tuberculosis* causes a dose-dependent decrease in the frequency of IFNγ, IL-6 and IL-1β positive cells. Along with reduced cytokine induction, DEP pre-treatment down-regulated *TLR3*, *TLR4*, *TLR7 and TLR10* gene expression in *M. tuberculosis* infected PBMC (Sarkar et al. 2012). Taken together, these findings demonstrate that DEP is a potent suppressor of bacterial responses.

Another model of bacterial infection that is known to be sensitive to DEP exposure is *Listeria monocytogenes*. Acute exposure to DEP increased bacterial burden in the lungs of *Listeria* infected rats at day 5 and 7 post infection, while exposure to carbon black did not affect bacterial burden at these time points (Yang et al. 2001). Yin et al. also reported elevated bacterial burden of *Listeria* in the lungs of rats exposed to DEP for 4 days prior to infection, with significantly more bacteria at day 7 post-infection (Yin et al. 2005). This study also found reduced numbers of $CD4^+$ and $CD8^+$ T cell in lung draining lymph nodes at this same time point. *Ex vivo* stimulation of lymphocytes from chronically exposed rats demonstrated reduced levels of both IL-2 and IFNγ, cytokines important to adaptive immunity against pathogens. Additionally, DEP exposure has been shown to cause dose-dependent decreases in both bactericidal function and bacterial uptake by rat alveolar macrophages (Yin et al. 2007). These studies suggest that DEP can impact both innate and adaptive immunity against bacteria, which taken together could explain the elevated bacterial burden following DEP exposure.

11.3.2.2 Viral Infection

DE exposure has also been shown to affect viral clearance in the lungs of experimental animals. Elevated respiratory syncytial virus (RSV) gene expression is detected in the lungs of high dose DE exposed mice (1,000 μg/m^3, 6 h/day, 7 days) compared to low dose (30 μg/m^3, 6 h/day, 7 days) and air exposed controls at 4 days post infection (Harrod et al. 2003). Further, increased inflammation is observed in lungs from the high dose group compared to air control as well as increased levels of both TNFα and IFNγ in both the low and high dose DE exposures.

Similar to what has been observed following RSV infections, DEP mediated effects are also observed in several models of influenza virus infection. Elevations in viral load up to day 8 post infection were observed in the lungs of influenza virus infected mice exposed to DE (500 mg/m^3 4 h/day, 13 days) when compared to infected air controls (Gowdy et al. 2010). Elevations in pulmonary inflammation have also been demonstrated in this model, as measured by elevated neutrophil numbers, protein concentration and histopathological score 4 days after infection (Gowdy et al. 2010). Also similar to what has been observed with *Listeria* infection, DE exposure also modulates key adaptive immune cytokines during influenza virus infection, including IFNγ and IL-4 (Gowdy et al. 2010). The impact of DE on influenza virus infection of human cells has also been investigated. Human epithelial cells

(nasal and immortalized A549) treated with DE had increased attachment of influenza virus compared to control (Jaspers et al. 2005). Additionally, DE treatment of A549 cells resulted in higher numbers of infected cells compared to control cells exposed to the virus (Jaspers et al. 2005). Finally, in human volunteers infected with live attenuated influenza virus, those exposed to DE had elevated IFNγ in nasal lavage fluid compared to air exposed individuals (Noah et al. 2012).

11.3.2.3 Asthma and Allergy

Over the past two decades, the level of ambient air pollution, including diesel exhaust emissions have been associated with the prevalence of asthma and allergy in industrialized nations (Riedl 2008; Clark et al. 2010; Ring et al. 2001; Riedl and Diaz-Sanchez 2005). DEP is known to exacerbate hallmark allergic phenotypes in sensitized mice including eosinophil and neutrophil recruitment, allergen-specific antibody levels (IgG and IgE), Th2 cytokines (IL-4, IL-5), airway inflammation and airway responsiveness to acetylcholine (Stevens et al. 2009; Kim et al. 2011; Matsumoto et al. 2006; Inoue et al. 2008). Mice sensitized to the house dust mite (HDM) allergen *Dermatophagoides pteronyssinus* (Der p1) and co-exposed to DEP had elevated airway responsiveness to acetylcholine, increased numbers of eosinophils and neutrophils in the lung and enhanced Der p1-specific IgG levels compared to mice sensitized with Der p1 alone (Takahashi et al. 2010). Additionally, these effects were observed when mice were dosed with DEP throughout sensitization and challenge with Der p1 as well as when exposure was only during the first 2 days of sensitization, suggesting that DEP may not only exacerbate pre-existing allergic phenotypes, but could act as an adjuvant to induce sensitization to novel allergens. This adjuvant effect of DEP and road traffic exposure has also been shown using other potential allergens, such as ovalbumin (OVA) and keyhole limpet hemocyanin (KLH) in both mice and humans (Samuelsen et al. 2008; Diaz-Sanchez et al. 1999).

11.3.3 Mechanistic Insights

It is abundantly clear that exposure to DEP or DE can impact immune function; however the exact mechanistic causes of immunomodulation are not entirely defined. However, mounting evidence suggest that oxidative stress caused by DEP exposure could play a significant role. Specifically, DEP exposure has been shown to increase production of reactive oxygen species (ROS) (Hiura et al. 1999; Baulig et al. 2003; Wu et al. 2012). Further, DEP-mediated increases in ROS have been linked to elevations in cytokines such as GM-CSF (Baulig et al. 2003), with free radical scavenger treatment abrogating DEP and DE extract specific increases in cytokine production in human epithelial cells (Boland et al. 2000). Importantly, in this study cells exposure to carbon black did not show elevations in GM-CSF, again illustrating the importance of the chemical composition of the DEP on immune outcome and

responses. DEP exposure may elicit effects through a number of cell signaling pathways critical to cytokine induction. Boland et al. demonstrated DEP-mediated increases in GM-CSF to be largely dependent on tyrosine kinase signaling (Boland et al. 2000). Other kinase pathways and related downstream signaling transcription factors have also been shown to be sensitive to DEP, including p38 mitogen activated protein kinase (MAPK), NF-κB, and AP-1 (Hiura et al. 1999; Boland et al. 2000; Xiao et al. 2003; Pourazar et al. 2005; Hashimoto et al. 2000; Takizawa et al. 1999). Finally, some DEP induced changes in cytokine induction may be caused indirectly through miRNA induction (Jardim et al. 2009). DEP induces both thymic stromal lymphopoietin (TSLP) and the human microRNA (hsa-miR)-375 in primary human bronchial epithelial cells (pHBEC). In this same study, synthetic has-mir-375 was also shown to induce TSLP, suggesting a possible mechanistic link between DEP exposure and TSLP synthesis (Bleck et al. 2013). As noted throughout this section, it is clear the DEP-mediated effects on immune function are complex and the specific mechanisms through which DEP exert their effects are likely related to the dose and source DEP, but also the specific cell type and pathogen challenge.

11.4 Ambient Ozone

Ground level ambient ozone is generated through the interaction between pollutants generated by the combustion of fossil fuels which are then exposed to UV light. Inhalation of ambient ozone can impact pulmonary health through changes in pulmonary function, bronchial reactivity, epithelial permeability (Que et al. 2011), airway inflammation (Basha et al. 1994; Koren and Bromberg 1995; Scannell et al. 1996; Balmes et al. 1997; Peden et al. 1997), and altered clearance of live pathogens (Goldstein et al. 1971; Aranyi et al. 1983; Gilmour et al. 1993; Miller and Ehrlich 1958; Thomas et al. 1981; Van Loveren et al. 1988). In epidemiological studies, exposure to increased levels of ambient ozone has been associated with increased mortality (Bell et al. 2004; Jerrett et al. 2009). Susceptibility to ozone has been associated with young age (Sheffield et al. 2011; Strickland et al. 2010), elderly with predisposing cardiopulmonary diseases (Zanobetti and Schwartz 2011), and individuals participating in outdoor physical exercise (Carlisle and Sharp 2001). While there are likely multiple mechanisms that ozone adversely impacts health, growing evidence to support that ambient ozone can modify host immune function.

11.4.1 Ozone and Innate Immunity

We now recognize that inhalation of ambient ozone can alter many components of innate immunity (Hollingsworth et al. 2007; Li et al. 2013; Al-Hegelan et al. 2011) and alters host defense to live pathogens (Goldstein et al. 1971; Aranyi et al. 1983; Gilmour et al. 1993; Miller and Ehrlich 1958; Thomas et al. 1981; Van Loveren

et al. 1988). Innate immunity is an evolutionarily conserved first line of defense against pathogens, which consists of anatomical barriers, a variety of cell types, and specific proteins (soluble proteins and receptors) that recognize pathogen-associated molecular patterns. It is also recognized that innate immune signaling can regulate adaptive immunity.

11.4.1.1 Airway Epithelia

Exposure to ambient ozone alters the airway epithelia resulting in loss of ciliary function (Stephens et al. 1974) that is associated with defects in mucociliary clearance (Foster et al. 1987). Inhalation of ozone additionally results in disruption of the epithelial barrier as measured by increased airway epithelial permeability (Foster and Stetkiewicz 1996; Kehrl et al. 1987). Both intact barrier and mucociliary function are required to provide innate protection against numerous pathogens and helps facilitate clearance of inhaled foreign material. We now recognize that the airway epithelia can additionally function as an initial sensor resulting in signal transduction and in some contexts can function as a regulator of immune function (Hippenstiel et al. 2006; Diamond et al. 2000). Direct ozone challenge of human epithelial cell lines in culture conditions resulted in release of pro-inflammatory mediators under most (Devlin et al. 1994; Nichols et al. 2001; Rusznak et al. 1996; Jaspers et al. 1998; Voynow et al. 2009), but not all experimental conditions (Wang et al. 2006; Manzer et al. 2006). Similar increases in pro-inflammatory cytokines in the airspace are observed in human exposures to ozone under controlled experimental conditions (Devlin et al. 1991). Recent work support that direct exposure of human airway epithelia to ozone induces a pro-inflammatory response in a manner dependent on activation of MAP kinases, but not NF-κB (McCullough et al. 2014). However, it is most likely that there are both direct and indirect effects of ozone on airway epithelia. Indirect effects of ozone need to be considered as it remains unclear whether reactive ozone can diffuse across the airway and alveolar lining fluid (Pryor 1992). It has been recognized that ozone exposure can result in formation of biologically active oxysterols during ozonolysis of cholesterol present in lung surfactant (Pulfer and Murphy 2004). It is now recognized that some lipid ozonation products can function as signal transduction molecules (Pryor et al. 1995; Leikauf et al. 1995). In addition to ozone modifying soluble factors, ozone can induce macrophage-derived IL-1α that can indirectly activate lung epithelia (Manzer et al. 2008). Together these findings support both direct and indirect effects of ambient ozone on epithelial cell function.

11.4.1.2 Myeloid Immune Cells

Resident interstitial and alveolar macrophages are central to innate immunity. Macrophage function in the lung can be regulated by either activation status or newly recruited sub-populations of macrophages in the lung after inhalation

exposure. While cell culture studies cannot reconstitute the complex cell networks and microenvironment in the lung, cell culture studies do support that macrophages are highly sensitive to direct challenge with ozone (Janic et al. 2003). Macrophage function is significantly altered after exposure to ozone including; decreased phagocytosis of particulate immune complexes, enhanced production of prostaglandin E2, increased superoxide production. These functional changes in macrophages are associated with impaired antimicrobial host defense (Gilmour et al. 1993; Gilmour et al. 1991). However, additional studies of alveolar macrophage exposure to ozone generate inconsistent results with either enhanced (Arsalane et al. 1995; Ishii et al. 1997; Larini and Bocci 2005) or reduced cytokine production (Devlin et al. 1994; Becker et al. 1991; Janic et al. 2005). Reported findings may be resultant from differences in experimental protocols. However, numerous pro-inflammatory cytokines that can be produced by macrophages have been associated with functional response to ozone in mouse models.

Inhalation of ozone induces the release of a number of cytokines into the lung that are largely derived from myeloid lineages of cells including; TNFα, IL-1, IL-6, keratinocyte chemo-attractant (KC), and osteopontin. Elegant pioneering work demonstrate that TNFα release after inhalation of ozone in mice contributes to both neutrophil influx into the lung (Kleeberger et al. 1997) and AHR (Cho et al. 2001; Shore et al. 2001), in part, through activation of both NF-κB and MAPK / AP-1 signaling pathways (Cho et al. 2007). The role of TNFα is further supported by evidence supporting that human genetic variation within the promoter of TNFα (G-308A) was associated with the response to ozone in both adult human subjects (Yang et al. 2005) and children with asthma (Li Kam Wa et al. 1999). In addition to TNFα, IL-1 signaling contributes to innate immune response. IL-1ß is released by macrophages after direct exposure to ozone (Arsalane et al. 1995) and studies in mouse models support the role of IL-1 signaling in both ozone-induced lung injury and AHR (Wu et al. 2008; Park et al. 2004a; Johnston et al. 2007; Verhein et al. 2008). IL-6 is released into the airspace after inhalation of ozone in mice (Vincent et al. 1996; Shore et al. 2002; Samet et al. 2001) and evidence support a role for IL-6 in airway epithelial injury (Yu et al. 2002), recruitment of neutrophils into the airspace, and expression of TNFα (Johnston et al. 2005). Interestingly, there also appears to be a relationship between the role of IL-6 in response to ozone and adiponectin (Kasahara et al. 2014). KC, the mouse counterpart to human IL-8, is released after inhalation of ozone (Park et al. 2004a) or stimulation by TNFα (Ohmori et al. 1993). While the specific functional role of KC in response to ozone remains unknown, CXCR2 (*a receptor for KC*) is required for PMN recruitment and AHR after exposure to ozone (Johnston et al. 2005). Osteopontin is both an intracellular and secreted protein released in many inflammatory conditions with divergent functions including immune modulation. Recent work demonstrates that osteopontin contributes to both neutrophilic inflammation and AHR after ozone exposure (Barreno et al. 2013). The functional relevance of these pro-inflammatory cytokines in human ozone-induced airways disease remains poorly understood. Together, these findings support that TNFα, IL-1, IL-6, KC, and OPN may contribute to the biological response to ambient ozone.

In addition to specific cytokines, progress has been made to identify some of the mechanisms that regulate macrophage function after ozone. For example, previous work in an animal model demonstrates through identification of enhanced expression after ozone exposure a novel functional consequence for the macrophage receptor with collagenous structure (*marco*). *Marco* functions by promoting the uptake of ozone-derived surfactant oxidation products in the airspace (Pulfer and Murphy 2004; Dahl et al. 2007). Marco-deficient mice (when compared to wild-type) demonstrate enhanced oxidative stress after exposure to either ozone or oxidized pro-inflammatory lipids. This finding suggests that inhalation of ozone can directly alter macrophage phenotype. This response reflects either activation of resident macrophages or introduction a new population of macrophages into the lung. Data are emerging that support macrophage sub-populations have functional consequences in lung host defense. Whether there is a role for activation of resident macrophages or for newly recruited macrophages into the lung after ozone exposure remain poorly understood. In summary, the role of macrophages in context of ambient ozone remains complex and incompletely understood. Appreciation of the functional role of specific sub-populations of lung macrophages may provide insight into human health and disease.

While classically associated with adaptive immune response, lymphocytes contribute to the release of soluble cytokines that could impact the functional response to ozone. Similar to other cell types, studies in animal models demonstrate exposure to ozone appears to have a toxic effect on lymphocytes as measured by reduced weights of lymphoid organs (Fujimaki et al. 1984; Dziedzic and White 1986a, b) and attenuated immunologic responses of T-lymphocytes (Van Loveren et al. 1988; Fujimaki et al. 1987). Several compelling studies in animal models support the functional role of T cells in response to ozone. CD4+ lymphocytes are required for the complete inflammatory response to ozone (Chen et al. 1995). γδ T cells appear to prevent epithelial injury (King et al. 1999) and contribute to AHR in a manner partially dependent on TNFα (Matsubara et al. 2009). iNKT-cells appear to be an important source of pro-inflammatory cytokines (Kronenberg 2005) and are required for ozone-induced AHR in a manner dependent on IL-17 (Pichavant et al. 2008). Emerging evidence suggests other host factors including obesity and level of adiponectin may influence the IL-17 / γδ T cell axis (Kasahara et al. 2012). The IL-17 receptor contributes to AHR after chronic exposure to inhaled ozone in a manner likely related to activation of p38 MAPK (Pinart et al. 2013). The translational relevance of these findings in humans remains poorly understood. For example, a recent study in healthy human volunteers did not demonstrate a benefit of anti-IL-17 antibody therapy to attenuate ozone-induced airway neutrophilia (Kirsten et al. 2013). Direct exposure of human peripheral blood mononuclear cells to ozone demonstrates increased natural killer (NK) cell cytotoxicity (Kucuksezer et al. 2014). However, ozone modifications of human NK function may be dependent on complex interaction with airway epithelia (Muller et al. 2013b). Together these findings support a role for lymphocytes in the biological response to ozone.

11.4.1.3 Innate Immunity

A number of genes that regulate innate immune responses are critical to the biological response to ozone. The response to inhalation of ozone is now recognized to be dependent on surfactant proteins (SP-A, SP-D), complement, and toll-like receptor (TLR2, TLR4) signaling pathways.

Surfactant Proteins

SP-A and SP-D are C-type lectins (collectins) that are considered pattern recognition receptors of the innate immune system. Susceptibility to ozone-induced airway inflammation is associated with low levels of SP-D (Kierstein et al. 2006). Numerous studies support that SP-A deficient mice are more susceptible to detrimental effects after inhalation of ozone (Mikerov et al. 2008a, b, 2012; Haque et al. 2007). Both SP-A and SP-D are well recognized as potent inhibitors of oxidative cellular injury and lipid peroxidation (Bridges et al. 2000). As previously mentioned, some lipid ozonation products can function as signal transduction molecules (Pryor et al. 1995; Leikauf et al. 1995). Collectin protection against lipid ozonation could provide an explanation for the observed phenotypes in SP-A and SP-D knock-out animals (Pryor et al. 1995; Leikauf et al. 1995). Furthermore, the function of SP-A can be altered by ozone-induced oxidation of itself (Wang et al. 2002; Mikerov et al. 2008c; Stagos et al. 2007) possibly contributing to ineffective macrophage phagocytosis of bacterial pathogens (Mikerov et al. 2005, 2007, 2008a, b). Together, these findings support that intact SP-A and SP-D provide protection against ozone-induced lung injury and maintain antibacterial host defense.

Complement

The complement system is composed of approximately 30 proteins and protein fragments which can initiate innate immune response through recognition of pathogens or tissue injury. Central to the complement-derived immune responses are the cleavage C3 and C5 to generate immune activating intermediates C3a/b, and C5a/b (Janeway et al. 2001). Human exposure to ozone resulted in an increased level of C3a in bronchoalveolar lavage (BAL) fluid (Devlin et al. 1996). Studies in a mouse model support that complement activation is critical to airway hyperresponsiveness, neutrophil recruitment, and cytokine production after inhalation of ozone (Park et al. 2004b). The role of complement in airways disease is quite complex (Schmudde et al. 2013). Both C3a and C5a are recognized as pro-inflammatory. However, C5 can also function to protect against the development of airway disease in an allergic mouse model (Karp et al. 2000). The mechanisms, by which, complement regulates the response to ambient ozone and the subsequent impact on adaptive immunity remains incompletely understood.

TLRs

Toll-like receptors are a highly evolutionarily conserved family of pattern recognition receptors (PRR) that recognize either pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Studies utilizing mice deficient in TLR4 support an important role of innate immunity in ozone-induced lung injury (Kleeberger et al. 2001; Connor et al. 2012) and AHR (Hollingsworth et al. 2004; Williams et al. 2007b). Activation of the TLR4 receptor results in activation of a pro-inflammatory signaling cascade (Kawai and Akira 2010). In addition to TLR4, TLR2 is functionally important to the complete response after ozone inhalation (Williams et al. 2007b). MyD88 is a shared downstream adaptor molecule for both TLR4 and TLR2. Previous work supports that the response to ozone is partially dependent on the conserved downstream adaptor molecule MyD88 (Williams et al. 2007b). It remains possible that additional TLR receptors contribute to the response to ozone. The NF-κB pathway is activated with inhalation of ozone, as well as, signaling that is dependent on NF-κB (Chung and Adcock 2000; Punjabi et al. 1994; Xie et al. 1994). NF-κB is composed of multiple subunits including p50 and p65. Upon activation the subunits translocate to the nucleus and bind DNA resulting in gene expression. Ozone inhalation in mice results in activation of NF-κB in multiple cell types. Previous work using the p50−/− reported reduction in lung injury supporting that NF-κB activation is functionally important after ozone inhalation (Fakhrzadeh et al. 2004). Together, these findings support an evolving paradigm where ozone (either directly or indirectly) activates the surface receptors TLR2 and TLR4 resulting in MyD88-dependent activation of NF-κB with transcription of pro-inflammatory factors (including TNFα, IL-1, IL-6), which contribute to the complete response to ozone.

11.4.2 Ozone and Adaptive Immunity

In this section, we briefly highlight asthma as an example of a prototypic adaptive immunological response that has been well studied in context of exposure to ambient ozone (see Chap. 4). There is considerable overlap between the biological response to ozone and ambient particles (PM and DEP) with regard to the impact of air pollution on adaptive response. The allergic response in the lung is dependent on initial sensitization to an otherwise inert antigen and an immunological memory response (or adaptive immunological response) to subsequent exposure by that same antigen. Results from animal studies remain conflicting with regard to the overall impact of air pollution on allergic asthma, which is likely related to differences in the timing, dose, and duration of co-exposures (Last et al. 2004; Depuydt et al. 2002; Wagner et al. 2002; Ozawa et al. 1985). However, it appears that ozone may have a dual effect of both functioning as an adjuvant facilitating sensitization to potential allergens, as well as, exacerbating existing allergic airways disease. Given

changes in global climate and predictions of elevated levels of ozone, understanding the relationship between ozone and allergic asthma could have important implications to global health.

First, we will consider the evidence supporting that ozone could modify sensitization to otherwise inert antigen. To our knowledge, these studies are exclusively performed in animal model systems. Using the OVA model of allergic airways, ozone has been demonstrated to enhance the priming effect in mice to both inhaled (Neuhaus-Steinmetz et al. 2000) and systemic OVA challenge (Osebold et al. 1980, 1988; Gershwin et al. 1981). In monkeys, ozone exposure enhances allergen sensitization to both house dust mite allergen (Schelegle et al. 2003) and platinum (Biagini et al. 1986). Cumulatively, these data support that ambient ozone can enhance priming effect to otherwise inert antigens. The mechanisms that ozone alters sensitization to antigens remains poorly understood. However, it is now recognized that activation of innate immune response contributes to airway sensitization to otherwise inert antigens (Wilson et al. 2009; Eisenbarth et al. 2002). Previous work supports that ozone can both activate innate immunity and can function as a weak adjuvant during airway sensitization to OVA in a manner partially dependent on TLR4 (Hollingsworth et al. 2010). Together animal studies suggest that inhalation of ozone could enhance airway priming of allergic responses.

Second, there are considerably more evidence supporting that inhalation of ozone can enhance the severity of existing asthma (Bernstein et al. 2004; Bartoli et al. 2013). Epidemiological studies in asthmatic patients support a relationship between levels of ambient ozone and respiratory symptoms (Stenfors et al. 2010). High levels of ambient ozone have been associated with enhanced the frequency of emergency room visits and hospitalizations in patients with asthma (Ji et al. 2011; Glad et al. 2012). Associations in epidemiological studies are supported by numerous animal models that demonstrate worsening severity of allergic disease with ozone co-exposure (Steerenberg et al. 1996; Van Loveren et al. 1996). Multiple controlled ozone exposure studies in human with asthma demonstrate worsening of symptoms associated with challenge. Many studies in humans demonstrate enhanced number of inflammatory cells (neutrophils, monocytes, and mast cells) (Basha et al. 1994; Koren and Bromberg 1995; Scannell et al. 1996; Balmes et al. 1997; Peden et al. 1997) and markers of antigen presentation by flow cytometry (Meunier et al. 1994; Lenschow et al. 1993; Freeman et al. 1993; Koike and Kobayashi 2004; Koike et al. 2004). Similar to animal models, human subjects have increased markers of activation of innate immunity after inhalation of ozone (mCD14, CD11b, CD16) (Lay et al. 2007; Alexis et al. 2010). Additionally, airway epithelia isolated from allergic asthmatic subjects exposed to ozone display enhanced mRNA expression of genes of innate immunity (Hernandez et al. 2012). Together these data support that inhalation of ozone can contribute to the pathogenesis of allergic airways disease.

11.5 Overall Conclusions

The epidemiologic link between environmental pollutants and adverse health effects is well-document and led to extensive investigation into mechanisms that may help to explain these effects. Strict regulation of pulmonary immunity facilitates appropriate host response to both microbial pathogens and antigens. We now recognize that common environmental air pollutants can alter host immunity through disruption of alterations in both innate and adaptive immune responses. Current evidence supports that common environmental air pollutants including ambient particulate matter, vehicle exhaust/diesel particles and ozone, can modify host immunological response. Common inhaled pollutants can either dampen or exacerbate the intensity of immune function. While there are clear differences among each of these air pollutants, there are also common mechanisms that contribute to altered host immunity. Each pollutant can impact both lung epithelia and macrophage function and alter host innate and adaptive immune function (Figs. 11.1 and 11.2). We highlighted the conserved role of specific signaling pathways including generation of reactive oxygen species, toll-like receptors, and NF-κB signaling. The body of available literature generally supports that common air pollutants can alter the host response to both pathogens and modify allergic disease. Common air pollutants may enhance the prevalence of allergic disease through facilitating adjuvancy to otherwise inert antigens and can enhance the severity pre-existing airways disease by contributing to exacerbations. Clear appreciation of the mechanisms that air pollution alters host immune function will provide mechanistic insight into the complex relationship between host genetics, common environmental exposures, and human disease. Novel insight into the mechanisms that air pollution can alter host immunity could result in novel therapeutic approaches to the numerous human conditions that are impacted by alterations in host immunity.

Acknowledgements Investigators are supported by National Institutes of Health Grants ES016126, & ES020350 (to J.W.H.) and HL105537 (to R.M.T.). Portions of this chapter were adapted from published review articles on the topic (Li et al. 2013; Al-Hegelan et al. 2011) with kind permission from Springer Science + Business Media and John Wiley & Sons, Inc.

References

Alexis NE, Lay JC, Zeman K, Bennett WE, Peden DB, Soukup JM, Devlin RB, Becker S (2006) Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers. J Allergy Clin Immunol 117:1396–1403

Alexis NE, Lay JC, Hazucha M, Harris B, Hernandez ML, Bromberg PA, Kehrl H, Diaz-Sanchez D, Kim C, Devlin RB et al (2010) Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. Inhal Toxicol 22:593–600

Al-Hegelan M, Tighe RM, Castillo C, Hollingsworth JW (2011) Ambient ozone and pulmonary innate immunity. Immunol Res 49:173–191

Aranyi C, Vana SC, Thomas PT, Bradof JN, Fenters JD, Graham JA, Miller FJ (1983) Effects of subchronic exposure to a mixture of O3, SO2, and (NH4)2SO4 on host defenses of mice. J Toxicol Environ Health 12:55–71

Arredouani MS, Yang Z, Imrich A, Ning Y, Qin G, Kobzik L (2006) The macrophage scavenger receptor SR-AI/II and lung defense against pneumococci and particles. Am J Respir Cell Mol Biol 35:474–478

Arsalane K, Gosset P, Vanhee D, Voisin C, Hamid Q, Tonnel AB, Wallaert B (1995) Ozone stimulates synthesis of inflammatory cytokines by alveolar macrophages in vitro. Am J Respir Cell Mol Biol 13:60–68

Atkinson RW, Anderson HR, Sunyer J, Ayres J, Baccini M, Vonk JM, Boumghar A, Forastiere F, Forsberg B, Touloumi G et al (2001) Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. Air Pollution and Health: a European Approach. Am J Respir Crit Care Med 164:1860–1866

Aust AE, Ball JC, Hu AA, Lighty JS, Smith KR, Straccia AM, Veranth JM, Young WC (2002) Particle characteristics responsible for effects on human lung epithelial cells. Res Rep 1–65; discussion 110:67–76.

Balmes JR, Aris RM, Chen LL, Scannell C, Tager IB, Finkbeiner W, Christian D, Kelly T, Hearne PQ, Ferrando R et al (1997) Effects of ozone on normal and potentially sensitive human subjects. Part I: airway inflammation and responsiveness to ozone in normal and asthmatic subjects. Res Rep Health Eff Inst 1–37; discussion 78:81–99

Barreno RX, Richards JB, Schneider DJ, Cromar KR, Nadas AJ, Hernandez CB, Hallberg LM, Price RE, Hashmi SS, Blackburn MR et al (2013) Endogenous osteopontin promotes ozone-induced neutrophil recruitment to the lungs and airway hyperresponsiveness to methacholine. Am J Physiol Lung Cell Mol Physiol 305:L118–L129

Bartoli ML, Vagaggini B, Malagrino L, Bacci E, Cianchetti S, Dente FL, Novelli F, Costa F, Paggiaro P (2013) Baseline airway inflammation may be a determinant of the response to ozone exposure in asthmatic patients. Inhal Toxicol 25:127–133

Basha MA, Gross KB, Gwizdala CJ, Haidar AH, Popovich J Jr (1994) Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest 106:1757–1765

Baulig A, Garlatti M, Bonvallot V, Marchand A, Barouki R, Marano F, Baeza-Squiban A (2003) Involvement of reactive oxygen species in the metabolic pathways triggered by diesel exhaust particles in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 285:L671–L679

Beamer CA, Holian A (2005) Scavenger receptor class A type I/II (CD204) null mice fail to develop fibrosis following silica exposure. Am J Physiol Lung Cell Mol Physiol 289:L186–L195

Becker S, Soukup JM (1998) Decreased CD11b expression, phagocytosis, and oxidative burst in urban particulate pollution-exposed human monocytes and alveolar macrophages. J Toxicol Environ Health A 55:455–477

Becker S, Soukup J (2003) Coarse(PM(2.5-10)), fine(PM(2.5)), and ultrafine air pollution particles induce/increase immune costimulatory receptors on human blood-derived monocytes but not on alveolar macrophages. J Toxicol Environ Health A 66:847–859

Becker S, Madden MC, Newman SL, Devlin RB, Koren HS (1991) Modulation of human alveolar macrophage properties by ozone exposure in vitro. Toxicol Appl Pharmacol 110:403–415

Becker S, Fenton MJ, Soukup JM (2002) Involvement of microbial components and toll-like receptors 2 and 4 in cytokine responses to air pollution particles. Am J Respir Cell Mol Biol 27:611–618

Becker S, Soukup JM, Sioutas C, Cassee FR (2003) Response of human alveolar macrophages to ultrafine, fine, and coarse urban air pollution particles. Exp Lung Res 29:29–44

Becker S, Mundandhara S, Devlin RB, Madden M (2005a) Regulation of cytokine production in human alveolar macrophages and airway epithelial cells in response to ambient air pollution particles: further mechanistic studies. Toxicol Appl Pharmacol 207:269–275

Becker S, Dailey L, Soukup JM, Silbajoris R, Devlin RB (2005b) TLR-2 is involved in airway epithelial cell response to air pollution particles. Toxicol Appl Pharmacol 203:45–52

Bell ML, McDermott A, Zeger SL, Samet JM, Dominici F (2004) Ozone and short-term mortality in 95 US urban communities, 1987–2000. JAMA 292:2372–2378

Bellmann B, Muhle H, Creutzenberg O, Mermelstein R (1992) Irreversible pulmonary changes induced in rat lung by dust overload. Environ Health Perspect 97:189–191

Bernstein JA, Alexis N, Barnes C, Bernstein IL, Bernstein JA, Nel A, Peden D, Diaz-Sanchez D, Tarlo SM, Williams PB (2004) Health effects of air pollution. J Allergy Clin Immunol 114:1116–1123

Bezemer GF, Bauer SM, Oberdorster G, Breysse PN, Pieters RH, Georas SN, Williams MA (2011) Activation of pulmonary dendritic cells and Th2-type inflammatory responses on instillation of engineered, environmental diesel emission source or ambient air pollutant particles in vivo. J Innate Immun 3:150–166

Biagini RE, Moorman WJ, Lewis TR, Bernstein IL (1986) Ozone enhancement of platinum asthma in a primate model. Am Rev Respir Dis 134:719–725

Bleck B, Tse DB, Jaspers I, Curotto de Lafaille MA, Reibman J (2006) Diesel exhaust particle-exposed human bronchial epithelial cells induce dendritic cell maturation. J Immunol 176:7431–7437

Bleck B, Grunig G, Chiu A, Liu M, Gordon T, Kazeros A, Reibman J (2013) MicroRNA-375 regulation of thymic stromal lymphopoietin by diesel exhaust particles and ambient particulate matter in human bronchial epithelial cells. J Immunol 190:3757–3763

Boland S, Bonvallot V, Fournier T, Baeza-Squiban A, Aubier M, Marano F (2000) Mechanisms of GM-CSF increase by diesel exhaust particles in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 278:L25–L32

Braun A, Bewersdorff M, Lintelmann J, Matuschek G, Jakob T, Gottlicher M, Schober W, Buters JT, Behrendt H, Mempel M (2010) Differential impact of diesel particle composition on pro-allergic dendritic cell function. Toxicol Sci 113:85–94

Brauner EV, Mortensen J, Moller P, Bernard A, Vinzents P, Wahlin P, Glasius M, Loft S (2009) Effects of ambient air particulate exposure on blood-gas barrier permeability and lung function. Inhal Toxicol 21:38–47

Bridges JP, Davis HW, Damodarasamy M, Kuroki Y, Howles G, Hui DY, McCormack FX (2000) Pulmonary surfactant proteins A and D are potent endogenous inhibitors of lipid peroxidation and oxidative cellular injury. J Biol Chem 275:38848–38855

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA et al (2010) Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121:2331–2378

Brunekreef B, Forsberg B (2005) Epidemiological evidence of effects of coarse airborne particles on health. Eur Respir J 26:309–318

Caraballo JC, Yshii C, Westphal W, Moninger T, Comellas AP (2011) Ambient particulate matter affects occludin distribution and increases alveolar transepithelial electrical conductance. Respirology 16:340–349

Carlisle AJ, Sharp NC (2001) Exercise and outdoor ambient air pollution. Br J Sports Med 35:214–222

Castranova V, Ma JY, Yang HM, Antonini JM, Butterworth L, Barger MW, Roberts J, Ma JK (2001) Effect of exposure to diesel exhaust particles on the susceptibility of the lung to infection. Environ Health Perspect 109(Suppl 4):609–612

Chaudhuri N, Jary H, Lea S, Khan N, Piddock KC, Dockrell DH, Donaldson K, Duffin R, Singh D, Parker LC et al (2012) Diesel exhaust particle exposure in vitro alters monocyte differentiation and function. PLoS One 7:e51107

Chen X, Gavett SH, Wills-Karp M (1995) CD4+ T lymphocyte modulation of ozone-induced murine pulmonary inflammation. Am J Respir Cell Mol Biol 12:396–403

Chen Y, Wermeling F, Sundqvist J, Jonsson AB, Tryggvason K, Pikkarainen T, Karlsson MC (2010) A regulatory role for macrophage class A scavenger receptors in TLR4-mediated LPS responses. Eur J Immunol 40:1451–1460

Cho HY, Zhang LY, Kleeberger SR (2001) Ozone-induced lung inflammation and hyperreactivity are mediated via tumor necrosis factor-alpha receptors. Am J Physiol Lung Cell Mol Physiol 280:L537–L546

Cho HY, Morgan DL, Bauer AK, Kleeberger SR (2007) Signal transduction pathways of tumor necrosis factor–mediated lung injury induced by ozone in mice. Am J Respir Crit Care Med 175:829–839

Chung KF, Adcock IM (2000) Induction of nuclear factor-kappa B by exposure to ozone and inhibition by glucocorticoids. Methods Enzymol 319:551–562

Churg A, Xie C, Wang X, Vincent R, Wang RD (2005) Air pollution particles activate NF-kappaB on contact with airway epithelial cell surfaces. Toxicol Appl Pharmacol 208:37–45

Clark NA, Demers PA, Karr CJ, Koehoorn M, Lencar C, Tamburic L, Brauer M (2010) Effect of early life exposure to air pollution on development of childhood asthma. Environ Health Perspect 118:284–290

Connor AJ, Laskin JD, Laskin DL (2012) Ozone-induced lung injury and sterile inflammation. Role of toll-like receptor 4. Exp Mol Pathol 92:229–235

D'Amato G, Liccardi G, D'Amato M, Holgate S (2005) Environmental risk factors and allergic bronchial asthma. Clin Exp Allergy 35:1113–1124

Dagher Z, Garcon G, Billet S, Verdin A, Ledoux F, Courcot D, Aboukais A, Shirali P (2007) Role of nuclear factor-kappa B activation in the adverse effects induced by air pollution particulate matter (PM2.5) in human epithelial lung cells (L132) in culture. J Appl Toxicol 27:284–290

Dahl M, Bauer AK, Arredouani M, Soininen R, Tryggvason K, Kleeberger SR, Kobzik L (2007) Protection against inhaled oxidants through scavenging of oxidized lipids by macrophage receptors MARCO and SR-AI/II. J Clin Invest 117:757–764

Depuydt PO, Lambrecht BN, Joos GF, Pauwels RA (2002) Effect of ozone exposure on allergic sensitization and airway inflammation induced by dendritic cells. Clin Exp Allergy 32:391–396

Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schreinemachers D, Koren HS (1991) Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4:72–81

Devlin RB, McKinnon KP, Noah T, Becker S, Koren HS (1994) Ozone-induced release of cytokines and fibronectin by alveolar macrophages and airway epithelial cells. Am J Physiol 266:L612–L619

Devlin RB, McDonnell WF, Becker S, Madden MC, McGee MP, Perez R, Hatch G, House DE, Koren HS (1996) Time-dependent changes of inflammatory mediators in the lungs of humans exposed to 0.4 ppm ozone for 2 hr: a comparison of mediators found in bronchoalveolar lavage fluid 1 and 18 hr after exposure. Toxicol Appl Pharmacol 138:176–185

Diamond G, Legarda D, Ryan LK (2000) The innate immune response of the respiratory epithelium. Immunol Rev 173:27–38

Diaz-Sanchez D, Garcia MP, Wang M, Jyrala M, Saxon A (1999) Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. J Allergy Clin Immunol 104:1183–1188

Dziedzic D, White HJ (1986a) Thymus and pulmonary lymph node response to acute and subchronic ozone inhalation in the mouse. Environ Res 41:598–609

Dziedzic D, White HJ (1986b) T-cell activation in pulmonary lymph nodes of mice exposed to ozone. Environ Res 41:610–622

Eftim SE, Samet JM, Janes H, McDermott A, Dominici F (2008) Fine particulate matter and mortality: a comparison of the six cities and American Cancer Society cohorts with a medicare cohort. Epidemiology 19:209–216

Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K (2002) Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. J Exp Med 196:1645–1651

Fakhrzadeh L, Laskin JD, Laskin DL (2004) Ozone-induced production of nitric oxide and TNF-alpha and tissue injury are dependent on NF-kappaB p50. Am J Physiol Lung Cell Mol Physiol 287:L279–L285

Foster WM, Stetkiewicz PT (1996) Regional clearance of solute from the respiratory epithelia: 18–20 h postexposure to ozone. J Appl Physiol 81:1143–1149
Foster WM, Costa DL, Langenback EG (1987) Ozone exposure alters tracheobronchial mucociliary function in humans. J Appl Physiol 63:996–1002
Freeman GJ, Borriello F, Hodes RJ, Reiser H, Gribben JG, Ng JW, Kim J, Goldberg JM, Hathcock K, Laszlo G et al (1993) Murine B7-2, an alternative CTLA4 counter-receptor that costimulates T cell proliferation and interleukin 2 production. J Exp Med 178:2185–2192
Fujii T, Hayashi S, Hogg JC, Vincent R, Van Eeden SF (2001) Particulate matter induces cytokine expression in human bronchial epithelial cells. Am J Respir Cell Mol Biol 25:265–271
Fujimaki H, Ozawa M, Imai T, Shimizu F (1984) Effect of short-term exposure to O3 on antibody response in mice. Environ Res 35:490–496
Fujimaki H, Shiraishi F, Ashikawa T, Murakami M (1987) Changes in delayed hypersensitivity reaction in mice exposed to O3. Environ Res 43:186–190
Gershwin LJ, Osebold JW, Zee YC (1981) Immunoglobulin E-containing cells in mouse lung following allergen inhalation and ozone exposure. Int Arch Allergy Appl Immunol 65:266–277
Gilmour MI, Hmieleski RR, Stafford EA, Jakab GJ (1991) Suppression and recovery of the alveolar macrophage phagocytic system during continuous exposure to 0.5 ppm ozone. Exp Lung Res 17:547–558
Gilmour MI, Park P, Selgrade MK (1993) Ozone-enhanced pulmonary infection with Streptococcus zooepidemicus in mice. The role of alveolar macrophage function and capsular virulence factors. Am Rev Respir Dis 147:753–760
Glad JA, Brink LL, Talbott EO, Lee PC, Xu X, Saul M, Rager J (2012) The relationship of ambient ozone and PM(2.5) levels and asthma emergency department visits: possible influence of gender and ethnicity. Arch Environ Occup Health 67:103–108
Goldstein E, Tyler WS, Hoeprich PD, Eagle C (1971) Ozone and the antibacterial defense mechanisms of the murine lung. Arch Intern Med 127:1099–1102
Gowdy KM, Krantz QT, King C, Boykin E, Jaspers I, Linak WP, Gilmour MI (2010) Role of oxidative stress on diesel-enhanced influenza infection in mice. Part Fibre Toxicol 7:34
Grunwald U, Fan X, Jack RS, Workalemahu G, Kallies A, Stelter F, Schutt C (1996) Monocytes can phagocytose Gram-negative bacteria by a CD14-dependent mechanism. J Immunol 157:4119–4125
Haque R, Umstead TM, Ponnuru P, Guo X, Hawgood S, Phelps DS, Floros J (2007) Role of surfactant protein-A (SP-A) in lung injury in response to acute ozone exposure of SP-A deficient mice. Toxicol Appl Pharmacol 220:72–82
Harrod KS, Jaramillo RJ, Rosenberger CL, Wang SZ, Berger JA, McDonald JD, Reed MD (2003) Increased susceptibility to RSV infection by exposure to inhaled diesel engine emissions. Am J Respir Cell Mol Biol 28:451–463
Hashimoto S, Gon Y, Takeshita I, Matsumoto K, Jibiki I, Takizawa H, Kudoh S, Horie T (2000) Diesel exhaust particles activate p38 MAP kinase to produce interleukin 8 and RANTES by human bronchial epithelial cells and N-acetylcysteine attenuates p38 MAP kinase activation. Am J Respir Crit Care Med 161:280–285
Hernandez M, Brickey WJ, Alexis NE, Fry RC, Rager JE, Zhou B, Ting JP, Zhou H, Peden DB (2012) Airway cells from atopic asthmatic patients exposed to ozone display an enhanced innate immune gene profile. J Allergy Clin Immunol 129(259–261):e251–e252
Hippenstiel S, Opitz B, Schmeck B, Suttorp N (2006) Lung epithelium as a sentinel and effector system in pneumonia–molecular mechanisms of pathogen recognition and signal transduction. Respir Res 7:97
Hiraiwa K, van Eeden SF (2013) Contribution of lung macrophages to the inflammatory responses induced by exposure to air pollutants. Mediators Inflamm 2013:10
Hiramatsu K, Saito Y, Sakakibara K, Azuma A, Takizawa H, Sugawara I (2005) The effects of inhalation of diesel exhaust on murine mycobacterial infection. Exp Lung Res 31:405–415
Hiura TS, Kaszubowski MP, Li N, Nel AE (1999) Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J Immunol 163:5582–5591

Hiura TS, Li N, Kaplan R, Horwitz M, Seagrave JC, Nel AE (2000) The role of a mitochondrial pathway in the induction of apoptosis by chemicals extracted from diesel exhaust particles. J Immunol 165:2703–2711

Hollingsworth JW 2nd, Cook DN, Brass DM, Walker JK, Morgan DL, Foster WM, Schwartz DA (2004) The role of Toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 170:126–132

Hollingsworth J, Kleeberger SR, Foster WM (2007) Ozone and pulmonary innate immunity. Proc Am Thorac Soc 4:240–246

Hollingsworth JW, Free ME, Li Z, Andrews LN, Nakano H, Cook DN (2010) Ozone activates pulmonary dendritic cells and promotes allergic sensitization through a Toll-like receptor 4-dependent mechanism. J Allergy Clin Immunol 125:1167–1170

Inoue K, Koike E, Yanagisawa R, Takano H (2008) Impact of diesel exhaust particles on th2 response in the lung in asthmatic mice. J Clin Biochem Nutr 43:199–200

Ishii Y, Yang H, Sakamoto T, Nomura A, Hasegawa S, Hirata F, Bassett DJ (1997) Rat alveolar macrophage cytokine production and regulation of neutrophil recruitment following acute ozone exposure. Toxicol Appl Pharmacol 147:214–223

Janeway CA Jr (1989) Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54(Pt 1):1–13

Janeway C, National Center for Biotechnology Information (U.S.), and National Institutes of Health (U.S.). PubMed (2001) Immunobiology 5 the immune system in health and disease. Garland Pub., NCBI, New York, Bethsada

Janic B, Umstead TM, Phelps DS, Floros J (2003) An in vitro cell model system for the study of the effects of ozone and other gaseous agents on phagocytic cells. J Immunol Methods 272:125–134

Janic B, Umstead TM, Phelps DS, Floros J (2005) Modulatory effects of ozone on THP-1 cells in response to SP-A stimulation. Am J Physiol Lung Cell Mol Physiol 288:L317–L325

Jardim MJ, Fry RC, Jaspers I, Dailey L, Diaz-Sanchez D (2009) Disruption of microRNA expression in human airway cells by diesel exhaust particles is linked to tumorigenesis-associated pathways. Environ Health Perspect 117:1745–1751

Jaspers I, Chen LC, Flescher E (1998) Induction of interleukin-8 by ozone is mediated by tyrosine kinase and protein kinase A, but not by protein kinase C. J Cell Physiol 177:313–323

Jaspers I, Ciencewicki JM, Zhang W, Brighton LE, Carson JL, Beck MA, Madden MC (2005) Diesel exhaust enhances influenza virus infections in respiratory epithelial cells. Toxicol Sci 85:990–1002

Jerrett M, Burnett RT, Pope CA 3rd, Ito K, Thurston G, Krewski D, Shi Y, Calle E, Thun M (2009) Long-term ozone exposure and mortality. N Engl J Med 360:1085–1095

Ji M, Cohan DS, Bell ML (2011) Meta-analysis of the association between short-term exposure to ambient ozone and respiratory hospital admissions. Environ Res Lett 6

Johnston RA, Mizgerd JP, Shore SA (2005) CXCR2 is essential for maximal neutrophil recruitment and methacholine responsiveness after ozone exposure. Am J Physiol Lung Cell Mol Physiol 288:L61–L67

Johnston RA, Mizgerd JP, Flynt L, Quinton LJ, Williams ES, Shore SA (2007) Type I interleukin-1 receptor is required for pulmonary responses to subacute ozone exposure in mice. Am J Respir Cell Mol Biol 37:477–484

Karp CL, Grupe A, Schadt E, Ewart SL, Keane-Moore M, Cuomo PJ, Kohl J, Wahl L, Kuperman D, Germer S et al (2000) Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. Nat Immunol 1:221–226

Kasahara DI, Kim HY, Williams AS, Verbout NG, Tran J, Si H, Wurmbrand AP, Jastrab J, Hug C, Umetsu DT et al (2012) Pulmonary inflammation induced by subacute ozone is augmented in adiponectin-deficient mice: role of IL-17A. J Immunol 188:4558–4567

Kasahara DI, Kim HY, Mathews JA, Verbout NG, Williams AS, Wurmbrand AP, Ninin FM, Neto FL, Benedito LA, Hug C et al (2014) Pivotal role of IL-6 in the hyperinflammatory responses to subacute ozone in adiponectin-deficient mice. Am J Physiol Lung Cell Mol Physiol 306:L508–L520

Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11:373–384
Kehrl HR, Vincent LM, Kowalsky RJ, Horstman DH, O'Neil JJ, McCartney WH, Bromberg PA (1987) Ozone exposure increases respiratory epithelial permeability in humans. Am Rev Respir Dis 135:1124–1128
Kennedy T, Ghio AJ, Reed W, Samet J, Zagorski J, Quay J, Carter J, Dailey L, Hoidal JR, Devlin RB (1998) Copper-dependent inflammation and nuclear factor-kappaB activation by particulate air pollution. Am J Respir Cell Mol Biol 19:366–378
Kierstein S, Poulain FR, Cao Y, Grous M, Mathias R, Kierstein G, Beers MF, Salmon M, Panettieri RA Jr, Haczku A (2006) Susceptibility to ozone-induced airway inflammation is associated with decreased levels of surfactant protein D. Respir Res 7:85
Kim J, Natarajan S, Vaickus LJ, Bouchard JC, Beal D, Cruikshank WW, Remick DG (2011) Diesel exhaust particulates exacerbate asthma-like inflammation by increasing CXC chemokines. Am J Pathol 179:2730–2739
King DP, Hyde DM, Jackson KA, Novosad DM, Ellis TN, Putney L, Stovall MY, Van Winkle LS, Beaman BL, Ferrick DA (1999) Cutting edge: protective response to pulmonary injury requires gamma delta T lymphocytes. J Immunol 162:5033–5036
Kirsten A, Watz H, Pedersen F, Holz O, Smith R, Bruin G, Koehne-Voss S, Magnussen H, Waltz DA (2013) The anti-IL-17A antibody secukinumab does not attenuate ozone-induced airway neutrophilia in healthy volunteers. Eur Respir J 41:239–241
Kleeberger SR, Levitt RC, Zhang LY, Longphre M, Harkema J, Jedlicka A, Eleff SM, DiSilvestre D, Holroyd KJ (1997) Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17:475–478
Kleeberger SR, Reddy SP, Zhang LY, Cho HY, Jedlicka AE (2001) Toll-like receptor 4 mediates ozone-induced murine lung hyperpermeability via inducible nitric oxide synthase. Am J Physiol Lung Cell Mol Physiol 280:L326–L333
Kleinman MT, Sioutas C, Froines JR, Fanning E, Hamade A, Mendez L, Meacher D, Oldham M (2007) Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice. Inhal Toxicol 19(Suppl 1):117–126
Kobzik L (1995) Lung macrophage uptake of unopsonized environmental particulates. Role of scavenger-type receptors. J Immunol 155:367–376
Koike E, Kobayashi T (2004) Ozone exposure enhances antigen-presenting activity of interstitial lung cells in rats. Toxicology 196:217–227
Koike E, Watanabe H, Kobayashi T (2004) Exposure to ozone enhances antigen-presenting activity concentration dependently in rats. Toxicology 197:37–46
Koren HS, Bromberg PA (1995) Respiratory responses of asthmatics to ozone. Int Arch Allergy Immunol 107:236–238
Kronenberg M (2005) Toward an understanding of NKT cell biology: progress and paradoxes. Annu Rev Immunol 23:877–900
Kucuksezer UC, Zekiroglu E, Kasapoglu P, Adin-Cinar S, Aktas-Cetin E, Deniz G (2014) A stimulatory role of ozone exposure on human natural killer cells. Immunol Invest 43:1–12
Larini A, Bocci V (2005) Effects of ozone on isolated peripheral blood mononuclear cells. Toxicol In Vitro 19:55–61
Last JA, Ward R, Temple L, Pinkerton KE, Kenyon NJ (2004) Ovalbumin-induced airway inflammation and fibrosis in mice also exposed to ultrafine particles. Inhal Toxicol 16:93–102
Laumbach RJ, Kipen HM (2012) Respiratory health effects of air pollution: update on biomass smoke and traffic pollution. J Allergy Clin Immunol 129:3–11, quiz 12–13
Lay JC, Alexis NE, Kleeberger SR, Roubey RA, Harris BD, Bromberg PA, Hazucha MJ, Devlin RB, Peden DB (2007) Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals. J Allergy Clin Immunol 120:719–722
Leikauf GD, Zhao Q, Zhou S, Santrock J (1995) Activation of eicosanoid metabolism in human airway epithelial cells by ozonolysis products of membrane fatty acids. Res Rep Health Eff Inst 1–15; discussion 71:19–26

Lenschow DJ, Su GH, Zuckerman LA, Nabavi N, Jellis CL, Gray GS, Miller J, Bluestone JA (1993) Expression and functional significance of an additional ligand for CTLA-4. Proc Natl Acad Sci U S A 90:11054–11058

Li Kam Wa TC, Mansur AH, Britton J, Williams G, Pavord I, Richards K, Campbell DA, Morton N, Holgate ST, Morrison JF (1999) Association between – 308 tumour necrosis factor promoter polymorphism and bronchial hyperreactivity in asthma. Clin Exp Allergy 29:1204–1208

Li N, Wang M, Bramble LA, Schmitz DA, Schauer JJ, Sioutas C, Harkema JR, Nel AE (2009) The adjuvant effect of ambient particulate matter is closely reflected by the particulate oxidant potential. Environ Health Perspect 117:1116–1123

Li Z, Tighe RM, Feng F, Ledford JG, Hollingsworth JW (2013) Genes of innate immunity and the biological response to inhaled ozone. J Biochem Mol Toxicol 27:3–16

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224–2260

Lippmann M (2007) Health effects of airborne particulate matter. N Engl J Med 357:2395–2397

Manzer R, Wang J, Nishina K, McConville G, Mason RJ (2006) Alveolar epithelial cells secrete chemokines in response to IL-1beta and lipopolysaccharide but not to ozone. Am J Respir Cell Mol Biol 34:158–166

Manzer R, Dinarello CA, McConville G, Mason RJ (2008) Ozone exposure of macrophages induces an alveolar epithelial chemokine response through IL-1alpha. Am J Respir Cell Mol Biol 38:318–323

Matsubara S, Takeda K, Jin N, Okamoto M, Matsuda H, Shiraishi Y, Park JW, McConville G, Joetham A, O'Brien RL et al (2009) Vgamma1+ T cells and tumor necrosis factor-alpha in ozone-induced airway hyperresponsiveness. Am J Respir Cell Mol Biol 40:454–463

Matsumoto A, Hiramatsu K, Li Y, Azuma A, Kudoh S, Takizawa H, Sugawara I (2006) Repeated exposure to low-dose diesel exhaust after allergen challenge exaggerates asthmatic responses in mice. Clin Immunol 121:227–235

McCullough SD, Duncan KE, Swanton SM, Dailey LA, Diaz-Sanchez D, Devlin RB (2014) Ozone induces a proinflammatory response in primary human bronchial epithelial cells through mitogen-activated protein kinase activation without nuclear factor-kappaB activation. Am J Respir Cell Mol Biol 51:426–435

McDonald JD, Campen MJ, Harrod KS, Seagrave J, Seilkop SK, Mauderly JL (2011) Engine-operating load influences diesel exhaust composition and cardiopulmonary and immune responses. Environ Health Perspect 119:1136–1141

Meunier L, Bohjanen K, Voorhees JJ, Cooper KD (1994) Retinoic acid upregulates human Langerhans cell antigen presentation and surface expression of HLA-DR and CD11c, a beta 2 integrin critically involved in T-cell activation. J Invest Dermatol 103:775–779

Mikerov AN, Umstead TM, Huang W, Liu W, Phelps DS, Floros J (2005) SP-A1 and SP-A2 variants differentially enhance association of Pseudomonas aeruginosa with rat alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 288:L150–L158

Mikerov AN, Wang G, Umstead TM, Zacharatos M, Thomas NJ, Phelps DS, Floros J (2007) Surfactant protein A2 (SP-A2) variants expressed in CHO cells stimulate phagocytosis of Pseudomonas aeruginosa more than do SP-A1 variants. Infect Immun 75:1403–1412

Mikerov AN, Haque R, Gan X, Guo X, Phelps DS, Floros J (2008a) Ablation of SP-A has a negative impact on the susceptibility of mice to Klebsiella pneumoniae infection after ozone exposure: sex differences. Respir Res 9:77

Mikerov AN, Umstead TM, Gan X, Huang W, Guo X, Wang G, Phelps DS, Floros J (2008b) Impact of ozone exposure on the phagocytic activity of human surfactant protein A (SP-A) and SP-A variants. Am J Physiol Lung Cell Mol Physiol 294:L121–L130

Mikerov AN, Gan X, Umstead TM, Miller L, Chinchilli VM, Phelps DS, Floros J (2008c) Sex differences in the impact of ozone on survival and alveolar macrophage function of mice after Klebsiella pneumoniae infection. Respir Res 9:24

Mikerov AN, Hu S, Durrani F, Gan X, Wang G, Umstead TM, Phelps DS, Floros J (2012) Impact of sex and ozone exposure on the course of pneumonia in wild type and SP-A (–/–) mice. Microb Pathog 52:239–249

Miller S, Ehrlich R (1958) Susceptibility to respiratory infections of animals exposed to ozone. I. Susceptibility to Klebsiella pneumoniae. J Infect Dis 103:145–149

Miyata R, van Eeden SF (2011) The innate and adaptive immune response induced by alveolar macrophages exposed to ambient particulate matter. Toxicol Appl Pharmacol 257:209–226

Mukhopadhyay S, Varin A, Chen Y, Liu B, Tryggvason K, Gordon S (2011) SR-A/MARCO-mediated ligand delivery enhances intracellular TLR and NLR function, but ligand scavenging from cell surface limits TLR4 response to pathogens. Blood 117:1319–1328

Muller L, Chehrazi CV, Henderson MW, Noah TL, Jaspers I (2013a) Diesel exhaust particles modify natural killer cell function and cytokine release. Part Fibre Toxicol 10:16

Muller L, Brighton LE, Jaspers I (2013b) Ozone exposed epithelial cells modify cocultured natural killer cells. Am J Physiol Lung Cell Mol Physiol 304:L332–L341

Neuhaus-Steinmetz U, Uffhausen F, Herz U, Renz H (2000) Priming of allergic immune responses by repeated ozone exposure in mice. Am J Respir Cell Mol Biol 23:228–233

Nichols BG, Woods JS, Luchtel DL, Corral J, Koenig JQ (2001) Effects of ozone exposure on nuclear factor-kappaB activation and tumor necrosis factor-alpha expression in human nasal epithelial cells. Toxicol Sci 60:356–362

Noah TL, Zhou H, Zhang H, Horvath K, Robinette C, Kesic M, Meyer M, Diaz-Sanchez D, Jaspers I (2012) Diesel exhaust exposure and nasal response to attenuated influenza in normal and allergic volunteers. Am J Respir Crit Care Med 185:179–185

Oberdorster G (1996) Significance of particle parameters in the evaluation of exposure-dose–response relationships of inhaled particles. Inhal Toxicol 8(Suppl):73–89

Oberdorster G, Ferin J, Gelein R, Soderholm SC, Finkelstein J (1992) Role of the alveolar macrophage in lung injury: studies with ultrafine particles. Environ Health Perspect 97:193–199

Oberdorster G, Oberdorster E, Oberdorster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839

Ohmori Y, Wyner L, Narumi S, Armstrong D, Stoler M, Hamilton TA (1993) Tumor necrosis factor-alpha induces cell type and tissue-specific expression of chemoattractant cytokines in vivo. Am J Pathol 142:861–870

Ohtoshi T, Takizawa H, Okazaki H, Kawasaki S, Takeuchi N, Ohta K, Ito K (1998) Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation in vitro. J Allergy Clin Immunol 101:778–785

Osebold JW, Gershwin LJ, Zee YC (1980) Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. J Environ Pathol Toxicol 3:221–234

Osebold JW, Zee YC, Gershwin LJ (1988) Enhancement of allergic lung sensitization in mice by ozone inhalation. Proc Soc Exp Biol Med 188:259–264

Ozawa M, Fujimaki H, Imai T, Honda Y, Watanabe N (1985) Suppression of IgE antibody production after exposure to ozone in mice. Int Arch Allergy Appl Immunol 76:16–19

Palecanda A, Paulauskis J, Al-Mutairi E, Imrich A, Qin G, Suzuki H, Kodama T, Tryggvason K, Koziel H, Kobzik L (1999) Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles. J Exp Med 189:1497–1506

Park JW, Taube C, Swasey C, Kodama T, Joetham A, Balhorn A, Takeda K, Miyahara N, Allen CB, Dakhama A et al (2004a) Interleukin-1 receptor antagonist attenuates airway hyperresponsiveness following exposure to ozone. Am J Respir Cell Mol Biol 30:830–836

Park JW, Taube C, Joetham A, Takeda K, Kodama T, Dakhama A, McConville G, Allen CB, Sfyroera G, Shultz LD et al (2004b) Complement activation is critical to airway hyperresponsiveness after acute ozone exposure. Am J Respir Crit Care Med 169:726–732, Epub 2003 Dec 30

Peden DB, Boehlecke B, Horstman D, Devlin R (1997) Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. J Allergy Clin Immunol 100:802–808

Pichavant M, Goya S, Meyer EH, Johnston RA, Kim HY, Matangkasombut P, Zhu M, Iwakura Y, Savage PB, DeKruyff RH et al (2008) Ozone exposure in a mouse model induces airway hyper-

reactivity that requires the presence of natural killer T cells and IL-17. J Exp Med 205:385–393
Pinart M, Zhang M, Li F, Hussain F, Zhu J, Wiegman C, Ryffel B, Chung KF (2013) IL-17A modulates oxidant stress-induced airway hyperresponsiveness but not emphysema. PLoS One 8:e58452
Pope CA 3rd, Ezzati M, Dockery DW (2009) Fine-particulate air pollution and life expectancy in the United States. N Engl J Med 360:376–386
Porter M, Karp M, Killedar S, Bauer SM, Guo J, Williams D, Breysse P, Georas SN, Williams MA (2007) Diesel-enriched particulate matter functionally activates human dendritic cells. Am J Respir Cell Mol Biol 37:706–719
Pourazar J, Mudway IS, Samet JM, Helleday R, Blomberg A, Wilson SJ, Frew AJ, Kelly FJ, Sandstrom T (2005) Diesel exhaust activates redox-sensitive transcription factors and kinases in human airways. Am J Physiol Lung Cell Mol Physiol 289:L724–L730
Provoost S, Maes T, Willart MA, Joos GF, Lambrecht BN, Tournoy KG (2010) Diesel exhaust particles stimulate adaptive immunity by acting on pulmonary dendritic cells. J Immunol 184:426–432
Provoost S, Maes T, Joos GF, Tournoy KG (2012) Monocyte-derived dendritic cell recruitment and allergic T(H)2 responses after exposure to diesel particles are CCR2 dependent. J Allergy Clin Immunol 129:483–491
Pryor WA (1992) How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic Biol Med 12:83–88
Pryor WA, Squadrito GL, Friedman M (1995) The cascade mechanism to explain ozone toxicity: the role of lipid ozonation products. Free Radic Biol Med 19:935–941
Pulfer MK, Murphy RC (2004) Formation of biologically active oxysterols during ozonolysis of cholesterol present in lung surfactant. J Biol Chem 279:26331–26338
Punjabi CJ, Laskin JD, Pendino KJ, Goller NL, Durham SK, Laskin DL (1994) Production of nitric oxide by rat type II pneumocytes: increased expression of inducible nitric oxide synthase following inhalation of a pulmonary irritant. Am J Respir Cell Mol Biol 11:165–172
Quay JL, Reed W, Samet J, Devlin RB (1998) Air pollution particles induce IL-6 gene expression in human airway epithelial cells via NF-kappaB activation. Am J Respir Cell Mol Biol 19:98–106
Que LG, Stiles JV, Sundy JS, Foster WM (2011) Pulmonary function, bronchial reactivity, and epithelial permeability are response phenotypes to ozone and develop differentially in healthy humans. J Appl Physiol 111:679–687
Rabinovitch N, Strand M, Gelfand EW (2006) Particulate levels are associated with early asthma worsening in children with persistent disease. Am J Respir Crit Care Med 173:1098–1105
Renwick LC, Brown D, Clouter A, Donaldson K (2004) Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occup Environ Med 61:442–447
Riedl MA (2008) The effect of air pollution on asthma and allergy. Curr Allergy Asthma Rep 8:139–146
Riedl M, Diaz-Sanchez D (2005) Biology of diesel exhaust effects on respiratory function. J Allergy Clin Immunol 115:221–228, quiz 229
Ring J, Kramer U, Schafer T, Behrendt H (2001) Why are allergies increasing? Curr Opin Immunol 13:701–708
Rusznak C, Devalia JL, Sapsford RJ, Davies RJ (1996) Ozone-induced mediator release from human bronchial epithelial cells in vitro and the influence of nedocromil sodium. Eur Respir J 9:2298–2305
Samet JM, Hatch GE, Horstman D, Steck-Scott S, Arab L, Bromberg PA, Levine M, McDonnell WF, Devlin RB (2001) Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med 164:819–825
Samuelsen M, Nygaard UC, Lovik M (2008) Allergy adjuvant effect of particles from wood smoke and road traffic. Toxicology 246:124–131

Sarkar S, Song Y, Sarkar S, Kipen HM, Laumbach RJ, Zhang J, Strickland PA, Gardner CR, Schwander S (2012) Suppression of the NF-kappaB pathway by diesel exhaust particles impairs human antimycobacterial immunity. J Immunol 188:2778–2793

Saxon A, Diaz-Sanchez D (2005) Air pollution and allergy: you are what you breathe. Nat Immunol 6:223–226

Scannell C, Chen L, Aris RM, Tager I, Christian D, Ferrando R, Welch B, Kelly T, Balmes JR (1996) Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154:24–29

Schelegle ES, Miller LA, Gershwin LJ, Fanucchi MV, Van Winkle LS, Gerriets JE, Walby WF, Mitchell V, Tarkington BK, Wong VJ et al (2003) Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. Toxicol Appl Pharmacol 191:74–85

Schmudde I, Laumonnier Y, Kohl J (2013) Anaphylatoxins coordinate innate and adaptive immune responses in allergic asthma. Semin Immunol 25:2–11

Schwartz J (1995) Short term fluctuations in air pollution and hospital admissions of the elderly for respiratory disease. Thorax 50:531–538

Sheffield PE, Knowlton K, Carr JL, Kinney PL (2011) Modeling of regional climate change effects on ground-level ozone and childhood asthma. Am J Prev Med 41:251–257; quiz A253

Shoenfelt J, Mitkus RJ, Zeisler R, Spatz RO, Powell J, Fenton MJ, Squibb KA, Medvedev AE (2009) Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. J Leukoc Biol 86:303–312

Shore SA, Schwartzman IN, Le Blanc B, Murthy GG, Doerschuk CM (2001) Tumor necrosis factor receptor 2 contributes to ozone-induced airway hyperresponsiveness in mice. Am J Respir Crit Care Med 164:602–607

Shore SA, Johnston RA, Schwartzman IN, Chism D, Krishna Murthy GG (2002) Ozone-induced airway hyperresponsiveness is reduced in immature mice. J Appl Physiol 92:1019–1028

Soberanes S, Panduri V, Mutlu GM, Ghio A, Bundinger GR, Kamp DW (2006) p53 mediates particulate matter-induced alveolar epithelial cell mitochondria-regulated apoptosis. Am J Respir Crit Care Med 174:1229–1238

Soukup JM, Becker S (2001) Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse material, including particulate endotoxin. Toxicol Appl Pharmacol 171:20–26

Spira-Cohen A, Chen LC, Kendall M, Lall R, Thurston GD (2011) Personal exposures to traffic-related air pollution and acute respiratory health among Bronx schoolchildren with asthma. Environ Health Perspect 119:559–565

Stagos D, Umstead TM, Phelps DS, Skaltsounis L, Haroutounian S, Floros J, Kouretas D (2007) Inhibition of ozone-induced SP-A oxidation by plant polyphenols. Free Radic Res 41:357–366

Steerenberg PA, Garssen J, van Bree L, van Loveren H (1996) Ozone alters T-helper cell mediated bronchial hyperreactivity and resistance to bacterial infection. Exp Toxicol Pathol 48:497–499

Steerenberg PA, van Dalen WJ, Withagen CE, Dormans JA, van Loveren H (2003a) Optimization of route of administration for coexposure to ovalbumin and particle matter to induce adjuvant activity in respiratory allergy in the mouse. Inhal Toxicol 15:1309–1325

Steerenberg PA, Withagen CE, Dormans JA, van Dalen WJ, van Loveren H, Casee FR (2003b) Adjuvant activity of various diesel exhaust and ambient particles in two allergic models. J Toxicol Environ Health A 66:1421–1439

Stenfors N, Bosson J, Helleday R, Behndig AF, Pourazar J, Tornqvist H, Kelly FJ, Frew AJ, Sandstrom T, Mudway IS et al (2010) Ozone exposure enhances mast-cell inflammation in asthmatic airways despite inhaled corticosteroid therapy. Inhal Toxicol 22:133–139

Stephens RJ, Evans MJ, Sloan MF, Freeman G (1974) A comprehensive ultrastructural study of pulmonary injury and repair in the rat resulting from exposures to less than one PPM ozone. Chest 65(Suppl):11S–13S

Stevens T, Cho SH, Linak WP, Gilmour MI (2009) Differential potentiation of allergic lung disease in mice exposed to chemically distinct diesel samples. Toxicol Sci 107:522–534

Strickland MJ, Darrow LA, Klein M, Flanders WD, Sarnat JA, Waller LA, Sarnat SE, Mulholland JA, Tolbert PE (2010) Short-term associations between ambient air pollutants and pediatric asthma emergency department visits. Am J Respir Crit Care Med 182:307–316

Takahashi G, Tanaka H, Wakahara K, Nasu R, Hashimoto M, Miyoshi K, Takano H, Yamashita H, Inagaki N, Nagai H (2010) Effect of diesel exhaust particles on house dust mite-induced airway eosinophilic inflammation and remodeling in mice. J Pharmacol Sci 112:192–202

Takizawa H, Ohtoshi T, Kawasaki S, Kohyama T, Desaki M, Kasama T, Kobayashi K, Nakahara K, Yamamoto K, Matsushima K et al (1999) Diesel exhaust particles induce NF-kappa B activation in human bronchial epithelial cells in vitro: importance in cytokine transcription. J Immunol 162:4705–4711

Thakur SA, Beamer CA, Migliaccio CT, Holian A (2009) Critical role of MARCO in crystalline silica-induced pulmonary inflammation. Toxicol Sci 108:462–471

Thomas GB, Fenters JD, Ehrlich R, Gardner DE (1981) Effects of exposure to ozone on susceptibility to experimental tuberculosis. Toxicol Lett 9:11–17

Totlandsdal AI, Cassee FR, Schwarze P, Refsnes M, Lag M (2010) Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses via differential pathways in human bronchial epithelial cells. Part Fibre Toxicol 7:41

Totlandsdal AI, Herseth JI, Bølling AK, Kubátová A, Braun A, Cochran RE, Refsnes M, Øvrevik J, Låg M (2012) Differential effects of the particle core and organic extract of diesel exhaust particles. Toxicol Lett 208:262–268

Urich D, Soberanes S, Burgess Z, Chiarella SE, Ghio AJ, Ridge KM, Kamp DW, Chandel NS, Mutlu GM, Budinger GR (2009) Proapoptotic Noxa is required for particulate matter-induced cell death and lung inflammation. FASEB J 23:2055–2064

Van Loveren H, Rombout PJ, Wagenaar SS, Walvoort HC, Vos JG (1988) Effects of ozone on the defense to a respiratory Listeria monocytogenes infection in the rat. Suppression of macrophage function and cellular immunity and aggravation of histopathology in lung and liver during infection. Toxicol Appl Pharmacol 94:374–393

Van Loveren H, Steerenberg PA, Garssen J, Van Bree L (1996) Interaction of environmental chemicals with respiratory sensitization. Toxicol Lett 86:163–167

Verhein KC, Jacoby DB, Fryer AD (2008) IL-1 receptors mediate persistent, but not acute, airway hyperreactivity to ozone in guinea pigs. Am J Respir Cell Mol Biol 39:730–738

Vincent R, Vu D, Hatch G, Poon R, Dreher K, Guenette J, Bjarnason S, Potvin M, Norwood J, McMullen E (1996) Sensitivity of lungs of aging Fischer 344 rats to ozone: assessment by bronchoalveolar lavage. Am J Physiol 271:L555–L565

von Klot S, Wolke G, Tuch T, Heinrich J, Dockery DW, Schwartz J, Kreyling WG, Wichmann HE, Peters A (2002) Increased asthma medication use in association with ambient fine and ultrafine particles. Eur Respir J 20:691–702

Voynow JA, Fischer BM, Zheng S, Potts EN, Grover AR, Jaiswal AK, Ghio AJ, Foster WM (2009) NAD(P)H quinone oxidoreductase 1 is essential for ozone-induced oxidative stress in mice and humans. Am J Respir Cell Mol Biol 41:107–113

Wagner JG, Hotchkiss JA, Harkema JR (2002) Enhancement of nasal inflammatory and epithelial responses after ozone and allergen coexposure in Brown Norway rats. Toxicol Sci 67:284–294

Wang G, Umstead TM, Phelps DS, Al-Mondhiry H, Floros J (2002) The effect of ozone exposure on the ability of human surfactant protein a variants to stimulate cytokine production. Environ Health Perspect 110:79–84

Wang J, Wang S, Manzer R, McConville G, Mason RJ (2006) Ozone induces oxidative stress in rat alveolar type II and type I-like cells. Free Radic Biol Med 40:1914–1928

Wang T, Moreno-Vinasco L, Huang Y, Lang GD, Linares JD, Goonewardena SN, Grabavoy A, Samet JM, Geyh AS, Breysse PN et al (2008) Murine lung responses to ambient particulate matter: genomic analysis and influence on airway hyperresponsiveness. Environ Health Perspect 116:1500–1508

Williams MA, Porter M, Horton M, Guo J, Roman J, Williams D, Breysse P, Georas SN (2007a) Ambient particulate matter directs nonclassic dendritic cell activation and a mixed TH1/TH2-like cytokine response by naive CD4+ T cells. J Allergy Clin Immunol 119:488–497

Williams AS, Leung SY, Nath P, Khorasani NM, Bhavsar P, Issa R, Mitchell JA, Adcock IM, Chung KF (2007b) Role of TLR2, TLR4, and MyD88 in murine ozone-induced airway hyperresponsiveness and neutrophilia. J Appl Physiol 103:1189–1195

Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN (2009) Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. Am J Respir Crit Care Med 180:720–730

Wu ZX, Barker JS, Batchelor TP, Dey RD (2008) Interleukin (IL)-1 regulates ozone-enhanced tracheal smooth muscle responsiveness by increasing substance P (SP) production in intrinsic airway neurons of ferret. Respir Physiol Neurobiol 164:300–311

Wu W, Peden DB, McConnell R, Fruin S, Diaz-Sanchez D (2012) Glutathione-S-transferase M1 regulation of diesel exhaust particle-induced pro-inflammatory mediator expression in normal human bronchial epithelial cells. Part Fibre Toxicol 9:31

Xiang Q, Wen L, Liu MH, Zhang Y, Qu JF, Tian J (2009) Endotoxin tolerance of RAW264.7 correlates with p38-dependent up-regulation of scavenger receptor-A. J Int Med Res 37:491–502

Xiao GG, Wang M, Li N, Loo JA, Nel AE (2003) Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line. J Biol Chem 278:50781–50790

Xie QW, Kashiwabara Y, Nathan C (1994) Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem 269:4705–4708

Yang HM, Antonini JM, Barger MW, Butterworth L, Roberts BR, Ma JK, Castranova V, Ma JY (2001) Diesel exhaust particles suppress macrophage function and slow the pulmonary clearance of Listeria monocytogenes in rats. Environ Health Perspect 109:515–521

Yang IA, Holz O, Jorres RA, Magnussen H, Barton SJ, Rodriguez S, Cakebread JA, Holloway JW, Holgate ST (2005) Association of tumor necrosis factor-alpha polymorphisms and ozone-induced change in lung function. Am J Respir Crit Care Med 171:171–176

Yin XJ, Dong CC, Ma JY, Antonini JM, Roberts JR, Barger MW, Ma JK (2005) Sustained effect of inhaled diesel exhaust particles on T-lymphocyte-mediated immune responses against Listeria monocytogenes. Toxicol Sci 88:73–81

Yin XJ, Dong CC, Ma JY, Roberts JR, Antonini JM, Ma JK (2007) Suppression of phagocytic and bactericidal functions of rat alveolar macrophages by the organic component of diesel exhaust particles. J Toxicol Environ Health A 70:820–828

Yu M, Zheng X, Witschi H, Pinkerton KE (2002) The role of interleukin-6 in pulmonary inflammation and injury induced by exposure to environmental air pollutants. Toxicol Sci 68:488–497

Zanobetti A, Schwartz J (2011) Ozone and survival in four cohorts with potentially predisposing diseases. Am J Respir Crit Care Med 184:836–841

Zhou H, Kobzik L (2007) Effect of concentrated ambient particles on macrophage phagocytosis and killing of Streptococcus pneumoniae. Am J Respir Cell Mol Biol 36:460–465

Chapter 12
Genetics

Talat Islam and Frank Gilliland

12.1 Introduction

In previous chapters we discussed the chemical and physical properties of the different airborne pollutants (AP) and their potential health effects. Air pollutants with potential detrimental health effects can be gasses (i.e., Ozone, oxides of nitrogen (NOx)) or solid particles (i.e., PM_{10}, $PM_{2.5}$) that can elicit myriad of biological responses and affect cardio-respiratory system, immune system, CNS, and reproductive system. Despite the epidemiological and toxicological evidence of AP-related health effects, the biological mechanisms and pathways involved are not well established. The particular biological pathways and process involved in AP-mediated health effects can vary by the involved organs/systems and may include oxidative stress, local or systemic inflammation, neural-mediated responses, and oxygen insufficiency. Many of these pathways have been identified through toxicological studies and discussed in the earlier chapters. However, toxicological studies often involve exposures that are limited in reproducing exposures encountered in the real world. One approach to investigating the biological pathways has been to identify the genes that modify sensitivity to AP.

If a *specific* pathway is important for *specific* air pollutant mediated health effects, then functional polymorphisms in gene(s) involved in the pathway can affect the magnitude of the health effects of that air pollutant. Therefore, gene-environment interaction (GxE) studies can confirm or identify the important mediating biological pathways, as well as, serve to identify susceptible groups. Multiple genes are likely to be involved in a single biological pathway and functional polymorphisms of the different genes in the pathway might provide synergistic or antagonistic effect on the air pollutant mediated health effect.

T. Islam, M.B.B.S., Ph.D. (✉) • F. Gilliland, M.D., M.P.H., Ph.D.
Division of Environmental Health, Department of Preventive Medicine, Keck School of Medicine, University of Southern California (USC), Los Angeles, CA, USA
e-mail: islam@usc.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_12

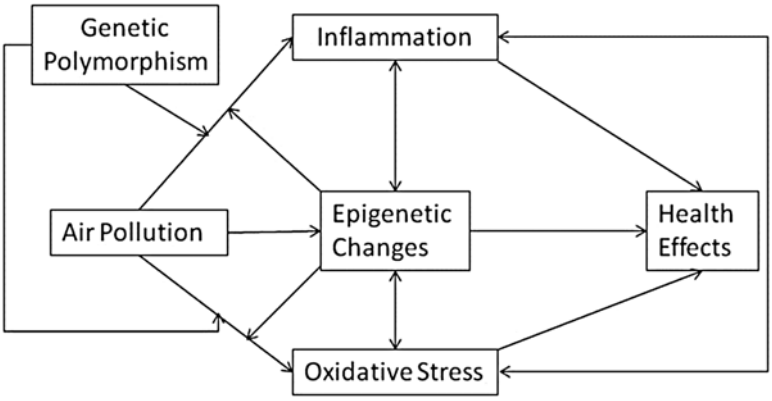

Fig. 12.1 Schematic representation of genetics and air pollution on human health. The health effects of AP exposure can be mediated through inflammation, oxidative stress and epigenetic changes; all of which are interrelated. Furthermore the effects of AP can be modified by genetic and epigenetic variants

Beyond identifying the biological pathways, a better knowledge of GxE can serve multiple purposes in public health and medicine as has been enumerated by Hunter in his review (Hunter 2005). Some of the rationale for studying gene-AP interactions are as follow:

- To have a better estimate of the population attributable risk considering the joint effect of genes and air pollution.
- To identify the susceptible population to AP-mediated disease (i.e., increased susceptibility to allergy due to exposure to diesel exhaust among population with GSTM1 deletion (~50 % Caucasian) (Gilliland et al. 2004))
- Identify the specific factor from a complex pollutant mix that can have a causal effect (i.e., polycyclic aromatic hydrocarbon in traffic exposure for asthma risk) (Salam et al. 2007)
- Utilize the information to develop better preventive and therapeutic approaches

In this chapter, we will review the complex interplay between air pollution, genetic variants and epigenetic changes on human health outcomes. Our discussion will focus on genetic polymorphisms that can modify the effect of AP on local and systemic inflammation and oxidative stress (Fig. 12.1). We will also discuss contributions of AP-induced epigenetic changes that could contribute to AP exposure related health effects.

12.2 Identification of Gene-Environment Interactions

In epidemiological studies, the identification of the genetic polymorphisms that modify the effect of AP (GxAP) involves mainly three major approaches: (i) hypothesis driven candidate gene approach: selecting genes with main effect on the health

outcome or selecting genes that are involved in a purported biological pathway, (ii) agnostic genome-wide interaction studies (GWIS), and (iii) hypothesis driven pathway based approaches. Each of these approaches has merits and limitations (Thomas 2010).

Because the candidate gene approach is hypothesis driven, we often have better study designs that are less subject to bias and residual confounding. These studies require a relatively small sample size compared to GWIS and have sufficient statistical power to detect moderate level of gene-environment interaction. It also provides a better chance for cross validation across studies as researchers are more likely to use the same genetic variants to address similar hypothesis. One of the major limitations of candidate gene approach is, by definition, it will miss any novel genetic pathway. Using candidate GxE approach from the onset can limit the identification of new genes as researchers focus on validating the initial finding and genes that might not have reached threshold p-value in some studies may not surface at all. In the candidate gene approach, we are likely to miss genetic factors that do not have any main effect especially when genetic variants have a 'flip-flop' effect where the effect of the genetic variants on disease is opposite based on presence or absence of environmental factor. This approach is also susceptible to researcher bias as those variants are not agnostically chosen. Many of the limitations of candidate gene approach are adequately addressed using the agnostic GWIS approach. The current availability of 2.5 million SNP chips at a reasonable price has supported the era of genome-wide association studies (GWAS) as well as for GWIS. The major concerns for the GWIS approach are related to exposure assessment, multiple testing and sample size issues. Considering the issues of multiple testing, the common cut-off p-value for GWAS/GWIS level of significance is 10^{-8}. A study would require almost 40,000 participants to have 80 % power to see a moderate level of effect (i.e., both the marginal effects and interaction odds ratio being 1.5) for a genetic variant with minor allele frequency (MAF) of 0.05 and exposure prevalence of 10 %. This is often a challenge for any single study and requires collaboration among multiple studies. Although, the collaboration approach in current state of information technology is quite feasible and beneficial to all involved, methodological challenges is the major hindrance for such approach. Differences in study design as well as availability of the appropriate exposure metrics and covariates limits collaborative GxE work. The participating studies are required to have similar genetic and environmental information that, to date, is often not available. Genetic data often varies between groups based on the choice of array (i.e., half-a-million/million or other, Illumina or Affymetrix platform). Although this may raise technical issues, most of them can be addressed. A greater concern is often the complete lack of, or variability in the collection of exposure data. For example some studies use model based estimate of oxides of Nitrogen (NOx) as a proxy measure for traffic exposure; whereas, another study can use residential distance to freeway. Even the model used by different groups to estimate NOx may not be similar. Often studies have to rely on some basic measure that is available in all of the groups, i.e. residential distance from freeway or major roads, instead of more refined measures. Therefore, measurement misclassification is a major concern in GxE collaborative studies that can result in biased effect estimates, reduced power and or null findings.

12.2.1 An Example of Genetic Polymorphism Modifying the Effect of AP

The gene-AP interactions discussed in this chapter are mostly identified through candidate gene approach in human studies. The following example from Salam et al. (2007) illustrates how candidate gene approach can be utilized in the identification of the modifying effect of multiple genes in the PAH metabolism pathway on the association between traffic related air pollutant (**TRAP**) and asthma prevalence (Salam et al. 2007).

Multiple epidemiologic studies have reported that high level of TRAP exposure is associated with asthma (Chap. 4). The investigators hypothesized that PAHs that are formed due to incomplete combustion of fuel and have been observed to be associated with asthma, might be the major component of TRAP that contributes to TRAP-Asthma association. The researchers hypothesized that genetic polymorphisms of microsomal epoxide hydrolase (EPHX1) and glutathione S-transferase (GST) can modify the effect of traffic exposure on asthma prevalence.

Once PAHs from TRAP enter the body through inhalation, it undergoes metabolic transformation that can lead to detoxification or activation (Fig. 12.2). Reactive PAHs are initially converted to active PAH-epoxide that serves as substrate for

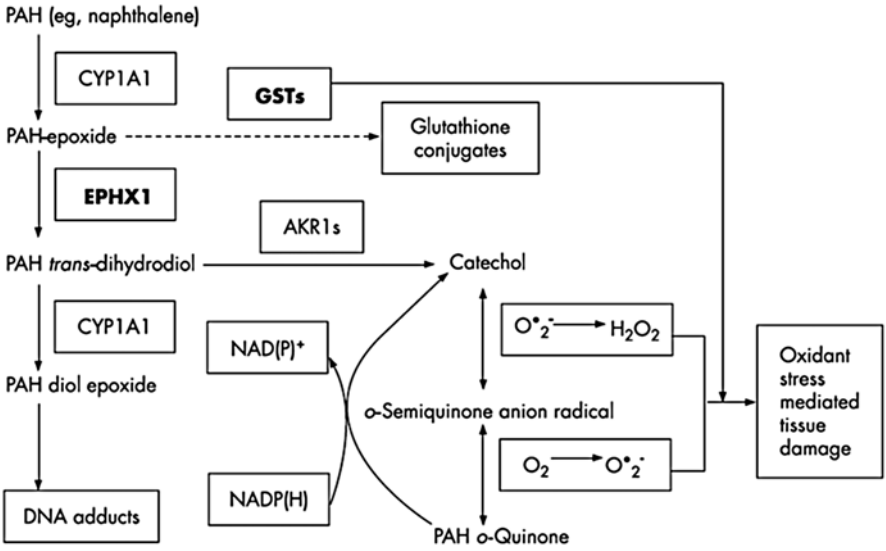

Fig. 12.2 Schematic pathway of PAH metabolism. After inhalation, polycyclic aromatic hydrocarbon (PAH) undergoes metabolic changes with formation of toxic metabolites that can lead to oxidative changes and formation of DNA adducts. PAHs can also be sequestered by the formation of GST–conjugates. Therefore, the activity level of the different involved enzymes, i.e., epoxides or GSTs, can affect the biological effective dose of PAH exposure (Reproduced from Salam et al. (2007), with permission from BMJ Publishing Group Ltd)

GSTs and epoxide hydrolase. GSTs detoxify PAHs by forming epoxide-conjugates and rendering them inactive. Epoxide hydrolases on the other hand form PAH-*trans*-dihyrodiol from PAH-epoxide. The PAH-trans-hydrodiols can be further metabolized to form semiquinones that generate reactive oxygen species (ROS) leading to oxidative stress (Bolton et al. 2000). Based on this pathway it can be purported that people with low level of GST activity and higher level of EPHX1 activity will be more susceptible to TRAP exposure compared to those with high activity of GST activity and low EPHX1 activity. The researchers identified a genetic polymorphism of *GSTP1* (*ile105val)*, where the variant (*val*) is associated with decreased conjugation of PAH-epoxide and functional polymorphisms of EPHX1 are associated with increased activity of epoxide hydrolase.

The joint effect of residential distance from a major road (exposure to TRAP decreases with increased distance), *Ile105Val* polymorphism, and two *EPHX1* polymorphisms that defined three metabolic phenotypes (low, medium, and high activity) was investigated in 2,700 children (Table 12.1). They reported statistically significant three way interaction showing that those children who were *GSTP1* variant homozygous (val105 val) with the high metabolic phenotype of EPHX1 and lived within 75 m of a major road, had almost nine times increased risk of asthma

Table 12.1 Functional polymorphisms of GSTP1 and EPHX1 modify the effect of traffic related exposure on lifetime asthma prevalence in children (Salam et al. 2007)

Distance of residence from major road (metres)	GSTP1 ile 105Val	EPHX1 metabolic phenotypes	No asthma (N[a])	Lifetime asthma (N[a])	OR[b] (95 % CI)
≥75	Ile/Ile	Low/intermediate	589	94	1.0
≥75	Ile/Val	Low/intermediate	720	132	1.19 (0.88–1.61)
≥75	Val/Val	Low/intermediate	215	29	0.94 (0.59–1.50)
≥75	Ile/Ile	High	141	23	1.03 (0.61–1.71)
≥75	Ile/Val	High	158	31	1.35 (0.85–2.15)
≥75	Val/Val	High	38	14	2.63 (1.34–5.18)
<75	Ile/Ile	Low/intermediate	144	23	1.01 (0.60–1.69)
<75	Ile/Val	Low/intermediate	171	28	0.89 (0.54–1.44)
<75	Val/Val	Low/intermediate	48	11	1.46 (0.71–3.03)
<75	Ile/Ile	High	31	9	1.71 (0.75–3.87)
<75	Ile/Val	High	30	12	2.61 (1.22–5.58)
<75	Val/Val	High	5	6	8.91 (2.40–33.12)
					p = 0.04[c]

Reprinted from Salam et al. (2012), Copyright (2012), with permission from Elsevier

[a]Children with missing data on distance of residence from major road and GSTP1 Ilel05Val were excluded

[b]ORs adjusted for age, sex, race/ethnicity, in utero exposure to maternal smoking, number of smokers at home, community of residence, parental education, health insurance and parental history of asthma

[c]p value for EPHX1 activity phenotype by distance of residence from a major road and by GSTP1 Ile105Val genotype interaction was obtained from likelihood ratio test from a non-stratified model with appropriate interaction terms and was based on 7df

compared to children who lived beyond 75 m of a major road and were GSTP1 wild type homozygous (*ile105ile)* with low/intermediate activity EPHX1 metabolic phenotype. In fact a dose dependent effect of the genetic polymorphisms and TRAP exposure was observed for asthma prevalence in children. The finding was replicated in a cross-sectional study of Taiwanese children (Tung et al. 2011). This study exemplifies many of the rationale of GxE studies we have mentioned earlier:

- Demonstrates the importance of PAH metabolism pathway in TRAP mediated risk of asthma
- Identification of susceptible population to TRAP exposure: children who are GSTP1 variant (Val) homozygous and EPHX1 high metabolic phenotype (~11 % of US children)
- Identifies PAH as the important chemical of TRAP, which is a complex mix of chemicals, as the risk factor for asthma following TRAP exposure.

12.3 Modification of AP Effect on Respiratory Outcomes by Genetic Factors

Inflammation, both chronic and acute, are central features of many common AP mediated lung conditions, such as asthma, COPD, or decline in lung function (Rahman et al. 2006; Adcock et al. 2005; Rahman and Adcock 2006; Adcock and Lee 2006). The respiratory tract endures the primary insult from air pollutants as air is inhaled into the lungs, thus it also provides the first line of defense against the air pollutants, as has been discussed in Chap. 5. In response to the environmental insults, as well as endogenous metabolism, reactive oxygen species (ROS) and free radicals are formed in the airway (Rahman et al. 2006). The airway, specially the lungs serves as the first line of defense against the effects of air pollutants. The fluid lining of the lung tissue is rich in enzymatic anti-oxidants (i.e., Glutathione-S-transferases (GSTs), heme oxugenase-1 (HMOX1), catalase (CAT), superoxide dismutases (SODs) and others) and non-enzymatic (i.e., vitamin C & E, glutathione (GSH) and other low molecular weight particles) that can neutralize the oxidative effects of air pollutants. Normally a balance is attained between the oxidative and anti-oxidative forces; however, when the anti-oxidative activity is overwhelmed due to exogenous (i.e., high level of air pollutants) or endogenous (i.e., respiratory infection) factors, oxidative stress ensues. The excess ROS can cause local damage to the lung parenchyma leading to a pro-inflammatory state in the lung tissue. Inflammation itself being an oxidative process starts a vicious cycle of oxidative stress-inflammation-oxidative stress (Fig. 12.1). Therefore, healthy abundance of anti-oxidant and anti-inflammatory repertoire in the lung tissue can provide great defense against the air pollutants mediated oxidative stress in the airway; whereas, a lack of such defense can make the lungs susceptible to the air pollutants.

Most of the genetic and gene-environmental works related to AP and respiratory outcomes have focused on the oxidative and inflammatory pathway in the airways.

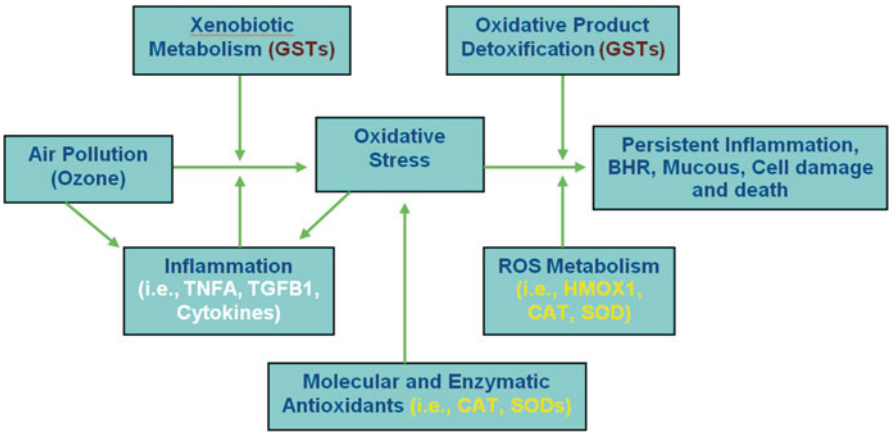

Fig. 12.3 Schematic representation of AP mediated health effects in lung tissue and its possible mediators. Inhalation of air pollutants such as ozone, PM, NOx, or PAHs, can lead to oxidative stress and inflammation of the lung tissue

The framework for AP mediated respiratory health outcomes and it's interaction with the genetic factors is depicted in Fig. 12.3. Air pollutants such as ozone or diesel exhaust upon entering the airway can cause oxidative stress in the lung tissue that if uncontrolled can lead to acute and chronic inflammation of the lung leading to asthma, COPD, decline in lung functions and other ailments. The detrimental effect of the air pollutant depends on the level of exposure and the susceptibility of the lung tissue. The availability of enzymatic and not-enzymatic anti-oxidants in the lung tissue and the inflammatory response mechanism are the major factors in the determination of the individual susceptibility to the effects of air pollutants.

High level of exposure to AP may cause little detrimental effect in presence of abundant anti-oxidants (i.e, HMOX-1, CAT, SODs, & GSTs) repertoire in the lung fluid as well as a well controlled inflammatory mechanism. On the contrary, low level exposure to AP can lead to substantial damage to the lung tissue if there is deficiency in the anti-oxidant activity and hyperactivity of the inflammatory mechanism. Therefore, genetic factors that can affect the level of anti-oxidative activity or inflammation level in the lung have been the genes of choice to investigate gene-environment interaction in the candidate GxE approach.

12.3.1 Anti-oxidant Genes and AP

12.3.1.1 Overview

The most commonly studied genes in the anti-oxidant pathway are the GSTs, HMOX1, NAD(P) Dehydrogenase Quinone 1 (NQO1), CAT and SODs. Figure 12.4 presents a schematic representation of the role of these enzymes in the anti-oxidative

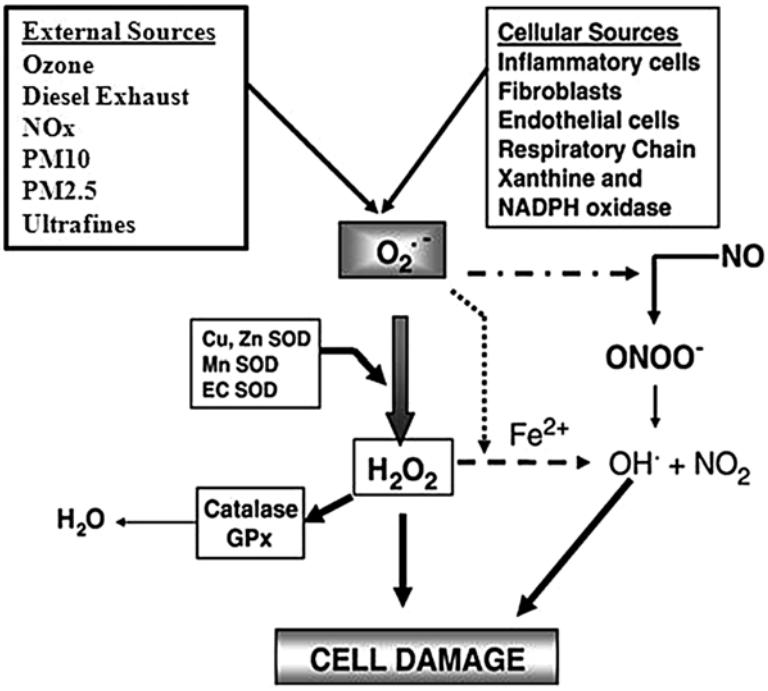

Fig. 12.4 Endogenous and exogenous sources of ROS and cellular anti-oxidative machinery. Intracellular reactive oxygen species $O_2^{\cdot -}$ = superoxide anion, NO = nitric oxide, H_2O_2 = hydrogen peroxide, ·OH = hydroxyl radical, NO_2 = nitrogen dioxide, ONOO– = peroxynitrite, Fe2+= Ferrous oxide, GPx= Glutathione peroxidase (Reprinted from Rahman et al. (2006), Copyright (2006), with permission from Elsevier)

defenses in response to the formation of different intracellular reactive oxygen species. The physiologically important ROSs are superoxide anion ($O_2^{\cdot -}$), hydroxyl radical (OH^-), nitric oxide (NO) and hydrogen peroxide (H_2O_2) with OH^- being most reactive and damaging to the cells (Rahman et al. 2006). The SODs, GSTs, and possibly HMOX-1 act as ROS scavengers in the cell and protects the cell by reducing the availability of ROS to cause cellular damage. SODs form H_2O_2 from two superoxide anions and two hydrogen ion. Catalase neutralizes H_2O_2 by converting it to water. Therefore the anti-oxidative activity of SOD is dependent on catalase/GPx availability and activity. The role of HMOX1 as an anti-oxidant and cytoprotective factor is more complex and enigmatic (Rahman et al. 2006). HMOX1 is purported to exerts its anti oxidant effect by (i) formation of bilirubin from heme protein which is readily converted to biliverdin, potent anti-oxidant (Stocker and Ames 1987; Stocker et al. 1987), (ii) regulating Fe^{2+} transfer (Ferris et al. 1999), and (iii) formation of CO that stimulates a number of anti-oxidant activity (Otterbein et al. 2000).

