



# Air Pollution Toxicology—A Brief Review of the Role of the Science in Shaping the Current Understanding of Air Pollution Health Risks

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Human and animal toxicology has had a profound impact on our historical and current understanding of air pollution health effects. Early animal toxicological studies of air pollution had distinctively military or workplace themes. With the discovery that ambient air pollution episodes led to excess illness and death, there became an emergence of toxicological studies that focused on industrial air pollution encountered by the general public. Not only did the pollutants investigated evolve from ambient mixtures to individual pollutants but also the endpoints and outcomes evaluated became more sophisticated, resulting in our present state of the science. Currently, a large toxicological database exists for the effects of particulate matter and ozone, and we provide a focused review of some of the major contributions to the biological understanding for these two “criteria” air pollutants. A limited discussion of the toxicological advancements in the scientific knowledge of two hazardous air pollutants, formaldehyde and phosgene, is also included. Moving forward, the future challenge of air pollution toxicology lies in the health assessment of complex mixtures and their interactions, given the projected impacts of climate change and altered emissions on ambient conditions. In the coming years, the toxicologist will need to be flexible and forward thinking in order to dissect the complexity of the biological system itself, as well as that of air pollution in all its varied forms.

**Key Words:** particulate matter; ozone; hazardous air pollutants; toxicity; historical perspective.

The goal of this review is to discuss the evolving role and significance of human and animal toxicology in the development of our understanding of air pollution impacts on health. The

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toxicological database that constitutes this history is, by its nature, diverse and ever expansive. We provide a selective view of this science from the perspective of the evolution of the field from its early days in the appreciation of the hazards of air pollution to the current state of the science. As such, we first provide a background perspective that is followed by a focused review of our current knowledge based on what are considered the two major “criteria” pollutants, particulate matter (PM) and ozone (O<sub>3</sub>), with a limited review of two selected, notable hazardous air pollutants (HAPs). We chose to focus on formaldehyde because of its widespread use, high reactivity, and established toxicity, with the nasal cavity as its main target. In contrast, phosgene is less reactive, exerts its effects in the deep lung, and follows Haber’s “rule” (concentration × time relationship) for these effects. We end with a view to the future role of air pollution toxicology, the impact of new technology and thinking, and its importance to the regulatory process.

## A BRIEF HISTORY OF AIR POLLUTION

Whereas “natural” hazy smogs have long hung over heavily forested and sunlit topographies like that of the Smoky Mountains of Western North Carolina or the basins of southern California, air pollution as we think of it with all its complexities and intensity has evolved along with human exploitation of combustion. The smoky fires of early cave and hut dwellers choked the air inside their abodes and often blanketed their villages. As communities grew and exhausted available wood supplies, peat and coal came into use to provide fuel for heating and cooking. Access to cheap sulfurous coal launched soot throughout the local ambient environment, fouling the air and settling as a shadowy coat on local structures. As towns and cities grew, kilns and metal smelters

that provided for the urban economic infrastructure added their combustion effluents as an acrid mix. As long ago as AD 61, Seneca, the Roman philosopher wrote: “As soon as I had gotten out of the heavy air of Rome, and from the stink of the chimneys thereof . . . I felt an alteration to my disposition” (Miller and Miller, 1993). Ironically, it was that same sooty, hazy air shrouding major cities that captured the attention of writers like Dickens and painters like Moliere who used fogs like those of London for artistic inspiration. Furthermore, the benefits of the job explosion that came with the industrial revolution of the late 18th and 19th centuries outweighed the yoke of air pollution as people saw the smokes of industry as symbols of prosperity and assumed the pollution was just another of life’s burdens.

A series of air pollution events in the 20th century have come to mark the public realization that air pollution had become a problem that should be limited. Three acute episodes of urban air pollution are considered classic (London, UK; Meuse Valley, Belgium; and Donora, PA). In each event, community inhabitants were clearly affected adversely; hospitalizations were concomitant with an elevated mortality rate. In London, people and animals literally died in the streets. In each case, a meteorological inversion (cold air capped above by a blanket of warm air, with little or no vertical air mixing) that trapped combustion emissions prevailed for multiple days, during which time the concentration of pollutants rose well above the normal levels for these already heavily polluted cities. Few actual measurements of pollution were made in the Meuse Valley and Donora, but crude measurements of the London fog estimated daily average concentrations of suspended PM to be  $\sim 4.5 \text{ mg/m}^3$  and sulfur dioxide ( $\text{SO}_2$ ) to be  $\sim 1.34 \text{ ppm}$ . Hourly peak concentrations were likely much higher. The Meuse Valley episode killed 65 people, whereas in Donora, the number was 20. The infamous “London smog” of 1952 is estimated to have resulted in 4000 excess deaths during the event with a doubling of this number during its aftermath influenza epidemic. In each of these, hospital admissions increased dramatically, mainly among the elderly and those with preexisting cardiac and/or respiratory disease. Even otherwise, healthy people on the street fell to choking spells and sudden death.