The GSTs are a group of Phase II iosenzymes that removes secondary ROSs, including products of lipid peroxidation and xenobiotics (i.e., PAH from diesel

exhaust), by catalyzing their conjugation with reduced GSH. The conjugates are sequestered and removed from the cell. The GST superfamily is highly diverse. Based on their substrate specificity, structure, and kinetic behavior eight distinct classes are defined: Alpha (GSTA), Kappa (GSTK), Mu (GSTM), Omega (GSTO), Pi (GSTP), Sigma (GSTS), Theta (GSTT) and Zeta (GSTZ). Each of these classes has subgroups. GSTM1, GSTP1 and GSTT1 have been most studied in the context of AP and environmental effect (Minelli et al. 2011).

12.3.1.2 Genes and the Commonly Studied Polymorphism

GSTs: The GST genes are located in different chromosomes and these are highly polymorphic. The commonly studied *GSTM1*, *GSTP1*, and *GSTT1* is located in the 1p13.3, 11q13, and 22q11.2 region. The most commonly studied functionally relevant polymorphisms are deletion allele of *GSTM1* and *GSTT1* and Ile105Val (rs1695) polymorphism of *GSTP1*. The *GSTM1* and *GSTT1* deletion is often studied as homozygous deletion ('null' genotype) that is associated with total absence of enzymatic activity. The functional effect of *GSTP1*-val variant in more complex as the increased or decreased activity of the polymorphism is substrate specific (Hu et al. 1997; Sundberg et al. 1998) and its effect on asthma can be age specific (Islam et al. 2009). Early onset asthma (by 6 years of age) appears to be associated with the *val*-allele; whereas late onset asthma (after 9) appears to be associated with *Ile*-allele. The inducibility of inducible *HMOX-1*, located in 22q12, is inversely associated with the length of a $(GT)_n$ tandem repeat in the 5′ flanking (promoter) region of the gene (Chen et al. 2002; Hirai et al. 2003; Yamada et al. 2000). The functional 609C>T polymorphism of *NQO1*(16q22.1) results in a serine variant that reduces the activity of the enzyme. Subjects with homozygous variant (Ser/Ser) has no detectable enzyme activity and the enzymatic activity among the heterozygous (Ser/Pro) is significantly lower to those with wild type homozygous (Pro/Pro) (Siegel et al. 1999). Paradoxically, epidemiologic studies observed that Ser allele renders protection against ozone. The observed effect of this polymorphism initially appears paradoxical as the Ser allele provides protection against asthma among children with *GSTM1*-null genotype (David et al. 2003). This is biologically plausible as beside its anti-oxidant activity, NQO1 also catalyzes the bioactivation of quinones to more reactive hydroquinones which are potent oxidants (Minelli et al. 2011). Because NQO1 can activate nitroaromatic compounds and heterocyclic amines present in diesel exhaust (Hajos and Winston 1991a, b; Nakagawa et al. 1983), Ser allele that reduces the enzymatic activity of NQO1 might be protective under certain conditions. Similar to oxidative stress, nitrosative stress mediated through NO can also play role in airway inflammation (Baraldi et al. 2006; Ricciardolo et al. 2006a, b). Genes encoding nitric oxide synthase (NOS) and arginases (ARG) are of particular interest. (Islam et al. 2010; Salam et al. 2009)

12.3.1.3 Gene, AP on Respiratory Health Outcomes

There has been extensive interest in the scientific community to identify genetic variants modifying the effect of air pollutants on health. As the field is quite young, there have been a limited number of studies providing evidence for gene-AP interaction. In 2011, Minelli et al. systemically reviewed 17 articles from 15 different studies that were published prior to April 30 2009 (Minelli et al. 2011). Of the 17 articles reviewed, five were published between 2001–2005 and the other 12 between 2006–2010. Romieu et al. also reviewed 13 articles on children gene-AP interaction that were published prior to April 2009 (Romieu et al. 2010), many of these studies were also in the review of Minelli et al. Following the same MEDLINE search strategy as Minelli et al., a total of 32 studies could be identified with five publications between 2001–2005, 18 between 2006–2010, and 9 between 2011–2013. This shows a rapid growth in this nascent field of gene-AP.

GSTs (*GSTM1, GSTP1 or GSTT1*) were investigated in 18 of those 32 studies. An array of APs were considered including indoor particulate matter to outdoor ozone, particulate matter and TRAP often based on modeled oxides of Nitrogen (Gauderman et al. 2009). Bergamaschi and colleagues (Bergamaschi et al. 2001) first reported on the possible modifying effect *GSTM1* of ozone on lung function. They performed a cross-over experimental study on 24 healthy non-smoker participants. The participants were randomly exposed to above and below 80 ppb ozone. Exposure to ozone level above 80 ppb was associated with decrease in lung function measures and increase in oxidative stress marked by high level of Clara cell (CC18) protein and decreased 8-Hydroxy-2′-deoxyguanosine a biomarker of ROS-DNA interaction. All those associations were much stronger in *GSTM1* and *NQO1 wild type.* Similar joint modifying effect of *GSTM1 null* and *NQO1 wild type* in modifying the effect of biomarker of oxidative stress (Corradi et al. 2002) and lung function (Chen et al. 2007) was substantiated by other studies (Corradi et al. 2002; Chen et al. 2007). Based on their study of young college students, Chen et al. (2007) reported a sex specific modifying effect of the anti-oxidant genes. The detrimental effect of chronic ozone exposure on lung function was modified by *GSTP1 val105* allele in males and the *GSTM1 null-NQO1* genotype in the male. It should be noted that 19 of the 24 participants in the study reported by Bergamaschi et al. (2001) were female. David et al. reported from their 218 case-triads (case + parents) a protective role of the variant *NQO1(Ser)* genotype against asthma among *GSTM1-null* carriers. Although, there was no formal test of interaction for any air pollutants, the authors argued that the observed effect is possibly due to the high background ozone level and particulate mass in Mexico city (David et al. 2003). Although, David et al. did not provide relevant pollution data with appropriate statistical testing to validate their claim; the overall hypothesis is supported by experimental findings of Gilliland et al. (2004). Gilliland and colleagues conducted a single blind placebo controlled cross over study exposing ragweed sensitive study participants in presence of clean air and diesel exhaust. The researchers observed increased level of nasal allergic responses following exposure to diesel exhaust with further amplification of the effects of diesel exhaust among carriers of *GSTM1-null* or *GSTP1-ile*

genotypes. Most pronounced effect was observed among subjects who were both *GSTM1-null* and *GSTP1-ile* genotype carriers. The finding certainly supports the claim by David et al. (2003) that the observed effect in children of highly polluted Mexico City might be due to the effect of ozone and traffic exposure. The group did a follow up study that sheds more light on the purported gene-environment interaction in childhood asthma. Among children with asthma, the researchers observed that recent exposure to high level of ozone was associated with increased breathing difficulties in children with *GSTP1-val* genotype and most pronounced among those with *GSTM1-null* and *GSTP1-val* genotype (Romieu et al. 2006). The group also conducted a clinical trial investigating whether anti-oxidant supplementation (Vit-C and Vit-E) can protect against ozone induced lung function decline. The detrimental effect of ozone was most pronounced among children with *GSTM1-null* genotype who most benefitted from the anti-oxidant supplementation (Romieu et al. 2004). This was confirmed in a recent follow up study (Moreno-Macias et al. 2013).

It is interesting to note that only a few studies reported that *GSTM1*-null alone modifying the effect of AP on respiratory outcomes (Romieu et al. 2004; Moreno-Macias et al. 2013; Alexis et al. 2009). Kim and colleagues (2011) conducted a double blinded experimental study to investigate the effect of ozone exposure on lung function and the possible modifying effect *GSTM1*. They selected 59 healthy adults 19–35 years of age without any history of smoking and performed a double blinded experiment to investigate the effect of ozone exposure on lung function (FEV1 and FVC). Spirometry and blood samples were taken after study participants were exposed to clean air (0.00 ppm ozone) or 0.06 ppm ozone randomly for a period of 6.6 h following moderate level of exercise. Compared to clean air, exposure to 0.06 ppm ozone for 6.6 h lead to decrease in both FEV1 and FVC, pulmonary inflammation marked by increase in polymorphonuclear neutrophils. No statistically significant modifying effect of *GSTM1* was observed for either lung function measures or pulmonary inflammation. Although, in the *GSTM1 null* showed a detrimental effect following ozone exposure compared to clean air, the detrimental effect was no different from that observed for *GSTM1 present* group. Also other studies involving children with asthma (Newcomb et al. 2012; Vagaggini et al. 2010) did not show any modifying effect of *GSTM1-null* on ozone induced airway responses. The modifying effect of *GSTM1* appears to be dependent on health status, exposure conditions, health outcome, and the overall anti-oxidant repository.

The experimental work by Gilliland and colleagues was further followed through utilizing the resources of Children Health Study, a large cohort (~6,000 starting in 1993 (Peters et al. 1999a, b) and another ~5,000 in 2003 (McConnell et al. 2006)) of school aged children (6–18 years of age). Given the large sample size, extensive genetic and exposure data, and longitudinal nature of the study we could investigate gene-AP interaction on both prevalent and incident asthma as well as lung function in this study. In 2002, McConnell et al., reported an important but controversial finding that participating in high level of sports activity (>2 team sports) in high ozone communities (24-h ozone > 30.7 ppb and 8-h ozone (10 am-6 pm) >55.8 ppb) was associated with increased incidence of asthma in children (50/1,000 person year) compared to low ozone communities (asthma incidence ~ 19–33/1,000 person

year) (McConnell et al. 2002). This was followed up to investigate whether the joint effect of participating in team sports and exposure to high level of ambient ozone is further modified by genetic susceptibility (Islam et al. 2009). In this study, both *GSTM1-null* and *GSTP1-Ile* genotype was associated with increased risk of incident asthma; however, the modifying effect was observed only for *GSTP1-Ile* genotype. The increased risk of incident asthma for high outdoor sports mostly restricted to those who were *GSTP1*-Ile *homozygous* (p-value of interaction = 0.02). The carriers of *GSTP1 Ile/Ile* who lived in high ozone communities and were involved in high sports activity were almost sixfold more likely to develop asthma compared to any other groups (Fig. 12.5). As we discussed earlier, the direction of effect for *GSTP-1* Ile105Val might be age and exposure dependent. In a cross sectional study from Taiwan, *GSTP-1* Ile allele appeared to provide protection against asthma and wheeze following exposure to $PM_{2.5}$ and ozone (Hwang et al. 2013). In this study, a strong correlation between $PM_{2.5}$ and ozone ($r = 0.73$) was observed and the interaction appeared to be stronger for $PM_{2.5}$ compared to ozone. Therefore, the observed effect might be due to $PM_{2.5}$, rather than ozone. In a birth cohort study, allergic sensitization in the first four years of life following exposure to modeled NO_x (surrogate measure of TRAP) was most apparent among carriers of *GSTP1-val* allele (Melen et al. 2008). Other studies involving TRAP exposure also noted *GSTP1-val* allele as the risk allele (Salam et al. 2007; Tung et al. 2011).

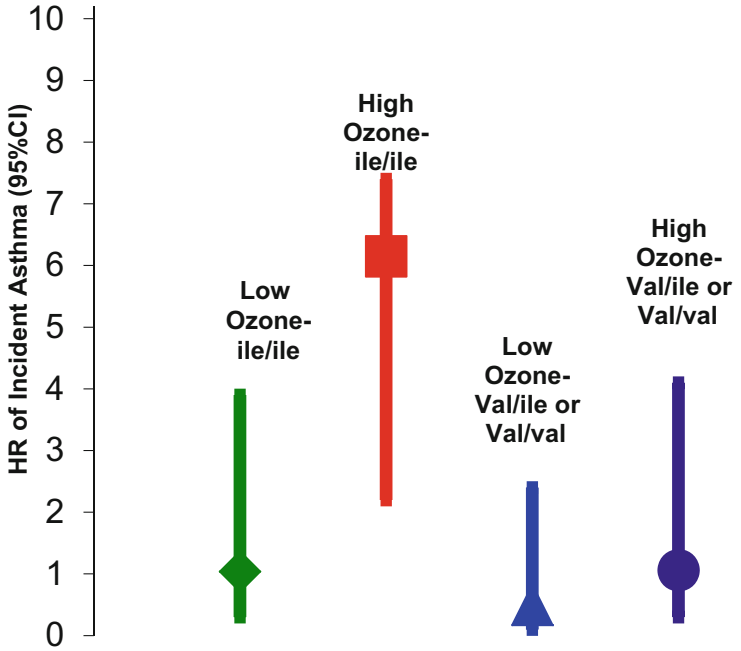

Fig. 12.5 Effect of ozone and GSTP1-Ile allele on asthma incidence among children participating in >2 team sports

Another anti-oxidant polymorphism that has shown some consistent effect have been the (GT)n tandem repeat of *HMOX1*. In the Children Health Study, an ethnicity specific effect of the tandem repeat was observed (Islam et al. 2008). Based on functionality, the *HMOX1* with alleles 23 or less $(GT)_n$ repeats were defined as 'Short' (S) in the study. Non-Hispanic Whites (40 %) were more likely to have one *S-allele*, compared to Hispanic Whites (28 %). Although, no association was observed between the *S-allele* and asthma, *S-allele* appeared to be protective against new onset asthma in non-Hispanic children (Hazard ratio (HR): 0.64, 95 % confidence interval (CI):0.41–0.99) but not among Hispanic Whites (HR: 1.25, 95%CI: 0.64–2.47). Among the non-Hispanic Whites, the *S-allele* appears to modify the effect of ozone on asthma risk (interaction p-value = 0.003). The *S-allele* appears to be more protective in the low-ozone communities (HR = 0.44) compared to high-ozone communities (HR: 0.88). Although, PM_{10} exposure appeared to be associated with increased risk of asthma in children without any *S-allele* (HR: 1.79, 95%CI: 0.69–4.61) compared to those with *S-allele* (HR: 0.62, 95%CI: 0.20–1.87); no statistically significant interaction was observed (p-value of interaction: 0.18). A large cohort study (N = 4,365) of Swiss Caucasian adults (18–65 years of age) (the SAPALDIA study) reported that the beneficial effect of PM_{10} reduction on lung function was modified by *HMOX1* polymorphisms including the $(GT)_n$ repeat (Curjuric et al. 2010). Each 10 μgm–m^3 decrease of PM_{10} level was associated with a 26.5 (11.7–41.2) mL-sec^{-1}-$year^{-1}$ improvement of $FEF_{25–75}$ values compared to 11.7 (95%CI: 4.5–19.2) mL-sec^{-1}-$year^{-1}$ among those without any long allele. Although the definition of short and long allele differed between the CHS and SAPALDIA study, both noted the presence of shorter allele in each population to be protective against the detrimental effect of air pollutants (ozone and PM_{10}).

Although, GSTT1 (Hong et al. 2007; Castro-Giner et al. 2009), CAT (Islam et al. 2008; Wenten et al. 2009), and *SOD* (superoxide dismutase) (Islam et al. 2008; Yang et al. 2005) have been studied in the context of gene-environmental interaction for respiratory health outcomes, currently limited evidence exists for such modifying effect. In the CHS cohort, a statistically significant three-way interaction was observed between a haplotype (based on 8 tag-SNPs) of *ARG1*, atopy, and ozone exposure for asthma risk (Salam et al. 2009). Presence of each copy of the *ARG1* haplotype was associated with 0.55 fold reduction in asthma risk among children; however the protective effect was much larger among children with atopy and living in high ozone communities (Odds Ratio (OR):0.12, p-value of interaction = 0.008).

12.3.2 Inflammatory Genes and AP

Exposure to air pollutants leads to airway inflammation either indirectly due to oxidative stress or directly due to induction of inflammation that plays an important role in air pollutant induced detrimental respiratory health effects (Kelly 2003; Tubby et al. 2011). Air pollutants like ozone, particulate matter or oxides of nitrogen cause activation of transcription factors such as nuclear factor – κB (NF-κB),

resulting in increased expression of different cytokines, chemokines, and adhesion molecules that can lead to a proinflammatory state. Both innate and adaptive immune responses within the lung contribute to the inflammatory process (Tubby et al. 2011). Therefore, genes of the inflammatory pathway have often been considered as potential modifier of AP effect on respiratory health. Animal studies suggest that tumor necrosis factor α (TNF-α) and toll like receptor (TLR4) are possible susceptible region to ozone effect (Cho and Kleeberger 2007; Hollingsworth et al. 2004; Kleeberger et al. 1997).

A functional polymorphism of TNF-α, *TNF-α G308A* (rs1800629), where the variant allele can lead to higher level of TNF-α: a proinflammatory cytokine, has been reported to modify the effect of air pollutants (Melen et al. 2008; Yang et al. 2005; Li et al. 2006; Lee et al. 2009). Yang et al. reported that there is a decline in FEV_1 following acute exposure to varying level of ozone (400 ppb for 2 h to 200 ppb for 24 h) during moderate exercise, and the decline differed by *TNFαG308A* status (Yang et al. 2005). The percentage decline following the ozone exposure was about 3 % among those with at least one *A*-allele; whereas the decline was almost 9 % among *G/G* individuals. On the contrary, in the CHS the *G*-allele was identified as a protective factor for asthma and wheezing in children (Li et al. 2006). The protective effect of the *G*-allele was most pronounced among children living in the low ozone communities. The GG homozygous children were 50 % less likely to ever suffer from wheezing compared to GA or AA carriers in the low ozone communities; however, there was no difference in risk of wheezing based on *TNFG308A* status in the high ozone communities. Similar protective effect of the *GG* allele for bronchitic symptoms in children with asthma living in low zone communities but not in high ozone communities have been reported from Taiwan (Lee et al. 2009). Melena et al. reported that children with *GSTP1-val* allele had almost 2.5 fold increased risk of sensitization when exposed to elevated level of NOx; whereas, no heighted risk of sensitization was reported for *GSTP1-Ile* homozygous (Melen et al. 2008). This GSTP1-NOx interaction was mostly limited to those with *TNF-308 A* allele (OR = 22), with no substantial effect among the *GG* homozygous. Except for the study reported by Yang et al., which was unique being an experimental study in adults, a consistent protective effect of *TNF-308 GG* homozygous against air pollutant mediated health effect is observed.

Toll like receptors are important gatekeepers of host immunity in response to gram-positive (TLR2) and gram-negative (TLR4) bacteria. Therefore they can play important role in AP mediated airway inflammation as pathogens can get access to lower airway with inhaled particulate matter. Kerkhof and colleagues (2010) investigated whether *TLR2* and *TLR4* modifies the susceptibility to AP mediated asthma in the well established birth cohort of Prevention and Incidence of Asthma and Mite Allergy (PIAMA). They identified multiple polymorphisms of *TLR2* and *TLR4* to increase the susceptibility of prevalent asthma following exposure to $PM_{2.5}$ and soot.

12.3.3 Summary

The respiratory tract being the first system to deal with the onslaught of air pollutants, gene-AP studies have mostly focused on respiratory diseases. In the process multiple genes such as *GSTM1, GSTP1, HMOX1, NQO1, TNF-α* and *TLRs(TLR2 & TLR4)* have been identified to modify individual susceptibility to air pollutants in respect to airway diseases. The observations from the epidemiological studies often fall short to provide conclusive evidence, owing to methodological and biological issues discussed later in this chapter. The genetic factors discussed in the context of AP and respiratory health also plays a role in other health outcomes because the systemic effect of air pollutants often starts from lung parenchyma (Brook et al. 2009, 2010).

12.4 Gene, AP and Cardiovascular Health

Over time epidemiological studies have shown the impact of AP on cardiovascular health as has been discussed in Chaps. 8 and 9. Acknowledging the importance of AP, specially particulate matter in cardiovascular morbidity and mortality, the American Heart Society first issued scientific statement underlining the importance of a better understanding of the role of particulate matter in cardiovascular disease (CVD) (Brook et al. 2010). Despite the accumulating evidence of AP mediated cardiovascular health effects; the biological mechanism is not well understood. The possible biological pathway for AP mediated health effects include (i) systemic inflammation following oxidative stress and inflammation in the lungs (Systemic spillover effect), (ii) imbalance of the automatic nervous system (ANS) following activation of respiratory ANS reflex arc and (iii) direct effect of the ultra-fine particles (UFP) that can pass from through the alveolar membrane and reach blood from alveolar air (Brook et al. 2009, 2010).

12.4.1 Gene, AP, and CVD: Human Studies

Unlike respiratory health outcomes, the gene-AP studies in CVD are extremely limited. In a review by Zanobetti et al. (2011) reviewed 16 publications in the context of gene-AP interaction in CVD, were from three cohort studies. Thirteen were from the Normative Aging Study (NAS), two from AIRGENE, and one from MESA study. Only the MESA study had racial/ethnic diversity with NAS and AIRGENE being totally Caucasian population. Sex representation were also highly skewed in those studies, with 100 % of NAS participants being male, 80 % of AIREGENE and about 48 % of MESA. Average age range in these studies was 40–85. Most of the studies investigated the effect of short term pollutant exposure rather than chronic AP exposure (Van Hee et al. 2010). The health endpoints also varied widely in these

studies, ranging from biomarkers such as plasma homocysteine (Ren et al. 2010a) or heart rate variability (HRV) (Ren et al. 2010b; Park et al. 2006) to disease endpoints such as blood pressure (Wilker et al. 2009) and left ventricular mass (Van Hee et al. 2010). Homocysteine concentrations at high levels are an independent risk factor for cardiovascular disease. Elevated levels in the blood may be associated with atherosclerosis, stroke, blood clot and heart attacks. Exposure to air pollutants are associated with decreased heart rate variability and related markers of oxidative stress may play an important role in cardiac toxicity of particles.

The oxidative stress genes had been the early focus of gene-AP studies in CVD. In 2005, Schwartz and colleagues reported an increased susceptibility to $PM_{2.5}$ mediated decreased variability in HRV among *GSTM1-null* individuals with no apparent effect of $PM_{2.5}$ on HRV among *GSTM1-positive* individuals (Schwartz et al. 2005). Each 10μgm-m^{-1} increase in $PM_{2.5}$ was associated with 34 % (95%CI: −9 to −52 %) decrease in HRV among *GSTM1-null* carriers; whereas, no effect was observed among *GSTM1-positive* individuals. The detrimental effect of $PM_{2.5}$ was even more prominent among *GSTM1-null* individuals who did not use statin or had high level of neutrophil or were obese. This initial provocative finding was followed up by multiple investigations in the NAS exploring the role of oxidative-stress genes as potential modifiers for AP mediated CV outcomes (Ren et al. 2010a; Park et al. 2006, 2009; Baja et al. 2010; Mordukhovich et al. 2009; Chahine et al. 2007). Chahine et al. (2007) investigated the role of the *HMOX1* promoter region (GT)n repeat in addition to *GSTM1*, modifying the effect of $PM_{2.5}$ on HRV. They observed no statistically significant interaction for *GSTM1* (interaction p-value: 0.13, although HRV decrease in response to $PM_{2.5}$ was significant only in the *null* group) and the interaction p-value for *HMOX1* was 0.06. The largest detrimental effect of $PM_{2.5}$ was observed among the *GSTM1-null* individuals with long(GT)n repeats ($\geq$25) (p-value of interaction $<$ 0.04). This modifying effect of *HMOX1* was supported by two other studies from the same group in the context of tibia lead level and QT interval (Park et al. 2009) and $PM_{2.5}$ and plasma level of homocystiene (Ren et al. 2010a); however, not in the context of exposure to black carbon (BC)/$PM_{2.5}$ and blood pressure (Mordukhovich et al. 2009). In the elderly male population of NAS, 1-standard deviation increase in 7-day moving average of BC from the time of clinical examination was associated with 1.46 mmHg increase in systolic blood pressure (SBP) and 0.87 mmHg increase in diastolic blood pressure (DBP); however, this detrimental effect of BC was not modified by any of the tested polymorphisms of the anti-oxidant genes: namely, *GSTM1-null*, *GSTT1-null*, *GSTP(Ile105Val)*, *HMOX1 tandem repeat, NQO1(C609T)* and three SNPs of *CAT* (Mordukhovich et al. 2009). In another study from the NAS, the effect of ambient $PM_{2.5}$ on changes in the postural blood pressure was modified by genetic variants of genes (PHD finger protein 11 (PHF11), matrix metalloprotease 1 (MMP1) and inositol 1,4,5-triphosphate receptor, type 2 (ITPR2) in the renin-angiotensin pathway (Wilker et al. 2009).

The AIRGENE study recruited 1,031 survivors of myocardial infarctions (MI) to investigate the role of particulate exposure on inflammation in this vulnerable population. The researchers were interested in fibrinogen and IL-6 as fibrinogen is

an essential component in cardiovascular related inflammation and coagulation and established risk factor of CVD (Danesh et al. 2005) and IL-6 is key regulator of fibrinogen (Fuller and Zhang 2001). Among these survivors of MI, different measures of TRAP related pollutants were associated with elevated plasma level of IL-6 and fibrinogen, underlining the acute effect of particulate matter in MI related inflammation (Ruckerl et al. 2007; Peters et al. 2009; Ljungman et al. 2009). This effect of the TRAP on plasma IL-6 and fibrinogen level was modified by multiple genetic variants of fibrinogen beta-chain gene (FBG). In a sub-cohort of 2,880 of the MESA study, researchers investigated the possible modifying effect of genetic variants of 12 different genes on the detrimental effect of TRAP on left ventricular mass, a strong marker of adverse cardiovascular outcomes (Bluemke et al. 2008). Of the 12 different genes investigated, genetic variants of type 1 angiotensin II receptor (*AGTR1*) gene and 15-lipoxygenase gene (*ALOX15*), modified the effect of residential proximity, a marker of TRAP, on LVM (Van Hee et al. 2010). Those genes are involved in vascular inflammation, oxidative stress, and vasoconstriction.

12.4.2 Summary

One of the major concerns regarding the current state of evidence for gene-AP interactions for CVD is lack of replication of findings across studies. The lack of diversity and the different endpoints and exposures of interest in the studies are certainly a major limitation in the replication of findings across studies. While NAS and AIRGENE were optimized to identify acute (hours) to sub-chronic (days) effect of air pollutants, MESA is best positioned to investigate chronic exposure (i.e., residential distance from major roads). The endpoints investigated also differed considerably between the studies, ranging from HRV, blood pressure, QT intervals and markers of inflammation. Although, these outcomes are related to each other on a continuum, the genes and pathways involved can differ considerably thus making it difficult to have replication across these studies. There is also considerable variation between study population make replication of findings quite difficult between those studies.

Despite those limitations, both the NAS and the AIRGENE study provided evidence that genetic variants of genes in the rennin-angiotensin pathway may modify the effect of air pollutant on CV outcomes. All these studies investigated the short term effect of air pollutant on CV health outcomes. The observations of a consistent effect of *GSTM1(null)* and *HMOX1 (GT)n* repeat in modifying the effect of different air pollutants on multiple CV health outcomes within NAS provides confidence to the observed effect, specially as the findings were in line with the reported respiratory effects (Sect. 12.3.1). Further studies investigating gene-AP for CV outcomes utilizing both candidate gene approach and GWIS are required to reach a definitive conclusion.

12.5 Gene, AP and Non Cardio-respiratory Health Outcomes

Understanding the role of AP in the causation of health effects beyond cardio-respiratory health outcomes is relatively new and there are only a limited number of studies investigating gene-AP interaction on such health outcomes. One major interest has been the role of genes and AP in pregnancy outcomes.

There is substantial body of evidence noting the importance of AP specially ozone, TRAP, or CO in adverse pregnancy outcomes, such as low birth weight (LBW), preterm birth or intrauterine growth retardation (IUGR) (Backes et al. 2013; Stieb et al. 2012; Misra et al. 2012; Shah and Balkhair 2011; Bonzini et al. 2010). Because oxidative stress as well as reduced oxygen supply to the fetus are the presumed mechanism for AP mediated adverse pregnancy health outcomes, the aforementioned sets of genes (Sect. 12.3.1.2) have been investigated as the usual suspects for gene-AP interaction. In 2008, Suh et al. (2008) reported on the possible modifying effect of three xenobiotic genes, *GSTM1 (null), GSTT1(null),* and cytochrome P450IA1 (CYP1A1) on PM_{10} mediated preterm delivery (before 37th week). The researchers recruited 117 women with preterm delivery and living in Seoul during 2003–2007 and matched them randomly with 118 women who had full term delivery and were living in Seoul. All women were recruited from the same hospital. An increased risk for preterm delivery was observed for women with *GSTM1(null)* genotype compared to positives (OR > 2.0, p-value < 0.008) and women who were exposed to high level of ambient PM_{10} (≥75th percentile) during the 3rd trimester compared to those with lower level of exposure (OR > 2.3, p-value 0.02). The effect of PM_{10} on preterm birth appeared to be modified by maternal *GSTM1* status. The risk of preterm delivery was almost 6 fold greater (OR: 6.22, p-value = 0.001) among women who were *GSTM1(null)* and were exposed to high level of PM_{10} compared to those who were *GSTM1* positives and were exposed to low level of PM_{10} during the last trimester; however, no overall p-value for test of interaction was not available. Although the data has been adjusted for a number of confounders, it was not adjusted for maternal respiratory infection or asthma/wheeze status. It is possible that *GSTM1(null)* women exposed to high level of pollutant experienced more respiratory problems leading to oxygen deficiency to both the mother and the fetus resulting in preterm birth. In 2011, Rossner and colleagues (2011) also reported possible gene-AP interaction on pregnancy outcomes. Data on 1200 women were randomly selected from a large case control study, but, genetic (placental), AP and covariate information was available only for 891 women. Unlike the South Korean study, the researchers investigated the role of placental genes in modifying the effect of $PM_{2.5}$ and PAH on IUGR and LBW in this study. Besides *GSTM1 a*nd *GSTT1*, they also investigated the possible role of 94 (577 SNPs) other genes using an *Illumina* array. They failed to observe any statistically significant interaction between AP and *GSTM1* or other genetic polymorphism in this study. The identification of gene-AP interaction for pregnancy outcomes are specially

challenging as both maternal, placental and fetal physiology and genetics can play important role as well as the time and type of exposure. Therefore, well designed studies are needed.

12.6 AP and Epigenetics

Epigenetics is the study of mitotically or meiotically heritable states of gene expression potential that occur without directly altering the DNA sequence. DNA methylation, histone modifications and miroRNAs are major types of epigenetic variations that are currently being investigated in relation to AP and health outcomes. The epigenetic process is schematically presented in Fig. 12.6, and briefly described in the following paragraphs. (for further reading: Yang and Schwartz (2012)).

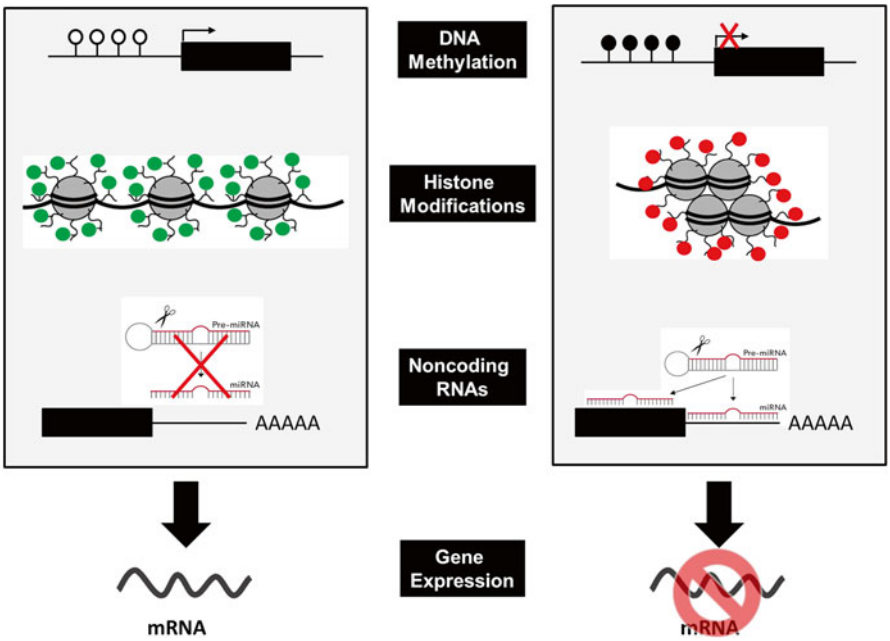

Fig. 12.6 Effect of epigenetic marks (DNA methylation, histone modifications, and miRNAs) on gene expression. *White circles* denote unmethylated CpGs, and *black circles* denote methylated CpGs. *Green circles* refer to permissive histone modifications, and *red circles* indicate repressive histone marks. miRNAs can affect gene expression through either RNA degradation (perfect complementarity and binding) or inhibition of protein translation (imperfect complementarity and partial binding) (Reprinted from Yang and Schwartz (2012), Copyright (2012), with permission from Elsevier)

DNA methylation refers to the covalent addition of a methyl group to cytosine nucleotides (5-methylcytosine or 5mC) adjacent to guanine residues in the DNA sequence – so-called CpG sites (Irizarry et al. 2009). Methylation leads to 'switching off' of the genes and thus inhibiting expression of the involved genes

Histone modifications refers to the epigenetic changes that controls the accessibility of a particular gene during transcription. In cells, the DNA is tightly packaged and ordered in nucleosomes by histone proteins (H2A, H2B, H3 and H4) to form chromatin structure. Posttranslational histone modifications such as acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and ADP-ribosylation on the tails of core histones are important epigenetic modifications for gene transcription. For example, acetylation of histone tails often increases the accessibility of binding sites to transcription factors and thus the activation of gene expression. The status of histone acetylation is reversibly regulated by two distinct enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC). Increased histone acetylation by HATs leads to the unwinding of chromatin structure and transcriptional activation, whereas removal of acetyl groups by HDACs causes chromatin condensation and transcriptional silencing.

MicroRNAs (miRNAs) are small (~22 nucleotides), non-coding RNAs that negatively regulate gene expression at posttranscriptional level by RNA degradation or inhibition of protein translation (Fig. 12.6). The miRNAs are generated from much longer primary miRNA by a multi-step process which is regulated by RNase III endonuclease (Drosha and Dicer). One miRNA sequence can regulate the expression of multiple genes in a coordinated fashion.

There is a growing body of evidence that exposure to air pollutants such as particulate matter or diesel exhaust can lead to epigenetic changes that can have potential detrimental health effects (Sonkoly and Pivarcsi 2011; Jardim 2011; Jardim et al. 2012). A pubmed search for articles on AP (or particulate matter, ozone and traffic exposure) and epigenetics identified 50 articles (2013, September) with 27 of them being review articles. Most of the original research articles are focused on cardio-respiratory health outcomes.

12.6.1 AP and Methylation

There is a growing body of evidence that exposure to AP results in epigenetic changes in human (Jardim 2011; Jardim et al. 2012; Bellavia et al. 2013; Hou et al. 2012; Bind et al. 2012; Carugno et al. 2012; Cantone et al. 2011; Baccarelli and Bollati 2009; Bollati et al. 2007). Multiple studies have noted that exposure to AP, specially **particulate matter or diesel exposure** can lead to ***methylation*** changes that can have potential health effects. These findings have generated great interest in identifying epigenetic changes that can modify or mediate AP effect on health outcomes. A study by Bollati et al. demonstrated that exposure to low level of benzene among gas station attendants and traffic police in Italy were associated with both genome-wide and gene-specific changes in methylation (Bollati et al. 2007). In controlled experiment, short term exposure to particulate matter was associated

with both genome-wide (Alu) and gene-specific (TLR4) hypomethylation (Bellavia et al. 2013). Recent findings in the Children Health study (Breton et al. 2012; Salam et al. 2012) also demonstrates that exposure to particulate matter can result in changes in methylation in the inducible nitric oxide synthase promoter region that can lead to health effects (Breton et al. 2011). These findings provide the impetus to investigate the effect of AP on epigenetic changes that can explain the observed health effects of AP.

Perera and colleagues reported that maternal exposure to polyaromatic hydrocarbon (PAH; a constituent found in diet, tobacco smoke and traffic-related pollution) was associated with DNA methylation level in a CpG site in *ACSL3* in umbilical cord blood white cells (Perera et al. 2009) and that DNA methylation level was associated with increased asthma risk in children (odds ratio [OR] = 3.9; 95 % confidence interval (CI): 1.14–14.3).

A recent study involving 141 participants of NAS observed that ambient SO_2 and black carbon level was associated with methylation pattern in the asthma pathway suggesting possible mediative role of methylation in AP related respiratory effect (Sofer et al. 2013).

Another study investigated the possible differential pattern of global methylation in placenta due to $PM_{2.5}$ exposure during pregnancy (Janssen et al. 2013). The investigators reported that $PM_{2.5}$ was associated with global hypomethylation of the placental tissue. Each 5μgm/m^3 increase in $PM_{2.5}$ was associated with 2.13 % (95%CI: −3.71, −0.54) decrease in placental global methylation. Strongest association was observed for 1st trimester especially during the period of implantation. This finding suggests that the observed effects of AP on pregnancy outcomes can be mediated through epigenetic changes of the placental tissue. The major limitations in the interpretation of the study finding is due to (i) unavailability of gene-specific methylation, & (ii) lack of cell specificity. Further studies are required to identify the genes that undergo epigenetic changes and specify the cells where those changes are most marked.

12.6.2 AP, Histone Modification and miRNA

Currently there is very limited data linking AP to histone modification or microRNA and health outcomes. Most of the evidence is indirect and based on *in vitro* studies. Two different i*n vitro* studies have noted increased gene expression of *IL8* and *COX2* genes leading to increased inflammatory activity in human airway epithelial cells exposed to DEP or PM due to increased H4-acetylation (Gilmour et al. 2003; Cao et al. 2007). The few studies investigated the effect of AP on microRNA expression profile have also indicated the involvement of oxidative stress and inflammatory pathway. Jardim et al. investigated the effect of diesel exposure microRNA expression in human bronchial epithelial cells (HBEC) (Jardim et al. 2009). Following exposure to diesel exhaust particle, 1.5 fold change in expression was observed for 63 % (197 of 313) of the detectable microRNAs in the HBECs. Based on bioinformatics analysis, the microRNAs with highest level of changes

were identified to be mostly involved in the regulation of inflammatory and tumorigenic pathways. Inhalation of DEP has also been shown to result in changes in microRNA expression in peripheral blood (Yamamoto et al. 2013). Yamamoto and colleagues investigated the effect of DEP exposure on the microRNA expression in the peripheral blood of 13 participants with asthma, after exposing them to filtered air with placebo (FAP), diesel exhaust (300 μg/l $PM_{2.5}$) with placebo (DEP) and diesel exhaust with anti-oxidant supplementation (DEN) in a double blinded cross-over experiment. The researchers initially identified differential expression of miR-21, miR30e, miR215 and miR-144 in the peripheral blood following DEP exposure and validated increased expression of miR-144 through RT-qPCR. The increased expression of miR-144 was associated with decreased activity of NRF2 and other downstream anti-oxidant genes (NQO1 and GCLC) and increase in oxidative stress measured by 8-hydroxy 2′-deoxyguanosine inn plasma. This finding further provides evidence that exposure to diesel exhaust can result in differential microRNA expression may regulate genes of the oxidative pathway. The different sets of microRNAs identified in HBEC (Jardim et al. 2009) and peripheral blood (Yamamoto et al. 2013) is expected given tissue specificity of microRNA. Although miR-21 was only marginally (p-value = 0.06, one-sided test) associated with DEP exposure in this study, a body of evidence suggests it could be associated with inflammation, oxidative stress, atherosclerosis, carcinogenesis, asthma and allergic pathway and toll-receptor pathway (Yamamoto et al. 2013). mir21 and two other micro RNA (miR222 and miR-146a) was also found to be differentially exposed following three days exposure to particulate matter at workplace among foundry workers (Bollati et al. 2010). Another line of evidence for the possible role of microRNA in AP mediated health effect comes from the NAS population, where genes involved in microRNA processing (DICER, Gem-associated Protein 4(*GEMIN4*), & Diegeorge syndrome critical region 8 (*DGCR8*)) was found to modify the effect of exposure to black carbon on blood pressure (Wilker et al. 2010) and one of the SNPs of *GEMIN4*, rs1062923, also modified the effect of particulate matter on the level of soluble intracellular and vascular adhesion molecules (sICAM & sVCAM, respectively), that are markers of inflammation and endothelial function (Wilker et al. 2011). However, the observed effect modification of the rs1062923 was paradoxical. Exposure to BC was associated with increase in diastolic blood pressure among homozygous carriers whereas; $PM_{2.5}$ was associated with lower level of sICAM and sVCAM, compared to homozygous variant and heterozygous. Further studies are required to provide credence to the findings and address any apparent inconsistencies.

12.7 Challenges and Limitations

Given that most of the diseases are considered to be a complex interplay between individual susceptibility and environmental exposure, the role of gene-environmental interaction in the causation or exacerbation of disease has gained momentum in the

last decade. Genetics certainly plays an important role in defining individual susceptibility to both disease and effect of the environmental exposure. This has ushered in new wave of research in AP and health studies, investigating the joint and interactive effect of AP and genetics on different health outcomes. Given the scientific importance of, and interest in genetics in AP related health effects, this is a burgeoning field that is currently at its infancy facing many challenges that needs to be addressed in future.

As discussed earlier, currently only a **limited number of studies** are available investigating the interplay between AP-Gene in health outcomes and majority of these studies are focused on cardio-respiratory health. Furthermore, most of the findings are based on couple of studies (i.e., CHS, NAS, SAPALDIA and others) thus limiting the external validity and generalizability of the findings. **Lack of replication** of the findings has been one major concern. The published evidence of lack of replication can be considered as the tip of iceberg given the publication bias against ***null*** findings. Reviewing the studies that have reported gene-AP interactions, it is evident that many of the studies had similar gene, environment and outcome information to replicate findings from other studies; however, only few such replications are reported. One of the major challenges in replicating the gene-AP interaction studies is the availability of similar exposure matrix given the intrinsic and extrinsic **heterogeneity in exposure assessment**. Population from different region, age group, and birth cohort can have substantially different level of exposure to the same pollutant; therefore, it can be quite challenging in replicating GxE findings. Furthermore, the exposure assessment of the same pollutant was carried out differently in these studies, i.e. using residential distance to major roads, traffic density, or NOx as a measure of '***traffic exposure***', making replication challenging. Sample size is another major concern in investigating GxAP models (Fig. 12.7). It is evident that to observe modest GxE effect (OR ~ 1.2) quite a substantial sample size (N > 5,000) is required unless the minor allele frequency is high (MAF > 0.4) or there is substantial variability in exposure. If the standard deviation of the outcome variable is large, the required sample size will also increase substantially. The sample size calculation in the table considered that the outcome variable is continuously distributed with the standard deviation being 1/10th of the mean; however, if it is even 1/5th of the mean, then the required sample size will almost double. This illustrates the challenges faced in identifying GxAP health effects majority of these studies. Moreover, if we are to consider possible multiple GxG interactions in GxAP models the required sample size can increase substantially. This sample size estimations are based on hypothesis based GxAP models; however, for agnostic approach of GWIS will increase substantially. Currently there is also scarcity of studies that fully utilizes **different genomic approaches in a single study**, i.e. investigating the GxAP effect including possible epigenetic changes and gene expression data to clearly elucidate the biological pathway involved in GxAP health effects. The availability of tissue specific epigenetic and gene expression data is specially challenging in large epidemiological studies. To fill the paucity in data for demonstration of Gene-AP interactions observed in epidemiological studies warrant additional research including using non-invasive approaches such as buccal and blood samples for biomarker identification.

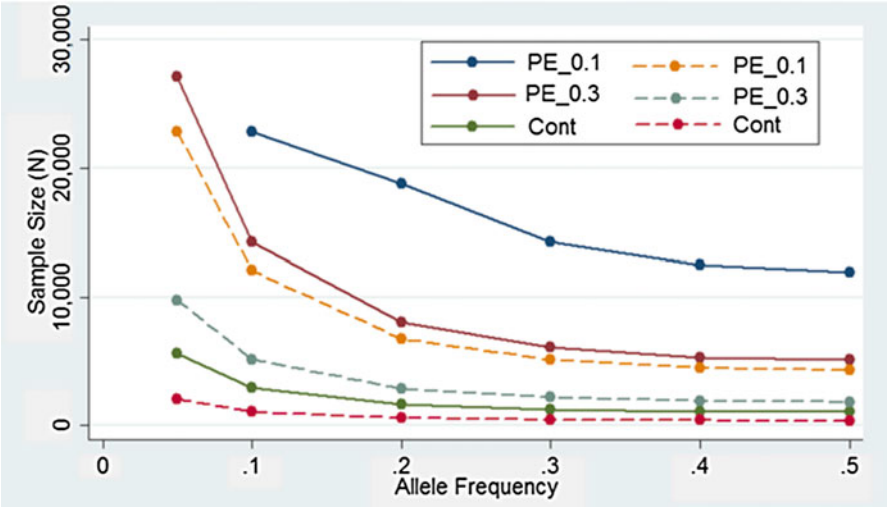

Fig. 12.7 Sample size calculations are based on using individual subject study design for additive effects of genes, with gene and AP marginal effect being 1.5 for continuous outcome with mean = 100 and std = 10. For dichotomous exposure, exposure prevalence was 10 % (PE_0.1) and 30 % (PE_0.3). Type I and II errors were 0.05 (2-df) and 0.20, respectively. *Solid* and *Dash lines* represent GxE interaction OR = 1.2 and 2, respectively for dichotomous exposure and interaction $\beta = 1.2$ and 2, respectively for continuous variable

12.8 Summary

The availability of new technologies and tools paves the way for innovative GxAP studies in future that may identify the underlying biological pathway in disease causation or exacerbation. Gene-AP studies in addition to identifying susceptible populations will also guide developing preventive and intervention strategies. With all the limitations discussed above, we are already witnessing the intricate interplay between AP and genomics in wide range of health outcomes starting from cardio-respiratory health outcomes to neurological conditions and pregnancy outcomes. These initial findings should motivate the development of larger new studies or consortiums among studies that collect consistent exposure and outcome data.

References

Adcock IM, Lee KY (2006) Abnormal histone acetylase and deacetylase expression and function in lung inflammation. Inflamm Res 55(8):311–321. doi:10.1007/s00011-006-0081-1

Adcock IM, Ito K, Barnes PJ (2005) Histone deacetylation: an important mechanism in inflammatory lung diseases. COPD 2(4):445–455

Alexis NE, Zhou H, Lay JC, Harris B, Hernandez ML, Lu TS et al (2009) The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 124(6):1222–1228.e5. doi:10.1016/j.jaci.2009.07.036, S0091-6749(09)01145-2 [pii]

Baccarelli A, Bollati V (2009) Epigenetics and environmental chemicals. Curr Opin Pediatr 21(2):243–251

Backes CH, Nelin T, Gorr MW, Wold LE (2013) Early life exposure to air pollution: how bad is it? Toxicol Lett 216(1):47–53. doi:10.1016/j.toxlet.2012.11.007, S0378-4274(12)01380-X [pii]

Baja ES, Schwartz JD, Wellenius GA, Coull BA, Zanobetti A, Vokonas PS et al (2010) Traffic-related air pollution and QT interval: modification by diabetes, obesity, and oxidative stress gene polymorphisms in the normative aging study. Environ Health Perspect 118(6):840–846. doi:10.1289/ehp.0901396

Baraldi E, Giordano G, Pasquale MF, Carraro S, Mardegan A, Bonetto G et al (2006) 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. Allergy 61(1):90–96. doi:10.1111/j.1398-9995.2006.00996.x, ALL996 [pii]

Bellavia A, Urch B, Speck M, Brook RD, Scott JA, Albetti B et al (2013) DNA hypomethylation, ambient particulate matter, and increased blood pressure: findings from controlled human exposure experiments. J Am Heart Assoc 2(3):e000212. doi:10.1161/JAHA.113.000212, jah3232 [pii]

Bergamaschi E, De Palma G, Mozzoni P, Vanni S, Vettori MV, Broeckaert F et al (2001) Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. Am J Respir Crit Care Med 163(6):1426–1431. doi:10.1164/ajrccm.163.6.2006056

Bind MA, Baccarelli A, Zanobetti A, Tarantini L, Suh H, Vokonas P et al (2012) Air pollution and markers of coagulation, inflammation, and endothelial function: associations and epigene-environment interactions in an elderly cohort. Epidemiology 23(2):332–340. doi:10.1097/EDE.0b013e31824523f0

Bluemke DA, Kronmal RA, Lima JA, Liu K, Olson J, Burke GL et al (2008) The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study. J Am Coll Cardiol 52(25):2148–2155. doi:10.1016/j.jacc.2008.09.014, S0735-1097(08)03231-2 [pii]

Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D et al (2007) Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res 67(3):876–880. doi:10.1158/0008-5472.CAN-06-2995, 67/3/876 [pii]

Bollati V, Marinelli B, Apostoli P, Bonzini M, Nordio F, Hoxha M et al (2010) Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes. Environ Health Perspect 118(6):763–768. doi:10.1289/ehp.0901300

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of quinones in toxicology. Chem Res Toxicol 13(3):135–160. doi:tx9902082 [pii]

Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC (2010) Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. Med Lav 101(5):341–363

Breton CV, Byun HM, Wang X, Salam MT, Siegmund K, Gilliland FD (2011) DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. Am J Respir Crit Care Med 184(2):191–197. doi:10.1164/rccm.201012-2029OC, 201012-2029OC [pii]

Breton CV, Salam MT, Wang X, Byun HM, Siegmund KD, Gilliland FD (2012) Particulate matter, DNA methylation in nitric oxide synthase, and childhood respiratory disease. Environ Health Perspect 120(9):1320–1326. doi:10.1289/ehp.1104439

Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G et al (2009) Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. Hypertension 54(3):659–667. doi:10.1161/HYPERTENSIONAHA.109.130237, HYPERTENSIONAHA.109.130237 [pii]

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV et al (2010) Particulate matter Air pollution and cardiovascular disease. An update to the Scientific Statement from the American Heart Association. Circulation. doi:10.1161/CIR.0b013e3181dbece1

Cantone L, Nordio F, Hou L, Apostoli P, Bonzini M, Tarantini L et al (2011) Inhalable metal-rich air particles and histone H3K4 dimethylation and H3K9 acetylation in a cross-sectional study of steel workers. Environ Health Perspect 119(7):964–969. doi:10.1289/ehp.1002955

Cao D, Bromberg PA, Samet JM (2007) COX-2 expression induced by diesel particles involves chromatin modification and degradation of HDAC1. Am J Respir Cell Mol Biol 37(2):232–239. doi:10.1165/rcmb.2006-0449OC, 2006-0449OC [pii]

Carugno M, Pesatori AC, Dioni L, Hoxha M, Bollati V, Albetti B et al (2012) Increased mitochondrial DNA copy number in occupations associated with low-dose benzene exposure. Environ Health Perspect 120(2):210–215. doi:10.1289/ehp.1103979

Castro-Giner F, Kunzli N, Jacquemin B, Forsberg B, de Cid R, Sunyer J et al (2009) Traffic-related air pollution, oxidative stress genes, and asthma (ECHRS). Environ Health Perspect 117(12):1919–1924. doi:10.1289/ehp.0900589

Chahine T, Baccarelli A, Litonjua A, Wright RO, Suh H, Gold DR et al (2007) Particulate air pollution, oxidative stress genes, and heart rate variability in an elderly cohort. Environ Health Perspect 115(11):1617–1622. doi:10.1289/ehp.10318

Chen YH, Lin SJ, Lin MW, Tsai HL, Kuo SS, Chen JW et al (2002) Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. Hum Genet 111(1):1–8

Chen C, Arjomandi M, Tager IB, Holland N, Balmes JR (2007) Effects of antioxidant enzyme polymorphisms on ozone-induced lung function changes. Eur Respir J 30(4):677–683. doi:10.1183/09031936.00160806, 09031936.00160806 [pii]

Cho HY, Kleeberger SR (2007) Genetic mechanisms of susceptibility to oxidative lung injury in mice. Free Radic Biol Med 42(4):433–445. doi:10.1016/j.freeradbiomed.2006.11.021, S0891-5849(06)00758-1 [pii]

Corradi M, Alinovi R, Goldoni M, Vettori M, Folesani G, Mozzoni P et al (2002) Biomarkers of oxidative stress after controlled human exposure to ozone. Toxicol Lett 134(1–3):219–225. doi:S0378427402001698 [pii]

Curjuric I, Imboden M, Schindler C, Downs SH, Hersberger M, Liu SL et al (2010) HMOX1 and GST variants modify attenuation of FEF25-75 % decline due to PM10 reduction. Eur Respir J 35(3):505–514. doi:10.1183/09031936.00044309, 35/3/505 [pii]

Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB et al (2005) Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 294(14):1799–1809. doi:10.1001/jama.294.14.1799, 294/14/1799 [pii]

David GL, Romieu I, Sienra-Monge JJ, Collins WJ, Ramirez-Aguilar M, del Rio-Navarro BE et al (2003) Nicotinamide adenine dinucleotide (phosphate) reduced: quinone oxidoreductase and glutathione S-transferase M1 polymorphisms and childhood asthma. Am J Respir Crit Care Med 168(10):1199–1204. doi:10.1164/rccm.200305-684OC, 200305-684OC [pii]

Ferris CD, Jaffrey SR, Sawa A, Takahashi M, Brady SD, Barrow RK et al (1999) Haem oxygenase-1 prevents cell death by regulating cellular iron. Nat Cell Biol 1(3):152–157

Fuller GM, Zhang Z (2001) Transcriptional control mechanism of fibrinogen gene expression. Ann N Y Acad Sci 936:469–479

Gauderman W, Vora H, Avol E, McConnell R (2009) Predictors of intra-community variation in air quality. Technical report. Report No.: 180. University of Southern California Keck School of Medicine Division of Biostatistics – Research Section 2009, Los Angeles.

Gilliland FD, Li YF, Saxon A, Diaz-Sanchez D (2004) Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. Lancet 363(9403):119–125. doi:10.1016/S0140-6736(03)15262-2, S0140-6736(03)15262-2 [pii]

Gilmour PS, Rahman I, Donaldson K, MacNee W (2003) Histone acetylation regulates epithelial IL-8 release mediated by oxidative stress from environmental particles. Am J Physiol Lung Cell Mol Physiol 284(3):L533–L540. doi:10.1152/ajplung.00277.2002, 00277.2002 [pii]

Hajos AK, Winston GW (1991a) Dinitropyrene nitroreductase activity of purified NAD(P)H-quinone oxidoreductase: role in rat liver cytosol and induction by Aroclor-1254 pretreatment. Carcinogenesis 12(4):697–702

Hajos AK, Winston GW (1991b) Purified NAD(P)H-quinone oxidoreductase enhances the mutagenicity of dinitropyrenes in vitro. J Biochem Toxicol 6(4):277–282

Hirai H, Kubo H, Yamaya M, Nakayama K, Numasaki M, Kobayashi S et al (2003) Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. Blood 102(5):1619–1621

Hollingsworth JW 2nd, Cook DN, Brass DM, Walker JK, Morgan DL, Foster WM et al (2004) The role of Toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 170(2):126–132. doi:10.1164/rccm.200311-1499OC, 200311-1499OC [pii]

Hong YC, Hwang SS, Kim JH, Lee KH, Lee HJ, Yu SD et al (2007) Metals in particulate pollutants affect peak expiratory flow of schoolchildren. Environ Health Perspect 115(3):430–434. doi:10.1289/ehp.9531

Hou L, Zhang X, Wang D, Baccarelli A (2012) Environmental chemical exposures and human epigenetics. Int J Epidemiol 41(1):79–105. doi:10.1093/ije/dyr154, dyr154 [pii]

Hu X, Xia H, Srivastava SK, Herzog C, Awasthi YC, Ji X et al (1997) Activity of four allelic forms of glutathione S-transferase hGSTP1-1 for diol epoxides of polycyclic aromatic hydrocarbons. Biochem Biophys Res Commun 238(2):397–402. doi:10.1006/bbrc.1997.7311, S0006-291X(97)97311-3 [pii]

Hunter DJ (2005) Gene-environment interactions in human diseases. Nat Rev Genet 6(4):287–298. doi:10.1038/nrg1578, nrg1578 [pii]

Hwang BF, Young LH, Tsai CH, Tung KY, Wang PC, Su MW et al (2013) Fine particle, ozone exposure, and asthma/wheezing: effect modification by glutathione S-transferase P1 polymorphisms. PLoS One 8(1):e52715. doi:10.1371/journal.pone.0052715, PONE-D-12-03383 [pii]

Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P et al (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41(2):178–186. doi:10.1038/ng.298, ng.298 [pii]

Islam T, McConnell R, Gauderman WJ, Avol E, Peters JM, Gilliland FD (2008) Ozone, oxidant defense genes, and risk of asthma during adolescence. Am J Respir Crit Care Med 177(4):388–395. doi:10.1164/rccm.200706-863OC, 200706-863OC [pii]

Islam T, Berhane K, McConnell R, Gauderman WJ, Avol E, Peters JM et al (2009) Glutathione-S-transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. Thorax 64(3):197–202. doi:10.1136/thx.2008.099366, thx.2008.099366 [pii]

Islam T, Breton C, Salam MT, McConnell R, Wenten M, Gauderman WJ et al (2010) Role of inducible nitric oxide synthase in asthma risk and lung function growth during adolescence. Thorax 65(2):139–145. doi:10.1136/thx.2009.114355, thx.2009.114355 [pii]

Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A et al (2013) Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 10(1):22. doi:10.1186/1743-8977-10-22, 1743-8977-10-22 [pii]

Jardim MJ (2011) microRNAs: implications for air pollution research. Mutat Res 717(1–2):38–45. doi:10.1016/j.mrfmmm.2011.03.014, S0027-5107(11)00080-7 [pii]

Jardim MJ, Fry RC, Jaspers I, Dailey L, Diaz-Sanchez D (2009) Disruption of microRNA expression in human airway cells by diesel exhaust particles is linked to tumorigenesis-associated pathways. Environ Health Perspect 117(11):1745–1751. doi:10.1289/ehp.0900756

Jardim MJ, Dailey L, Silbajoris R, Diaz-Sanchez D (2012) Distinct microRNA expression in human airway cells of asthmatic donors identifies a novel asthma-associated gene. Am J Respir Cell Mol Biol 47(4):536–542. doi:10.1165/rcmb.2011-0160OC, rcmb.2011-0160OC [pii]

Kelly FJ (2003) Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med 60(8):612–616. doi:10.1136/oem.60.8.612

Kerkhof M, Postma DS, Brunekreef B, Reijmerink NE, Wijga AH, de Jongste JC et al (2010) Toll-like receptor 2 and 4 genes influence susceptibility to adverse effects of traffic-related air pollution on childhood asthma. Thorax 65(8):690–697. doi:10.1136/thx.2009.119636, 65/8/690 [pii]

Kim CS, Alexis NE, Rappold AG, Kehrl H, Hazucha MJ, Lay JC et al (2011) Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183(9):1215–1221. doi:10.1164/rccm.201011-1813OC, 201011-1813OC [pii]

Kleeberger SR, Levitt RC, Zhang LY, Longphre M, Harkema J, Jedlicka A et al (1997) Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17(4):475–478. doi:10.1038/ng1297-475

Lee YL, McConnell R, Berhane K, Gilliland FD (2009) Ambient ozone modifies the effect of tumor necrosis factor G-308A on bronchitic symptoms among children with asthma. Allergy 64(9):1342–1348. doi:10.1111/j.1398-9995.2009.02014.x, ALL2014 [pii]

Li YF, Gauderman WJ, Avol E, Dubeau L, Gilliland FD (2006) Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. Am J Respir Crit Care Med 173(9):970–976. doi:10.1164/rccm.200508-1256OC, 200508-1256OC [pii]

Ljungman P, Bellander T, Schneider A, Breitner S, Forastiere F, Hampel R et al (2009) Modification of the interleukin-6 response to air pollution by interleukin-6 and fibrinogen polymorphisms. Environ Health Perspect 117(9):1373–1379. doi:10.1289/ehp.0800370

McConnell R, Berhane K, Gilliland F, London SJ, Islam T, Gauderman WJ et al (2002) Asthma in exercising children exposed to ozone: a cohort study. Lancet 359(9304):386–391. doi:10.1016/S0140-6736(02)07597-9, S0140-6736(02)07597-9 [pii]

McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F et al (2006) Traffic, susceptibility, and childhood asthma. Environ Health Perspect 114(5):766–772

Melen E, Nyberg F, Lindgren CM, Berglind N, Zucchelli M, Nordling E et al (2008) Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. Environ Health Perspect 116(8):1077–1084. doi:10.1289/ehp.11117

Minelli C, Wei I, Sagoo G, Jarvis D, Shaheen S, Burney P (2011) Interactive effects of antioxidant genes and air pollution on respiratory function and airway disease: a HuGE review. Am J Epidemiol 173(6):603–620. doi:10.1093/aje/kwq403, kwq403 [pii]

Misra P, Srivastava R, Krishnan A, Sreenivaas V, Pandav CS (2012) Indoor air pollution-related acute lower respiratory infections and low birthweight: a systematic review. J Trop Pediatr 58(6):457–466. doi:10.1093/tropej/fms017, fms017 [pii]

Mordukhovich I, Wilker E, Suh H, Wright R, Sparrow D, Vokonas PS et al (2009) Black carbon exposure, oxidative stress genes, and blood pressure in a repeated-measures study. Environ Health Perspect 117(11):1767–1772. doi:10.1289/ehp.0900591

Moreno-Macias H, Dockery DW, Schwartz J, Gold DR, Laird NM, Sienra-Monge JJ et al (2013) Ozone exposure, vitamin C intake, and genetic susceptibility of asthmatic children in Mexico City: a cohort study. Respir Res 14:14. doi:10.1186/1465-9921-14-14, 1465-9921-14-14 [pii]

Nakagawa R, Kitamori S, Horikawa K, Nakashima K, Tokiwa H (1983) Identification of dinitropyrenes in diesel-exhaust particles. Their probable presence as the major mutagens. Mutat Res 124(3–4):201–211. doi:0165-1218(83)90191-X [pii]

Newcomb P, Hunt A, Rast P, Cauble D, Rowe N, Li J (2012) Acute effects of walking environment and GSTM1 variants in children with asthma. Biol Res Nurs 14(1):55–64. doi:10.1177/1099800410389167, 1099800410389167 [pii]

Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M et al (2000) Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 6(4):422–428

Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D et al (2006) HFE genotype, particulate air pollution, and heart rate variability: a gene-environment interaction. Circulation 114(25):2798–2805. doi:10.1161/CIRCULATIONAHA.106.643197, CIRCULATIONAHA.106.643197 [pii]

Park SK, Hu H, Wright RO, Schwartz J, Cheng Y, Sparrow D et al (2009) Iron metabolism genes, low-level lead exposure, and QT interval. Environ Health Perspect 117(1):80–85. doi:10.1289/ehp.11559

Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R et al (2009) Relation of DNA methylation of 5′-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One 4(2):e4488. doi:10.1371/journal.pone.0004488

Peters JM, Avol E, Gauderman WJ, Linn WS, Navidi W, London SJ et al (1999a) A study of twelve Southern California communities with differing levels and types of air pollution. II. Effects on pulmonary function. Am J Respir Crit Care Med 159(3):768–775. doi:10.1164/ajrccm.159.3.9804144

Peters JM, Avol E, Navidi W, London SJ, Gauderman WJ, Lurmann F et al (1999b) A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. Am J Respir Crit Care Med 159(3):760–767. doi:10.1164/ajrccm.159.3.9804143

Peters A, Greven S, Heid IM, Baldari F, Breitner S, Bellander T et al (2009) Fibrinogen genes modify the fibrinogen response to ambient particulate matter. Am J Respir Crit Care Med 179(6):484–491. doi:10.1164/rccm.200805-751OC, 200805-751OC [pii]

Rahman I, Adcock IM (2006) Oxidative stress and redox regulation of lung inflammation in COPD. Eur Respir J 28(1):219–242. doi:10.1183/09031936.06.00053805, 28/1/219 [pii]

Rahman I, Biswas SK, Kode A (2006) Oxidant and antioxidant balance in the airways and airway diseases. Eur J Pharmacol 533(1–3):222–239. doi:10.1016/j.ejphar.2005.12.087, S0014-2999(05)01384-1 [pii]

Ren C, Park SK, Vokonas PS, Sparrow D, Wilker E, Baccarelli A et al (2010a) Air pollution and homocysteine: more evidence that oxidative stress-related genes modify effects of particulate air pollution. Epidemiology 21(2):198–206. doi:10.1097/EDE.0b013e3181cc8bfc

Ren C, Baccarelli A, Wilker E, Suh H, Sparrow D, Vokonas P et al (2010b) Lipid and endothelium-related genes, ambient particulate matter, and heart rate variability–the VA Normative Aging Study. J Epidemiol Community Health 64(1):49–56. doi:10.1136/jech.2008.083295, jech.2008.083295 [pii]

Ricciardolo FL, Nijkamp FP, Folkerts G (2006a) Nitric oxide synthase (NOS) as therapeutic target for asthma and chronic obstructive pulmonary disease. Curr Drug Targets 7(6):721–735

Ricciardolo FL, Di Stefano A, Sabatini F, Folkerts G (2006b) Reactive nitrogen species in the respiratory tract. Eur J Pharmacol 533(1–3):240–252. doi:10.1016/j.ejphar.2005.12.057, S0014-2999(05)01387-7 [pii]

Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, Moreno-Macias H, Reyes-Ruiz NI (2004) Estela del Rio-Navarro B et al. Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 59(1):8–10

Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ, Moreno-Macias H, del Rio-Navarro BE, David G et al (2006) GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28(5):953–959. doi:10.1183/09031936.06.00114905, 09031936.06.00114905 [pii]

Romieu I, Moreno-Macias H, London SJ (2010) Gene by environment interaction and ambient air pollution. Proc Am Thorac Soc 7(2):116–122. doi:10.1513/pats.200909-097RM, 7/2/116 [pii]

Rossner P Jr, Tabashidze N, Dostal M, Novakova Z, Chvatalova I, Spatova M et al (2011) Genetic, biochemical, and environmental factors associated with pregnancy outcomes in newborns from the Czech Republic. Environ Health Perspect 119(2):265–271. doi:10.1289/ehp.1002470

Ruckerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T et al (2007) Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ Health Perspect 115(7):1072–1080. doi:10.1289/ehp.10021

Salam MT, Lin PC, Avol EL, Gauderman WJ, Gilliland FD (2007) Microsomal epoxide hydrolase, glutathione S-transferase P1, traffic and childhood asthma. Thorax 62(12):1050–1057. doi:10.1136/thx.2007.080127, thx.2007.080127 [pii]

Salam MT, Islam T, Gauderman WJ, Gilliland FD (2009) Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol 123(3):596–602.e1–8. doi:10.1016/j.jaci.2008.12.020, S0091-6749(08)02435-4 [pii]

Salam MT, Byun HM, Lurmann F, Breton CV, Wang X, Eckel SP et al (2012) Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. J Allergy Clin Immunol 129(1):232–239e1–7. doi:10.1016/j.jaci.2011.09.037, S0091-6749(11)01560-0 [pii]

Schwartz J, Park SK, O'Neill MS, Vokonas PS, Sparrow D, Weiss S et al (2005) Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. Am J Respir Crit Care Med 172(12):1529–1533. doi:10.1164/rccm.200412-1698OC, 200412-1698OC [pii]

Shah PS, Balkhair T (2011) Air pollution and birth outcomes: a systematic review. Environ Int 37(2):498–516. doi:10.1016/j.envint.2010.10.009, S0160-4120(10)00225-4 [pii]

Siegel D, McGuinness SM, Winski SL, Ross D (1999) Genotype-phenotype relationships in studies of a polymorphism in NAD(P)H:quinone oxidoreductase 1. Pharmacogenetics 9(1):113–121

Sofer T, Baccarelli A, Cantone L, Coull B, Maity A, Lin X et al (2013) Exposure to airborne particulate matter is associated with methylation pattern in the asthma pathway. Epigenomics 5(2):147–154. doi:10.2217/epi.13.16

Sonkoly E, Pivarcsi A (2011) MicroRNAs in inflammation and response to injuries induced by environmental pollution. Mut Res 717(1–2):46–53. doi:http://dx.doi.org/10.1016/j.mrfmmm.2011.02.002

Stieb DM, Chen L, Eshoul M, Judek S (2012) Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ Res 117:100–111. doi:10.1016/j.envres.2012.05.007, S0013-9351(12)00176-4 [pii]

Stocker R, Ames BN (1987) Potential role of conjugated bilirubin and copper in the metabolism of lipid peroxides in bile. Proc Natl Acad Sci U S A 84(22):8130–8134

Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. Science 235(4792):1043–1046

Suh YJ, Ha EH, Park H, Kim YJ, Kim H, Hong YC (2008) GSTM1 polymorphism along with PM10 exposure contributes to the risk of preterm delivery. Mutat Res 656(1–2):62–67. doi:10.1016/j.mrgentox.2008.07.006, S1383-5718(08)00204-0 [pii]

Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B et al (1998) Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 19(3):433–436

Thomas D (2010) Methods for investigating gene-environment interactions in candidate pathway and genome-wide association studies. Annu Rev Public Health 31(1):21–36. doi:10.1146/annurev.publhealth.012809.103619

Tubby C, Harrison T, Todd I, Fairclough L (2011) Immunological basis of reversible and fixed airways disease. Clin Sci 121(7):285–296. doi:10.1042/cs20110062

Tung KY, Tsai CH, Lee YL (2011) Microsomal epoxide hydroxylase genotypes/diplotypes, traffic air pollution, and childhood asthma. Chest 139(4):839–848. doi:10.1378/chest. 10-2479, chest.10-2479 [pii]

Vagaggini B, Bartoli ML, Cianchetti S, Costa F, Bacci E, Dente FL et al (2010) Increase in markers of airway inflammation after ozone exposure can be observed also in stable treated asthmatics with minimal functional response to ozone. Respir Res 11:5. doi:10.1186/1465-9921-11-5, 1465-9921-11-5 [pii]

Van Hee VC, Adar SD, Szpiro AA, Barr RG, Diez Roux A, Bluemke DA et al (2010) Common genetic variation, residential proximity to traffic exposure, and left ventricular mass: the multi-ethnic study of atherosclerosis. Environ Health Perspect 118(7):962–969. doi:10.1289/ehp.0901535

Wenten M, Gauderman WJ, Berhane K, Lin PC, Peters J, Gilliland FD (2009) Functional variants in the catalase and myeloperoxidase genes, ambient air pollution, and respiratory-related school absences: an example of epistasis in gene-environment interactions. Am J Epidemiol 170(12):1494–1501. doi:10.1093/aje/kwp310, kwp310 [pii]

Wilker E, Mittleman MA, Litonjua AA, Poon A, Baccarelli A, Suh H et al (2009) Postural changes in blood pressure associated with interactions between candidate genes for chronic respiratory diseases and exposure to particulate matter. Environ Health Perspect 117(6):935–940. doi:10.1289/ehp.0800279

Wilker EH, Baccarelli A, Suh H, Vokonas P, Wright RO, Schwartz J (2010) Black carbon exposures, blood pressure, and interactions with single nucleotide polymorphisms in MicroRNA processing genes. Environ Health Perspect 118(7):943–948. doi:10.1289/ehp.0901440

Wilker EH, Alexeeff SE, Suh H, Vokonas PS, Baccarelli A, Schwartz J (2011) Ambient pollutants, polymorphisms associated with microRNA processing and adhesion molecules: the Normative Aging Study. Environ Health 10:45. doi:10.1186/1476-069X-10-45, 1476-069X-10-45 [pii]

Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S et al (2000) Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 66(1):187–195

Yamamoto M, Singh A, Sava F, Pui M, Tebbutt SJ, Carlsten C (2013) MicroRNA expression in response to controlled exposure to diesel exhaust: attenuation by the antioxidant N-acetylcysteine in a randomized crossover study. Environ Health Perspect 121(6):670–675. doi:10.1289/ehp.1205963

Yang IV, Schwartz DA (2012) Epigenetic mechanisms and the development of asthma. J Allergy Clin Immunol 130(6):1243–1255. doi:10.1016/j.jaci.2012.07.052

Yang IA, Holz O, Jorres RA, Magnussen H, Barton SJ, Rodriguez S et al (2005) Association of tumor necrosis factor-alpha polymorphisms and ozone-induced change in lung function. Am J Respir Crit Care Med 171(2):171–176. doi:10.1164/rccm.200402-194OC, 200402-194OC [pii]

Zanobetti A, Baccarelli A, Schwartz J (2011) Gene-air pollution interaction and cardiovascular disease: a review. Prog Cardiovasc Dis 53(5):344–352. doi:10.1016/j.pcad.2011.01.001, S0033-0620(11)00015-6 [pii]

Chapter 13
Urban Air Pollution and Health in Developing Countries

Junfeng (Jim) Zhang and Drew Day

13.1 Introduction

A number of countries have rapidly industrialized in the past few decades, leading to severe decreases in air quality associated with industrial processes, urbanization, and population growth. Meanwhile, indoor air pollution resulting from household combustion of solid fuels remains severe, affecting more than half of the population. Each of these two problems contributes greatly to the global burden of disease, as outdoor air pollution and household air pollution from solid fuels were responsible for 3.4 and 3.5 million premature deaths, respectively, worldwide in 2010 (Lim et al. 2012). The majority of this disease burden due to air pollution, however, occurs in developing countries. Since indoor air pollution from household biomass combustion is covered in Chap. 14, the goal of this chapter is to describe outdoor air pollution problems in developing countries with a focus on urban areas.

Wealthier nations underwent similar processes decades earlier in a much slower fashion, and most have since at least partially addressed the severe air pollution that used to afflict their urban areas. In cities such as Beijing and many northern Chinese cities, severe winter smog episodes have frequently occurred in recent years, replaying the historical air pollution episodes of the twentieth century (e.g., Meuse River Valley, Belgium in 1930; Donora, Pennsylvania in 1948; and London in 1952). For example, daily $PM_{2.5}$ concentrations measured on the grounds of the U.S. Embassy in Beijing in the month of January from 2010 to 2014 frequently exceeded 100 μg/m^3 and reached as high as 552 μg/m^3, about 16 times the US EPA's 24-h standard for $PM_{2.5}$ of 35 μg/m^3 (see Fig. 13.1). Given that the highest air pollutant concentrations

J. Zhang, Ph.D. (✉) • D. Day, B.A., B.S.
Nicholas School of the Environment & Duke Global Health Institute, Duke University, LSRC A309, 308 Research Drive, Durham, NC 27708, USA
e-mail: junfeng.zhang@duke.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_13

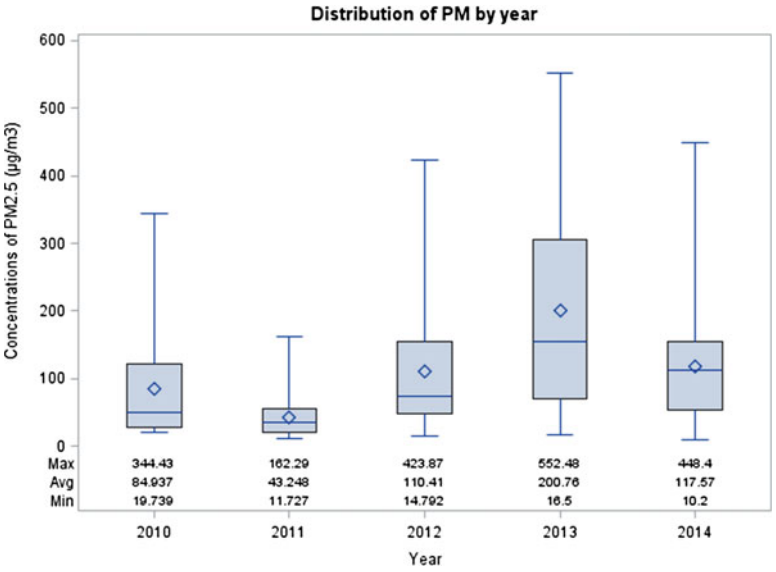

Fig. 13.1 January $PM_{2.5}$ concentrations measured at the U.S. Embassy in Beijing 2010–2014. Winter $PM_{2.5}$ concentrations in Beijing far exceed international standards. In this *boxplot*, the *top whisker line* corresponds with the maximum value, the *top line* of the *box* corresponds with the 75th percentile, the *blue diamond* is the mean, the *middle box line* is the median, the *bottom box line* is the 25th percentile, and the *bottom whisker line* is the minimum (Data were retrieved from the Beijing U.S. Embassy air monitoring Twitter feed at https://twitter.com/BeijingAir)

are found in rapidly industrializing developing nations, it is important to understand what types of exposures are occurring there and why. The following specific questions will be addressed:

1. What are the levels and composition of the urban air pollution mixture in developing countries in comparison to those in developed countries? What are the trends in emissions?
2. What are the major sources that contribute to urban air pollution in developing countries?
3. What are the disease burdens attributable to outdoor air pollution in developing countries? Is it appropriate to use concentration-response relationships derived from studies at lower concentrations in health risk assessment for developing countries?
4. What are the recommendations for reducing health risks associated with urban outdoor pollution in developing countries?

13.2 Pollution Levels and Emission Trends

Urbanization has been a dominant trend in the developing world that has expanded at a much higher rate than what occurred previously in the developed world. For example, it took the United States 90 years to go from 40 % urbanized in 1900 to over 75 % urbanized in 1990, but the same process took only 20 years in South Korea and only 30 years in Brazil (Henderson 2002). This urbanization can lead to worsening air pollution as increased vehicle traffic, household emissions, and power plant and factory emissions resulting from the tightly packed population centers create areas of high emissions and high exposure. Currently, 19 of the top 25 most populous cities in the world are in developing countries, and many of these megacities have annual average concentrations of air pollutants in excess of health-based standards and guidelines, as can be seen in Fig. 13.2. In particular, megacities in developing countries tend to have higher air pollution concentrations than those in developed countries, consistent with the PM_{10} emission trends described below.

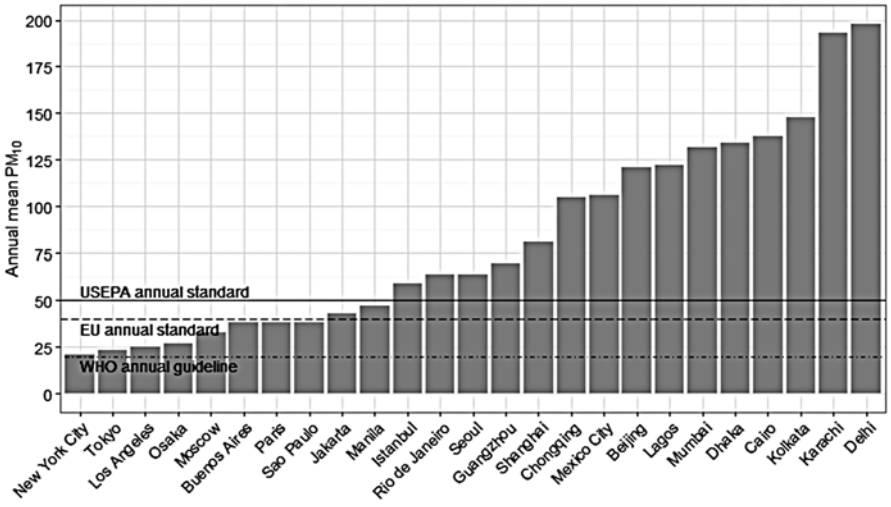

Fig. 13.2 Annual mean urban PM_{10} levels versus international standards in the 25 most populous cities. Many of the top 25 most populous cities in the world have annual PM_{10} levels above national and international standards. The cities are ordered in terms of increasing annual mean PM_{10} concentrations, which are based on WHO data from 2003 to 2010. The top 25 most populous cities were determined based on the 2011 population in the "urban agglomeration" as defined by the UN (UNDESA 2011). Shenzhen was in the top 25, but PM_{10} data was not available for that city. Therefore we used Jakarta (#26) instead. The US EPA annual standard for PM_{10} was obtained from their website, but this standard was revoked in 2006 in favor of only having a 24-h average standard of 150 μg/m^3 for PM_{10} that is not to be exceeded more than once a year on average. The EU annual standard of 40 μg/m^3 was established in 2005 and obtained from the European Commission website. The WHO annual guideline of 20 μg/m^3 was obtained from their website and is up to date as of 2014

Over the past four decades, the developed countries in North America, Europe, and the Pacific have implemented policies to curtail the severe air pollution events that had plagued them during the early and mid-twentieth century. One of the consequences of the increased stringency of pollution control regulations in the developed, industrialized world is the outsourcing of more polluting manufacturing jobs into the developing world by multi-national corporations. This process was coupled with domestically driven industrial development by a number of countries that saw industry as a path to national prosperity. Increased national wealth is coupled with a desire for increased living standards among the population. For example, the use of cars as a means of personal transportation rose rapidly with per capita income. Many of these developing countries have among the highest population density in the world, and so the rise of personal vehicles has become a major source of air pollution and transportation congestion in the developing world. Furthermore, these higher living standards led to increased energy consumption. These trends coupled with higher rates of garbage and agricultural combustion, low quality fuel use, coal combustion and other major polluting energy sources, and construction-related dust have led to much higher air pollutant concentrations in developing countries in comparison with the developed world. Figure 13.3 shows how drastic the air pollution

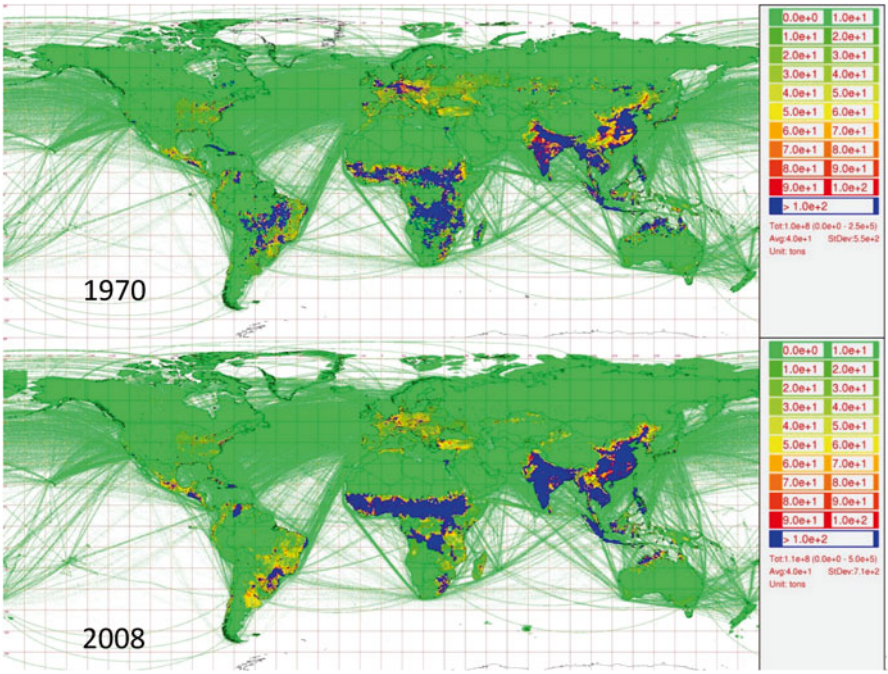

Fig. 13.3 Global annual PM_{10} emissions in 1970 and 2008. The annual emissions scale ranges from *green*, marking 0 to 10 tons, to *blue*, marking greater than 100 tons. The *lines* over bodies of water reflect the PM_{10} contribution of major international shipping and aviation routes (These maps were obtained from the European Commission Joint Research Centre Emission Database for Global Atmospheric Research (EDGAR) http://edgar.jrc.ec.europa.eu/overview.php?v=42)

burden shifts of the past four decades have been, using PM_{10} emissions as a surrogate marker for other major air pollutants in terms of global trends ($PM_{2.5}$ data are largely unavailable historically).

There are many noticeable differences between these two time periods. PM_{10} distributed across the eastern area of the United States and Canada diminished over the decades. Southern Mexico, Central America, and the Caribbean have increased their air pollution emissions, though Cuba produces much less PM now. PM_{10} emissions in South America attributable to wide-scale forest burning have abated, though the population centers of the continent in Venezuela, Brazil, and Argentina have much worse air quality currently. Europe has generally seen remarkable improvements in air quality, though Asia Minor and parts of Northern Africa have gotten worse. Most of the line of PM_{10} across the former Soviet Union and Kazakhstan has disappeared with the collapse of the USSR. A thick belt of heavy PM pollution has developed between the Saharan Desert and the equator in Africa, though Southern Africa has improved. The already thick pockets of air pollution over South, East, and South-East Asia have intensified in the past four decades. Finally, it is apparent that the developed countries of the Pacific, namely Australia, New Zealand, Japan, and South Korea, have experienced air quality improvements. These global trends only account for PM_{10}, but there are similar patterns of other major air pollutants on the global scale.

To get a clearer picture of just how the contribution of different countries to global air pollution burdens has changed, Fig. 13.4 maps out the regional trends in annual PM_{10} emissions from 1970 to 2008.

The starkest trend in this figure is that of the Northern and Western African regions, which have dramatically increased particulate emissions in the latter half of the 2000s. This has been driven by rapid industrialization in the coastal West African countries and the belt of Central and Eastern African nations just south of the Sahel Desert, where some of the fastest urbanization rates in the world are occurring. On the other end of the emissions scale, the Middle Eastern countries have gone from being barely visible on the figure in the 1970s to just becoming apparent in the mid-1990s and 2000s. The regions that are steadily increasing include Northern and Western Africa, China and surrounding countries, India and surrounding countries, and the Middle East. There have been increases that seem to oscillate in a less discernible pattern for Eastern and Southern Africa, Southeast Asia and Indonesia +, and South America. Asia-Stan and Russia + and Central America peaked in the late 1990s and are on the decline now. The regions that have experienced a steady decline in air pollutant emissions are North America, OECD Europe, Central Europe, and Japan and Korea. Finally, Oceania, Turkey and Ukraine +, and international shipping and aviation have been relatively steady emitters over these past four decades. These trends show that the greatest burden of air pollution exposure falls on the developing countries, in particular those in the process of massive urbanization and industrialization.

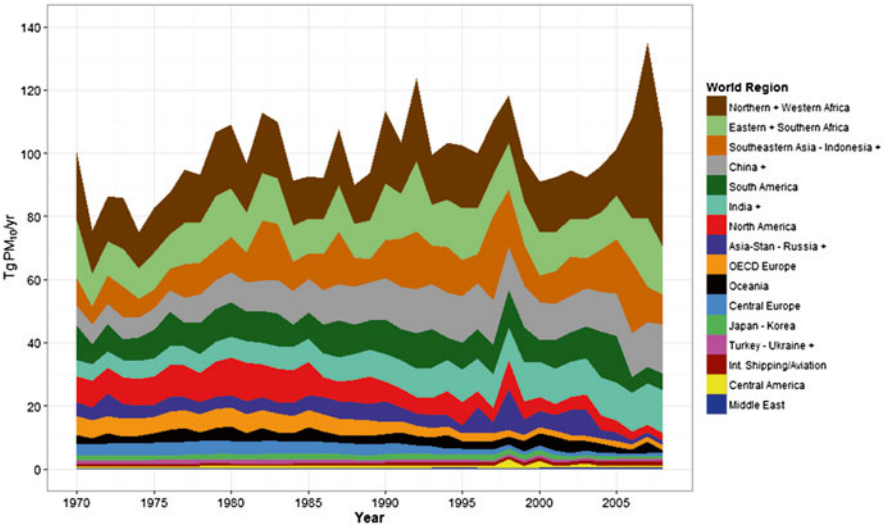

Fig. 13.4 Global PM_{10} emission trends by region from 1970 to 2008. The regions with more developing nations have grown to comprise a greater share of the global PM_{10} emissions. The portion of total PM_{10} emissions contributed by each world region is shown by the thickness of the space between *lines* that represents each region. The regions are organized from highest total PM_{10} emissions from 1970 to 2008 on *top* to the lowest total emission regions on *bottom*. Each *line* represents the trend in total PM_{10} annual emissions for that region and all the ones below it. All data were obtained from the European Commission Joint Research Centre Emission Database for Global Atmospheric Research (EDGAR) (http://edgar.jrc.ec.europa.eu/overview.php?v=42). **Asia-Stan**: Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan; **China +**: China, Hong Kong, Macao, Mongolia, Taiwan; **India +**: Afghanistan, Bangladesh, Bhutan, India, British Indian Ocean Territory, Sri Lanka, Maldives, Nepal, Pakistan; **Indonesia +**: Indonesia, Papua New Guinea; **Russia +**: Armenia, Azerbaijan, Georgia, Russian Federation; **Ukraine +**: Belarus, Republic of Moldova, Ukraine (For more information on the regional categories, please visit http://edgar.jrc.ec.europa.eu/methodology.php)

13.3 Criteria Air Pollutants in Developing Countries

The urban air pollution mixture is very complex in terms of its chemical composition. In the United States, common pollutants in this mixture that affect the general population are regulated as criteria air pollutants, including particulate matter (PM_{10} and $PM_{2.5}$), lead, ozone, nitrogen dioxide, carbon monoxide, and sulfur dioxide. Similarly, some or all of these pollutants have national standards in many other countries and have guidelines by WHO. In the previous section, we have described emission trends and typical ambient concentrations of PM_{10}, because PM (especially $PM_{2.5}$) has been most commonly linked to various adverse health effects. Although $PM_{2.5}$ is a more health-relevant measure of PM, historical data are less available for $PM_{2.5}$ than for PM_{10}. Given the focus on PM in the previous section, the following section will not emphasize that criteria pollutant. Below we describe

how developing countries differ in other common pollutants of the urban air pollution mixture from developed countries.

13.3.1 Lead

Lead can enter the air through a variety of natural processes, such as volcanic eruptions, forest fires, and soil erosion. The natural background concentration of lead in the air can vary due to local natural sources. The background lead concentration was 5–15 ng/m^3 in Central Europe in 2003 (UNEP 2010), but it was approaching 100 ng/m^3 in 2005 in Australia due to a higher prevalence of brush fires (Heritage 2005). The corresponding background child blood lead levels worldwide are generally in the range of 10–30 μg/l (WHO 2000). Anthropogenic sources include lead smelters, waste incineration, battery recycling, mining operations, lead paint dust, and leaded gasoline. The latter two sources have been reduced significantly through legislation either banning or significantly reducing lead content in certain countries. Today only some of the developed countries have significant regulations on paint lead content, whereas all but six countries in the world have bans on leaded gasoline (see the Sect. 13.4.3 for more information on leaded gasoline). Nevertheless, some illegal production of leaded gasoline still occurs. The widespread banning of leaded gasoline has reduced the impact of this air pollutant, particularly in developed countries. Finland began phasing out leaded gasoline in the early 1980s, and from 1980 to 1991 the mean ambient air lead concentrations dropped from 0.335 to 0.041 μg/m^3 in Helsinki (Ponka et al. 1993). Though lead air concentrations have dropped in developed countries, they are still an issue due to mining and industrial processes. The United States banned lead paint in 1977 and leaded gasoline in 1995, but 21 cities or counties exceeded the 3 month rolling average standard of 0.15 μg/m^3 as of 2014. Lead exposure through ambient air may still be an issue in some developed countries, but the problem is more severe in developing countries.

Although the US EPA monitors ambient air concentrations of lead, many countries do not, and so it is difficult to assess lead exposure via air in developing countries. More countries monitor blood lead levels (BLLs) in children, and so this can be used as a marker of the total exposure that a child receives in a given country. The WHO based the establishment of its 2000 air lead concentration guideline for Europe, 0.5 μg/m^3, on the assumption that 1 μg/m^3 lead in the air corresponds to 50 μg/l lead in the blood through inhalation exposure and other indirect routes (WHO 2000). The U.S. phasing out and eventual ban of leaded gasoline contributed to an 84 % reduction (from 8.6 to 1.4 %) in the percentage of children with elevated blood lead levels (≥100 μg/l) from 1988 to 2004 (Jones et al. 2009). The banning of leaded gasoline in 2000 in China was a large factor in the 29 % reduction (from 33.8 to 23.9 %) in the mean percentage of children with elevated blood lead levels from the 1995–2003 sampling period to the 2001–2007 period (He et al. 2009), but the fraction of children with elevated blood lead levels still remain much higher in China than in the U.S.

Similarly, blood lead levels measured in Kinshasa, the capital of the Democratic Republic of the Congo, are much higher than those measured in the U.S., even after the banning of leaded gasoline. In 2008, the ambient lead concentration in the urban air ranged from 0.57 to 5.22 μg/m^3. Sixty three percent of children measured in 2004 and 71 % of children measured in 2008 had elevated BLLs (Tuakuila et al. 2013a). Leaded gasoline was banned in 2009, and by 2011 only 41 % of children measured had elevated BLLs, a 42 % reduction. Nevertheless, this is still a very high incidence of hazardous lead exposure, indicating that the children of Kinshasa are still being exposed to significant amounts of lead through other routes (e.g., inhalation and ingestion of lead-contaminated soil, the use of fired clay as a traditional treatment for gastritis during pregnancy, household paint chips, and household Portland cement) (Tuakuila et al. 2013b). The post-leaded gasoline phase-out incidence of elevated BLLs is higher in Kinshasa in comparison to other areas of Africa. In 1991, over 90 % of the children attending inner city schools in the Cape Peninsula of South Africa had elevated BLLs, but by 2002, when leaded gasoline represented only 30 % of the market, only 10 % of children in those same schools had elevated BLLs (Mathee et al. 2006). Leaded gasoline was phased out of Uganda in 2005, and a 2009 study of schoolchildren in Kampala found that 20.5 % had elevated BLLs, less than the percentage found in Kinshasa and China but still far greater than that found in the USA (Graber et al. 2010). These examples indicate that the phasing out of leaded gasoline has significantly reduced ambient air lead concentrations and children's blood lead levels, but lead exposure through airborne and other routes still remains much more of a problem in the developing world.

13.3.2 Sulfur Dioxide

SO_2 is used as an indicator for the sulfur oxides (SO_X), as it is the sulfur oxide with the highest concentration in the atmosphere. SO_2 is also a precursor of particulate sulfate formed photochemically in the atmosphere. SO_2 has been dramatically reduced in developed countries due to regulations concerning industrial emission controls and other important sources. The US EPA Clean Air Act resulted in a 78 % reduction in national SO_2 levels (CAI-Asia 2010). On the whole, global SO_2 emissions have declined from 1990 to 2010 (from 121 to 103 Tg SO_2), led mostly by declines in the developed world (Klimont et al. 2013). The greatest sources for 2011 in order of contribution are the energy sector (40 Tg; primarily coal with petroleum combustion as the next largest source), industry (38.7 Tg), shipping (13.6 Tg), residential sources (6.4 Tg), transportation (1.6 Tg), and waste (0.3 Tg).

China contributed 29.1 % of global SO_2 emissions in 2011, showing a peak in 2005, after which the control measures in the 11th Five Year Plan (2006–2010) resulted in a 14 % reduction in SO_2 emissions and a concomitant 13–15 % and 8–10 % reduction in ambient SO_2 and SO_4^{2-}concentrations, respectively, over eastern China (Wang et al. 2014). Nevertheless, China is still the largest contributor to global SO_2 emissions of any country. India is the next largest contributor, just

behind total international shipping, and it has been steadily increasing SO_2 emissions from 1990 to 2011, rising from 2.8 to 10 % of the global total (Klimont et al. 2013). Much higher coal usage in China and India is the main factor leading to their relatively high SO_2 levels. Coal use in U.S. is relatively high for an industrialized nation, making it the next greatest contributor to emissions behind India (6.2 % of the global total in 2011). Over 70 % of total SO_2 emissions in the U.S. came from coal-fired power plants in 2008 (USEPA 2012). However, cleaner coal processing and increased removal of gaseous byproducts from flue gas in the U.S. have made it so that SO_2 ambient levels in 2012 are nearly all below the US EPA 1 h maximum standard (99th percentile of 1 h daily maximum concentrations, averaged over 3 years) of 75 ppb (196 μg/m^3) (USEPA 2013a). For most other countries in the world, regardless of economic status, SO_2 is a relatively minor pollutant, though it may still have a significant health impact below national standards. A recent study in Seoul on ambient pollution levels and tuberculosis incidence found that SO_2 was the only pollutant correlated with a significantly increased risk of TB in males, even though SO_2 levels (annual average of 6.1 ppb) were below Korean standards (annual average of 20 ppb) (Hwang et al. 2014). It is not clear if the health effects of SO_2 are mainly attributable to the pollutant itself or to the secondary pollutants it creates through atmospheric chemical reactions.

13.3.3 Nitrogen Dioxide

NO_2 is used as an indicator of the nitrogen oxides (NO_X) due to its more common prevalence than NO and N_2O, much like SO_2 and SO_X. NO_2 is formed by high temperature combustion, in which nitrogen reacts with oxygen to form NO and then NO_2. NO_2 and other NO_X contribute to the formation of secondary pollutants such as particulate nitrate (He et al. 2014). For example, due to increasing emissions of NOx, the nitrate/sulfate ratio in $PM_{2.5}$ increased from 0.43 to 0.75 from 2000 to 2009 in Shanghai, China (Huang et al. 2012a).

In many areas the greatest source of NO_X is traffic-related pollution. Overall, mobile sources (57.5 %), fuel combustion from stationary sources (24.2 %), and industrial processes (8.4 %) account for the majority of the 14.1 Tg of NO_X emissions in the U.S. (USEPA 2014a). As a result, the cities with the greatest concentrations of NO_2 in the U.S. often have the worst traffic problems, such as downtown Los Angeles, which had an annual 98th percentile of daily 1-h maximum average of 67 μg/m^3 in 2011 (USEPA 2013b). In the U.S., over 90 % of NO_2 concentrations have been below the national annual 98th percentile of daily maximum 1-h average standard of 100 ppb (188 μg/m^3) at least since the early 2000s, and they continue to show a decline. The Japanese government enacted the Automobile NO_X Law in 1992 to ban vehicles in certain areas not conforming to emission standards and strengthened it to include PM considerations and stricter standards in 2001. Areas in which the law was enforced had half the average annual NO_2 concentration that unenforced areas did (21.8 versus 39.3 μg/m^3 for 2006–2009), and this was

correlated with a reduction in asthma and atopic dermatitis prevalence even after controlling for PM (Hasunuma et al. 2014).

China is the largest producer of NO_X, emitting 23.4 Tg in 2012. However, according to the Chinese Ministry of Environmental Protection, 70.9 % of this was from industrial sources, with motor vehicles only accounting for 27.4 % (MEP 2013a). Beijing has a high concentration of vehicle traffic, but it also receives about 50 % of its NO_X from regional and not local sources (Ma et al. 2014). The high industrial contribution to Beijing NO_X levels is likely due to sources in the greater Beijing/Tianjin/Hebei area, which accounts for 43 % of national coal consumption, 30 % of national thermal power consumption, and 50 % of national steel and coke production. Beijing had a monthly mean concentration of 100 ppb NO_X in January 2013 (He et al. 2014). During this time levels spiked to around 350 ppb a few times and over 200 ppb several times, and so the 98th percentile of daily maximum 1-h average would likely be much higher than the monthly mean. Nationwide average concentrations of NO_2 show a slightly increasing trend.

In India, NO_2 pollution shows increasing trends in many areas. In the National Capital Region and surrounding states, increasing trends from 2007 to 2011 have been seen in the annual mean NO_2 concentrations of the National Capital Territory of Delhi (49.67–57 μg/m^3, 15 % increase), Uttar Pradesh (36.17–44 μg/m^3, 22 % increase), and Haryana (25–54 μg/m^3, 116 % increase) (Board, N.C.R.P 2013). By 2011, each of these regions exceeded the national annual average NO_2 air quality standard of 40 μg/m^3.

13.3.4 Carbon Monoxide

As a principal product of incomplete combustion, CO has global background concentrations ranging from 0.06 to 0.14 mg/m^3 depending on natural sources such as photochemical synthesis, volcanic eruptions, and forest fires (WHO 2000). Mobile sources account for the bulk of CO emissions (50.8 % of total U.S. emissions in 2011 (USEPA 2014b)), and so concentrations of this pollutant tend to be higher in urban environments with dense traffic. Vehicle emission controls greatly influence CO emissions, as average Pakistani vehicles emitted 25 times more CO than the average U.S. vehicle in 2000 (Barletta et al. 2002). The EU has made great strides to reduce CO emissions from 1990 to 2010, with all but one member country showing reductions ranging from 92 % in Luxembourg to 18 % in Romania, in large part through vehicle emission regulation (EEA 2010).

CO pollution has been very effectively curbed in the U.S., so much so that no counties exceeded the 8-h average standard of 9 ppm (10.3 mg/m^3) more than once per year in 2012 (USEPA 2014c). The last nonattainment area for CO in the U.S. was redesignated as being in attainment in September 2010. CO concentrations dropped 83 % from 1980 to 2012 in the U.S., from a mean annual second maximum 8-h average of 8.9 ppm (10.2 mg/m^3) to 1.5 ppm (1.7 mg/m^3). CO levels have also been dropping in Chinese cities. In Shanghai, CO annual averages have dropped

from about 1.4 mg/m^3 in 2008 to 0.88 mg/m^3 (37 % decrease) in 2012 (EPB-Shanghai 2013), and Beijing annual averages have dropped from about 2.5 mg/m^3 in 2006 to 1.4 mg/m^3 (44 % decrease) in 2012 (EPB-Beijing 2013). However, increasing vehicle traffic in East Asia, South Asia, Africa, and other rapidly urbanizing environments may lead to increased CO exposure to urban populations. In Lagos, short-term air monitoring along a busy road found CO values exceeding the US EPA 8-h standard (Olajire et al. 2011). The average roadside concentration seen in Lagos, 19.27 ppm, is much higher than that seen roadside in central London, 0.53 ppm (von Schneidemesser et al. 2010). Furthermore, a global comparison found higher CO concentrations in Latin America and Asia when compared to the United States and U.K. (von Schneidemesser et al. 2010).

13.3.5 Ozone

Ozone is a major component of photochemical smog. Concentrations of ozone typically have large daily variations depending on variations in heat, sunlight, and precursor gas concentrations. Monitoring in Agra, India found a diurnal ozone cycle with an average maximum concentration of 117 μg/m^3 in the peak noontime and an average minimum concentration of 23.2 μg/m^3 at sunrise (Saini et al. 2005). Though ozone concentrations can be high in urban environments, the presence of NO in the local urban air can "quench" ozone, causing there to be lower concentrations of O_3 in urban areas and possibly higher O_3 in non-urban areas where NO concentrations are low.

Ozone is still a major problem in both the developing and developed world. In July 2006, afternoon mean surface ozone concentrations show the highest levels between the 30° N and 60° N parallels, especially the Eastern and Western U.S., Europe, the Middle East, Central Asia, and the Yellow Sea area (WHO 2007). Other areas of high ozone include the Arabian Peninsula and southern Africa. In Delhi, average hourly concentrations measured in 2008 ranged from about 20 μg/m^3 at 9:00 pm to over 70 μg/m^3 at 3:00 pm (Guttikunda 2009). Ozone concentrations are much higher in the summer, and so there is considerable seasonal variation. 2010 peak pre-monsoon summer average ozone concentrations in the twin Pakistani cities of Islamabad and Rawalpindi were about 42.7 μg/m^3, as compared with about 31.8 μg/m^3 in the deep winter (Ahmad and Aziz 2013). In the United States, ozone levels decreased the least of all the criteria air pollutants following the Clean Air Act and other relevant air pollution legislation. There was only a 9 % reduction in O_3 from 2000 to 2012, compared to the next smallest reduction, 27 % for PM_{10}, and the largest reduction, 57 % for CO (USEPA 2014c). Furthermore, 94 % of people in the United States estimated to live in counties with at least one air pollutant concentration exceeding its relevant standard in 2012 live in counties with ozone exceedances, far greater than for any other criteria pollutant. The high burden of ozone in the U.S. is apparent in that the US EPA 1-h and 8-h ozone standards are higher than those for most Asian nations or territories with the exception of the Hong Kong

SAR (1-h), Indonesia (1-h), Singapore (8-h), and Bangladesh (1 and 8-h) (CAI-Asia 2010). Interestingly, unlike other directly emitted air pollutants, ozone is not necessarily higher in developing countries.

13.4 Air Pollution Sources More Common in Developing Countries

Common anthropogenic sources of air pollution in urban atmospheres in both developed and developing countries include fuel combustion for energy production (e.g., power plants, steam generation, household and commercial boilers/heaters), industrial processes (e.g., oil refinery), solvent utilization, gasoline or diesel powered vehicles, and fugitive dust (for PM only). In addition, the pollutants generated outside a city can be transported, along with secondary pollutants that are formed via photochemical reactions, contributing to the complex mixture of urban air pollution. Here we describe some unique air pollution sources in developing countries.

13.4.1 Industrial Emissions Especially from Coal Combustion

Industries emit air pollutants in every country, but more developed countries have much more stringent standards in regards to point source emission control. For instance, the EU has experienced significant reductions in industrial air pollutants as a result of its Large Combustion Plant (LCP) Directive, implemented in 2001, which required that new plants follow strict standards and that old plants exhibit significant reductions in criteria pollutants by 2008 (Commission 2014). From 2007 to 2009, LCPs reduced emissions of SO_2 by 44 %, NO_X by 27 %, and dust by 44 % (Grebot et al. 2012). Nearly all member nations showed negative trends for these pollutants, with the exceptions of Romania, Sweden, and Slovakia for SO_2; Greece, Hungary, and Sweden for NO_X; and Cyprus, Hungary, Lithuania, and Latvia for dust. In terms of meeting emissions ceilings, compliance was exceedingly good, with only Estonia's and Romania's SO_2 emissions as well as Bulgaria's SO_2, NO_X, and dust levels exceeding their limits. Even with a number of developing countries in its member nations, the EU was able to successfully curb industrial emissions with good compliance rates using well-enforced legislation. Many developing countries suffer much higher air pollution due to a lack of enforcement of emission standard laws.

In more developed industrialized nations, policies requiring flue gas scrubbers and other technologies have been instrumental in reducing industrial sources of air pollution. The common technologies used to clean flue gases include electrostatic precipitators and fabric filters for reducing PM; flue-gas desulfurization (FGD) for reducing SO_2; flue-gas denitrification for reducing NO_X through selective catalytic reduction (SCR) and selective non-catalytic reduction (SNCR); and wet and dry

scrubbing, absorbers, flue gas recirculation, etc. for a variety of air pollutants. China has adopted most of these technologies to varying extents, including circulating fluidized beds (CFB), in which a gas or fluid is passed through a high kinetic energy solid-fluid mixture to increase gas/fluid-solid contact, to increase coal combustion efficiency and reduce emissions (Chen and Xu 2010). In addition, they have extensive FGD system use in coal-fired plants due to the 11th Five Year Plan, which has helped to reduce SO_2 emissions from the 2005 peak though enforcement and implementation across provinces is still an issue. However, the relatively high operating costs of NO_X removal systems such as SNCR/SCR, which inject urea or ammonia into the flue gas to reduce NO_X into N_2, have impeded its large scale installation in China until the 12th Five Year Plan (2011–2015) required that all new thermal power plants be fitted and old, large plants be retrofitted with SCR/SNCR (Chen and Xu 2010; Zhao et al. 2013). Though China has issued laws requiring the use of these technologies, enforcement appears to be too relaxed given the number of plants and factories above emission standards.

The severe, prolonged smog episodes in Northern China have major inputs from industrial plants in Hebei and Shandong Province as well as other northern and central provinces. Unprecedented transparency in air pollutant monitoring results ordered by the Ministry of Environmental Protection (MEP) in July 2013 revealed that several large scale steel factories and thermal power plants were consistently breaching discharge standards. From October to December 2013, a comparison of eight major pollution sources each in Hebei and Shandong showed that NO_X emissions were 30 and 37 times greater, respectively, than eight major sources in Beijing (Ma et al. 2014). This contributed to Hebei and Shandong being the No. 1 and No. 2 greatest emitters of NO_X of any Chinese province in 2012 (MEP 2013b). Industrial emissions accounted for 84–91 % of total national SO_2 emissions for China in 2011, and the industries monitored in these provinces also showed a high frequency of exceeding SO_2 standards. During a month and a half period in fall 2013, 13 cities in Shandong had a 24-h moving average air quality index over 200, and many industries in those cities were exceeding NO_X and SO_2 emission standards every hour during the worst days (Ma et al. 2014). Shandong has already implemented more stringent emission standards, but most companies will require significant measures to bring their emissions down. Thermal power and cement production facility emission standards are lacking in Hebei, and so are municipal standards in other major polluting provinces, such as Jiangsu, Zhejiang, and Liaoning. The use of coal to power industrial and power plants is a major source of many air pollutants. Coal use is extremely high in the Yangtze River Delta region (Jiangsu/Zhejiang/Shanghai, 1.2 billion metric tons in 2011), with the industrial sector being the greatest consumer (39 %). This region consumes more coal than the entire U.S. (807 million metric tons in 2012 (USEIA 2014)), which contributed to it being the area of China with the greatest discharge intensity (tons per km^2 land area) for SO_2, NO_X, and VOCs (Ma et al. 2014). Though China has adopted some technologies to clean flue gas on a case by case basis, it lacks widespread legislation and enforcement measures to ensure these technologies are used across the country. Issues with power plant and factory emissions is not a problem unique to China, but it provides an extreme

example of how a lack of regulation enforcement can contribute significantly to ambient pollution. Other less industrialized developing countries also lack legislation concerning industrial emissions.

13.4.2 Open Burning

The widespread burning of trash and other waste can be a major contributor to ambient air pollution, particularly on a local scale. Garbage burning releases particulate matter, carbon monoxide, toxic organics from plastics, toxic metals, PAHs, and to a lesser extent SO_X and NO_X. Many developing countries lack regulations or sufficient enforcement concerning garbage disposal or other forms of open burning. Open burning is defined as combustion that releases emissions directly into the open air without passing through an adequate chimney or duct. This is an issue that affects both rural and urban settings in a local sense, but the health burden of this practice is especially severe and wide reaching in crowded urban settings where more people are exposed to these emissions. However, no data are available on the quantitative assessment of the health impact from such unique sources.

Open burning exists even where it is illegal when laws are not sufficiently enforced. This practice is illegal in Mumbai, but about 2 % of the solid waste generated in each of the city's wards is disposed of in this fashion (NEERI 2010). Open burning in Mumbai accounts for 8.7 % of all annual combustion-related emissions of PM, CO, NO_X, SO_2, and hydrocarbons (HC). More specifically, it accounts for 23.9 %, 15.7 %, and 26.1 % of all annual combustion-related PM, CO, and HC emissions, respectively. This open burning includes the combustion of municipal waste, auto parts, wood refuse, small-scale industrial waste, and leaves. One of the more distinctive components of garbage is plastic materials, which give off some unique compounds when combusted. Plastic combustion mainly produces non-specific even-carbon-chain alkanes, terephthalic acid, phthalates, and 4-hydroxybenzoic acid. Minor products include PAHs (in particular triphenylbenzenes) and tris(2,4-di-tert-butylphenyl)-phosphate, and air sampling in Chile suggested that 1,3,5-triphenylbenzene and tris(2,4-di-tert-butylphenyl)-phosphate could be used as unique markers of plastic burning (Simoneit et al. 2005).

These trash-burning operations can be a significant source of fine particulate matter in certain areas. For example, a study in Accra, Ghana found that an increase of 5 open trash burning spots per hectare was associated with a 27 % increase in local $PM_{2.5}$ concentrations (Rooney et al. 2012). Garbage burning in the home in Accra was also associated with a 195 % increased risk of low birth weight (Amegah et al. 2012). There is not only an increase in fine particulate matter surrounding the burning sites, but also an increase for the entire urban environment. An analysis of monitoring stations around Hyderabad, India found that waste burning accounted for 12 % of $PM_{2.5}$ in the summer and winter (7 % during rain), and 4–6 % of PM_{10} depending on weather and season conditions (Guttikunda et al. 2013). A study of the Mexico City Metropolitan Area found that there were about 25 tons of primary

organic aerosols (POAs) emitted in the area each day as a result of trash burning, which is a similar amount to fossil fuel POA emissions for that city (Hodzic et al. 2012). The authors concluded that reducing or eliminating trash burning could reduce ambient POA concentrations by 2–40 % and $PM_{2.5}$ concentrations by 1–15 % depending on the location within the city.

In contrast, developed countries do use burning as a means of eliminating waste, but this is usually done in incineration plants with much higher temperatures and scrubbers to remove pollutants from the emissions. These strategies lead to a much lower exposure burden for the local population. In terms of particulate matter pollution, sampling and modeling of British incineration plants found that the levels of PM_{10} immediately at the mouth of the flue, or waste gas duct, varied between 0 and 10 μg/m^3 depending on use, and ground concentrations were modelled to not exceed 0.01 μg/m^3 even under the most intense use conditions (Ashworth et al. 2013). Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are carcinogenic byproducts of combustion that can be associated with waste incineration, especially if it is performed at lower temperatures or if the flue contains electrostatic precipitators that can provide a surface that catalyzes these compounds' formation. However, commonly employed wet scrubbers in the flue are efficient at removing these pollutants (Karademir and Korucu 2013). Even workers inside of the incineration plants are exposed to metal, VOC, PM, and airborne microorganism levels well below international occupational exposure standards (Sabatini et al. 2013). The contribution of open waste burning to air pollution in developing countries is largely absent from developed countries, and this leads not only to higher levels of general combustion products such as CO, PM, PAHs, but also some unique pollutants such as chlorinated compounds.

13.4.3 Low Quality Gasoline and Diesel

Mobile sources are significant contributors to urban air pollution in megacities. Emission rates of pollutants depend on engine combustion efficiency, whether there is a catalytic converter or other pollution control device, and fuel quality. The composition of gasoline and diesel can vary significantly based on refining processes, and these variations affect the emissions produced through gasoline combustion. Various regulations in developed and developing countries have phased out harmful additives and components. However, much of the gasoline in developing countries still has not caught up with the standards of developing countries.

One of the first initiatives to improve gasoline quality was the international effort to phase out tetraethyl lead (TEL) as an anti-knocking agent in gasoline. Knocking is a process by which small pockets of the air/fuel mixture in gasoline combust outside of the normal combustion front caused by spark plug ignition, and the effects of this phenomenon were potentially destructive to early engines. TEL was not only able to reduce knocking, but also to boost octane and subsequently improve engine efficiency. The use of TEL as a gasoline additive began in the 1920s, but its

contribution to neurotoxic lead exposures and coating and inactivating of catalytic converters led to it being phased out in the U.S. beginning in the mid-1970s. Catalytic converters have been used starting in 1975 in the U.S. to catalyze redox reactions that decrease hydrocarbon, CO, and NO_X emissions, but they can become less useful if gasoline components coat the converter and prevent harmful chemicals from interacting with the catalytic surface. Japan enacted the first ban on leaded gasoline in 1986, and most countries had officially phased out TEL use by the early 2000s. Only six countries continue to use leaded gasoline today (2014): Afghanistan, Algeria, Iraq, Myanmar, North Korea, and Yemen. However, there is evidence that illegal leaded gasoline production in some developing nations such as China is being performed despite national regulations (Chung 2013). Though there has been dramatic international improvement in TEL regulation in the past decade, other potentially hazardous gasoline additives are not as controlled.

High sulfur content is another gasoline component that can increase harmful emissions through disruption of the catalytic converter and increasing SO_X emissions. Sulfur is a naturally occurring component of crude oil, and its levels vary by region. It is present in the fuel in the form of thiols, thiophenes, and disulfides. When combusted in a vehicle, SO_2 is formed, and this can adsorb onto palladium, platinum, or rhodium catalytic converters, in order of most to least sensitivity to sulfur adsorption (Truex 1999). This adsorbed sulfur both physically and electrically blocks the binding of other emission chemicals to the catalytic surface. The "dose-response" curve for fuel sulfur content and catalytic inhibition is supralinear, meaning that lower levels of SO_2 have a disproportionately high effect on inhibition. Refining processes are capable of reducing sulfur content in both gasoline and diesel fuels, resulting in a greater efficiency of the catalytic converter and a subsequent reduction in the emissions of several pollutants. The reduction of sulfur content in fuels from 450 to 50 ppm has led to reductions of 35 % for acetaldehyde, 21 % for benzene, 19 % for CO, 17 % for hydrocarbons, and 8 % for NO_X (Schuetzle et al. 1994). Though high sulfur content in fuel can lead to direct increases in SO_2 tailpipe emissions, traffic is generally a much lower source of SO_2 than energy sector or industrial emissions. SO_2 reacts with hydroxyl radicals in the air to form sulfuric acid (H_2SO_4) particles that can combine with each other in a rare process known as homogeneous nucleation. The EPA estimates that over 12 % of SO_2 emitted in the urban U.S. is converted into sulfate fine and ultrafine particulate matter, which indicates that gasoline and diesel vehicles may be responsible for eight times more than what is accounted for in direct diesel emission inventories of PM (Blumberg et al. 2003).

The most stringent sulfur standards for gasoline and diesel (below 15 ppm for diesel) tend to be in the wealthier nations, particularly those in Europe, North America, and Australia (Programme., P.f.C.F.a.V.U.N.E 2014). Moderate to high diesel sulfur standards (between 50 and 500 ppm) exist in Mexico, southern and parts of central Africa, Russia, South Asia, East Asia, and Southeast Asia. The least stringent standards (greater than 500 ppm) predominate in South America, Central Asia, the Middle East, and most of Africa. Hence, the relative contribution of fuel sulfur in gasoline and diesel to SO_2 and fine particles emissions would be higher in developing countries where fuel sulfur content is higher.

13.5 Disease Burdens Attributable to Air Pollution in Developing Countries

The disease burden attributable to air pollution is the product of population, exposure concentration, and the concentration-response relationship. Hence, large disease burdens are expected to occur in heavily polluted, populous urban areas of developing countries.

Figure 13.5 shows the relationship between regional PM_{10} concentration, gross national income per capita, and the PM_{10}-attributable disease burden in disability-

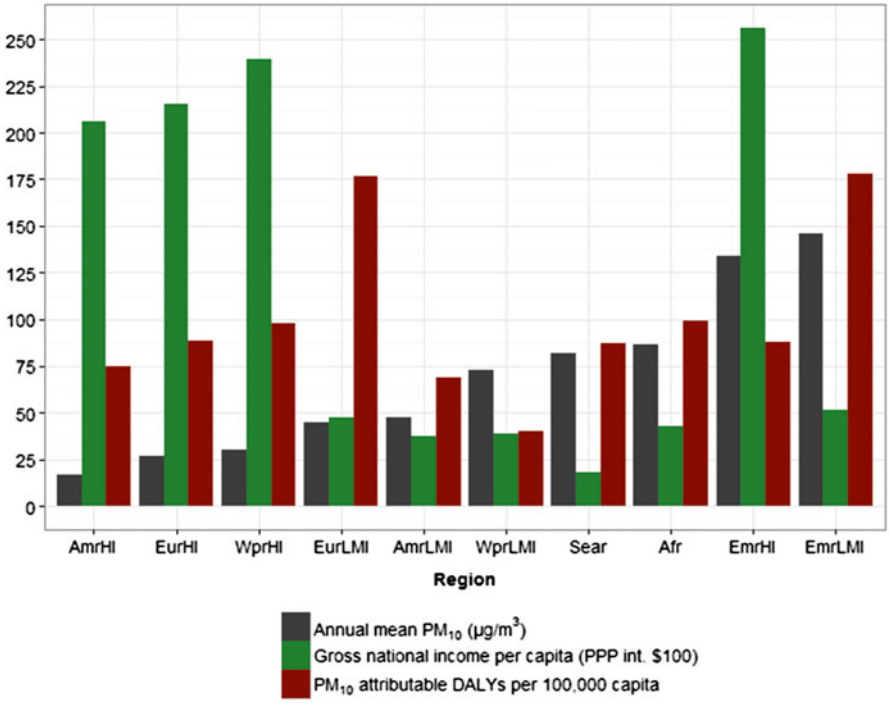

Fig. 13.5 Trends between annual mean urban PM10, GNI, and PM_{10}-attributable DALYs by region. Wealthier nations in more developed regions tend to have lower annual PM_{10} and DALYs attributable to air pollution. All data were obtained from the WHO Global Health Observatory Data Repository. The annual mean PM_{10} data was averaged over the period of 2003–2010, the regional mean GNI data is averaged over the period of 1980–2012, and the regional mean PM_{10} burden of disease data was collected in 2004. ***Amr*** Americas (includes North, Central, and South America and the Caribbean), ***Eur*** Europe (includes all of Europe, Israel, and some of Central Asia), ***Wpr*** Western Pacific (includes Pacific nations, East Asia, and eastern South-east Asia), ***Sear*** South-east Asia (includes South Asia, western South-east Asia, and some Pacific nations), ***Emr*** Eastern Mediterranean (includes the Middle East and some northern African nations), ***Afr*** Africa (includes most African nations), ***HI*** High income, ***LMI*** low-middle income (For more information on the WHO regions, please visit http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_AnnexC.pdf?ua=1)

adjusted life years (DALYs). DALYs can be defined as healthy years lost due to a disease-causing factor, and they are calculated as the sum of years of life lost (YLL) from premature mortality and years lost due to disability (YLD) from a disease or set of diseases (DALY = YLL + YLD). The WHO-defined world regions are organized by increasing annual mean PM_{10} levels, and in general all the high-income regions have the lowest PM_{10} concentrations. (The Eastern Mediterranean regions are an exception due to the contribution of sandstorms and desert dust to ambient PM_{10} levels.) The relationship between particulate matter concentrations and disease burden is less clear on the regional scale, as it is a function of air pollution levels, the size of the affected population, and the population of the WHO region. The low-middle income European region has a high disease burden despite having only moderate annual PM_{10} levels because those areas over which the PM_{10} is distributed are consistently highly populated across the region, and the relatively small population of the lower income European countries inflates the per capita DALY value. For the Western Pacific region, countries like China have high air pollution over densely populated areas, but the region also contains countries with sparse populations, such as Mongolia, and has a large total population, which dampens the effect of high air pollution there on DALYs. Generally, the high-income WHO regions tend to have smaller total populations than the low to middle income regions because there are fewer countries that meet the high income criteria, thus inflating their DALYs per capita values. Nevertheless, the global data shows that regions with lower average GNIs tend to have higher exposures to PM_{10} and higher overall burdens of disease attributable to PM_{10}. Two issues, however, arise when comparing DALYs between developing and developed countries, namely the nature of the concentration-response relationship and the influence of regional differences on air pollution composition.

13.5.1 Concentration-Response Relationship

A linear dose-response relationship (slope factor) has been applied to estimate the global burden of disease attributable to air pollution. This slope factor, however, has been largely derived from observations made in developed countries where the pollution level was substantially lower. Hence, questions arise as to whether this would have resulted in an overestimation or underestimation of the actual burden. Such questions are related to fundamentals on the shape of dose-response curves, as depicted in Fig. 13.6. The prevailing evidence, particularly in terms of PM effects on mortality, suggests that the response is linear or approximately linear across a wide range of concentrations measured in both developed and developing countries. Figure 13.7 shows that meta-analyses of multi-city studies of air pollution and mortality have found similar effect sizes of PM_{10} on mortality in all regions of the world, even though the concentration ranges differ greatly between these regions.

The studies summarized in Fig. 13.7 show an excess risk of all-cause mortality per 10 $\mu g/m^3$ increase in PM_{10} ranging from 0.27 % (95 % CI, 0.12–0.42 %) in the

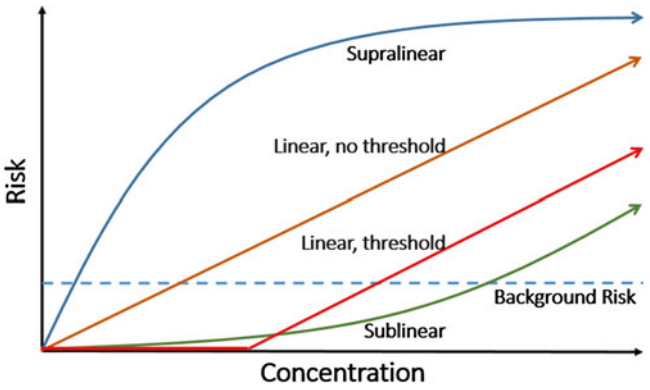

Fig. 13.6 Hypothetical shapes of concentration-response relationships

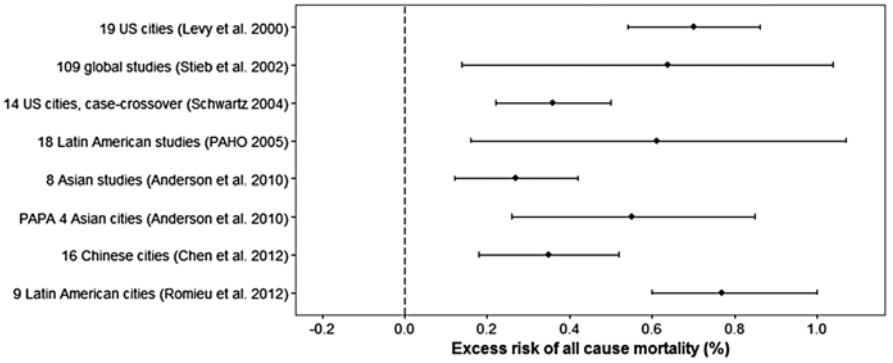

Fig. 13.7 Excess risk of mortality and 95 % confidence intervals associated with a 10 μg/m³ increase in PM_{10} concentration. There appear to be small variations between the increased all-cause mortality risk associated with unit increases in PM_{10} between countries and regions, suggesting a linear dose-response relationship between air pollutants and mortality. *Dots* represent mean increases in percent mortality risk per 10 μg/m³ increase in PM_{10} from multi-city or multi-study models. The *bars* represent 95 % confidence intervals. Any mean change in risk with confidence interval *bars* not overlapping the *dashed zero line* represents a significant change. This figure is based on a similar one in (Anderson et al. 2010) (Sources: Levy et al. 2000; Stieb et al. 2002; Schwartz 2004; PAHO 2005; Anderson et al. 2010; Chen et al. 2012; Romieu et al. 2012)

8 Asian studies reviewed by Anderson et al. to 0.77 % (95 % CI, 0.6–1 %) in the 2012 Latin American nine-city study conducted by Romieu et al. The variation in these estimates is not apparently correlated with PM_{10} levels, as similar estimates were reported in two studies that were conducted in areas with several-fold differences in PM_{10} (Schwartz 2004; Chen et al. 2012). Considering likely contributions of inter-population variability and differences in the statistical methods to the variations in the concentration-response relationship between different studies, the estimates are remarkably similar, suggesting that the dose-response relationship between PM_{10} exposure and mortality is fairly constant across air pollution levels.

However, from a toxicological standpoint, differences in PM_{10} chemical composition are expected to cause differences in PM toxicity. This is supported by limited epidemiological evidence. For example, $PM_{2.5}$ rich in secondary species, such as sulfate and nitrate, and certain organic compounds has been associated with greater effects on mortality. Specifically, it was found that transmural myocardial infarctions (MIs), blockages of major coronary arteries, were more associated with $PM_{2.5}$ that was composed mostly of nitrate, sulfate, and ammonium and had the least elemental carbon (Rich et al. 2013). The fact that similar PM_{10}-mortality relationships (effect size) were observed as shown in Fig. 13.7 may not reflect PM_{10} toxicity or effects. In these studies, PM_{10} concentrations may have simply served as a surrogate for exposure to the whole pollution mixture.

13.5.2 Composition of Pollution Mixture

Another issue that needs to be considered in understanding the health effects of air pollution in developing countries is the difference in the pollution mixture composition compared to that in developed countries. In most areas of developed countries, concentrations of sulfur dioxide and carbon monoxide, for example, are low and potentially below a threshold level for health effects. This is perhaps a major reason that PM has often been used in epidemiological studies and has been regarded as the outdoor air pollutant most relevant to health in estimating risk (as done in the global burden of disease assessment). In contrast, the gaseous pollutants in areas of developing countries are still high enough to cause significant health problems, or they may be a more accurate surrogate for certain air pollution sources (e.g., SO_2 for coal and high-sulfur oil combustion) than PM_{10} or $PM_{2.5}$. For example, ambient SO_2 concentrations in Chinese cities have been associated with various adverse health effects, such as pathophysiological biomarkers of adverse cardiopulmonary events (Huang et al. 2012b; Rich et al. 2012), respiratory symptoms and lung function (Roy et al. 2012; Zhang et al. 2002), and low birth weight (Wang et al. 1997). The associations were stronger with SO_2 than $PM_{2.5}$ or PM_{10} in some of these studies.

13.6 Historical Lessons and Recommendations

Since the Industrial Revolution, urban air pollution has been an incessant problem. Local air quality became so severe in the early and mid-twentieth century that industrialized countries such as the U.K. and the U.S. had to establish the Clean Air Act or similar laws to protect the public from the harmful impact of air pollution. Pollution control technologies have been developed and advanced consistently to meet the increasingly stringent air quality standards. For example, although gross domestic product (GDP) and vehicle miles traveled increased by 133 % and 92 %, respectively, from 1980 (10 years after the US Clean Air Act was passed) to 2012 in

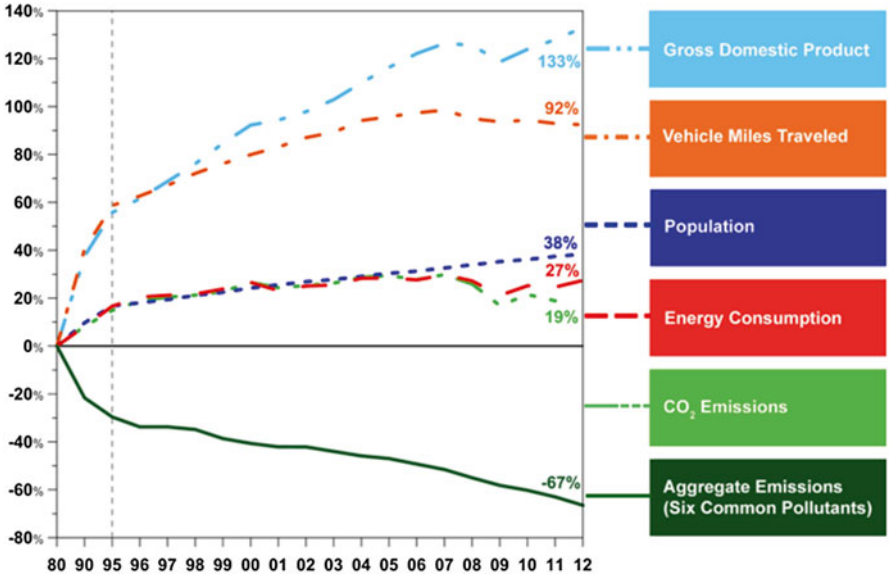

Fig. 13.8 The 1970 Clean Air Act lowered emissions of key air pollutants even as energy consumption, GDP, vehicle miles, and population increased (This graph was adapted directly from the US EPA website USEPA 2014c)

the U.S., the aggregate emissions of the six criteria pollutants decreased by 67 % in the same period (see Fig. 13.8). A recent analysis of the relationship between reductions in ambient $PM_{2.5}$ concentrations and increases in life expectancy in U.S. cities suggests that the 1970 Clean Air Act alone may have extended life expectancy by a half year to a year (Pope et al. 2009). The additional measures of the 1990 Clean Air Act Amendment have and will continue to reduce a number of disease burdens, as is shown in Table 13.1. The formula for this kind of success may be simply described as:

Legislation + Technology + Enforcement = Clean Air

On the contrary, air quality in some developing countries such as China has actually become worse long after the establishment of national air quality standards (China's first clean air law was passed in 1989). The key issue, hence, is not the lack of laws or regulations, but the effectiveness in enforcing the laws, pointing to the importance of "Enforcement" in the clean air success formula. Today in places like China, air pollution problems are so severe and widespread that they consistently become major topics in the press. During severe smog episodes schools are closed, people are advised to stay indoors, and people use air filters or purifiers and wear dust masks. There is strong public support to combat the air pollution. The timing for the above success formula is ideal, as developing countries do not have to go through the long history during which developed countries learned about various aspects of air pollution (e.g., sources, fate and transport, and health effects). Most importantly, developing countries have opportunities to utilize the most advanced

Table 13.1 The 1990 Clean Air Act Amendment improved a number of health outcomes associated with $PM_{2.5}$ and ozone

Health effect reductions (PM2.5 & Ozone only)	Pollutant(s)	Year 2010	Year 2020
PM2.5 adult mortality	PM	160,000	230,000
PM2.5 infant mortality	PM	230	280
Ozone mortality	Ozone	4,300	7,100
Chronic bronchitis	PM	54,000	75,000
Acute bronchitis	PM	130,000	180,000
Acute myocardial infarction	PM	130,000	200,000
Asthma exacerbation	PM	1,700,000	2,400,000
Hospital admissions	PM, ozone	86,000	135,000
Emergency room visits	PM, ozone	86,000	120,000
Restricted activity days	PM, ozone	84,000,000	110,000,000
School loss days	Ozone	3,200,000	5,400,000
Lost work days	PM	13,000,000	17,000,000

Numbers are presented as number of cases avoided. This table was adapted directly from (USEPA 2011)

emission control technologies that were nonexistent when developed countries experienced their worst air pollution problems.

References

Ahmad SS, Aziz N (2013) Spatial and temporal analysis of ground level ozone and nitrogen dioxide concentration across the twin cities of Pakistan. Environ Monit Assess 185(4):3133–3147

Amegah AK, Jaakkola JJ, Quansah R, Norgbe GK, Dzodzomenyo M (2012) Cooking fuel choices and garbage burning practices as determinants of birth weight: a cross-sectional study in Accra Ghana. Environ Health 11:78

Anderson HR, Atkinson R, Balbus J, Brauer M, Chapman R, Chowdhury Z, Cohen A, Demerjian KL, Ebi K, Favarato G, Greenbaum DS, Mehta S, North T, O'Keefe RM, Pandey KD, Pope CA III, Smith KR, Speizer F, Walsh M, Zhang J (2010) Outdoor air pollution and health in the developing countries of Asia: a comprehensive review. Health Effects Institute, Boston

Ashworth DC, Fuller GW, Toledano MB, Font A, Elliott P, Hansell AL, de Hoogh K (2013) Comparative assessment of particulate air pollution exposure from municipal solid waste incinerator emissions. J Environ Public Health 2013:560342

Barletta B, Meinardi S, Simpson IJ, Khwaja HA, Blake DR, Rowland FS (2002) Mixing ratios of volatile organic compounds (VOCs) in the atmosphere of Karachi Pakistan. Atmos Environ 36(21):3429–3443

Blumberg KO, Walsh MP, Pera C (2003) Low-sulfur gasoline & diesel: the key to lower vehicle emissions. Available online [April 24] at: http://www.theicct.org/documents/Low-Sulfur_Exec_Summ_ICCT_2003.pdf

Board, N.C.R.P (2013) Draft revised regional plan 2021: national capital region. Ministry of Urban Development, Government of India, New Delhi

CAI-Asia (2010) Air quality in Asia: status and trends 2010 edition. Clean Air Initiative for Asian Cities Center, Pasig City

Chen WY, Xu RN (2010) Clean coal technology development in China. Energy Policy 38(5):2123–2130
Chen R, Kan H, Chen B, Huang W, Bai Z, Song G, Pan G, Group CC (2012) Association of particulate air pollution with daily mortality: the China Air Pollution and Health Effects Study. Am J Epidemiol 175(11):1173–1181
Chung K (2013) TEL for MOGAS manufacture in China – LEAD project final report. The Lead Education and Abatement Design Group, Inc
Commission E (2014) Large combustion plants directive. Available from: http://ec.europa.eu/environment/air/pollutants/stationary/lcp/legislation.htm. 4 Nov 2014
EEA (2010) Change in CO emissions 1990–2010 (EEA member countries). Available from: http://www.eea.europa.eu/data-and-maps/figures/change-in-co-emissions-1
EPB-Beijing (2013) 2012北京市环境状况公报 2012 Beijingshi Huanjing Zhuangkuang Gongbao [Beijing environmental statement 2012]. Beijing Environmental Protection Bureau
EPB-Shanghai (2013) 2012上海市环境状况公报 2012 Shanghaishi Huanjing Zhuangkuang Gongbao [Shanghai environmental bulletin 2012]. Shanghai Environmental Protection Bureau
Graber LK, Asher D, Anandaraja N, Bopp RF, Merrill K, Cullen MR, Luboga S, Trasande L (2010) Childhood lead exposure after the phaseout of leaded gasoline: an ecological study of school-age children in Kampala, Uganda. Environ Health Perspect 118(6):884–889
Grebot B, Hill J, Lee J, Scarborough T (2012) Analysis and summary of the Member States' emission inventories 2007–2009 and related information under the LCP Directive (2001/80/EC): final report. European Commission
Guttikunda SK (2009) Photochemistry of air pollution in Delhi, India: a monitoring based analysis, in SIM-air working paper series: 25–2009. Simple Interactive Models for Better Air Quality
Guttikunda SK, Kopakka RV, Dasari P, Gertler AW (2013) Receptor model-based source apportionment of particulate pollution in Hyderabad, India. Environ Monit Assess 185(7):5585–5593
Hasunuma H, Ishimaru Y, Yoda Y, Shima M (2014) Decline of ambient air pollution levels due to measures to control automobile emissions and effects on the prevalence of respiratory and allergic disorders among children in Japan. Environ Res 131:111–118
He K, Wang S, Zhang J (2009) Blood lead levels of children and its trend in China. Sci Total Environ 407(13):3986–3993
He H, Wang Y, Ma Q, Ma J, Chu B, Ji D, Tang G, Liu C, Zhang H, Hao J (2014) Mineral dust and NOx promote the conversion of SO2 to sulfate in heavy pollution days. Sci Rep 4:4172
Henderson V (2002) Urbanization in developing countries. World Bank Res Obs 17:89–112
Heritage, D.o.t.E.a. (2005) Lead (Pb): air quality fact sheet
Hodzic A, Wiedinmyer C, Salcedo D, Jimenez JL (2012) Impact of trash burning on air quality in Mexico City. Environ Sci Technol 46(9):4950–4957
Huang K, Zhuang G, Lin Y, Fu JS, Wang Q, Liu T, Zhang R, Jiang Y, Deng C, Fu Q, Hsu NC, Cao B (2012a) Typical types and formation mechanisms of haze in an Eastern Asia megacity Shanghai. Atmos Chem Phys 12(1):105–124
Huang W, Wang G, Lu SE, Kipen H, Wang Y, Hu M, Lin W, Rich D, Ohman-Strickland P, Diehl SR, Zhu P, Tong J, Gong J, Zhu T, Zhang J (2012b) Inflammatory and oxidative stress responses of healthy young adults to changes in air quality during the Beijing Olympics. Am J Respir Crit Care Med 186(11):1150–1159
Hwang SS, Kang S, Lee JY, Lee JS, Kim HJ, Han SK, Yim JJ (2014) Impact of outdoor air pollution on the incidence of tuberculosis in the Seoul metropolitan area South Korea. Korean J Intern Med 29(2):183–190
Jones RL, Homa DM, Meyer PA, Brody DJ, Caldwell KL, Pirkle JL, Brown MJ (2009) Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988–2004. Pediatrics 123(3):e376–e385
Karademir A, Korucu MK (2013) Assessment of emissions and removal of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) at start-up periods in a hazardous waste incinerator. J Air Waste Manag Assoc 63(7):788–795

Klimont Z, Smith SJ, Cofala J (2013) The last decade of global anthropogenic sulfur dioxide: 2000–2011 emissions. Environ Res Lett 8(1):1–6

Levy JI, Hammitt JK, Spengler JD (2000) Estimating the mortality impacts of particulate matter: what can be learned from between-study variability? Environ Health Perspect 108(2):109–117

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, Atkinson C, Bacchus LJ, Bahalim AN, Balakrishnan K, Balmes J, Barker-Collo S, Baxter A, Bell ML, Blore JD, Blyth F, Bonner C, Borges G, Bourne R, Boussinesq M, Brauer M, Brooks P, Bruce NG, Brunekreef B, Bryan-Hancock C, Bucello C, Buchbinder R, Bull F, Burnett RT, Byers TE, Calabria B, Carapetis J, Carnahan E, Chafe Z, Charlson F, Chen H, Chen JS, Cheng AT, Child JC, Cohen A, Colson KE, Cowie BC, Darby S, Darling S, Davis A, Degenhardt L, Dentener F, Des Jarlais DC, Devries K, Dherani M, Ding EL, Dorsey ER, Driscoll T, Edmond K, Ali SE, Engell RE, Erwin PJ, Fahimi S, Falder G, Farzadfar F, Ferrari A, Finucane MM, Flaxman S, Fowkes FG, Freedman G, Freeman MK, Gakidou E, Ghosh S, Giovannucci E, Gmel G, Graham K, Grainger R, Grant B, Gunnell D, Gutierrez HR, Hall W, Hoek HW, Hogan A, Hosgood HD, Hoy D, Hu H, Hubbell BJ, Hutchings SJ, Ibeanusi SE, Jacklyn GL, Jasrasaria R, Jonas JB, Kan H, Kanis JA, Kassebaum N, Kawakami N, Khang YH, Khatibzadeh S, Khoo JP, Kok C, Laden F, Lalloo R, Lan Q, Lathlean T, Leasher JL, Leigh J, Li Y, Lin JK, Lipshultz SE, London S, Lozano R, Lu Y, Mak J, Malekzadeh R, Mallinger L, Marcenes W, March L, Marks R, Martin R, McGale P, McGrath J, Mehta S, Mensah GA, Merriman TR, Micha R, Michaud C, Mishra V, Mohd Hanafiah K, Mokdad AA, Morawska L, Mozaffarian D, Murphy T, Naghavi M, Neal B, Nelson PK, Nolla JM, Norman R, Olives C, Omer SB, Orchard J, Osborne R, Ostro B, Page A, Pandey KD, Parry CD, Passmore E, Patra J, Pearce N, Pelizzari PM, Petzold M, Phillips MR, Pope D, Pope CA, Powles J, Rao M, Razavi H, Rehfuess EA, Rehm JT, Ritz B, Rivara FP, Roberts T, Robinson C, Rodriguez-Portales JA, Romieu I, Room R, Rosenfeld LC, Roy A, Rushton L, Salomon JA, Sampson U, Sanchez-Riera L, Sanman E, Sapkota A, Seedat S, Shi P, Shield K, Shivakoti R, Singh GM, Sleet DA, Smith E, Smith KR, Stapelberg NJ, Steenland K, Stockl H, Stovner LJ, Straif K, Straney L, Thurston GD, Tran JH, Van Dingenen R, van Donkelaar A, Veerman JL, Vijayakumar L, Weintraub R, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams W, Wilson N, Woolf AD, Yip P, Zielinski JM, Lopez AD, Murray CJ, Ezzati M, AlMazroa MA, Memish ZA (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380(9859):2224–2260

Ma J, Gu B, He J, Shen S, Liu J, Sun Z, Yan Y, Yi Z, Lu X, Guo Y, Chang Z, Wang J, Song G, He W, Chen J, Xi T (2014) Blue sky roadmap report II: real-time disclosure begins. The Institute of Public and Environmental Affairs, Renmin University of China Institute of Environmental Policy and Planning, Society of Entrepreneurs and Ecology, Friends of Nature, Envirofriends, and Nature University Beijing, PRC

Mathee A, Rollin H, von Schirnding Y, Levin J, Naik I (2006) Reductions in blood lead levels among school children following the introduction of unleaded petrol in South Africa. Environ Res 100(3):319–322

MEP (2013a) 2012年中国环境状况公报:大气环境 2012 Nian Zhongguo Huanjing Zhuangkuang Gongbao: Daqi Huanjing [2012 Chinese environmental condition report: air environment]. Available from: http://jcs.mep.gov.cn/hjzl/zkgb/2012zkgb/201306/t20130606_253402.htm. 6 June 2013

MEP (2013b) 2012年环境统计年报:废气 2012 Nian Huanjing Tongji Nianbao: Feiqi [2012 annual environment statistical report: air emissions]. Ministry of Environmental Protection, People's Republic of China

NEERI (2010) Air quality assessment, emissions inventory and source apportionment studies: Mumbai. National Environmental Engineering Research Institute, New Delhi

Olajire AA, Azeez L, Oluyemi EA (2011) Exposure to hazardous air pollutants along Oba Akran road, Lagos-Nigeria. Chemosphere 84(8):1044–1051

PAHO (2005) An assessment of health effects of ambient air pollution in Latin America and the Caribbean. Pan American Health Organization (PAHO), Santiago

Ponka A, Salminen E, Ahonen S (1993) Lead in the ambient air and blood specimens of children in Helsinki. Sci Total Environ 138(1–3):301–308

Pope CA, Ezzati M, Dockery DW (2009) Fine-particulate air pollution and life expectancy in the United States. N Engl J Med 360(4):376–386

Programme., P.f.C.F.a.V.U.N.E. (2014) Diesel fuel sulfur levels: global status April 2014. Global Clean Fuels and Vehicles Database

Rich DQ, Kipen HM, Huang W, Wang G, Wang Y, Zhu P, Ohman-Strickland P, Hu M, Philipp C, Diehl SR, Lu SE, Tong J, Gong J, Thomas D, Zhu T, Zhang JJ (2012) Association between changes in air pollution levels during the Beijing Olympics and biomarkers of inflammation and thrombosis in healthy young adults. JAMA 307(19):2068–2078

Rich DQ, Ozkaynak H, Crooks J, Baxter L, Burke J, Ohman-Strickland P, Thevenet-Morrison K, Kipen HM, Zhang J, Kostis JB, Lunden M, Hodas N, Turpin BJ (2013) The triggering of myocardial infarction by fine particles is enhanced when particles are enriched in secondary species. Environ Sci Technol 47(16):9414–9423

Romieu I, Gouveia N, Cifuentes LA, de Leon AP, Junger W, Vera J, Strappa V, Hurtado-Diaz M, Miranda-Soberanis V, Rojas-Bracho L, Carbajal-Arroyo L, Tzintzun-Cervantes G, H.E.I.H.R. Committee (2012) Multicity study of air pollution and mortality in Latin America (the ESCALA study). Res Rep Health Eff Inst 171:5–86

Rooney MS, Arku RE, Dionisio KL, Paciorek C, Friedman AB, Carmichael H, Zhou Z, Hughes AF, Vallarino J, Agyei-Mensah S, Spengler JD, Ezzati M (2012) Spatial and temporal patterns of particulate matter sources and pollution in four communities in Accra, Ghana. Sci Total Environ 435–436:107–114

Roy A, Hu W, Wei F, Korn L, Chapman RS, Zhang JJ (2012) Ambient particulate matter and lung function growth in Chinese children. Epidemiology 23(3):464–472

Sabatini L, Pianetti A, Cecchetti G, Bruner A, Citterio B, Barbieri F, Bruscolini F (2013) Chemical and microbiological monitoring of air in two waste incineration plants. Ig Sanita Pubbl 69(1):13–37

Saini R, Masih A, Satsangi GS, Taneja A (2005) Surface levels of ozone and its precursor NO2 during winter season at urban and rural sites of a semi arid region in India. In: The atmospheric sciences and air quality conference. American Meteorological Society, San Francisco

Schuetzle D, Siegl WO, Jensen TE, Dearth MA, Kaiser EW, Gorse R, Kreucher W, Kulik E (1994) The relationship between gasoline composition and vehicle hydrocarbon emissions: a review of current studies and future research needs. Environ Health Perspect 102(Suppl 4):3

Schwartz J (2004) The effects of particulate air pollution on daily deaths: a multi-city case crossover analysis. Occup Environ Med 61(12):956–961

Simoneit BRT, Medeiros PM, Didyk BM (2005) Combustion products of plastics as indicators for refuse burning in the atmosphere. Environ Sci Technol 39(18):6961–6970

Stieb DM, Judek S, Burnett RT (2002) Meta-analysis of time-series studies of air pollution and mortality: effects of gases and particles and the influence of cause of death, age, and season. J Air Waste Manag Assoc 52(4):470–484

Truex TJ (1999) Interaction of sulfur with automotive catalysts and the impact on vehicle emissions – a review. Proceedings of the society of automotive engineers, SAE Paper 1999-01-1543, Detroit

Tuakuila J, Kabamba M, Mata H, Mata G (2013a) Blood lead levels in children after phase-out of leaded gasoline in Kinshasa, the capital of Democratic Republic of Congo (DRC). Arch Public Health 71(1):5

Tuakuila J, Lison D, Mbuyi F, Haufroid V, Hoet P (2013b) Elevated blood lead levels and sources of exposure in the population of Kinshasa, the capital of the Democratic Republic of Congo. J Expo Sci Environ Epidemiol 23(1):81–87

UNDESA (2011) On-line data: urban agglomerations, population of urban agglomerations with 750 000 or more in 2011. Available from: http://esa.un.org/unpd/wup/unup/index_panel2.html

UNEP (2010) Final review of scientific information on lead. United Nations Environment Programme. Chemicals Branch, DTIE, Geneva, Switzerland
USEIA (2014) Short-term energy and summer fuels outlook. US Energy Information Administration, Washington, DC
USEPA (2011) The benefits and costs of the Clean Air Act from 1990–2020. US Environmental Protection Agency, Washington, DC
USEPA (2012) 2008 national emissions inventory, version 2. Technical support document. U.S. Environmental protection agency. Office of air quality planning and standards. Air quality assessment division. Emissions Inventory and Analysis Group, Research Triangle Park
USEPA (2013a) Air trends: sulfur dioxide. Available from: http://www.epa.gov/air/airtrends/sulfur.html. 3 Sept 2013
USEPA (2013b) Air trends: nitrogen dioxide. Available from: http://www.epa.gov/airtrends/nitrogen.html. 3 Sept 2013
USEPA (2014a) Air emission sources: nitrogen oxides. Available from: http://www.epa.gov/cgi-bin/broker?_service=data&_debug=0&_program=dataprog.national_1.sas&polchoice=NOX 22 Apr 2014
USEPA (2014b) Air emission sources: carbon monoxide. Available from: http://www.epa.gov/cgi-bin/broker?_service=data&_debug=0&_program=dataprog.national_1.sas&polchoice=CO. 24 Apr 2014
USEPA (2014c) Air trends: air quality trends. Available from: http://www.epa.gov/airtrends/aqtrends.html. 21 Apr 2014
von Schneidemesser E, Monks PS, Plass-Duelmer C (2010) Global comparison of VOC and CO observations in urban areas. Atmos Environ 44(39):5053–5064
Wang X, Ding H, Ryan L, Xu X (1997) Association between air pollution and low birth weight: a community-based study. Environ Health Perspect 105(5):514
Wang S, Xing J, Zhao B, Jang C, Hao J (2014) Effectiveness of national air pollution control policies on the air quality in metropolitan areas of China. J Environ Sci (China) 26(1):13–22
WHO (2000) Air quality guidelines for Europe. In: WHO regional publications, European series, no. 91. World Health Organization Regional Office for Europe, Copenhagen
WHO (2006) Air quality guidelines: global update 2005. Particulate matter, ozone, nitrogen dioxide, and sulfur dioxide. World Health Organization
Zhang JJ, Hu W, Wei F, Wu G, Korn LR, Chapman RS (2002) Children's respiratory morbidity prevalence in relation to air pollution in four Chinese cities. Environ Health Perspect 110(9):961–967
Zhao B, Wang SX, Liu H, Xu JY, Fu K, Klimont Z, Hao JM, He KB, Cofala J, Amann M (2013) NOx emissions in China: historical trends and future perspectives. Atmos Chem Phys 13(19):9869–9897

Chapter 14
Indoor Biomass Burning and Health Consequences

John R. Balmes

14.1 Introduction

Inefficient cooking with biomass fuels in poorly ventilated homes is a major source of exposure to indoor air pollution in the developing world. The levels of exposure to particulate matter (PM) from biomass smoke in such homes are often at least an order of magnitude higher than the highest concentrations that occur in the ambient air of the developed world (Bruce et al. 2000; Diette et al. 2012). Household air pollution from cooking and heating with biomass fuels also is an important contributor to outdoor air pollution, accounting for an estimated 10 % of ambient fine PM ($PM_{2.5}$) (Smith et al. 2014).

Biomass fuel refers to any recently living plant- and/or animal-based material that is deliberately burned by humans as fuel, including wood, crop residues, and animal dung (Bruce et al. 2000). The number of people reliant on biomass fuels is projected to increase to 2.6 billion by 2030 (Smith et al. 2013). Most of these people live in rural areas of lesser-developed countries (LDCs), where some four-fifths of households rely on biomass fuels as their major or only source of domestic energy for cooking and sometimes space heating (World Health Organization 2009).

Cooking with biomass fuels is generally done on unvented stoves typically consisting of such simple arrangements as three rocks, a U-shaped hole in a block of clay, or a pit in the ground (Smith et al. 2013). Combustion under such conditions is inefficient and therefore incomplete products of combustion are generated. This typically leads to extremely high pollutant concentrations in the vicinity of the

J.R. Balmes, M.D. (✉)
Department of Medicine, Division of Occupational and Environmental Medicine, University of California, San Francisco, CA, USA

Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, Berkeley, CA, USA
e-mail: john.balmes@ucsf.edu

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_14

stove. Pollutant concentrations are further exacerbated by the lack of ventilation that characterizes many of the kitchens in rural areas of developing countries.

The high levels of smoke from cooking indoors with biomass fuels commonly contain up to 1,000 μg/m^3 particulate matter with a diameter of 5 μm or less (PM_5) and much higher values have been reported (Smith et al. 2013; Balakrishnan et al. 2013). These concentrations are orders of magnitude higher than either the U.S. Environmental Protection Agency national ambient air quality standard for PM10 or the World Health Organization (WHO) guideline. In addition to PM, carbon monoxide (CO), nitrogen oxides, formaldehyde, and a number of toxic organic compounds [e.g., benzene; 1,3 butadiene; benzo[α]pyrene and other polycyclic aromatic hydrocarbons (PAHs)] are present in biomass smoke, depending on the type of fuel that is burned (Warwick and Doig 2004). Readers are referred to Chap. 6 for details on potential carcinogenic effects of these constituents. The combustion of wood and other biomass is qualitatively similar to the burning of tobacco in terms of emissions of PM and gases, although without the nicotine.

In rural areas of lesser developed countries, women often spend many hours a day cooking so duration of exposure to biomass smoke is often considerable. The potential for exposure is further increased by common cultural practices. Infants and toddlers are often carried on the mother's back while she cooks so that from early infancy children spend hours breathing smoke from cooking or heating fires (Armstrong and Campbell 2009). In temperate climates and highland areas, people spend more time indoors to protect themselves from the cold, and the cold temperatures that characterize these areas require fires that burn over extended periods and tighter house construction (i.e., less ventilation) for space heating (Smith et al. 2013). Thus, both pollution levels and exposure times increase.

The public health impact of the relatively high exposures to PM in homes where cooking is done with inefficient, poorly ventilated stoves using biomass fuels is great. The latest version of the Global Burden of Disease comparative risk assessment undertaken in 2010 found household air pollution from solid fuel use to be responsible for approximately 4.8 % of the total global burden of disease disability-adjusted life years and 3.9 million premature deaths per year (Lim et al. 2012; Smith et al. 2014). Household air pollution ranked third on the examined list of risk factors globally and was the most important environmental risk factor. The estimates are based on the strength of the evidence, primarily meta-analyses of epidemiological studies of acceptable scientific quality, although for cardiovascular disease the evidence is more inferential (see Sect. 14.8). The greatest burden of household air pollution-related premature deaths is in children with pneumonia exposed to biomass smoke. The greatest burden in adults is for cardiovascular disease, but for non-smoking women, chronic obstructive pulmonary disease (COPD) is also an important cause of disability and death. Household air pollution also contributes to the burden of cataracts, the leading cause of blindness, and lung cancer, especially among women. It is also worthy of note that an estimated 16 % of the global burden of disease attributed to ambient PM pollution comes from household combustion of solid fuel. The burden of disease due to household air pollution falls hardest on poor populations in Africa, Latin America, and Asia.

In some parts of the world, poorly burned solid fuels are commonly used for space heating and/or lighting, as well as cooking. The 2010 comparative risk assessment for household air pollution was explicitly limited to exposures related to cooking with solid fuels, and did not include other sources of exposure, largely because of lack of data. Because exposures to pollutants do occur from combustion for space heating and lighting as well as cooking with non-solid fuels, such as kerosene, future assessments will consider these sources as better data accumulate.

14.2 Mechanisms

The mechanisms by which biomass smoke causes adverse health effects in humans are likely similar to those involved in tobacco smoke and combustion-generated PM pathogenic processes. Oxidative stress in the airways and alveoli leads to stimulation of alveolar macrophages and injury to the epithelial lining, which in turn attracts inflammatory cells from the circulation. This local lung inflammatory reaction can spill over into the systemic circulation and contribute to adverse effects in other organs, such as the cardiovascular system, and the fetus in pregnant women. Alveolar macrophages laden with carbon particles from biomass smoke may contribute to increased risk of respiratory tract infections. The pathways involved in biomass smoke-induced lung carcinogenesis are probably identical to those by which tobacco smoke induces lung cancers.

Although the toxicological literature for biomass smoke is much less rich than for tobacco smoke and PM, a number of animal and *in vitro* studies have been reported and were referenced in a recent review paper (Migliaccio and Mauderly 2010). Pulmonary outcomes have been best studied in animal models, including airway responsiveness, lung function, effects on surfactant, epithelial damage, inflammation, edema, and antibacterial activity. Extrapulmonary outcomes have included effects on the cardiovascular system, adjuvant activity, p450 enzyme activity, glutathione depletion, and tumor/cancer growth.

A growing number of reports of controlled human exposure studies of wood smoke have been published. These studies are limited to acute effects and are specific to the fuels and burn conditions used, but evidence of systemic oxidative stress and inflammation, airway inflammation, and arterial stiffness has been observed after short-term exposure to wood smoke (Barregard et al. 2008; Sehlstedt et al. 2010; Stockfelt et al. 2012; Unosson et al. 2013). Not all studies, however, have found adverse effects (Riddervold et al. 2012; Forchhammer et al. 2012; Stockfelt et al. 2013).

14.3 Low Birth Weight

Low birth weight was not one of the conditions considered in the 2010 Global Burden of Disease effort because of lack of data from many countries. That said, there is considerable evidence of an association between biomass smoke exposure and low birth weight. A systematic review on the risk of low birth weight and solid fuel use was published in 2010 (Pope et al. 2010). The meta-analysis was updated in 2012 for a WHO report on indoor air quality. One randomized controlled trial (RCT) (Thompson et al. 2011) and six observational studies (Mavalankar et al. 1992; Boy et al. 2002; Mishra et al. 2004; Siddiqui et al. 2008; Tielsch et al. 2009; Abusalah et al. 2011) on the risk of low birth weight (<2,500 g at term) were reviewed. The pooled odds ratio (OR) was 1.40 [95 % confidence interval (CI): 1.26–1.54].

The biological plausibility of a biomass smoke effect on birth weight is supported by a similar and well-documented effect of in utero exposure to secondhand tobacco smoke (SHS) as well as mechanistic evidence of association between components of household air pollution (HAP) (CO, PM, and PAHs) and low birth weight. The range of reported estimates are consistent with those for related exposures, including outdoor air pollution, second hand smoking (SHS) and active smoking. The mean decrease in birth weight for biomass smoke exposure (90–100 g) lies between published estimates for SHS and active smoking, as would be expected from the relative levels of exposure to $PM_{2.5}$ (Pope et al. 2010). The main deficiency of this literature is the lack of measurement of exposure to biomass smoke and thus the inability to demonstrate an exposure-response relationship. Nevertheless, the WHO report assessed the evidence of an association between biomass smoke exposure and low birth weight as moderately strong, applying Bradford Hill viewpoints, because of consistency, temporality, biological plausibility, and analogy. The one published RCT of a chimney stove provided some evidence in support of an intervention effect (Thompson et al. 2011).

14.4 Acute Lower Respiratory Infection (Childhood Pneumonia)

Acute lower respiratory infection (ALRI) is a leading contributor to the global burden of disease, accounting for 4.6 % of the total (Murray et al. 2012). Pneumonia is also the primary cause of death in children (1.4 million deaths in children younger than 5 years) and the incidence and mortality are generally highest in those countries and regions where solid fuel use is greatest (Nair et al. 2013). The relative risk of ALRIs for children exposed to household biomass smoke has been quantified in a number of studies, the majority from developing countries, but also from the United States (Torres-Duque et al. 2008). Most of these studies have used a case-control design, although several cohort studies have also been conducted. Taken

together, these studies show a consistent association between solid fuel use and an increase in the risk of ALRI in exposed children. The overall estimate of the risk of ALRI from 24 studies selected for a meta-analysis conducted in preparation for the 2010 Global Burden of Disease comparative risk assessment (Dherani et al. 2008) was a pooled OR of 1.79 (CI: 1.26–2.21) for children younger than 5 years. The OR for children younger than 2 years was 1.96 (CI: 1.36–2.82). Only three studies included direct measurement of exposure, the remainder used proxies (fuel type, etc.). Outcome definitions varied from parental recall of WHO signs of ALRI to chest X-ray confirmation, and included severe and fatal outcomes; included studies had to distinguish upper from lower respiratory infections. Some evidence of an increased risk for more severe pneumonia was found. Impaired respiratory tract defense mechanisms, such as decreased mucociliary clearance and alveolar macrophage function, provide biological plausibility for the observed association between biomass smoke exposure and ALRI risk in children (Siddiqui et al. 2008; Zelikoff et al. 2002).

Only one RCT of an intervention to reduce exposure to biomass smoke to prevent childhood ALRI has been completed (Smith et al. 2011), involving 514 Guatemalan children aged <16 months, randomized to use a chimney wood stove or traditional three-stone fire. The primary outcome was physician diagnosis, with pulse oximetry to define severity. Exposure was assessed for all children using repeated CO measurements, together with co-located kitchen CO and $PM_{2.5}$ measurements in a sub-sample to define the CO-$PM_{2.5}$ relationship. In intention to treat analysis, child exposure was reduced by 50 %, associated with a relative risk of 0.78 (CI: 0.59, 1.06) for all physician-diagnosed pneumonia, and of 0.67 (CI: 0.45, 0.98) for severe pneumonia (defined by low oxygen saturation). Adjusted exposure-response analysis found significant relationships for both all and severe pneumonia.

The randomized control trial to study the effect of reduction in household air pollution on childhood pneumonia in Guatemala (RESPIRE) results support a causal association between biomass smoke exposure and early childhood pneumonia and show that the greatest risk reduction occurs at lower exposure levels. The RESPIRE exposure-response data suggest that ALRI incidence would be reduced by around one-third with interventions that bring average $PM_{2.5}$ down from several hundreds of $\mu g/m^3$ to levels experienced by "unexposed" groups in the majority of epidemiological studies (Smith et al. 2014) (see Fig. 14.1). Reduction of exposure to biomass smoke levels at or below the WHO annual average guideline of 10 $\mu g/m^3$ $PM_{2.5}$ would theoretically result in larger risk benefit.

14.5 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease is a leading cause of morbidity and mortality (~4 million deaths/year) worldwide and results in an economic and social burden that is both substantial and increasing, especially among women (Global Initiative for Chronic Obstructive Lung Disease 2014; Lozano et al. 2012). In less developed countries, a substantial proportion of COPD occurs in people who have never

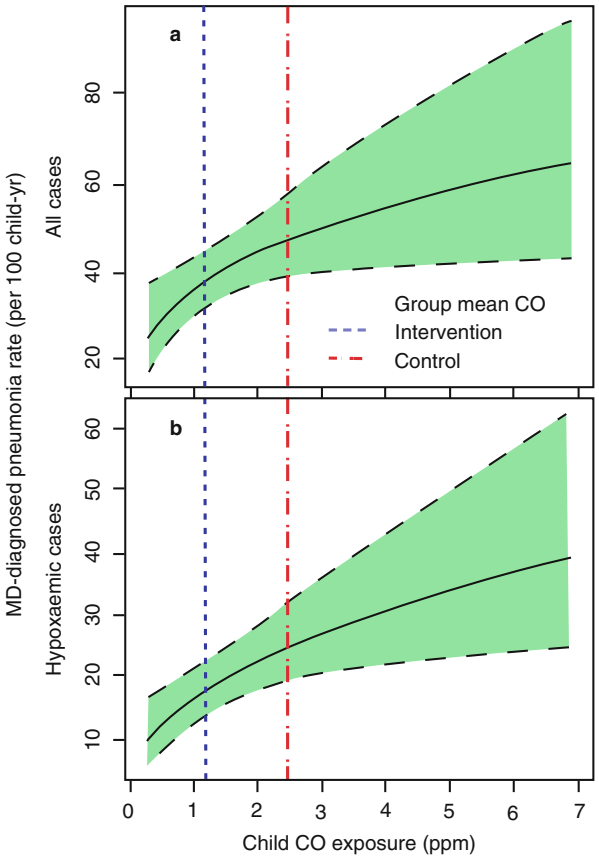

Fig. 14.1 Exposure-response from the RESPIRE study for wood smoke exposure as assessed by average personal monitoring data and the rate of physician-diagnosed severe pneumonia as assessed by oxygen saturation (Reprinted from Smith et al. (2011) with permission from Elsevier)

smoked tobacco products, especially among women cooking with stoves that emit high levels of a wide variety of pollutants similar to those present in tobacco smoke (Lim et al. 2012). Multiple case-control and cross-sectional studies have found an association between cooking with biomass fuel and chronic bronchitis or COPD when compared with lower exposure/cleaner fuels (Akhtar et al. 2007; Albalak et al. 1999; Caballero et al. 2008; Dutt et al. 1996; Ellegard 1996; Menezes et al. 1994; Qureshi 1994; Regalado et al. 2006; Saha et al. 2005; Dennis et al. 1996; Dossing et al. 1994; Ekici et al. 2005; Kiraz et al. 2003; Orozco-Levi et al. 2006; Perez-Padilla et al. 2006). Exposure was often assessed as present or absent or by daily hours spent by the stove. Most studies did not include direct measurements of specific pollutants. Studies that have measured kitchen particulate levels from biomass fuel use have confirmed very high concentrations (Albalak et al. 1999; Regalado et al. 2006), but personal measurements of exposure have not been used.

Only a few cross-sectional studies have measured lung function and these studies have shown an association between biomass fuel use and chronic airflow obstruction (Caballero et al. 2008; Regalado et al. 2006). Taken together, the results of previous studies show a strong association between cooking with biomass fuel and COPD among women (Balmes 2010).

Several systematic reviews and meta-analyses have been published, including the one conducted for the 2010 Global Burden of Disease comparative risk assessment (Smith et al. 2014; Kurmi et al. 2010; Hu et al. 2010; Po et al. 2011). In the Smith et al. (2014) review 25 studies were included in the final analysis. All but seven included in the meta-analysis were cross-sectional in design. One study was a retrospective cohort (Chapman et al. 2005) and six were case-control (Dennis et al. 1996; Dossing and Khan 1994; Orozco-Levi et al. 2006; Perez-Padilla et al. 2006; Sezer et al. 2006; Xu et al. 2007). Case-control studies recruited controls from various sources, including visitors to the hospital, patients from other hospital services without presence of pulmonary disease, and population-based selection. Heterogeneous exposure measures were used in these studies, including rural-urban comparisons (where data supported the use of place of residence as a proxy for fuel use), outdoor versus indoor cooking, fuel type for cooking and/or heating, stove type and time-exposed to biomass fuel combustion. The pooled OR was 1.94 (CI: 1.62–2.33). Stratified analysis by gender showed a stronger association between exposure to biomass smoke and COPD in women [OR, 2.30 (CI: 1.73-3.06)] than in men [OR, 1.90 (CI: 1.15–3.13)]. The association in women remained significant even after adjusting for both age and smoking, while this was not the case for men.

Three other recently published systematic reviews and meta-analyses reported similar summary estimates for the effect of biomass smoke on risk of COPD. Kurmi et al. (2010) reported a summary OR (CI) for the use of solid fuels and COPD from 23 studies of 2.80 (CI: 1.85–4.0) and for biomass smoke and chronic bronchitis of 2.32 (1.92–2.80). Pooled estimates for different types of fuel showed that exposure to wood smoke carried a greater risk than coal (also seen in the Global Burden of Disease meta-analysis). Hu et al. (Hu et al. 2010) reported a summary OR from 15 studies of 2.44 (CI: 1.9–3.33) for the risk of developing COPD with biomass smoke exposure. Po et al. (Po et al. 2011) included data from 12 studies of female populations and reported a summary OR for chronic bronchitis of 2.52 (CI: 1.88–3.38) and for COPD of 2.40 (CI: 1.47–3.93). Applying the Bradford-Hill viewpoints (consistency, exposure-duration evidence, analogous evidence of the effect of smoking, and biological plausibility), the case for a causal association between biomass smoke and COPD is moderately strong, especially among women (Eisner et al. 2010).

Longitudinal studies of the impact of biomass smoke exposure on the development of COPD are lacking, but one study did follow women with COPD associated with biomass smoke exposure in terms of mortality risk (Ramirez-Venegas et al. 2006). Survival analysis over a 7-year follow-up period showed that women with COPD associated with biomass smoke exposure had mortality rates similar to those of men with COPD due to tobacco smoking.

Intervention studies to prevent COPD from biomass smoke exposure have been limited. A retrospective Chinese study found significant reductions in COPD incidence in homes where coal was used in improved stoves with chimneys, the effect increasing with time since adoption (Chapman et al. 2005). Two recently published studies of the efficacy of reducing wood smoke exposure with the use of an improved chimney stove were limited by short follow-up time (18 months) (Smith-Sivertsen et al. 2009; Romieu et al. 2009). Thus, although studies suggest lower biomass smoke exposure may be associated with lower COPD risk, several key questions remain, including can the development or progression of COPD be prevented by reducing biomass smoke exposure in these settings and what is the exposure-response relationship between biomass smoke and the rate of decline in lung function? Prospective cohort studies that have adequate statistical power, follow participants for sufficient duration to accurately measure rate of decline in lung function, and actual exposure measures are needed to answer these questions. Ideally, intervention studies with clean stoves will be conducted.

14.6 Lung Cancer

The emissions generated of combustion of the main solid fuels used for cooking and space heating, biomass and coal, may be associated with differential cancer risk because of differences in chemical composition. The International Agency for Research on Cancer (IARC) has concluded household use of coal is a Group 1 carcinogen, while biomass is classified as a Group 2(a) or probable carcinogen, due to more limited epidemiological evidence (International Agency for Research on Cancer 2010).

Two recent systematic reviews and meta-analyses of household use of coal and lung cancer risk have been conducted, including the one used as the basis for the 2010 Global Burden of Disease comparative risk assessment (Hosgood et al. 2011) and an additional effort by Kurmi et al. (2012). The Hosgood et al. review identified 25 case-control studies investigating household coal use with seven provided cooking-specific estimates. Exposure was assessed by fuel type, and lung cancer confirmed by pathology for most cases, otherwise by chest X-ray. Only five studies were conducted in countries other than mainland China and Taiwan. Household coal use for cooking and heating was associated with a pooled OR for lung cancer of 2.15 (CI: 1.61–2.89). The second review by Kurmi et al. pooled a total of 22 studies to generate an OR of 1.82 (CI: 1.60–2.06). In both reviews, the risk for women was somewhat greater than the risk for men.

Since the great majority of the 2.8 billion solid fuel users globally is exposed to biomass rather than coal smoke, the issue of the carcinogenic risk associated with the former is an important one. A systematic review for the 2010 Global Burden of Disease comparative risk assessment (Smith et al. 2014) identified 14 eligible

studies of cooking and/or heating with biomass. Most of the studies conducted in Asia, although there were studies from Europe and North America as well. Biomass fuel was defined as including wood, straw, grass, crop waste, animal dung, and charcoal; only household use was considered, whether for cooking or heating. Exposure was determined by fuel type and no study directly measured exposure. One European study had exposure-response data available (Lissowska et al. 2005). Most cases of lung cancer were confirmed by pathology. The pooled OR for all 14 studies was 1.18 (CI: 1.03–1.35), but the most reliable estimate OR of 1.22 (CI: 1.08–1.37) from well-adjusted studies with clean fuel comparisons. The review by Kurmi et al. pooled seven studies and only used adjusted estimates to generate an OR that was somewhat higher, 1.50 (CI: 1.17–1.94). (Kurmi et al. 2012). In both the 2010 Global Burden of Disease and Kurmi et al. meta-analyses, women had somewhat higher risk than men. Applying the Bradford-Hill viewpoints (including consistency, exposure-response, biological plausibility), there does appear to be a moderately strong case for a causal association.

14.7 Cataracts

Cataract or severe lens opacification is one of the leading causes of blindness in the developing world. The 2010 Global Burden of Disease project (Murray et al. 2012) estimated that cataracts accounted for 0.2 % of the total disease burden, the majority of which is seen in the developing regions of sub-Saharan Africa and Southeast Asia alone where cooking with solid fuels is most common. The toxicological evidence from animal studies and epidemiological studies of other types of smoke (e.g., active smoking and secondhand smoke) suggest biological plausibility for an association between exposure to biomass smoke and risk of cataract. Condensates of both tobacco and biomass fuel smoke enhance the formation of superoxide radicals, which decrease antioxidants and cause lens discoloration and cataract. A component of biomass smoke is naphthalene, which has been shown to cause cataract in laboratory animal studies (van Heyningen and Pirie 1976).

The systematic review and meta-analysis for the 2010 Global Burden of Disease comparative risk assessment identified seven eligible studies, most of which had case-control designs, and all were from Southeast Asia (Smith et al. 2014). The pooled OR was 2.46 (CI: 1.74–3.50) and sensitivity analysis revealed no major confounding due to smoking or UV exposure. The results of the meta-analysis, the known risks from smoking, the consistency of results across studies, the toxicological data (especially regarding naphthalene), and some exposure-response evidence (Pokhrel et al. 2013) support causal inference.

14.8 Cardiovascular Disease

In contrast to the other health outcomes discussed above, at the time of writing this chapter, there were no epidemiological studies of the relationship between exposure to biomass smoke and cardiovascular disease. That said, a remarkably consistent, nonlinear relationship between estimated inhaled dose of combustion-sourced particles measured as $PM_{2.5}$ (particulate matter with aerodynamic diameter $\leq$2.5 μm) and cardiovascular disease mortality was first demonstrated by Pope et al. (Pope et al. 2009). This dose-response relationship was remarkable for a non-cancer outcome in that it covered at least three orders of magnitude of dose from lowest to highest (exposure to ambient air pollution, exposure to secondhand tobacco smoke exposure, and active cigarette smoking). It then struck Peel and Smith that exposures to $PM_{2.5}$ from solid fuel cooking lay in the range between those from secondhand smoke and active smoking (Smith and Peel 2010) and, given that tobacco is a kind of biomass, they postulated that the relative risk for CVD from biomass fuel smoke exposure would be in the order of 1.3–1.6 (Fig. 14.2).

Because the Global Burden of Disease project is designed to provide useful information for public health policy decisions regarding interventions to reduce important risks, the 2010 comparative risk assessment for household air pollution included identification of attributable risk for cardiovascular disease, the number one cause of death and disability on a global scale. If there were no available epidemiological studies that directly assessed the association between biomass smoke exposure and cardiovascular disease risk, then how could any risk be attributed to this exposure? An effort was made by an expert group to interpolate the risk due to biomass smoke by generating what has been termed an "integrated exposure-response curve." The curve derived for risk of ischemic heart disease in relation to combustion-sourced fine particles is similar to that suggested by Pope et al 2011 (Pope et al. 2009), i.e., supralinear in shape from low exposures through the household air pollution exposure range, where it reaches a relative risk of 1.5 for high biomass fuel smoke exposure and a maximum of 2.5 for high active smoking exposure (Smith et al. 2014; Burnett et al. 2014). The integrated exposure-response curve for stroke is similar, but appears to flatten off at levels well within the household air pollution range at a maximum relative risk of just over 2.0.

Subsequent to the preparation of the 2010 Global Burden of Disease comparative risk assessment, the results of a cross-sectional Chinese study involving over 14,000 men and women aged 18 and over were reported (Lee et al. 2012). Solid fuel use (biomass and coal) for heating and/or cooking was assessed by questionnaire, and categorized according to ever use, duration, total amount and lifetime use. Outcomes, including ischemic heart disease, stroke and diabetes mellitus, were assessed by self-report of physician-diagnosed conditions, while blood pressure was measured during the study. The results showed elevated adjusted odds ratios for ischemic heart disease forever vs. never use of solid fuels, and significant trends across duration of use for stroke, hypertension and diabetes. The OR for ischemic heart disease 2.6 is somewhat higher than predicted from the integrated exposure–response curve; the

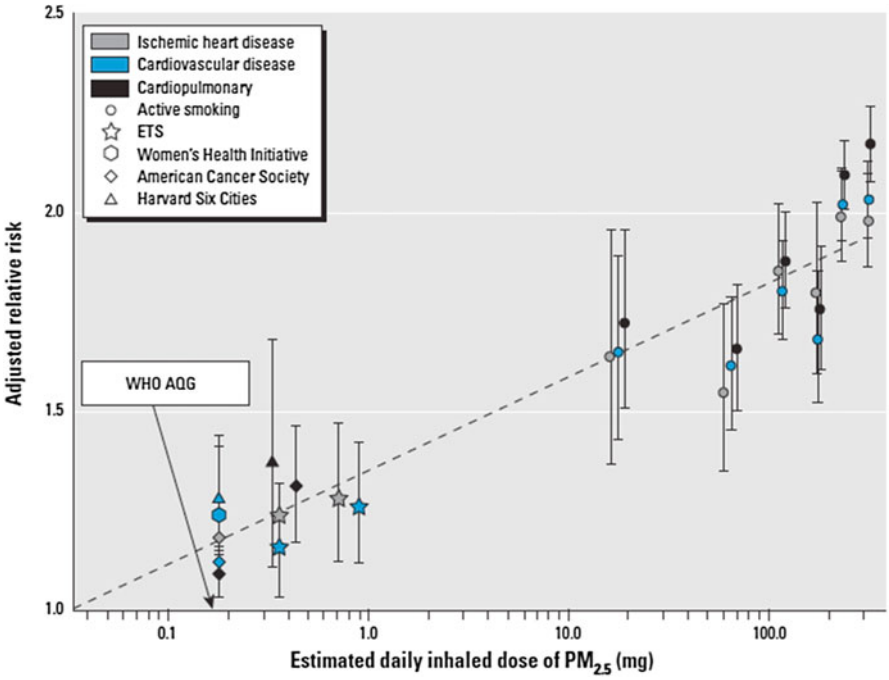

Fig. 14.2 Adjusted relative risks (95 % confidence intervals) of cardiovascular and cardiopulmonary mortality and estimated dose of PM2.5 across studies of outdoor air pollution, ETS, and active cigarette smoking (Adapted with permission from (Pope et al. 2009), their Fig. 2). Data on active smoking are from Pope et al. (Pope et al. 2009); on ETS are from the 2006 Surgeon General's Report (U.S. Department of Health and Human Services 2006) and INTERHEART study (Teo et al. 2006); on air pollution are from the Women's Health Initiative cohort (Miller et al. 2007), the American Cancer Society cohort (Pope et al. 1995, 2002, 2004), and the Harvard Six Cities cohort (Dockery et al. 1993; Laden et al. 2006). Exposure was measured as daily inhaled dose of $PM_{2.5}$ (plotted on a log scale), calculated assuming 18 m^3/day breathing rate. Active cigarette smoking was quantified as ≤3, 4–7, 8–12, 13–17, 18–22, and ≥23 cigarettes/day (relative to never-smokers). Also shown is the equivalent dose for the World Health Organization (WHO) (2006) Air Quality Guidelines (AQG) for $PM_{2.5}$ (10 μg/m3 annual average) (Smith and Peel 2010)

OR for stroke, 1.6, was in the range expected from the curve although it was not statistically significant. Sex-stratified analysis found stronger effects for women, consistent with their higher exposure, and among non-smokers.

Although there is little epidemiological literature on the effect of biomass smoke exposure on cardiovascular disease risk, there are studies that show effects on blood pressure (McCracken et al. 2007; Baumgartner et al. 2011) and the ST-segment of the electrocardiogram (ECG) (McCracken et al. 2011). For blood pressure, there are five studies, four observational and one intervention (the latter with both RCT and before and after components). All have found that increased exposure was associated with higher systolic and/or diastolic blood pressure, with most findings being statistically significant. In the intervention trial (RESPIRE), McCracken et al. found

the chimney stove intervention group to have statistically significant lower systolic (−3.7 mmHg) and diastolic (−3.0 mmHg) blood pressure in an intention-to-treat analysis, and similar sized effects when open-fire control subjects received the chimney stove at the end of follow-up (McCracken et al. 2007). In the same RESPIRE study subjects significant effects of the chimney stove intervention on subjects' ECGs were demonstrated. During the trial, the stove intervention was associated with a significant protective effect (OR 0.26) for ST-segment depression and a similar effect with the before-and-after comparison (McCracken et al. 2011).

The population attributable risk for ischemic heart disease associated with biomass smoke exposure estimated by the integrated exposure-response curve method was 18 % compared to 53 % for hypertension, 29 % for hypercholesterolemia, and 31 % for tobacco smoke (both active smoking and secondhand exposure) (Lim SS et al. 1990). Despite the dearth of epidemiological studies, the overall evidence, including from the related combustion-sourced particles of smoking (active and second-hand) and outdoor air pollution, together with the observed blood pressure and electrocardiographic effects, suggests that exposure to biomass smoke can increase cardiovascular disease risk.

14.9 Other Diseases (Tuberculosis, Adult Pneumonia, Asthma)

Three other diseases for which the weight of evidence was judged not strong enough to attribute risk of disability-adjusted life years to household air pollution in the 2010 Global Burden of Disease comparative risk assessment are tuberculosis, adult pneumonia, and asthma. Nevertheless, there is some evidence and biological plausibility for an association between exposure to biomass smoke and increased risk for each of these diseases.

14.9.1 Tuberculosis

Tuberculosis (TB) was responsible for 1.4 million deaths in 2011, and over 80 % of the global burden of TB lies in the Western Pacific, South-East Asia and Africa; China and India together have 40 % of the world's cases (Organization and Report 2013). The close association of tuberculosis with poverty, and use of solid fuel for cooking and heating in the home, makes the impact of exposure to household air pollution on risk of TB is a major concern. Because active tobacco smoking has been associated with increased risk for TB, it is again biologically plausible that exposure to biomass fuel smoke would also increase risk. Carbon particle–laden alveolar macrophages, for example, have been shown to be less effective in phagocytosis of microorganisms than those from subjects not exposed to smoke (Zhou and Kobzik 2007; Fick et al. 1984; Migliaccio et al. 2013).

Several reviews have been done over the past decade with the most recent, a systematic review and meta-analysis published in 2013 (Lin et al. 2007; Slama et al. 2010; Sumpter and Chandramohan 2013). In the 2013 review, 10 case-control and three cross-sectional studies were included. The outcome was sputum positive TB in all of the case-control studies, and two of the cross-sectional studies. None of the studies directly measured exposure to household air pollution so exposure assessment was only by survey of fuel type. Adjustment for smoking and crowding was done in most, but not all studies. Considerable study heterogeneity was found and study design issues were deemed problematic (e.g., hospital-based controls and over-representation of men). The pooled OR for the 10 case-control studies was 1.3. The six studies of women only had a pooled OR of 1.7.

A study by Pokhrel et al. also examined the risk of TB associated with the use of kerosene as well as solid biomass fuel (Pokhrel et al. 2010). Kerosene use was shown to have a much stronger effect on TB risk, for cooking with kerosene, the OR was 3.36 (CI: 1.01–11.22) and for lighting the OR was 9.43 (CI: 1.45–61.32). For cooking with a biomass fuel stove, the OR was a non-significant 1.21.

As noted above, the most up-to-date review and meta-analysis of the solid fuel-TB risk association reported a significantly increased pooled OR. However, both exposure-response evidence and intervention studies are lacking. Nevertheless, together with the well-established evidence that smoking increases risk of TB, findings from studies of animals exposed to solid fuel smoke (Zhou and Kobzik 2007; Fick et al. 1984; Migliaccio et al. 2013), and the plausible mechanism through impaired immunity from solid fuel exposure (Dutta et al. 2012), there is now a moderately strong case for a causal association between exposure to household air pollution and TB risk. Given the high prevalence of both exposure to household air pollution and TB across the developing world (and in the same geographic regions), reduction in household air pollution could make an important contribution to control of TB. Further research is needed to confirm that the association is causal rather than due to confounding by poverty-related factors, to better quantify exposure-response, and to assess potential interventions to reduce exposure.

14.9.2 Adult Acute Lower Respiratory Infection (Pneumonia)

In the 2010 Global Burden of Disease report, acute lower respiratory infections caused 2.8 million deaths annually (Lozano et al. 2012). Slightly over half of these deaths occur among adults (15 years and above), and most in those aged 60 years and over. While the majority of the adult deaths due to ALRI occur in developed countries, a substantial proportion of adult deaths in developing countries is due to ALRI/pneumonia. Given the strong evidence that exposure to biomass smoke causes ALRI in children (most probably through reducing immune defense mechanisms), as well as the established association between tobacco smoking and ALRI in adults, a biomass smoke-ALRI in adults is plausible.

A systematic review of adult ALRI was carried out for the 2010 Global Burden of Disease comparative risk assessment (Smith et al. 2014). From the many papers reviewed, only three were selected to have adequate data to assess the potential association of exposure to household air pollution and ALRI, and there is considerable heterogeneity among the three (Ezzati and Kammen 2001; Levesqu et al. 2001; Shen et al. 2009). The study by Ezzati and Kammen is a small cohort study in rural Kenya where various forms of biomass are used (wood, charcoal); home visits were used for assessment of community-acquired pneumonia (Ezzati and Kammen 2001). The data analysis also demonstrated a significant exposure-response relationship. The study by Levesque et al. is from Quebec, where biomass is used for household heating rather than cooking and exposure is much lower; the ALRI outcomes were self-reported and likely subject to some misclassification (Levesqu et al. 2001). The study by Shen et al. used a case–control design to assess risks for pneumonia deaths in a coal-using area of China (Shen et al. 2009). All three studies reported significant ORs for exposed groups in the range of 2.0–3.0. Because of differences in outcome definitions and assessment, pooling was not done.

Although the epidemiological data are limited, the exposure-response relationship from the study by Ezzati and Kammen (2001), strong evidence of a biomass smoke effect in children, and the known effects of other sources of combustion-sourced particle exposure (e.g., smoking and ambient air pollution), together suggest that exposure to solid fuel smoke increases the risk of adult ALRI. Further research is required to confirm and quantify this risk.

14.9.3 Asthma

Given the airway irritating effects of biomass smoke, it is reasonable to suspect that exposure might increase risk for asthma, especially because exposure to secondhand tobacco smoke has been associated with the development of asthma in children (Vork et al. 2007). On the other hand, there is a rural-urban gradient for asthma. The disease is generally much more prevalent in urban areas than in the largely rural areas where exposure to biomass smoke is more common (Addo-Yobo et al. 2007). The protective effect of living on a farm, especially with livestock, has been well documented (Ege et al. 2011). Because the evidence for an effect of biomass smoke on COPD is strong, and both asthma and COPD involve airway inflammation, the relationship between exposure to biomass smoke and risk of asthma remains a topic of considerable research interest.

A systematic review and meta-analysis of the association between exposure to biomass smoke and asthma in children and women was published in 2011 (Po et al. 2011). Nine studies were selected for the meta-analysis, four involving children and five involving women. The pooled OR for the four studies on asthma in children exposed to biomass smoke was 0.50 (CI: 0.12–1.98) (Fagbule and Ekanem 1994; Noorhassim et al. 1995; Behera et al. 2001; Melsom et al. 2001). For the five studies of women, the pooled OR was 1.34 (CI: 0.93–1.93) (Qureshi 1994; Behera et al. 2001; Golshan and Faghihi 2002; Uzun et al. 2003; Mishra 2003).

Since the Po et al. review was published, the results of several new studies have been reported. A survey of 508 adults in southeastern Kentucky found a significant association with asthma when wood and coal were burned for cooking (Barry et al. 2010). A much larger population survey from India found that adult women living in households using biomass and solid fuels had a significantly higher risk of asthma than those living in households using cleaner fuels, even after controlling for the effects of a number of potentially confounding factors (Agrawal 2012).

The most robust evidence supporting an increased risk of asthma from cooking with solid fuels was reported by the International Study of Asthma and Allergy in Children (ISAAC) which surveyed almost 513,000 children from 1999–2004 (Wong et al. 2013). The sole use of an open fire for cooking (assessed by questionnaire) was associated with an increased risk of wheeze in the past year in both young children (ages 6–7 years), OR 2.17 (CI: 1.64–2.87), and in older children (ages 13–14 years), OR 1.35 (CI: 1.11–1.64). The ORs for wheeze in the past year and the use of open fire in combination with other fuels for cooking were slightly lower, but still significant for both age groups.

Not all recent studies have reported positive associations between exposure to biomass smoke and asthma outcomes. For example, a recent study of 299 children from rural villages in Nigeria, reported smoke exposure was not associated with an increased risk of asthma symptoms or airway obstruction (Thacher et al. 2013). Because the published evidence is mixed, further research is needed to better understand the nature of the relationship between exposure to solid fuel smoke and asthma in both children and adults and for both new onset and exacerbations of disease. Studies that actually measure exposure and which are thus able to develop an exposure-response relationship would be particularly helpful.

14.10 Research Gaps

While one RCT of the efficacy of a chimney stove compared to an open fire for the prevention of early childhood pneumonia has been published (Smith et al. 2011) and related studies have suggested benefit regarding low birth weight, cognitive development, and respiratory symptoms in adult women, more studies of stove interventions are needed. In particular, the exposure-response data from the RESPIRE trial provide evidence that stoves which are cleaner than the chimney stove studied would provide greater reduction in childhood pneumonia. Several other ongoing RCTs of cleaner stove interventions in Nepal and Africa should contribute data to fill the gap in our knowledge about the added benefit of cleaner burning stoves beyond those with chimneys.

Another gap is the lack of a well-designed longitudinal study of the impact of improved stoves on the development of COPD in adults exposed to biomass smoke form cooking and heating. One published study from Mexico conducted in the context of a RCT designed to prevent childhood pneumonia found a protective effect of a chimney stove on rate of decline of lung function in women who actually

used the stove (Romieu et al. 2009), but this study suffers from the limitation of too short of a follow-up period (1 year). Another paper from RESPIRE reports evidence that reduced exposure from use of a chimney stove is associated with decreased expression of metalloproteinase genes (Guarnieri et al. 2014).

Perhaps the greatest research gap involves the lack of epidemiological evidence to support the concept that biomass smoke exposure leads to increased risk of cardiovascular disease consistent with other combustion-sourced particle exposures, outdoor $PM_{2.5}$, secondhand tobacco smoke, and active smoking.

More research is needed with regard to the associations between biomass smoke exposure and adult pneumonia, asthma, and tuberculosis, either because of paucity of data (adult pneumonia) or conflicting results. Another unresolved issue is the toxicity of kerosene as an alternative fuel, especially given the recently published evidence that it substantially increases the risk of tuberculosis (Pokhrel et al. 2010)

Because adoption of new stoves can be a difficult transition for households in the developing world due to both financial and cultural factors, more research is needed to improve our understanding of these potential barriers to the effectiveness of intervention programs.

Toxicological studies (*in vitro*, animal, and controlled human exposure) are needed to improve our mechanistic understanding of the pathways by which biomass smoke causes or contributes to disease pathogenesis. Such studies can also assist in the development of biomarkers of both exposure and effects (Rylance et al. 2013).

14.11 Opportunities for Intervention

For many years, the major obstacle to implementing programs to reduce biomass smoke exposure from domestic cooking and heating with inefficient, poorly ventilated stoves was lack of data proving benefit. Now that at least one RCT has proved efficacy in reduction of early childhood pneumonia, comparable to that of vaccination, with results from several other trials on the way, the major issues are cost and effectiveness of stoves that can substantially reduce biomass emissions. Improved stoves still cost more than many households in the developing world can afford, maintenance can be difficult, and many households may not be willing to adopt new stoves that require changes to traditional cooking styles.

An exciting program that was started with the help of Hillary Clinton when she was US Secretary of State is the Global Alliance for Clean Cookstoves (GACC), under the auspices of the United Nations Foundation. The GACC is a public-private partnership that "seeks to save lives, improve livelihoods, empower women, and protect the environment by creating a thriving global market for clean and efficient household cooking solutions."(Global Alliance for Clean Cookstoves 2014) Its mission is to mobilize high-level national and donor commitments toward the goal of universal adoption of clean cookstoves and fuels. The primary goal of the GACC is to foster the adoption of clean cookstoves and fuels in 100 million households by 2020. Multiple demonstration and research projects have been funded by the

organization since it was established in 2010. In 2012, the following countries were prioritized for immediate GACC engagement: Bangladesh, China, Ghana, Kenya, Nigeria, and Uganda.

Although the GACC represents a major breakthrough for efforts to improve the health of the 45 % of the world's population that still cooks with solid fuel, even the most efficient stoves that burn biomass emit more $PM_{2.5}$ than the gas and electric stoves that are used by developed world households. This has led some experts to suggest that electrification of developing countries and the use of relatively inexpensive induction cookers may be the most effective way to reduce exposure to household air pollution.

A final policy note is that black carbon generated from biomass combustion is an important short-lived climate-forcing agent (Jacobson 2001). Interventions to improve the efficiency of combustion of biomass fuels or better, that eliminate combustion of biomass fuels altogether, would provide a major climate change mitigation co-benefit in addition to the public health benefits described above.

References

Abusalah A, Gavana M, Haidich AB et al (2011) Low birth weight and prenatal exposure to indoor pollution from tobacco smoke and wood fuel smoke: a matched case–control study in Gaza Strip. Matern Child Health J 16:1718–1727

Addo-Yobo E, Woodcock A et al (2007) Exercise-induced bronchospasm and atopy in Ghana: two surveys ten years apart. PLoS Med 4:355–360

Agrawal S (2012) Effect of indoor air pollution from biomass and solid fuel combustion on prevalence of self-reported asthma among adult men and women in India: findings from a nationwide large-scale cross-sectional survey. J Asthma 49:355–365

Akhtar T, Ullah Z, Khan MH et al (2007) Chronic bronchitis in women using solid biomass fuel in rural Peshawar, Pakistan. Chest 132:1472–1475

Albalak R, Frisancho AR, Keeler GJ (1999) Domestic biomass fuel combustion and chronic bronchitis in two rural Bolivian villages. Thorax 54:1004–1008

Armstrong JR, Campbell H (2009) Indoor air pollution exposure and lower respiratory infections in young Gambian children. Int J Epidemiol 20:424–429

Balakrishnan K, Ghosh S, Ganguli B et al (2013) State and national household concentrations of PM2.5 from solid cookfuel use: results from measurements and modeling in India for estimation of the global burden of disease. Environ Health 12:77

Balmes JR (2010) When smoke gets in your lungs. Proc Am Thorac Soc 7:98–101

Barregard L, Sällsten G, Andersson L et al (2008) Experimental exposure to wood smoke: effects on airway inflammation and oxidative stress. Occup Environ Med 65:319–324

Barry AC, Mannino DM, Hopenhayn C, Bush H (2010) Exposure to indoor biomass fuel pollutants and asthma prevalence in Southeastern Kentucky: results from the Burden of Lung Disease (BOLD) study. J Asthma 47:735–741

Baumgartner J, Schauer JJ, Ezzati M et al (2011) Indoor air pollution and blood pressure in adult women living in rural China. Environ Health Perspect 119:1390–1395

Behera D, Chakrabarti T et al (2001) Effect of exposure to domestic cooking fuels on bronchial asthma. Indian J Chest Dis Allied Sci 43:27–31

Boy E, Bruce N, Delgado H (2002) Birth weight and exposure to kitchen wood smoke during pregnancy in rural Guatemala. Environ Health Perspect 10:109–114

Bruce N, Perez-Padilla R, Albalak R (2000) Indoor air pollution in developing countries: a major environmental and public health challenge. Bull World Health Organ 78:1078–1092
Burnett RT, Pope CA 3rd, Ezzati M et al (2014) An integrated risk function for estimating the global burden of disease attributable to ambient fine particulate matter exposure. Environ Health Perspect 122:397–403
Caballero A, Torres-Duque CA, Jaramillo C et al (2008) Prevalence of COPD in five Colombian cities situated at low, medium, and high altitude (PREPOCOL study). Chest 133:343–349
Chapman RS, He X, Blair AE et al (2005) Improvement in household stoves and risk of chronic obstructive pulmonary disease in Xuanwei, China: retrospective cohort study. BMJ 331:1050–1055
Dennis RJ, Maldonado D, Norman S et al (1996) Woodsmoke exposure and risk for obstructive airways disease among women. Chest 109:115–119
Dherani M, Pope D, Mascarenhas M et al (2008) Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis. Bull World Health Organ 86:390–398C
Diette GB, Accinelli RA, Balmes JR et al (2012) Obstructive lung disease and exposure to burning biomass fuel in the indoor environment. Glob Heart 7:265–270
Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME et al (1993) An association between air pollution and mortality in six U.S. cities. N Engl J Med 329:1753–1759
Dossing M, Khan J, al-Rabiah F (1994) Risk factors for chronic obstructive lung disease in Saudi Arabia. Respir Med 88:519–522
Dutt D, Srinivasa DK, Rotti SB et al (1996) Effect of indoor air pollution on the respiratory system of women using different fuels for cooking in an urban slum of Pondicherry. Natl Med J India 9:113–117
Dutta A, Bhattacharya P, Lahiri T, Ray MR (2012) Immune cells and cardiovascular health in premenopausal women of rural India chronically exposed to biomass smoke during daily household cooking. Sci Total Environ 438:293–298
Ege M, Mayer M et al (2011) Exposure to environmental microorganisms and childhood asthma. N Engl J Med 364:701–709
Eisner MD, Anthonisen N, Coultas D et al (2010) An official American Thoracic Society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 82:693–718
Ekici A, Ekici M, Kurtipek E et al (2005) Obstructive airway diseases in women exposed to biomass smoke. Environ Res 99:93–98
Ellegard A (1996) Cooking fuel smoke and respiratory symptoms among women in low-income areas in Maputo. Environ Health Perspect 104:980–985
Ezzati M, Kammen DM (2001) Quantifying the effects of exposure to indoor air pollution from biomass combustion on acute respiratory infections in developing countries. Environ Health Perspect 109:481–488
Fagbule D, Ekanem E (1994) Some environmental risk factors for childhood asthma: a case-control study. Ann Trop Paediatr 14:15–19
Fick RB Jr, Paul ES, Merrill WW et al (1984) Alterations in the antibacterial properties of rabbit pulmonary macrophages exposed to wood smoke. Am Rev Respir Dis 129:76–81
Forchhammer L, Møller P, Riddervold IS et al (2012) Controlled human wood smoke exposure: oxidative stress, inflammation and microvascular function. Part Fibre Toxicol 9:7
Global Alliance for Clean Cookstoves (2014) Washington, DC http://www.cleancookstoves.org/the-alliance/. Accessed 26 Jan 2014
Global Initiative for Chronic Obstructive Lung Disease. Global strategy for diagnosis, management, and prevention of COPD. http://www.goldcopd.org/Guidelines/guidelines-resources.html Accessed 20 Jan 2014
Golshan M, Faghihi M (2002) Indoor women jobs and pulmonary risks in rural areas of Isfahan, Iran. Respir Med 96:382–388
Guarnieri MJ, Diaz JV, Basu C et al (2014) Effects of woodsmoke exposure on airway inflammation in rural Guatemalan women. PLoS One 9:e88455

Hosgood HD 3rd, Wei H, Sapkota A et al (2011) Household coal use and lung cancer: systematic review and meta-analysis of case control studies, with emphasis on geographic variation. Int J Epidemiol 40:719–728

Hu G, Zhou Y, Tian J et al (2010) Risk of COPD from exposure to biomass smoke: a metaanalysis. Chest 138:20–31

International Agency for Research on Cancer (2010) Household use of solid fuels and high-temperature frying: evaluation of carcinogenic risks to humans. International Agency for Research on Cancer, Lyon

Jacobson MZ (2001) Strong radiative heating due to the mixing state of black carbon in atmospheric aerosols. Nature 409:695–697

Kiraz K, Kart L, Demir R et al (2003) Chronic pulmonary disease in rural women exposed to biomass fumes. Clin Invest Med 26:243–248

Kurmi OP, Semple S, Simkhada P et al (2010) COPD and chronic bronchitis risk of indoor air pollution from solid fuel: a systematic review and meta-analysis. Thorax 65:221–228

Kurmi OP, Arya PH, Lam KB et al (2012) Lung cancer risk of solid fuel smoke exposure: a systematic review and meta-analysis. Eur Respir J 40:1228–1237

Laden F, Schwartz J, Speizer FE, Dockery DW (2006) Reduction in fine particulate air pollution and mortality: extended follow-up of the Harvard Six Cities Study. Am J Respir Crit Care Med 173:667–672

Lee MS, Hang JQ, Zhang FY et al (2012) In-home solid fuel use and cardiovascular disease: a cross-sectional analysis of the Shanghai Putuo study. Environ Heal 11:18

Levesqu B, Allaire S, Gauvin D et al (2001) Wood-burning appliances and indoor air quality. Sci Total Environ 281:47–62

Lim SS, Vos T, Flaxman AD et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380(9859):2224–2260

Lin H-H, Ezzati M, Murray M (2007) Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis. PLoS Med 4(1):e20

Lissowska J, Bardin-Mikolajczak A, Fletcher T et al (2005) Lung cancer and indoor pollution from heating and cooking with solid fuels: the IARC International Multicenter Case-control Study in Eastern/Central Europe and the United Kingdom. Am J Epidemiol 162:326–333

Lozano R, Naghavi M, Foreman K et al (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380(9859):2095–2128

Mavalankar DV, Gray RH, Trivedi CR (1992) Risk factors for preterm and term low birth weight in Ahmedabad, India. Int J Epidemiol 21:263–272

McCracken JP, Smith KR, Mittleman M et al (2007) Chimney stove intervention to reduce long-term woodsmoke exposure lowers blood pressure among Guatemalan women. Environ Health Perspect 115:996–1001

McCracken J, Smith K, Stone P et al (2011) Intervention to lower household woodsmoke exposure in Guatemala reduces ST-segment depression on electrocardiograms. Environ Health Perspect 119:1562–1568

Melsom T, Brinch L et al (2001) Asthma and indoor environment in Nepal. Thorax 56:477–481

Menezes AM, Victora CG, Rigatto M (1994) Prevalence and risk factors for chronic bronchitis in Pelotas, RS, Brazil: a population-based study. Thorax 49:1217–1221

Migliaccio CT, Mauderly JL (2010) Biomass smoke exposures: toxicology and animal study design. Inhal Toxicol 22:104–107

Migliaccio CT, Kobos E, King QO et al (2013) Adverse effects of wood smoke PM(2.5) exposure on macrophage functions. Inhal Toxicol 25:67–76

Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL et al (2007) Longterm exposure to air pollution and incidence of cardiovascular events in women. N Engl J Med 356:447–458

Mishra V (2003) Effect of indoor air pollution from biomass combustion on prevalence of asthma in the elderly. Environ Health Perspect 111:71–78

Mishra V, Dai X, Smith KR et al (2004) Maternal exposure to biomass smoke and reduced birth weight in Zimbabwe. Ann Epidemiol 14:740–747
Murray CJ, Vos T, Lozano R et al (2012) Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380(9859):2197–2223
Nair H, Simões EA, Rudan I et al (2013) Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. Lancet 381(9875):1380–1390
Noorhassim I, Rampal K et al (1995) The relationship between prevalence of asthma and environmental factors in rural households. Med J Malaysia 50:263–267
Orozco-Levi M, Garcia-Aymerich J, Villar J et al (2006) Wood smoke exposure and risk of chronic obstructive pulmonary disease. Eur Respir J 27:542–546
Perez-Padilla R, Regalado J, Vedal S et al (2006) Exposure to biomass smoke and chronic airway disease in Mexican women. A case-control study. Am J Respir Crit Care Med 154:701–706
Po J, FitzGerald J, Carlsten C (2011) Respiratory disease associated with solid biomass fuel exposure in rural women and children: systematic review and meta-analysis. Thorax 66:232–239
Pokhrel AK, Bates MN et al (2010) Tuberculosis and indoor biomass and kerosene use in Nepal: a case–control study. Environ Health Perspect 118:558–564
Pokhrel AK, Bates MN, Shrestha SP et al (2013) Biomass stoves and lens opacity and cataract in Nepalese women. Optom Vis Sci 90:257–268
Pope CA III, Thun MJ, Namboodiri MM, Dockery DW, Evans JS, Speizer FE et al (1995) Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. Am J Respir Crit Care Med 151:669–674
Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K et al (2002) Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287:1132–1141
Pope CA III, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D et al (2004) Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation 109:71–77
Pope CA 3rd, Burnett RT, Krewski D et al (2009) Cardiovascular mortality and exposure to airborne fine particulate matter and cigarette smoke: shape of the exposure-response relationship. Circulation 120:941–948
Pope DP, Mishra V, Thompson L et al (2010) Risk of low birth weight and stillbirth associated with indoor air pollution from solid fuel use in developing countries. Epidemiol Rev 32:70–81
Qureshi KA (1994) Domestic smoke pollution and prevalence of chronic bronchitis/asthma in a rural area of Kashmir. Indian J Chest Dis Allied Sci 36:61–72
Ramirez-Venegas A, Sansores RH, Pérez-Padilla R et al (2006) Survival of patients with chronic obstructive pulmonary disease due to biomass smoke and tobacco. Am J Respir Crit Care Med 173:393–397
Regalado J, Pérez-Padilla R, Sansores R et al (2006) The effect of biomass burning on respiratory symptoms and lung function in rural Mexican women. Am J Respir Crit Care Med 174:901–905
Riddervold IS, Bønløkke JH, Olin AC et al (2012) Effects of wood smoke particles from woodburning stoves on the respiratory health of atopic humans. Part Fibre Toxicol 9:12
Romieu I, Riojas-Rodríguez H, Marrón-Mares AT et al (2009) Improved biomass stove intervention in rural Mexico: impact on the respiratory health of women. Am J Respir Crit Care Med 180:649–656
Rylance J, Gordon SB, Naeher LP et al (2013) Household air pollution: a call for studies into biomarkers of exposure and predictors of respiratory disease. Am J Physiol Lung Cell Mol Physiol 304:L571–578
Saha A, Rao NM, Kulkarni PK et al (2005) Pulmonary function and fuel use: a population survey. Respir Res 6:127
Sehlstedt M, Dove R, Boman C et al (2010) Antioxidant airway responses following experimental exposure to wood smoke in man. Part Fibre Toxicol 7:21

Sezer H, Akkurt I, Guler N et al (2006) A case–control study on the effect of exposure to different substances on the development of COPD. Ann Epidemiol 16:59–62
Shen M, Chapman RS et al (2009) Coal use, stove improvement and adult pneumonia mortality in Xuanwei, China: a retrospective cohort study. Environ Health Perspect 117:261–266
Siddiqui AR, Gold EB, Yang X et al (2008) Prenatal exposure to wood fuel smoke and low birth weight. Environ Health Perspect 116:543–549
Slama K, Chiang CY et al (2010) Indoor solid fuel combustion and tuberculosis: is there an association? Int J Tuberc Lung Dis 14:6–14
Smith KR, Peel JL (2010) Mind the gap. Environ Health Perspect 118:1643–1645
Smith KR, McCracken JP, Weber MW et al (2011) Effect of reduction in household air pollution on childhood pneumonia in Guatemala (RESPIRE): a randomised controlled trial. Lancet 378:1717–1726
Smith KR, Frumkin H, Butler CD, Chafe Z et al (2013) Energy and human health. Annu Rev Public Health 34:1–30
Smith KR, Bruce N, Balakrishnan K et al (2014) Millions dead: how do we know and what does it mean? Methods used in the comparative risk assessment of household air pollution. Annu Rev Public Health 35:185–206
Smith-Sivertsen T, Díaz E, Pope D et al (2009) Effect of reducing indoor air pollution on women's respiratory symptoms and lung function: the RESPIRE Randomized Trial, Guatemala. Am J Epidemiol 170:211–220
Stockfelt L, Sallsten G, Olin AC et al (2012) Effects on airways of short-term exposure to two kinds of wood smoke in a chamber study of healthy humans. Inhal Toxicol 24:47–59
Stockfelt L, Sallsten G, Almerud P et al (2013) Short-term chamber exposure to low doses of two kinds of wood smoke does not induce systemic inflammation, coagulation or oxidative stress in healthy humans. Inhal Toxicol 25:417–425
Sumpter C, Chandramohan D (2013) Systematic review and meta-analysis of the associations between indoor air pollution and tuberculosis. Trop Med Int Health 18:101–108
Teo KK, Ounpuu S, Hawken S, Pandey MR, Valentin V, Hunt D et al (2006) Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case–control study. Lancet 368:647–658
Thacher JD, Emmelin A, Madaki AJ, Thacher TD (2013) Biomass fuel use and the risk of asthma in Nigerian children. Respir Med 107:1845–1851
Thompson LM, Bruce N, Eskenazi B et al (2011) Impact of reduced maternal exposures to wood smoke from an introduced chimney stove on newborn birth weight in rural Guatemala. Environ Health Perspect 119:1489–1494
Tielsch JM, Katz J, Thulasiraj RD et al (2009) Exposure to indoor biomass fuel and tobacco smoke and risk of adverse reproductive outcomes, mortality, respiratory morbidity and growth among newborn infants in south India. Int J Epidemiol 38:1351–1363
Torres-Duque C, Maldonado D, Pérez-Padilla R et al (2008) Biomass fuels and respiratory diseases: a review of the evidence. Proc Am Thorac Soc 5:577–590
Unosson J, Blomberg A, Sandström T et al (2013) Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans. Part Fibre Toxicol 10:20
U.S. Department of Health and Human Services (2006) The health consequences of involuntary exposure to tobacco smoke: a report of the surgeon general. Centers for Disease Control and Prevention, Atlanta
Uzun K, Ozbay B et al (2003) Prevalence of chronic bronchitis-asthma symptoms in biomass fuel exposed females. Environ Health Prev Med 8:13–17
van Heyningen R, Pirie A (1976) Naphthalene cataract in pigmented and albino rabbits. Exp Eye Res 22:393–394
Vork K, Broadwin R et al (2007) Developing asthma in childhood from exposure to secondhand tobacco smoke: insights from a meta-regression. Environ Health Perspect 115:1394–1400
Warwick H, Doig A (2004) Smoke – the killer in the kitchen. ITDG Publishing, London

Wong GW, Brunekreef B, Ellwood P, ISAAC Phase Three Study Group et al (2013) Cooking fuels and prevalence of asthma: a global analysis of phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). Lancet 1:386–394

World Health Organization (2006) WHO air quality guidelines: global update for 2005. World Health Organization Regional Office for Europe, Copenhagen

World Health Organization (2009) Quantifying environmental health impacts. Global estimates of burden of disease caused by environmental risks. World Health Organization, Geneva

World Health Organization (2013) Global tuberculosis report 2013. World Health Organization, Geneva

Xu F, Yin X, Shen H et al (2007) Better understanding the influence of cigarette smoking and indoor air pollution on chronic obstructive pulmonary disease: a case-control study in Mainland China. Respirology 12:891–897

Zelikoff JT, Chen LC, Cohen MD et al (2002) The toxicology of inhaled woodsmoke. J Toxicol Environ Health 5:269–282

Zhou H, Kobzik L (2007) Effect of concentrated ambient particles on macrophage phagocytosis and killing of Streptococcus pneumoniae. Am J Respir Cell Mol Biol 36:460–465

Chapter 15
Using Science to Shape Policy

Jason D. Sacks, Neal Fann, Elizabeth Oesterling Owens, and Daniel L. Costa

15.1 Introduction

The United States has seen markedly improved air quality since the passage of the Clean Air Act (CAA) amendments of 1970. While both the U.S. economy and population have each grown significantly since the Act was first passed, primary pollutants (i.e., those emitted directly from sources) have declined by two-thirds or more (Fig. 15.1). Cleaner air offers significant benefits to society, including: improved public health, increased visibility in recreational areas (e.g., national parks); and, lakes, streams and forests that are less subject to acidification. A recent report commissioned by the U.S. Environmental Protection Agency (EPA) estimated that hundreds of thousands of avoided premature deaths, heart attacks, hospital visits, and millions of avoided asthma attacks and work and school days lost can

J.D. Sacks, M.P.H. (✉)
National Center for Environmental Assessment, Office of Research and Development, B243-01, U.S. EPA, 109 TW Alexander Drive, Research Triangle Park, NC 27711, USA
e-mail: sacks.jason@epa.gov

E.O. Owens, Ph.D.
National Center for Environmental Assessment, Office of Research and Development, U.S. EPA, 26 West Martin Luther King Drive (MS A-110), Cincinnati, OH 45268, USA
e-mail:owens.beth@epa.gov

N. Fann, M.P.P.
Office of Air Quality Planning and Standards, Office of Air and Radiation, C539-07, U.S. EPA, 109 TW Alexander Drive, Research Triangle Park, NC 27711, USA
e-mail: fann.neal@epa.gov

D.L. Costa, Sc.D., DABT
E205-09, Office of Research and Development, U.S. EPA, 109 TW Alexander Drive, Research Triangle Park, NC 27711, USA
e-mail: costa.dan@epa.gov

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*, Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6_15

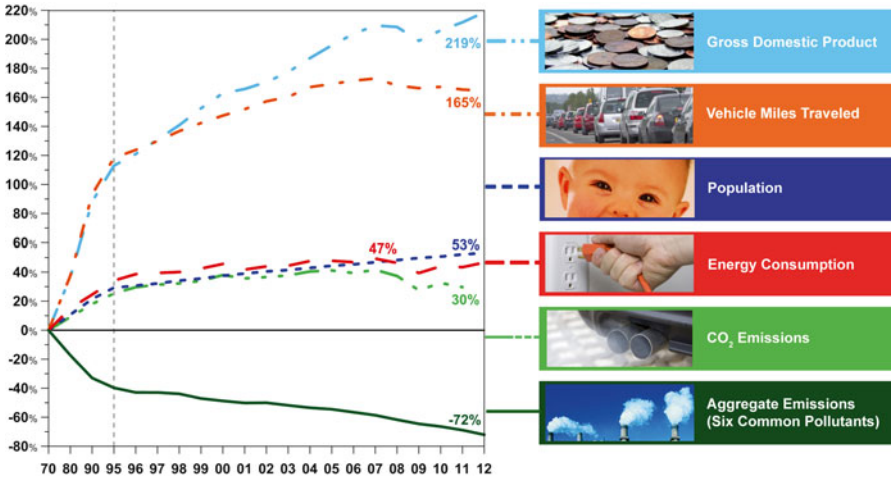

Fig. 15.1 The changing economic, population, and environmental status of the U.S., 1970–2012 (U.S. EPA 2013a)

directly be attributed to improved air quality (U.S. EPA 2011b). The economic value of these avoided deaths and illnesses is staggering—the U.S. has saved many millions of dollars avoiding air pollution-related illnesses, and the dollar value of the avoided premature deaths is many hundreds of billions of dollars. This study computed a return of ~$30 for every dollar spent in air pollutant reduction (U.S. EPA 2011b). Meanwhile, other studies have also shown a significant improvement in life-expectancy at birth as a result of improved air quality (Correia et al. 2013; Pope et al. 2009).

The impetus to form the U.S. Environmental Protection Agency (EPA) can be traced to both the environmental movement of the 1960s and 1970s, which responded in part to prominent environmental disasters, and the advent of focused health science research. Short-term and highly damaging air pollution episodes (e.g., London 1952) and gradually deteriorating air quality in urban centers demonstrated to the public that air quality was greatly influenced by expanding industrial activity and traffic volume; and, it was more than a passing nuisance—it could substantially affect public health and the environment over long periods of time. Initially, polluted air from industrial urban centers was commonly understood as the "reducing-type" and the "oxidant-type," each with unique chemistry profiles. The reducing-type of air pollution was typified by industrial sources and consisted of black, acrid smoke (soot particles and acid gases like oxides of sulfur). On the other hand, the oxidant-type air pollution was the prototype Los Angeles smog and consisted of photochemical oxidant gases (e.g., ozone [O_3]) and their precursors (nitrogen dioxide, carbon monoxide, and hydrocarbons).

Early epidemiologic studies suggested that elevated short-term concentrations of these pollutants could adversely affect health, primarily pulmonary outcomes and in some cases leading to death. This early literature suggested that those groups with pre-existent ailments, and the very young and old seemed to be at greatest risk of an air pollutant-related health effect. Additionally, because lead was present in gasoline

and vehicle exhaust at the time, the health community recognized that this pollutant merited special attention.

Health researchers in academia, federal institutes, and eventually the private sector saw the growing public concern and the early evidence of the potential for adverse health impacts of air pollution across all research disciplines, i.e., epidemiology, controlled human exposure, and animal and in vitro toxicology. While the epidemiologic tools available at that time were rather basic (e.g., not using the sophisticated statistical techniques now employed), it was the empirical health studies conducted in controlled human exposure facilities along with evidence from animal toxicological studies that showed the predominant air pollutants of the time might be linked to adverse health. These pollutants (photochemical oxidants [as ozone, O_3], suspended particles [as total suspended particles or TSP], oxides of sulfur [as sulfur dioxide, SO_2], oxides of nitrogen [as nitrogen dioxide, NO_2], carbon monoxide [CO], and hydrocarbons [as toluene equivalents]—had already been identified in 1971 as ubiquitous and suspect. They came to be called "criteria" (National Ambient Air Quality Standard, or NAAQS) pollutants because EPA is required to develop criteria for the air pollutants, including the health and environmental effects associated with each pollutant.

Scientists from a variety of disciplines have published an enormous amount of peer reviewed health research over the last 40 years since the 1970 CAA, and much of this literature has directly supported quantitative risk assessments. Over time, improved science and quantitative analytical methodologies have provided more refined empirical data upon which EPA relies for its science-policy decisions. The process the Agency follows for setting the NAAQS is widely acknowledged as among the best juried decision process of its kind, and it is this highly structured process that ensures these standards are informed by the best available health science. The CAA also brought renewed attention to the hazardous air pollutants (HAPS; air toxics) which tend to be less ubiquitous than the criteria pollutants described above and for which there is generally less complete data regarding human health risks.

The research linking air pollution to health outcomes has evolved in important ways, modifying our early understanding of how air quality affects specific organs. For example, we know now that the lung is not the sole—or necessarily primary—target of air pollutants. Rather, we now appreciate that the cardiovascular system is an extraordinarily sensitive target for some pollutants. Evidence has also emerged indicating that the nervous and reproductive systems may be important targets. Additionally, the advent of genome sciences shows that DNA is a major, perhaps malleable, determinant of risk and may be targeted by air pollutants and contribute to the development of cancer or heritable traits. Overall, the identification of populations or lifestages at increased risk of an air pollutant-related health effect embraces risk that is innate (genetics) or imposed (lifestyle, life-state or socioeconomic). These factors may come to be particularly important in determining response to air pollution, which could change over time and with exposure.

The science linking air quality and health is developing rapidly, and may yield even greater insight to the biological mechanisms and modes of action. Characterizing the human health exposure and risks associated with multiple pollutants remains a substantial challenge. Humans are rarely exposed to individual pollutants and as

both the absolute and relative levels of air pollutants change, it will become even more important to understand how pollutants within this complex mixture interact to affect human health. For example, some pollutants may prime responses to other pollutants or nonpollutant stressors—and indeed, some air pollution-related chronic or acute health outcomes may increase the risk of other air pollution-related outcomes. The next generation of health research requires innovative thinking (like that which revolutionized the field of air pollution epidemiology during the research of particulate matter), but must now encompass the biological and chemical sciences to more fully understand the complicated relationship between air pollution exposures and health effects. However, these and other new approaches may be challenging to apply within the policy constraints of the CAA.

The first 40 years of health research tended to be compartmentalized into digestible units where pollutant type or outcomes could be assessed using assumptions that now increasingly appear simplistic. Uncertainties regarding the relationship between ambient concentrations and population-level exposures to these pollutants, and understanding how these exposures resulted in human health effects were among the most challenging aspects of compiling comprehensive analyses for NAAQS development. Risk assessors appreciated the issues of spatial and temporal differences in air pollution within the exposure paradigm, as well as dose levels, durations of exposure, and time of day or frequency of exposure. In this early history of the NAAQS program, the comparative paucity of data meant that analysts at times had to draw upon all available data—even those studies with very different analytical approaches. In doing so, these analysts understood the uncertainties these methods introduced in exposure and health impact estimates, and noted these. In contrast to earlier literature, present day assessments are much more refined in terms of both the quality of the scientific studies used. Today, the accuracy and precision of bio-science tools, means that health outcome data can be even more highly refined across pollutants, health outcomes and population subgroups. As the quality and quantity of these data have improved, both risk analysts and managers must synthesize and reconcile this information. Likewise, the scientific literature has expanded to examine health outcomes that range from phenotypic descriptions to detailed mechanistic findings that inform mode-of-action evaluations, which subsequently allows for more detailed and relevant interpretations.

This chapter informs researchers on the processes whereby health science data is coalesced and translated for policy use with the added intention of helping researchers devise studies that could be informative for future policy decisions. This chapter reviews the current process for bringing the science together and translating it into policy. The desired qualities of the scientific information are noted, and for the purposes of this book, which is for the empiricist, special emphasis is placed on the empirical sciences because they target direct issues associated with examining whether a causal relationship exists between air pollutant exposures and health effects. While these types of data may allow for more direct association and incorporation into the regulatory science, the importance of population science—epidemiology—is also appreciated and folded into the full analytical circuit.

15.2 A Brief Legislative History of the Clean Air Act

As described above, a vast amount of scientific literature examining the relationship between air pollution exposures and human health, ecological health and public welfare has been published over the last 40 years, and much of it has been instrumental in shaping the policies that have improved U.S. air quality. Although the current regulatory form of the Clean Air Act (CAA) was not formally instituted until 1970, the U.S. Congress took a number of actions in the 15 years prior to highlight the importance of preserving and protecting the air quality of the nation. By passing the Air Pollution Control Act of 1955 (Air Pollution Control Act of 1955), the U.S. Federal government formally recognized, for the first time, the potential dangers associated with air pollution exposures on public health and welfare (e.g., visibility, agricultural impacts) of the U.S. This legislation requested state and local authorities to develop methods to measure and abate air pollution in their locales. While this Act was not very effective at improving air quality, growing public interest in improving air quality prompted Congress to enact the CAA of 1963 (Clean Air Act of 1963), also known as the "original" CAA, which improved the 1955 legislation with two key provisions: (a) initiate and accelerate a national research and development program to prevent and control air pollution; and (b) develop a grants program to support state and local governments as they develop and implement programs to prevent and control air pollution. The 1963 Act was strengthened somewhat by the Air Quality Act of 1967 (Air Quality Act of 1967), which continued to define the roles of the federal government and state governments in air pollution prevention and control including the development of State Implementation Plans (SIPS) to outline the approach each state would take to attain and maintain air quality standards and initiate approaches for dealing with the interstate transport of pollutants. Whereas state governments were responsible for maintaining clean air, the federal government was tasked with leading the research and development efforts, specifically on the effects of air pollution, monitoring methods, emissions characterization, and air pollution controls. For the first time, motor vehicle emission standards were established, but there were no standards for stationary sources (e.g., electric utilities, smoke stacks), nor was there identification of the pollutants of greatest concern.

The CAA Amendments of 1970 (Clean Air Act of 1970) represented the first environmental legislation that granted power to the federal government to regulate air pollution sources and also included the establishment of NAAQS. With the key provision of the CAA Amendments of 1970 giving the federal government the authority to regulate and enforce air pollution standards, Congress subsequently passed the National Environmental Policy Act, which formed the EPA, and tasked it with instituting this regulatory program (U.S. EPA 2010a). Additional amendments to the CAA (promulgated in 1977) (1977 Amendments to the Clean Air Act of 1970) added requirements to protect the air quality of areas already meeting the NAAQS. In 1978, EPA added lead (Pb), specifically Pb in Total Suspended Particulate (TSP), as the sixth criteria pollutant (Table 15.1). In 1979, hydrocarbons, which were instituted as a criteria pollutant to aid in O_3 control were deemed

Table 15.1 Evolution of the indicator used to define NAAQS criteria pollutants

Criteria Pollutant	Year				
	1970	1978	1979	1987	1997
Particulate matter (PM)	Total suspended particles (TSP)			PM_{10} [a]	PM_{10} $PM_{2.5}$ [b]
Photochemical oxidants (as O_3)	Total photochemical oxidants		Ozone (O_3)		
Oxides of nitrogen (NO_x)	Nitrogen dioxide (NO_2)				
Oxides of sulfur (SO_x)	Sulfur dioxide (SO_2)				
Carbon monoxide (CO)	Carbon monoxide (CO)				
Hydrocarbons (HC)	Hydrocarbons (nonmethane)[c]				
Lead (Pb)		Lead (Pb)[d]			

Note: Gray shading indicates time periods when indicator was not in use
[a]PM_{10} refers to particulate matter with an aerodynamic diameter ≤10 μm
[b]PM2.5 refers to particulate matter with an aerodynamic diameter ≤2.5 μm
[c]Non-methane hydrocarbons to help with O_3 control (Bachmann 2007)
[d]Specifically, Pb in suspended particles

unnecessary and removed as a criteria pollutant (Bachmann 2007). Finally, in 1987, instead of using TSP as a general indicator of particulate pollutants, a specific size fraction of particulate matter (PM) was designated as the indicator, PM_{10} (i.e., particulate matter with an aerodynamic diameter ≤10 μm).

The CAA Amendments of 1990 (1990 Amendments to the Clean Air Act of 1970) represent the last major additions to the CAA to date. They detailed how the federal government, and specifically the EPA, would address the issue of preventing and reducing air pollution (acid rain and its welfare impacts constituted a primary area of focus). Additionally, those chemicals previously regulated under National Emission Standards for Hazardous Air Pollutants (NESHAP), originally defined as 189 HAPs, were now controlled under a new program that focused on emission limits, utilizing Maximum Achievable Control Technology (MACT) standards.

Since the CAA Amendments of 1990, no significant changes have been made to the CAA. However, over time there have been modifications to the indicators used to characterize the criteria pollutant PM. In 1997 the PM NAAQS was further expanded to encompass a smaller size fraction of particles, $PM_{2.5}$ (i.e., particulate matter with an aerodynamic diameter ≤2.5 μm). This has resulted in a PM NAAQS that regulates two size fractions, larger particles known as coarse particles (i.e., PM_{10}) and smaller particles known as fine particles (i.e., $PM_{2.5}$) (Table 15.1).

15.3 The Current NAAQS Process

As designated in the CAA, the scientific basis underlying each of the NAAQS must be reviewed every 5 years. This review process comprises four key components that lead up to the rulemaking process (Fig. 15.2): (1) Planning/Integrated Review Plan (IRP), (2) Integrated Science Assessment (ISA), (3) Risk and Exposure Assessment (REA), and [4] Policy Assessment (PA) (4).

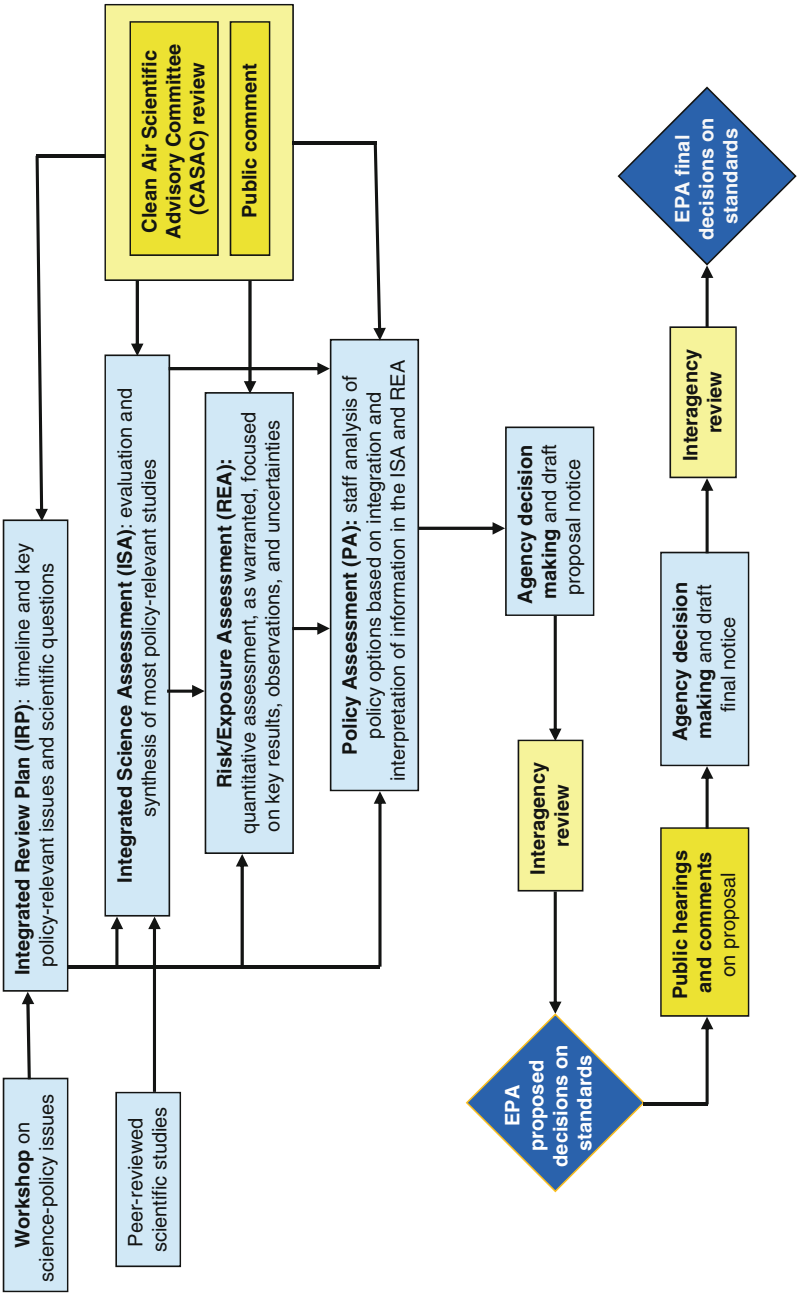

Fig. 15.2 Illustration of the key steps in the process of the review of National Ambient Air Quality Standards (NAAQS) (U.S. EPA 2013b)

The planning aspect of the NAAQS review process begins with a public workshop on the science-policy issues associated with the criteria pollutant of interest. This workshop brings together the scientific community and the public to discuss key research that has taken place since the previous review and to highlight important policy-relevant issues that need to be considered during the current review. The planning process culminates with the development of the IRP, which presents the schedule and details regarding the review process and discusses the key policy-relevant issues that will form the basis of the review.

The second step of the NAAQS review process is the development of the ISA, which establishes the scientific basis of each NAAQS. This effort is led by EPA's National Center of Environmental Assessment (NCEA), which is part of the Office of Research and Development (ORD). The ISA represents a thorough review, synthesis, and evaluation of the current state of the science, specifically the most policy-relevant science. Drafts of the ISA are thoroughly reviewed by the Clean Air Scientific Advisory Committee (CASAC), an independent panel of scientific experts that are vetted and selected by EPA's Science Advisory Board (SAB). Comments on the ISA from this CAA-mandated committee and the public are addressed in the final document.

15.4 Integrated Science Assessments

The ISA forms the scientific basis of each NAAQS review. The development of an ISA starts with a comprehensive literature search and the selection of studies to consider for inclusion in the document. This is facilitated by the publication of a call for information in the Federal Register that invites the public to provide information relevant to the assessment. The EPA maintains an ongoing literature search process for the identification of relevant scientific studies published since the last review of the NAAQS and maintains them in a database developed by the EPA, the Health and Environmental Research Online (HERO) database (U.S. EPA 2014). Published studies and studies accepted for publication that have undergone scientific peer review are considered for inclusion in the ISA.

Each of these studies is then individually evaluated with the aim to consider the strengths, limitations, and possible roles of chance, confounding and other biases that may affect its interpretation such that the final group of studies included in the ISA is deemed to represent those studies of highest quality and relevance. This collection of studies represents the body of scientific literature for a specific criteria pollutant, which spans studies of atmospheric science; human exposure, animal toxicology, controlled human exposure and epidemiology; and studies of ecological and welfare effects (e.g., visibility impairment). Although the ISA focuses on both health and ecological/welfare effects from this point forward the focus of this discussion is only on the health effects aspect of the ISA. It is important to note that the new studies evaluated in each review are done so in the context of the collective body of scientific evidence evaluated in previous reviews. Therefore, in many

instances studies evaluated in previous reviews are brought forward to more fully build upon the conclusions of previous assessments.

15.4.1 Development of Conclusions and Causal Determinations

EPA has developed a framework to integrate scientific evidence across disciplines and evaluate the causal nature of air pollution-related health or welfare effects. The standardized language used in each ISA was drawn from sources across the federal government and wider scientific community, especially the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005a), and National Academy of Sciences (NAS) Institute of Medicine (IOM) document, *Improving the Presumptive Disability Decision-Making Process for Veterans* (Samet and Bodurow 2008). Specifically, this framework is built upon four principles:

- describe the kinds of scientific evidence used to establish a general causal relationship between exposure and health effects;
- characterize the process for integration and evaluation of evidence necessary to reach a conclusion about the existence of a causal relationship;
- identify issues and approaches related to uncertainty;
- provide a means to classify and characterize the weight of evidence informing a determination of a general causal relationship.

Approaches to assessing the separate and combined lines of evidence (e.g., epidemiologic, controlled human exposure, and animal toxicological studies) have been formulated by a number of other regulatory and science agencies, including the IOM of the NAS (Samet and Bodurow 2008), International Agency for Research on Cancer (2006), U.S. EPA (2005a), and Centers for Disease Control and Prevention (CDC) (U.S. Department of Health and Human Services 2004). Causal inference criteria have also been described for ecological effects evidence (U.S. EPA 1998a; Fox 1991). These various frameworks are similar in nature, although adapted to different purposes. These four principles have proven effective in providing a uniform structure and language for EPA's causal determinations.

15.4.1.1 From Association to Causation

The 1964 Surgeon General's report defined "cause" as a "significant, effectual relationship between an agent and an associated disorder or disease in the host" (Public Health Service 1964). More generally, a cause is defined as an agent that brings about an effect or a result. An association is the statistical relationship among variables; alone, however, it is insufficient proof of a causal relationship between an exposure and a health outcome. Unlike an association, a causal claim supports the

creation of counterfactual claims, that is, a claim about what the world would have been like under different or changed circumstances (Samet and Bodurow 2008).

Moving from association to causation involves the elimination of alternative explanations for the association. Evidence from across scientific disciplines (e.g. epidemiology, animal toxicology, and controlled human exposure) for related and similar health effects is evaluated, synthesized, and integrated, including the evaluation of respective strengths and weaknesses in the overall collection of studies. Confidence in the collective body of evidence is evaluated based on study design and quality. The quality and strength of the input evidence varies by pollutant. Consideration of human health effects are informed by three general types of studies: controlled human exposure, epidemiologic and toxicological studies. Mechanistic and other evidence, such as toxicokinetics and exposure, relevant to the evaluation of health effects and of sufficient importance to affect the overall evaluation may be highlighted.

To aid judgment in interpreting scientific results, various "aspects" of causality have been discussed by many philosophers and scientists. For example, the 1964 Surgeon General's report on tobacco smoking discussed criteria for the evaluation of epidemiologic studies, focusing on consistency, strength, specificity, temporal relationship, and coherence (Public Health Service 1964). Sir Austin Bradford Hill (1965) articulated aspects of causality in epidemiology and public health that have been widely used in the scientific community (U.S. EPA 2005a; Samet and Bodurow 2008; International Agency for Research on Cancer 2006; U.S. Department of Health and Human Services 2004). The aspects to judging causality developed by Sir Austin Bradford Hill formed the basis for EPA's causal determination framework, but were modified to encompass a broader array of data (Table 15.2), and to be more consistent with EPA's *Guidelines for Carcinogen Risk Assessment*. This framework was developed to be specific to examining causality for health effects for pollutant exposures; however, it is important to note that in this process there are no simple formulas or fixed rules that can be used to evaluate the evidence and make conclusions regarding causality. For example, one cannot simply count the number of studies reporting statistically significant results or statistically non-significant results and reach credible conclusions about the relative weight of the evidence and the likelihood of causality. Rather, this framework supports a systematic appraisal of the body of evidence, informed by peer and public comment and advice, which includes weighing alternative views on controversial issues. The aspects noted in Table 15.2 do not constitute a strict checklist, but rather provide guidance to determine the weight of the evidence for inferring causality. As such, not meeting one or more of the principles does not automatically preclude a determination of causality [see discussion in (U.S. Department of Health and Human Services 2004)].

Table 15.2 Aspects to aid in judging causality (U.S. EPA 2013b)

Aspect	Description
Consistency of the observed association	An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered.
Coherence	An inference of causality from one line of evidence (e.g., epidemiologic, clinical, or animal studies) may be strengthened by other lines of evidence that support a cause—and—effect interpretation of the association. Evidence on ecological or welfare effects may be drawn from a variety of experimental approaches (e.g., greenhouse, laboratory, and field) and subdisciplines of ecology (e.g., community ecology, biogeochemistry, and paleontological/historical reconstructions). The coherence of evidence from various fields greatly adds to the strength of an inference of causality. In addition, there may be coherence in demonstrating effects across multiple study designs or related health endpoints within one scientific line of evidence.
Biological plausibility	An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A proposed mechanistic linking between an effect and exposure to the agent is an important source of support for causality, especially when data establishing the existence and functioning of those mechanistic links are available.
Biological gradient (exposure—response relationship)	A well—characterized exposure—response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times).
Strength of the observed association	The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. However, it is noted that a small magnitude in an effect estimate may represent a substantial effect in a population.
Experimental evidence	Strong evidence for causality can be provided through "natural experiments" when a change in exposure is found to result in a change in occurrence or frequency of health or welfare effects.
Temporal relationship of the observed association	Evidence of a temporal sequence between the introduction of an agent, and appearance of the effect, constitutes another argument in favor of causality.
Specificity of the observed association	Evidence linking a specific outcome to an exposure can provide a strong argument for causation. However, it must be recognized that rarely, if ever, does exposure to a pollutant invariably predict the occurrence of an outcome, and that a given outcome may have multiple causes.
Analogy	Structure activity relationships and information on the agent's structural analogs can provide insight into whether an association is causal. Similarly, information on mode of action for a chemical, as one of many structural analogs, can inform decisions regarding likely causality.

15.4.1.2 Determination of Causality

To draw conclusions on the causal relationships between criteria pollutant exposures and health effects, ISAs use a five-level hierarchy, to be consistent with EPA *Guidelines for Carcinogen Risk Assessment* that classifies the weight of evidence for causation. This weight of evidence evaluation is based on the integration of findings from various lines of evidence from across health effects disciplines and are integrated into a qualitative statement about the overall weight of evidence and causality. The five descriptors for causal determinations are described in Table 15.3.

Table 15.3 Weight of evidence for causal determination (U.S. EPA 2013b)

	Health effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in health effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example: (a) controlled human exposure studies that demonstrate consistent effects; or (b) observational studies that cannot be explained by plausible alternatives or are supported by other lines of evidence (e.g., animal studies or mode of action information). Evidence includes multiple high—quality studies.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures, but important uncertainties remain. That is, the pollutant has been shown to result in health effects in studies in which chance and bias can be ruled out with reasonable confidence but potential issues remain. For example: (a) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent; or (b) animal toxicological evidence from multiple studies from different laboratories that demonstrate effects, but limited or no human data are available. Evidence generally includes multiple high—quality studies.
Suggestive, but not sufficient, to infer a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures but is limited, and chance, confounding and other biases cannot be ruled out For example, [1] when the body of evidence is relatively small, at least one high—quality epidemiologic study shows an association with a given health and/or a high-quality toxicological study shows effects relevant to humans in animal species; or [2] when the body of evidence is relatively large, evidence from studies of varying quality is generally supportive but not entirely consistent, and there may be coherence across lines of evidence (e.g., animal studies or mode of action information) to support the determination.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations, are mutually consistent in not showing an effect at any level of exposure.

Determination of causality involves the evaluation and integration of evidence for different types of health effects associated with short- and long-term exposure periods. In making determinations of causality, evidence is evaluated for major outcome categories or groups of related endpoints (e.g., respiratory effects). To draw judgments regarding causality, the focus needs to be on the evidence of effects in the range of relevant pollutant exposures or doses, and not the determination of causality at any specific dose. Emphasis is placed on evidence of effects at doses (e.g., blood Pb concentration) or exposures (e.g., air concentrations) that are relevant to, or somewhat above, those currently experienced by the population. The extent to which studies of higher concentrations are considered varies by pollutant and major outcome category, but generally includes those with doses or exposures in the range of one to two orders of magnitude above current or ambient conditions. Studies that use higher doses or exposures may also be considered to the extent that they provide useful information to inform understanding of mode of action, interspecies differences, or factors that may increase the risk of a criteria pollutant-related health effect for) of a portion of the population. Studies which aid in understanding the quantitative relationships between pollutant exposures and health effects, including evaluation of the form of concentration-response or dose-response relationships particularly in populations that may be at greater risk, are quite valuable.

15.4.2 Public Health Impact

Under the CAA, the NAAQS are intended to protect public health with an adequate margin of safety (1990 Amendments to the Clean Air Act of 1970). To fully characterize the public health impact associated with exposures to a criteria pollutant, the ISA also evaluates the scientific evidence to address the following questions that represent key pieces of information used in the development of the REA and PA:

- What is the concentration-response, exposure-response, or dose-response relationship in the human population?
- What is the interrelationship between incidence and severity of effect?
- What exposure conditions (dose or exposure, duration and pattern) are important?
- What populations and lifestages appear to be differentially affected (i.e., at greater or less risk of experiencing effects)?

The first three questions require an evaluation of the quantitative evidence to characterize pollutant concentrations and exposure durations at which effects were observed for exposed populations. This involves considering evidence from both experimental and epidemiologic studies that examine the concentration-response relationship. Although often limited in number, controlled human exposure studies provide the most direct and quantifiable exposure-response data on the human health effects of pollutant exposures. Toxicological studies may also provide supporting information on the concentration-response relationship, specifically with regard to mode of action and characteristics of populations that may be at

increased risk of a criteria pollutant-related health effect. Additionally, epidemiologic studies, which examine the shape of the concentration-response relationship for an entire population, and can evaluate more severe outcomes (e.g., mortality) are added to the overall body of evidence.

An important consideration in characterizing the public health impacts associated with exposure to a criteria pollutant is whether the concentration-response relationship is linear across the range of concentrations or if nonlinear relationships exist. The shape of the concentration-response curve at and below the level of the current standard is of particular interest. Various sources of variability and uncertainty, such as low data density in the lower concentration range, possible influence of exposure measurement error, and variability between individuals to air pollution-related health effects, tend to smooth and "linearize" the concentration-response function, and thus can obscure the existence of a threshold or nonlinear relationship. Because individual thresholds vary from person-to-person due to differences such as genetic polymorphisms or preexisting diseases or conditions, it can be difficult to demonstrate that a single, population-level threshold exists in a population-level study. On the other hand, in animal and in vitro studies where there is substantial variable control, thresholds are quite typical and often suggest lower sensitivity to a pollutant challenge without incorporation of "risk" factors such as genetic factors that might affect mode of action. These sources of variability and uncertainty may explain why the available human data at ambient concentrations for some environmental pollutants (e.g., PM, O_3, Pb, environmental tobacco smoke [ETS], radiation) do not exhibit thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events.

Finally, the assessment of public health impacts of air pollutant exposures necessitates the identification of population groups or lifestages that may be at greater risk for an air pollutant-related health effect. In the ISA, the term "at-risk population" is used to broadly encompass populations or lifestages that have a greater likelihood of experiencing health effects related to exposure to an air pollutant. In the scientific literature, a number of terms have often been used to represent these factors. For example, "susceptibility" has often be used to refer to biological or intrinsic factors (e.g., lifestage, sex, preexisting disease/conditions) while "vulnerability" has often been used to refer to non-biological or extrinsic factors (e.g., socioeconomic status [SES]) (U.S. EPA 2010b; U.S. EPA 2009). Additionally, in some cases, the term "sensitive" has been used to encompass these concepts more generally, but the overarching term "at-risk" is all inclusive and includes interactions among these factors. The overall emphasis of this assessment is to identify and understand the factors that potentially increase the risk for air pollutant-related health effects, regardless of whether the increased risk is due to intrinsic factors, extrinsic factors, increased dose/exposure, or a combination due to the interconnectedness of factors.

To identify factors that potentially lead to increased risk for air pollution-related health effects, the evidence is systematically evaluated across all relevant scientific disciplines (i.e., exposure sciences, dosimetry, toxicology, and epidemiology). An evaluation of studies first consists of focusing on those studies that conducted

stratified analyses (i.e., epidemiologic or controlled human exposure) to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design. Epidemiologic studies can help identify populations potentially at increased risk for air pollutant-related health effects by evaluating whether health responses are modified in a subset of the study population. Examples include testing for interactions or effect modification by factors such as sex, lifestage, or health status. Experimental studies also provide important lines of evidence in the evaluation of factors that may lead to increased risk for an air pollutant related-health effect. For example, toxicological studies conducted using animal models of genetic predisposition (e.g., polymorphisms, gene knockouts) or disease and controlled human exposure studies that examine individuals with underlying disease or genetic polymorphisms may provide evidence in the absence of stratified epidemiologic analyses. These studies can also provide support for coherence with the health effects observed in epidemiologic studies as well as an understanding of biological plausibility. Building on the causal framework discussed in detail above, conclusions are reached regarding the strength of evidence across scientific disciplines for each factor that may contribute to increased risk for an air pollutant-related health effect. The conclusions drawn consider the "Aspects to aid in judging causality" discussed in Table 15.2. The categories considered for evaluating factors that may potentially increase the risk for an air pollutant-related health effect are "adequate evidence," "suggestive evidence," "inadequate evidence," and "evidence of no effect." They are described in more detail in Table 15.4.

Table 15.4 Classification of evidence for potential at-risk factors (U.S. EPA 2013b)

	Health effects
Adequate evidence	There is substantial, consistent evidence within a discipline to conclude that a factor results in a population or lifestage being at increased risk for an air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high—quality studies.
Suggestive evidence	The collective evidence suggests that a factor results in a population or lifestage being at increased risk for an air pollutant-related health effect relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines.
Inadequate evidence	The collective evidence is inadequate to determine if a factor results in a population or lifestage being at increased risk for an air pollutant-related health effect relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency, and/or statistical power to permit a conclusion to be drawn.
Evidence of no effect	There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased risk for an air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high—quality studies.

15.5 Translating the Science into Policy

Following ISA revisions based on CASAC and public reviews the Agency releases a Final ISA. The Office of Air Quality Planning and Standards (OAQPS) in the Office of Air and Radiation (OAR) is responsible for using the key science judgments to develop two critical documents: the Risk and Exposure Assessment (REA) and the Policy Assessment (PA) (U.S. EPA 2013c). These documents each inform the Administrator's final decisions on whether to revise or retain a NAAQS during rulemaking. Note that the Clean Air Act prohibits the Administrator from considering cost when making his or her decision regarding the appropriate standard level—though the Agency may (and indeed does) consider costs and other economic impacts when implementing the standard. OAQPS develops these documents partially in concurrence with the ISA; this ensures that the Agency adheres to the 5 year NAAQS review schedule. The NAAQS decision-making process is inherently collaborative and iterative, ensuring a coherent decision, although, accountability for the documents resides with the respective Offices. The final drafts and CASAC reviews of the REA and PA are completed after finalization of the ISA.

The REA presents a quantitative characterization of the exposures and risks to human health and ecology or public welfare in response to recent air quality conditions and air quality estimated to just meet (i.e. improve air quality to meet the standard, but no further) the existing or alternative air quality standards being considered. However, it is important to note that the REA is not required, and may not be warranted in cases where there is insufficient new scientific evidence or air quality data. The REA is reviewed by CASAC and in final form, provides a detailed discussion of the uncertainties associated with the estimates produced.

In addition to the REA, OAQPS also develops the PA representing the EPA staff analysis of the scientific basis for policy options that are then considered by senior EPA management prior to rulemaking (U.S. EPA 2013c). The policy considerations presented in the PA provide a linkage between the scientific conclusions discussed in the ISA and REA and the scientific judgments required of the EPA Administrator in determining whether the NAAQS for a criteria pollutant should be retained or revised. Development of the PA is also intended to facilitate CASAC's advice to EPA, including its recommendations to the Administrator with regard to the adequacy of the current standard and potential revisions that may be appropriate to consider. As such, the PA focuses on information that is pertinent to evaluating the four basic elements of a NAAQS: (a) "indicator" (i.e., what is the pollutant to be measured); (b) "averaging time" (i.e., defines the time period over which ambient measurements are averaged [e.g., 1-h maximum, 24-h average, annual]); (c) "form" (i.e., what air quality statistic should be used to identify the concentrations to be compared to the level [e.g., current O_3 NAAQS is 4th highest daily maximum 8-h concentration averaged over 3 years]); and (d) "level" (i.e., what is the allowable concentration of the indicator in the ambient air). These elements, which together serve to define each standard, are considered collectively in evaluating the health or welfare protection afforded by the standard.

Taking into consideration the information in the ISA, REA(s), and PA and the advice of CASAC, the EPA develops[1] and publishes a notice of proposed rulemaking that communicates the Administrator's proposed decisions regarding the review of the NAAQS, including preliminary decisions on the adequacy of the current standard(s) and, if appropriate, potential alternative standard(s). Proposed revisions may include revisions to one or all of the basic elements of a standard. The proposed rule presents the scientific basis for the options discussed. A public comment period, during which public hearings are generally held, follows publication of the notice of proposed rulemaking. Taking into account comments received on the proposed rule, the EPA develops and issues a final rule presenting the Administrator's final decision on whether the current NAAQS should be retained or revised. In addition, the Agency prepares a formal Response to Comments document to address substantive issues raised by the public during the public comment period on the proposed rule.

15.6 Hazardous Air Pollutants (HAPs)

As described briefly above, the approach for the regulation of air toxics, also referred to as hazardous air pollutants (HAPs), is different from that of the NAAQS. Rather than six pollutants, the CAA lists 187 pollutants and pollutant classes that the Agency must evaluate if they are emitted in certain amounts from stationary sources (U.S. EPA 2013d). The original list of 189 has since been reduced to 187 after formal petition-capalactum and methyl ethyl ketone (MEK) are no longer listed. A detailed description of the process for assessing HAPs and developing risk estimates can be found elsewhere (U.S. EPA 1998b). The assessment approach is fundamentally based on using computer models to estimate ambient air toxics concentrations and population exposures nationwide. The ability to directly measure ambient air toxics concentrations is currently limited. Such measurements are available for only a subset of air toxics in relatively few locations and for small study populations. Therefore, EPA estimates inhalation exposure concentrations and health risks from a given industry sector using the Human Exposure Model (Community and Sector HEM-3 version 1.1.0). The HEM-3 performs three primary risk assessment activities: (a) conducts dispersion modeling to estimate the concentrations of HAP in ambient air, (b) estimates long-term and short-term inhalation exposures to individuals residing within 50 kilometers (km) of the modeled sources, and (c) estimates individual- and population-level inhalation risks using the exposure estimates and quantitative dose-response information. To estimate potential ingestion impacts, EPA uses the Total Risk Integrated Methodology (U.S. EPA 2010c). Health benchmarks derived by the EPA Integrated Risk Information System (IRIS), the Agency for

[1] As with all major rulemaking, the development of a proposed and final rule undergoes extensive internal EPA review as well as interagency review. The interagency review is coordinated through the Office of Management and Budget and includes review from other agencies across the Federal government.

Toxic Substances and Disease Registry, and other appropriate agencies are used to estimate the maximum individual lifetime cancer risk, as well as noncancer and acute risk. If, as described below, the multipollutant realities of air pollution drive the next dimension of air pollution health science, there will be need for more refined HAPs toxicological data. Perhaps HAPs data coupled with mode-of-action and phenotypic outcomes attributed to NAAQS pollutants will allow for a more systematic perspective of the full public health impacts of air pollution.

15.7 Setting and Implementing Air Quality Standards Outside the U.S

Poor air quality affects populations throughout the world, particularly among developing countries. Indeed, a number of recent studies have highlighted the health burden posed by recent levels of ambient air pollution throughout the world (Burnett et al. 2014) as well as the extent to which air pollution from other countries may affect air quality and health in the U.S. (Anenberg et al. 2010).

While it is not possible to review systematically the approach that each country takes to managing its air quality in the space of this chapter, it is worth summarizing the most commonly used methods as well as the ambient standards. As an example, many countries throughout Southeast Asia specify a health-based air quality standard for the common, or criteria, air pollutants noted above; in certain cases these countries mirror the World Health Organization (WHO)-specified health-based standards or may set a standard that is more or less stringent than the WHO (Schwela 2006). As in the U.S., when designing emission control strategies to meet these standards, countries frequently implement technology-based standards affecting those emission sectors thought to most degrade air quality. As an example, emission control standards may require the use of specific fuels that reduce emissions of NO_x and CO, or may mandate that vehicle manufacturers install filters to trap particles. A comparison of U.S. air quality standards for the six criteria pollutants with those for the WHO and other countries including Japan, Korea, China, India and Singapore is detailed in Table 15.5. This table demonstrates how air quality standards vary according to both the absolute ambient level as well as the exposure duration.

15.8 The Role of Evolving Science in Shaping Future Air Quality Policy

Scientific research is inherently forward-looking and innovative, and fortunately the CAA is designed to accommodate this new knowledge. The CAA mandates EPA to periodically review the adequacy of existing NAAQS, and recognizes the importance of air pollution interactions. It clearly requires the Agency to set standards for

Table 15.5 Selected air quality standards worldwide (Scott 2014)

Air quality standards/guidelines/objectives for different countries (updated April 2014)											
Pollutant	Duration of exposure	WHO AQG (Interim targets)	US standards	EU standards	Japan standards	Korea standards	China standards		Hong Kong AQO (Proposed)	India standards	Singapore standards
							Class I[m]	Class II[m]			
Carbon monoxide (ppm)	24-h				10		3.49 (4 mg/m^3)				
	8-h	10	9[a]	8.73 (10 mg/m^3)	20	9			10 mg/m^3	2 mg/m^3	10 mg/m^3
	1-h	30	35[a]			25	8.73 (10 mg/m^3)		30 mg/m^3	4 mg/m^3	30 mg/m^3
	30-min	60									
	15-min	100									
Nitrogen dioxide (ppb)	Annual	40 ug/m^3	53	40 ug/m^3		30	40 ug/m^3 (NOx 50 ug/m^3)		40	40 ug/m^3	40 ug/m^3
	24-h				40–60	60	80 ug/m^3 (NOx 100 ug/m^3)			80 ug/m^3	
	1-h	200 ug/m^3	100[b]	200 ug/m^3[g]		100	200 ug/m^3 (NOx 250 ug/m^3)		200		200 ug/m^3
Ozone (ppm)	8-h	0.05 (100 ug/m^3) (160 ug/m^3 IT)	0.075[c] (150 ug/m^3)	0.06[h] (120 ug/m^3)		0.06	0.05 (100 ug/m^3)	0.08 (160 ug/m^3)	0.08 (160 ug/m^3)	0.05 (100 ug/m^3)	100 ug/m^3
	1-h				0.06[l]	0.10	0.08 (160 ug/m^3)	0.10 (200 ug/m^3)		0.09 (180 ug/m^3)	
Particulate matter-10 (μg/m^3)	Annual	20 (70-50-30 IT)		40		50	40	70	50	60	20
	24-h	50 (150-100-75 IT)	150[a]	50[i]	100	100	50	150	100	100	50
	1-h				200						
Particulate matter-2.5 (μg/m^3)	Annual	10 (35-25-15 IT)	12[d]	25	15[m]		15[o]	35[o]	35	40	12 → 10[p]
	24-h	25 (75-50-37.5 IT)	35[b]		35[m]		35[o]	75[o]	75	60	37.5 → 25[p]
	Annual (2°)		15[d]								

(continued)

Table 15.5 (continued)

Air quality standards/guidelines/objectives for different countries (updated April 2014)											
Pollutant	Duration of exposure	WHO AQG (Interim targets)	US standards	EU standards	Japan standards	Korea standards	China standards		Hong Kong AQO (Proposed)	India standards	Singapore standards
							Class I[m]	Class II[m]			
Sulfur dioxide (ppb)	Annual					20	7 (20 ug/m^3)	21 (60 ug/m^3)		17 (50 ug/m^3)	15 ug/m^3
	24-h	7 (20 ug/m^3) (125–50 IT)		48[j] (125 ug/m^3)	40	50	17 (50 ug/m^3)	52 (150 ug/m^3)	48 (125 ug/m^3)	28 (80 ug/m^3)	50 → 20 ug/m^3[p]
	3-h (2[o])		500[a]								
	1-h		75[o]	130[k] (350 ug/m^3)	100	150	52 (150 ug/m^3)	175 (500 ug/m^3)			
	10-min	190 (500 ug/m^3)							175 (500 ug/m^3)		

Note: Cross-country comparison is difficult, given inconsistent averaging times, units of measure, statistical adjustments, and numbers of allowed exceedances

[a]Not to be exceeded more than once per year

[b]The 3-year average of the 98th percentile

[c]The 3-year average of the fourth-highest daily maximum 8-h average ozone concentrations measured at each monitor within an area over each year must not exceed 0.075 ppm

[d]The 3-year average of the annual arithmetic mean

[e]The standard is attained when the expected number of days per calendar year with maximum hourly average concentrations above 0.12 ppm is ≤1, as determined by appendix H

[f]99th percentile of 1-h daily maximum concentrations, averaged over 3 years

[g]Allowed 18 exceedances

[h]Allowed 25 days averaged over 3 years

[i]Allowed 35 exceedances

[j]Allowed 3 exceedances

[k]Allowed 24 exceedances

[l]Defined as "photochemical oxidants" (ozone + PAN)

[m]In areas where public normally lives

[n]Class I = natural protection areas; Class II = residential, commercial, industrial, rural areas

[o]Effective nationally by 2016

[p]Target value by 2020 → Target value over "long term"

a single pollutant at a time, but also accounts for the fact that the atmosphere is comprised of pollutant mixtures (1990 Amendments to the Clean Air Act of 1970). Therefore, those performing the research must be aware of the need for basic health research regarding air pollutants and the way in which these pollutants—including both NAAQS and HAPs—interact in the atmosphere and affect public health jointly. As both absolute and relative levels of pollutants change, the potential for these pollutants to interact may change as well. Likewise, as pollutant levels fluctuate, exposures among certain population groups—particularly those who may be socio-economically or otherwise disadvantaged (e.g., compromised health)—may shift as well. As the science continues to evolve, the Agency's science-policy decisions must also evolve in concert. As evidence of the Agency's ability to adapt its policies to changing science, below we describe various approaches to managing air quality that account for a multipollutant atmosphere.

15.8.1 An Evolving Approach to Managing Air Quality in the U.S

While the statutes governing federal air quality policy have remained largely unchanged since the passage of the CAA Amendments of 1990, the EPA has over time evolved in its approach to implementing this law. As described in detail above, the CAA specified distinct regulatory approaches to reduce ambient concentrations and risks associated with the six so-called "criteria" pollutants and the 189 toxic air pollutants. Though largely treated by law as though they are independent, there are important interdependencies among these pollutants. For example:

- *A common array of sources may emit direct and precursor criteria and toxic air pollutants*. For example, a control strategy that focuses on reducing O_3 typically would affect both NO_x and volatile organic compound (VOC) emissions. Reduced levels of VOCs can also attenuate exposures to HAPS that are both primarily released (e.g. benzene, 1,3-butadience) and secondarily formed (e.g. formaldehyde, acetaldehyde).
- *Emission control technologies can reduce levels of both criteria air pollutants and HAPs*. Certain emission controls can affect the levels of criteria pollutants as well as HAPs.
- *Toxic and criteria air pollutants interact with one another in the atmosphere*. An air quality policy that reduces levels of air toxics that are also VOCs may also affect levels of O_3 and (to a lesser extent) $PM_{2.5}$ (Table 15.6).
- *Multiple pollutants affect human health*. Policies affecting the level of SO_2 in the atmosphere will influence $PM_{2.5}$ levels as well as both visibility and terrestrial acidity. Two or more pollutants may jointly contribute to population risks.

Table 15.6 Possible pollutant and atmospheric relationships associated with emission precursor reductions (Scheffe et al. 2007)

Reduction in pollutant emissions	Change in associated pollutant or atmospheric issue										
	Ozone	PM composition			$PM_{2.5}$	Visibility	HAP VOCs	HAP metals	Acid deposition	Watershed eutrophication	Hg—dep/ methylation
		Sulfate	Nitrate	Organic carbon							
SO_2	↓[a, b]	↓	↑[e]		↓	↓			↓		↓[g]
NO_x	↓[c]	↓[d]↑[e]	↓	↓[d]	↓	↓	↓		↓	↓	↑[g]
VOC HAPs	↓	↓	↑[f]↓[d]	↓	↓	↓	↓		–	↓	
CO	↓	↓	↓[d]	↓[d]	↓	↓	↓[d]		↓[d]		
nh_3	–	↓	↓		↓	↓				↓	↑[g]
Primary PM-organic C				↓	↓	↓					
Primary PM-black C					↓	↓					
Primary PM-(crustal/ metals)	↓[b]				↓	↓		↓			
Mercury											↓

[a]Arrow direction denotes relative increase ↑ or decrease ↓, of pollutant resulting from a decrease in associated emissions. Large arrow indicates either well established relationship and/or substantial magnitude of effect. Small arrow implies possible response that is likely to be of minimal magnitude
[b]Ozone reduction associated with decreased light scattering from decrease in fine particle levels
[c]NO_x titration effect on ozone largely limited to VOC-limited urban areas
[d]Associated with effect on decreasing OH and ozone levels
[e]Substitution effect m competition for NH_3 m NH_3-liinited regions (and increase in hydrogen peroxide leading to increased in-cloud SO_2 production)
[f]Associated with reduction of peroxyacetyl radicals leading to increased nitric acid formation
[g]Associated with nitrogen, sulfur, and mercury interactions within sediments

15.8.2 The Multipollutant, Risk-Based Approach to Managing Air Quality

Recognizing the multipollutant nature of ambient air sheds, in its 2004 report *Air Quality Management in the United States* (Air Quality Management in the United States: The National Academies Press 2004), the National Research Council (NRC) recommended that EPA transition from a "pollutant-by-pollutant" approach to managing air quality to a "multipollutant, risk-based approach." That is: design policies that account for the multipollutant nature of the atmosphere and thus reduce both the levels, and associated risks, of multiple pollutants. Since the NRC report, a number of studies have illustrated the advantages of applying this technique.

Perhaps the most complete example is the Detroit multipollutant pilot project (Wesson et al. 2010), whose goals were twofold: (a) demonstrate how such a multipollutant, risk-based approach could be performed using available tools and data; and (b) determine the benefits of this technique, relative to a more traditional air quality management plan. The pilot project employed two contrasting air quality management plans for Detroit. The first, termed the "Status Quo," aimed to attain the $PM_{2.5}$ and O_3 NAAQS at least cost, measured on the basis of the cost per ton of abating emissions. The second sought to improve air quality by at least the same degree as the "Status Quo," but to also maximize human health benefits and achieve air quality improvements among multiple pollutants. Wesson and colleagues employed well-established analytical tools and techniques to model changes in air pollutant emission levels, air quality, health impacts and monetized benefits (Fig. 15.3). When developing the multipollutant risk-based strategy, Wesson et al.

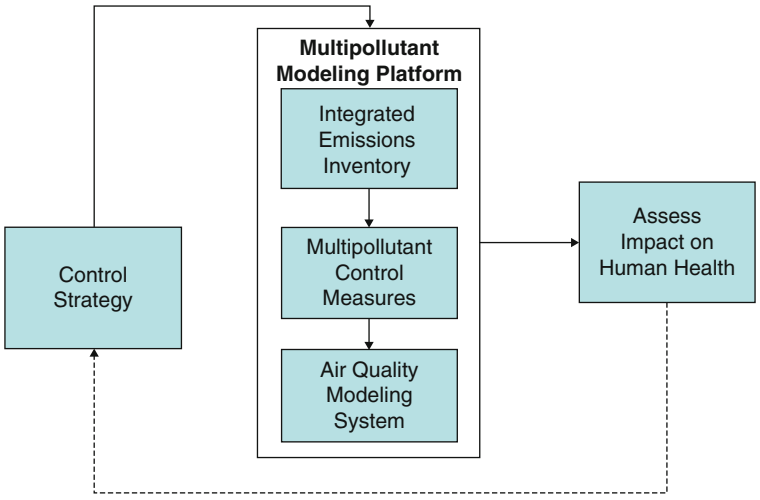

Fig. 15.3 Multipollutant analytical framework (Wesson et al. 2010) (Reprinted under the Creative Commons Attribution 3.0 License)

Table 15.7 Comparison of benefits and costs of the Status-Quo and the multipollutant risk-based approaches (Wesson et al. 2010)

		"Status Quo"	"Multi-pollutant, risk-based"
Total benefits (M 2006$)		$1, 127	$2, 385
Change in population-weighted $PM_{2.5}$ exposure ($\mu g/m^3$)	Regional	0.16	0.1666
	Local	0.2703	0.7211
Change in population-weighted O_3 exposure (ppb)	Regional	0.0005	0.0006
	Local	0.0318	0.0583
Total costs (M 2006$)		$56	$66
Cost per $\mu g/m_3$ $PM_{2.5}$ reduced		$0.50	$0.32
Cost per ppb O_3 reduced		$2.6	$0.58
Net benefits (M 2006$)		$1, 071	$2, 319
Benefit-cost ratio		20.1	36.1

(2010) preferentially selected emission control technologies that would reduce levels of multiple pollutants. For example, fabric filters, coal washing and electrostatic precipitators will each reduce emissions of total $PM_{2.5}$ mass as well as toxic metals.

When compared to the Status-Quo approach, the multipollutant risk-based plan was predicted to reduce O_3 and $PM_{2.5}$ levels throughout the Detroit metropolitan area to a greater degree, to yield twice the monetized health benefits in the form of avoided premature deaths and illnesses, and to lower the non-cancer risk from manganese. The overall projected cost of each strategy was roughly commensurate—the Status Quo was estimated to cost approximately $56M, while the multipollutant risk-based technique cost approximately $66M (2006 dollars). Moreover, the multipollutant risk-based approach was more cost effective compared to the status quo, costing less per $\mu g/m^3$ of $PM_{2.5}$ and ppb of ozone reduced. The net benefits (that is, benefits minus total costs) of the multipollutant risk-based strategy were roughly double those of the Status Quo (Table 15.7).

15.8.3 Characterizing Risk Among Certain Populations

As detailed above, when setting NAAQS, the EPA accounts for the fact that certain populations—due to lifestage, compromised baseline health status or other factors—may experience greater risks from air pollution than the general population. The EPA and others have increasingly sought to account for the elevated risks these subpopulations experience when both designing plans to attain (i.e., meet) the NAAQS and when performing benefits analyses in support of air pollution regulations.

15.8.3.1 Accounting for Specific Groups of the Population When Implementing the Standard

To address this first question of whether it is possible to account for differential risks among specific groups within the larger population when implementing an air quality standard, Fann et al. (2011) drew upon the results of the Detroit multipollutant pilot described above. The authors: (a) explored various techniques for both identifying, and characterizing the level of air pollution risk among specific groups of the population; and (b) tested whether it was possible to target air quality improvements among these groups and hence maximize their health benefits and more equitably distribute risk between these groups and the rest of the population.

Fann et al. (2011) first identified population subgroups that met various definitions of susceptibility and vulnerability and found that irrespective of how they operationalized these terms, the spatial distribution of these population subgroups were remarkably similar. When characterizing the change in air pollution exposure and risk among these vulnerable and susceptible groups, Fann et al. (2011) found that the multipollutant risk-based approach would yield greater health benefits in the form of fewer premature deaths and hospital visits. The multipollutant, risk-based approach also provided a more equitable distribution of air pollution risk among all populations, such that the risk among vulnerable and susceptible populations was more similar to the levels experienced by the rest of the population.

Taken together, the Detroit pilot project papers suggest that it is both technically feasible, and advantageous, to consider both the costs, as well as the size and distribution of benefits, when implementing air quality standards. Using readily available tools and data, policy makers can craft air quality management policy that: (a) reduces risks among multiple pollutants; (b) maximizes human health benefits among the total population as well as those most vulnerable and susceptible; and (c) more equitably distributes air pollution risk.

15.8.3.2 Accounting for Specific Populations When Developing Air Quality Rules

The U.S. EPA has historically described the benefits of air quality rules in terms of aggregate effects and total dollar benefits. For example, the 2005 Clean Air Interstate Rule (U.S. EPA 2005b) reported national monetized net benefits ranging from $71 to $60 billion per year (1999 dollars). That analysis quantified endpoints affecting certain populations, including aggravated asthma among asthmatics, and upper and lower respiratory symptoms among children, among other impacts. However, nowhere did this analysis describe how these impacts were distributed: (a) by place; or (b) by population subgroup.

Responding in part to the directives of Executive Order 12898, Environmental Justice for Low Income & Minority Populations (Executive Order 12898 1994), which requires Agencies to formally consider "disproportionately high and adverse impacts" of its policies, the U.S. EPA has sought to provide more detailed information

regarding the populations affected by air quality rules. Building upon the approach employed in the Detroit multipollutant pilot project, the Agency has recently begun accounting for impacts among certain populations in its national rules. For example, the Transport Rule (U.S. EPA 2011a) included a distributional analysis that aimed to answer two questions: (a) What is the baseline distribution of $PM_{2.5}$-related mortality risk according to race, income and education of the population; and (b) How did the rule affect the way in which this risk was distributed among those populations?

That analysis employed an array of metrics to answer these two questions, finding that the rule not only delivered substantial health benefits to the total population, but that it tended to improve air quality among those populations who were at greatest risk in the baseline. Figure 15.4 projects the change in annual mean $PM_{2.5}$ levels prior to, and after, the Transport Rule was to be implemented. The red outline encircles those counties that were at, or above, the median level of $PM_{2.5}$-related mortality risk in 2005. Note that the levels of $PM_{2.5}$ decline significantly after the Transport Rule is modeled to be implemented, and that the number of counties at or above the median 2005 risk level declines precipitously. These results suggest that the rule would deliver air quality benefits among those populations who were experiencing the highest levels of risk in the 2005 baseline.

In that analysis, we summarized the change in $PM_{2.5}$ mortality risk among populations who have attained three alternate levels of education—less than high school, high school and greater than high school (Fig. 15.5). This analysis applied education-stratified $PM_{2.5}$ mortality risk coefficients.[2] These results indicate that

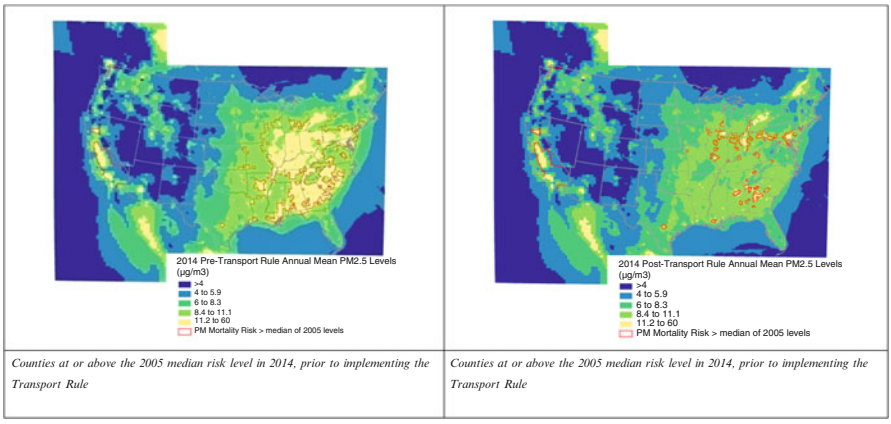

Fig. 15.4 Estimated change in ambient levels, and populations most at risk, of $PM_{2.5}$ in the U.S. prior to and after the Transport Rule is implemented (U.S. EPA 2011a)

[2] Analysis of the American Cancer Society cohort has found that education modifies the $PM_{2.5}$ mortality relationship, such that populations with less than a grade 12 education are at greater risk of $PM_{2.5}$-related death.

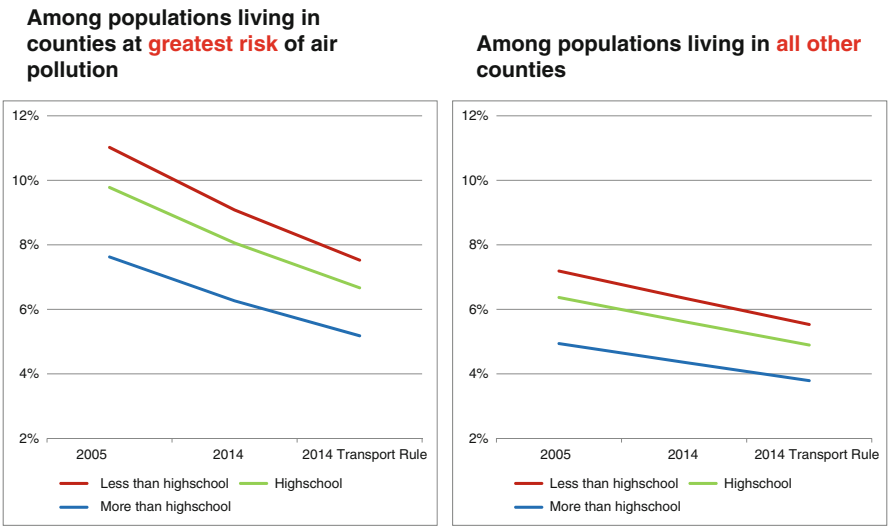

Fig. 15.5 $PM_{2.5}$-related mortality risk among populations of different levels of education attainment and levels of baseline risk (U.S. EPA 2011a)

populations with less than a high school education are at higher risk of $PM_{2.5}$ mortality in 2005, irrespective of whether these populations live in counties identified as being higher risk in the baseline. Between 2005 and the 2014 Transport Rule, all populations see their mortality risk fall, regardless of the level of education they attained.

Detailed results such as these can directly inform national air quality policy, and it is conceivable that as the empirical literature evolves, it may be possible to identify additional factors that affect population susceptibility. However, some of these factors, such as genotypic differences, are challenging to detect in large populations, and so it is likely that risk assessments will be constrained to categorize populations according to readily observable attributes such as those described above. Understanding how populations most susceptible and vulnerable to air pollution are spatially distributed can be useful to decision makers as they design air quality policy—both at the national and local level.

15.8.4 Characterizing the Human Health Burden Associated with Recent and Future Levels of Air Pollution

The substantial benefits of air pollution reductions since 1990 are noted above. Underscoring the impact of air pollution are additional analyses, including that by Fann et al. (2012), that estimated the overall health burden associated with recent levels of air pollution. They found that in 2005 over 100,000 premature deaths were

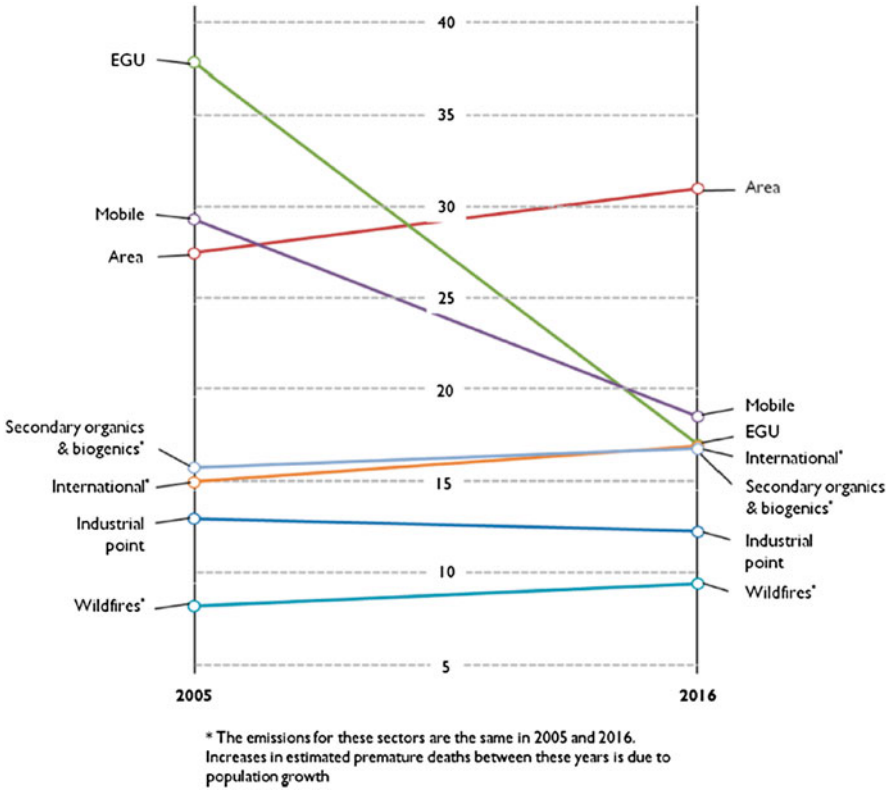

Fig. 15.6 Number of premature $PM_{2.5}$ and O_3-related deaths attributable to seven broad classes of sectors in 2016 (thousands of premature deaths) (Reprinted with permission from Fann et al. 2013, Copyright 2013, American Chemical Society) EGU = electric generating units

attributable to elevated levels of $PM_{2.5}$ and O_3, accounting for approximately six percent of all deaths. The mortality burden of air pollution was not shared equally by location; southern California had among the largest number of premature deaths and highest percentage of all-cause deaths attributable to air pollution. Refining this impact, Fann et al. (2013) used source apportionment modeling techniques to attribute recent (2005) and projected (2016) levels of $PM_{2.5}$ and O_3 to 23 different emission sectors. They found that while emissions from the Power Plant and Mobile sectors contributed to the greatest number of premature deaths in 2005, the health burden from these two sectors would drop significantly by 2016 (Fig. 15.6). A suite of federal and local air quality rules, they predicted, will have greatly reduced the level of $PM_{2.5}$ and O_3 precursors from these two sectors, in turn reducing the associated incidence of health impacts. Taken together, these analyses suggest that the overall health burden associated with recent, and projected, levels of $PM_{2.5}$ and O_3 is substantial and that federal and state air quality policy continues to improve public health.

15.9 Outstanding Science Questions

The CAA is designed in such a way that Agency air quality policy can (and should) adapt to, and directly incorporate, new scientific findings. Indeed, the move toward a multipollutant, risk-based approach and the focus on characterizing the burden of air pollution are each the direct result of advances in air pollution epidemiology, toxicology and human exposure studies. The following details several nascent areas of scientific research that is anticipated to directly influence future air quality policy.

15.9.1 How Do Pollutants, and Species of Pollutants, Interact to Affect Population Risk?

A large and growing body of epidemiologic literature has sought to explain whether population risk to pollutants such as $PM_{2.5}$ can be best explained by individual chemical species (Rohr and Wyzga 2012; Zanobetti et al. 2009; Ito et al. 2006), seasons (Bell et al. 2007, 2009), or region of the U.S. (Davis et al. 2011). Other literature has explored whether the pollutant-risk relationship might be modified, or mediated, by the presence of other pollutants or stressors. For example, researchers have hypothesized that elevated temperature can make O_3 more potent, such that populations exposed to O_3 in warmer climates may be at greater risk, with all other factors being equal (Ren et al. 2009).

Both the absolute and relative levels of air pollutants have changed over time. For example, the population-weighted levels of sulfate $PM_{2.5}$ represented roughly half of total $PM_{2.5}$ exposure in 2005, but are estimated to represent about a third of exposure in 2017 (Fig. 15.7). Changes in the absolute, and relative, levels of

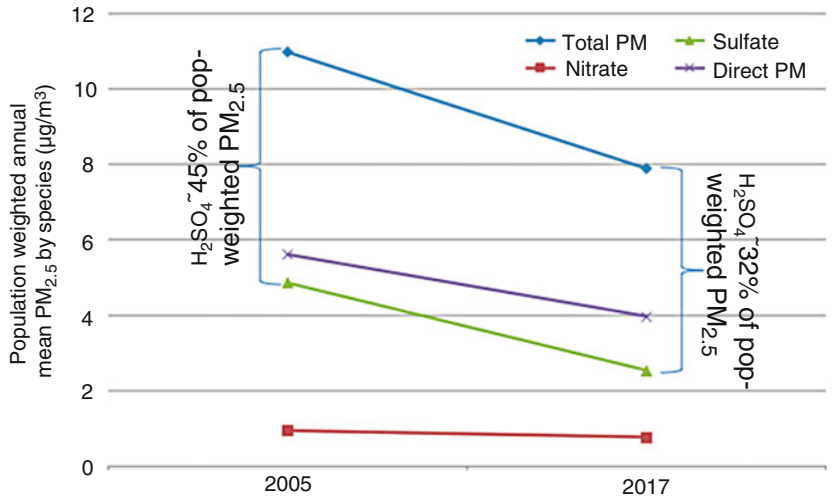

Fig. 15.7 Population-weighted $PM_{2.5}$ mass and species in 2005 and 2017 (U.S. EPA 2013e)

pollutants such as these may affect the findings of epidemiologic studies and may affect the fundamental interaction—chemically and physiologically—between pollutants. Hence the relative "potency" of the pollutants in the atmosphere may differ according to the mix; this may have regional implications as source contributions vary from one locale to another. To the extent that risks vary by location, as well as pollutant mixture, states may want to account for this information when designing emission control standards to meet the NAAQS. Therefore, if the literature finds that risks can be explained by specific pollutant species, combinations of pollutants or stressors, or relative levels of pollutants, air quality policy will need to account for this new area of concern.

15.9.2 To What Extent Do Certain Population Groups Respond to Air Pollutant Exposure Differently?

The literature has increasingly found that certain population groups respond to air pollution exposure to a greater degree than others. While there are not yet universally agreed-upon population attributes that constitute susceptibility or vulnerability (Sacks et al. 2011; American Lung Association 2001; Kleeberger and Ohtsuka 2005; Pope and Dockery 2006; Porta 2008), the empirical literature points to a number of factors that might increase the baseline level of risk to an individual: life stage; baseline exposure to pollution; genetic markers; obesity; compromised baseline health status; and, socio-economic status (SES). Moreover, the literature has suggested that air pollution may move populations from lower risk to higher risk over a lifetime (or less) of exposure. That is—repeated exposure to poor air quality may increase population frailty—thus making individuals more susceptible to future air pollution episodes, or other stressors.

These factors above are important to account for when both setting ambient air quality standards and characterizing population risk. When establishing NAAQS, the EPA determines whether the existing standard is sufficiently protective to sensitive population; further evidence suggesting that specific characteristics of these sensitive groups whose attributes place them at greater baseline risk can be used to inform the level and form of the standard.

15.9.3 How Are Acute and Chronic Air Pollution Effects Related?

Risk assessments performed by EPA and others generally treat air pollution-related health impacts as though they occur—independently—not accounting for the possibility that acute and chronic impacts may be directly related. For example, when estimating the number of avoided $PM_{2.5}$ and O_3-related deaths and illnesses

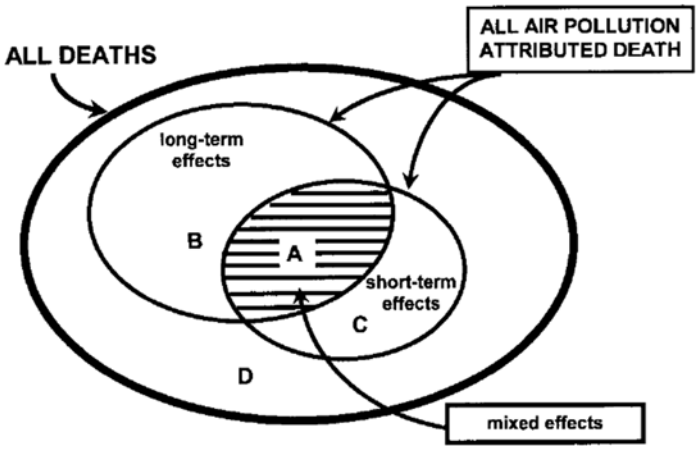

Fig. 15.8 Conceptual diagram illustrating the relationship between short- and long-term $PM_{2.5}$ exposure in contributing to the risk of premature death (Kunzli et al. 2001 by permission of Oxford University Press)

for the Clean Air Interstate Rule, the U.S. EPA reported the number of avoided acute and chronic impacts attributable to improved air quality in 2014 (U.S. EPA 2005b). This "pulse" approach to characterizing air pollution impacts does not account for the potential role that air pollution may have in both initiating and promoting acute and chronic diseases. Figure 15.8 illustrates the potential role that short- and long-term exposure to poor air quality may play in air pollution-related premature deaths; air pollution may affect the occurrence (event) of death ("short term effects") and/or increase the underlying frailty in the population ("long term effects"), leading to a shortening of lifetime (Kunzli et al. 2001). In this figure, the deaths identified by "A" are those whose underlying frailty (or susceptibility) was caused by air pollution, and for which air pollution caused the death. Future research may illuminate the role that air pollution plays in both initiating, as well as promoting, adverse health outcomes.

15.10 Conclusion

Reducing or preventing air pollution impacts on health and the environment is a central goal for all whom either do basic health research associated with air pollution or whom attempt to translate health science into policy. The science is fact-based and provides the intellectual structure upon which policy builds its argument that one approach or another to abatement or avoidance will have benefits in the real world. Historically, the science has been effects based, mostly descriptive but highly valuable for achieving seemingly clear-cut benefits. But as air pollution levels

continue to decline, the persistent public health impacts of air pollution call for more innovative strategies, potentially a move away from the traditional pollutant-by-pollutant approach to regulation. The science must embrace the complexities of the multipollutant real world and become less descriptive and more inquisitive, revealing essential truths that will fuel strategies for finding solutions and avoiding future problems. The CAA and the processes established in the regulatory science are flexible enough to evolve with the advances in science. However, science must also anticipate the future analytical methods needed to support air quality policy. That future has challenges underscored by the need for a common understanding of the respective ground rules and limitations of both the policy maker and health scientist.

Acknowledgments We are grateful to Steven Dutton, John Vandenberg, Bryan Hubbell, Beth Hassett-Sipple, and Kelly Rimer (U.S. EPA) for their critical review of this manuscript. The authors especially thank Ms. Dana Buchbinder for her editorial support and technical assistance.

Disclosures None

Disclaimer This book chapter has been reviewed by the Office of Research & Development (ORD) and the Office of Air Quality Planning and Standards (OAQPS/Office of Air and Radiation) of the U.S. Environmental Protection Agency and approved for publication. Although the research described in this article has been supported by the U.S. EPA, it does not necessarily reflect the views of the agency, and no official endorsement should be inferred.

References

1977 Amendments to the Clean Air Act of 1970. U.S. Government Printing Office; 1977
1990 Amendments to the Clean Air Act of 1970. U.S. Government Printing Office; 1990
Air Pollution Control Act of 1955. U.S. Government Printing Office; 1955
Air Quality Act of 1967. U.S. Government Printing Office; 1967
Air Quality Management in the United States: The National Academies Press; 2004
American Lung Association (2001) Urban air pollution and health inequities: a workshop report. Environ Health Perspect 109(Suppl 3):357–374
Anenberg SC, Horowitz LW, Tong DQ, West JJ (2010) An estimate of the global burden of anthropogenic ozone and fine particulate matter on premature human mortality using atmospheric modeling. Environ Health Perspect 118(9):1189–1195
Bachmann J (2007) Will the circle be unbroken: a history of the U.S. National Ambient Air Quality Standards. J Air Waste Manag Assoc 57(6):652–697
Bell ML, Dominici F, Ebisu K, Zeger SL, Samet JM (2007) Spatial and temporal variation in PM(2.5) chemical composition in the United States for health effects studies. Environ Health Perspect 115(7):989–995
Bell ML, Ebisu K, Peng RD, Samet JM, Dominici F (2009) Hospital admissions and chemical composition of fine particle air pollution. Am J Respir Crit Care Med 179(12):1115–1120
Burnett RT, Pope CA, Ezzati M, Olives C, Lim SS, Mehta S et al (2014) An integrated risk function for estimating the global burden of disease attributable to ambient fine particulate matter exposure. Environ Health Perspect 122(4):397–403
Clean Air Act of 1963. U.S. Government Printing Office; 1963
Clean Air Act of 1970 (1970) Clean Air Act of 1970. U.S. Government Printing Office, Washington, DC

Correia AW, Pope CA 3rd, Dockery DW, Wang Y, Ezzati M, Dominici F (2013) Effect of air pollution control on life expectancy in the United States: an analysis of 545 U.S. counties for the period from 2000 to 2007. Epidemiology 24(1):23–31

Davis JA, Meng Q, Sacks JD, Dutton SJ, Wilson WE, Pinto JP (2011) Regional variations in particulate matter composition and the ability of monitoring data to represent population exposures. Sci Total Environ 409(23):5129–5135

Executive Order 12898 (1994) Federal actions to address environmental justice in minority populations and low-income populations. Federal Register, Executive Order 12898. 59 FR 7629 (February 11, 1994)

Fann N, Roman HA, Fulcher CM, Gentile MA, Hubbell BJ, Wesson K et al (2011) Maximizing health benefits and minimizing inequality: incorporating local-scale data in the design and evaluation of air quality policies. Risk Anal Off Publ Soc Risk Anal 31(6):908–922

Fann N, Lamson AD, Anenberg SC, Wesson K, Risley D, Hubbell BJ (2012) Estimating the national public health burden associated with exposure to ambient PM2.5 and ozone. Risk Anal Off Publ Soc Risk Anal 32(1):81–95

Fann N, Fulcher CM, Baker K (2013) The recent and future health burden of air pollution apportioned across U.S. sectors. Environ Sci Technol 47(8):3580–3589

Fox GA (1991) Practical causal inference for ecoepidemiologists. J Toxicol Environ Health 33(4):359–373

Hill AB (1965) The environment and disease: association or causation? Proc R Soc Med 58:295–300

International Agency for Research on Cancer (2006) IARC monographs on the evaluation of carcinogenic risks to humans, preamble. World Health Organization, Lyon, France

Ito K, Christensen WF, Eatough DJ, Henry RC, Kim E, Laden F et al (2006) PM source apportionment and health effects: 2. An investigation of intermethod variability in associations between source-apportioned fine particle mass and daily mortality in Washington, DC. J Exposure Sci Environ Epidemiol 16(4):300–310

Kleeberger SR, Ohtsuka Y (2005) Gene-particulate matter-health interactions. Toxicol Appl Pharmacol 207(2 Suppl):276–281

Kunzli N, Medina S, Kaiser R, Quenel P, Horak F Jr, Studnicka M (2001) Assessment of deaths attributable to air pollution: should we use risk estimates based on time series or on cohort studies? Am J Epidemiol 153(11):1050–1055

Pope CA 3rd, Dockery DW (2006) Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc 56(6):709–742

Pope CA 3rd, Ezzati M, Dockery DW (2009) Fine-particulate air pollution and life expectancy in the United States. N Engl J Med 360(4):376–386

Porta M (2008) A dictionary of epidemiology. Oxford University Press, Oxford

Ren C, Williams GM, Mengersen K, Morawska L, Tong S (2009) Temperature enhanced effects of ozone on cardiovascular mortality in 95 large US communities, 1987–2000: assessment using the NMMAPS data. Arch Environ Occup Health 64(3):177–184

Rohr AC, Wyzga RE (2012) Attributing health effects to individual particulate matter constituents. Atmos Environ 62:130–152

Sacks JD, Stanek LW, Luben TJ, Johns DO, Buckley BJ, Brown JS et al (2011) Particulate matter-induced health effects: who is susceptible? Environ Health Perspect 119(4):446–454

Samet JM, Bodurow CC (2008) Improving the presumptive disability decision-making process for veterans. National Academies Press, Washington, DC

Scheffe R, Hubbell B, Fox T, Rao V, Pennell W (2007) The rationale for a multipollutant, multimedia air quality management framework. EM Mag 14–20

Schwela D (2006) Urban air pollution in Asian cities: status, challenges and management. Routledge, London

Scott Vorhees (2014) Selected air quality standards worldwide table

U.S. Department of Health and Human Services (2004) The health consequences of smoking: a report of the Surgeon General. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office of Smoking and Health, Atlanta, p 62

U.S. EPA (1998a) Guidelines for ecological risk assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC
U.S. EPA (1998b) Introduction to air toxics risk assessment. U.S. Environmental Protection Agency, Office of Air and Radiation, [April 2014]. Available from: http://www.epa.gov/ttnatw01/urban/appx1011.pdf
U.S. EPA (2005a) Guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency, Washington, DC
U.S. EPA (2005b) Regulatory impact analysis for the final Clean Air Interstate Rule. U.S. Environmental Protection Agency, Office of Air and Radiation, Air Quality Strategies and Standards Division, Emission, Monitoring, and Analysis Division, Clean Air Markets Division, Washington, DC
U.S. EPA (2009) Integrated science assessment for particulate matter [electronic resource]. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Research Triangle Park
U.S. EPA (2010a) National Environmental Policy Act (NEPA 1970, 42 U.S.C. 4321 et seq.): U.S. Environmental Protection Agency, [April 2014]. Available from: http://www.epa.gov/compliance/basics/nepa.html
U.S. EPA (2010b) Integrated science assessment for carbon monoxide Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. Available from: http://purl.fdlp.gov/GPO/gpo1732
U.S. EPA (2010c) Total risk integrated methodology (TRIM) – general information. U.S. Environmental Protection Agency, Office of Air and Radiation, [April 2014]. Available from: http://www.epa.gov/ttn/fera/trim_gen.html
U.S. EPA (2011a) Regulatory impact analysis for the final transport rule. U.S. Environmental Protection Agency, Research Triangle Park
U.S. EPA (2011b) The benefits and costs of the Clean Air Act from 1990 to 2020: final report. U.S. Environmental Protection Agency, Research Triangle Park
U.S. EPA (2013a) Air quality trends: U.S. Environmental Protection Agency, Office of Air and Radiation, [April 2014]. Available from: http://www.epa.gov/airtrends/aqtrends.html#comparison
U.S. EPA (2013b) Integrated science assessment for ozone and related photochemical oxidants. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Research Triangle Park
U.S. EPA (2013c) Process of reviewing the National Ambient Air Quality Standards U.S. Environmental Protection Agency, Office of Air and Radiation, [August 2013]. Available from: http://www.epa.gov/ttn/naaqs/review.html
U.S. EPA (2013d) EPA history: Clean Air Act Amendments of 1990. U.S. Environmental Protection Agency, [April 2014]. Available from: http://www2.epa.gov/aboutepa/epa-history-clean-air-act-amendments-1990
U.S. EPA (2013e) Designing research to assess air quality and health outcomes from air pollution regulations. U.S. Environmental Protection Agency, Research Triangle Park, [January 7, 2013]. Available from: http://www.cleanairinfo.com/finepmpolicy/
U.S. EPA (2014) Health and environmental research online (HERO): U.S. Environmental Protection Agency, [April 2014]. Available from: http://hero.epa.gov/
U.S. Public Health Service (1964) Smoking and health. Report of the advisory committee to the Surgeon General. DHEW publication (PHS), 1103 Washington, DC,
Wesson K, Fann N, Morris M, Fox T, Hubbell B (2010) A multi-pollutant, risk-based approach to air quality management: case study for Detroit. Atmos Pollut Res 1(4):296–304
Zanobetti A, Franklin M, Koutrakis P, Schwartz J (2009) Fine particulate air pollution and its components in association with cause-specific emergency admissions. Environ Health Glob Access Sci Sour 8:58

Index

S.S. Nadadur, J.W. Hollingsworth (eds.), *Air Pollution and Health Effects*,
Molecular and Integrative Toxicology, DOI 10.1007/978-1-4471-6669-6