The second half of the 20th century has seen ever-decreasing isolated air pollution events in the United States and Western Europe. However, industrialization of the developing world has, in many areas, been retracing the history just outlined. The difference today is that public tolerance of industrial pollution is less, and while rapid economic growth and technology in some parts of the world has brought the plight of dirty ambient air, public pressure on governments is accelerating this evolution toward cleaner air. Nevertheless, over the next decade or so, many problem areas persist around the world.

Early concerns about air pollution focused on domestic and industrial use of coal and growing smelter and manufacturing emissions of particles and gases. However, after World War II, the advent of highway systems to carry traffic to urban and

industrial centers from expanding suburbia added a new dimension to air pollution, one that was less sooty but more chemically reactive and irritating to the eyes and throat. This type of air pollution is driven by photochemical transformation of auto exhaust, yielding secondary oxidants that burned mucous membranes acutely, and over time can crack rubber hoses and gaskets. Los Angeles, with its lighter industry and intensely autocratic culture, became the poster child for this oxidizing pollution in the 1950’s and 1960’s, whereas the heavy industrial centers of Ohio and Pennsylvania retained their sooty reputations. Additional environmental catastrophes that released toxic gases, such as the 1984 methyl isocyanate disaster that killed thousands and injured hundreds of thousands in Bhopal, India, further heightened public awareness. Public annoyance and pressures led to local ordinances, which grew to the state and federal levels (Bachmann, 2007). However, behind this public annoyance, there were seedbeds of toxicological research focusing on these sooty and the oxidizing modes of air pollution providing early fodder to those who feared for their health and that of their children.

#### EARLY TOXICOLOGY AND ITS IMPACT ON PUBLIC SENTIMENT TOWARD AIR POLLUTION

Prior to the London smog incident, animal toxicological studies of air pollution had distinctively military or workplace themes. Studies focused on gases like phosgene with endpoints of lethality or lung pathology (Boyland *et al.*, 1946) or analogous studies with particulate inhalants found in industrial settings, like mining (Ray *et al.*, 1951). However, the 1950’s post-London Smog saw the emergence of toxicological studies focusing on the industrial air pollution as encountered by the public. Although, on the one hand, these studies focused on high concentrations with mortality and pathology outcomes, many of these studies involved “mixed” exposures of particles and gases as one actually finds in ambient air. These mixtures involving smokes generated from fuels like kerosene ( $354 \text{ mg/m}^3$ ) (Pattel and Burgess, 1957) or combinations of coal fire emissions like sulfuric acid mist ( $8 \text{ mg/m}^3$ ) and  $\text{SO}_2$  (89 ppm) imposed for several hours on rodents elicited a range of respiratory effects, including visible impacts on respiration (Amdur, 1954). Interestingly, some of these studies examined repeated exposures and surprisingly found tolerance to otherwise lethal secondary exposures from gases like  $\text{SO}_2$  (1100 ppm) (Pattel and Burgess, 1957). On the other hand, some combined exposures like that of mineral oil aerosol combined with formaldehyde, acrolein, or nitric acid could shorten survival times in exposed mice (Labelle and Brieger, 1955). Subsequently, other particulate exposures used in combination with these gases variably affected their toxicities, suggesting both physical and chemical interactions between the particle and gas phases (Amdur, 1961).

As Mary O. Amdur had seen in early studies evidence for interactions between sulfuric acid and  $\text{SO}_2$ , she began a long

career studying gas-particle interactions between SO<sub>2</sub> (and other water soluble gases like formaldehyde) and water soluble salts (metal salts) and acids (sulfuric and acetic acid) that carried through the 1980's (Costa and Gordon, 2000). Working in concert with the renowned physiologist, Jere Mead, in 1955, Amdur developed a lung function bioassay in guinea pigs, whereby airway resistance and lung compliance could be measured during exposures as indices of irritancy. This bioassay was the mainstay for her career and much of the coal emission studies for many years. The strength of this bioassay was its use in parallel with chemical studies, which provided a body of evidence that SO<sub>2</sub> could be oxidized in acidic aerosols by catalytic metals like Mn, Cu, etc., common to industrial coal and oil emissions. Insoluble particles had no effect, and oily particles tended to suppress irritancy. The oxidized form of SO<sub>2</sub>, sulfuric acid, resulting from metal catalysis was its most irritant form, and its production in the gas-aerosol phase was evidenced in the guinea pig physiology bioassay. Through the late 1950's and 1960's, these studies significantly contributed to growing concerns over industrial air pollution. Cancer studies in animals, on the other hand, were not particularly demonstrative although a chronic study of benzo(a)pyrene (BaP) and SO<sub>2</sub> did show potentiation of BaP carcinogenicity in the SO<sub>2</sub>-exposed rats (Laskin *et al.*, 1970).

Epidemiologic studies at the time appeared to lack the sensitivity to detect either excess cancer or significant mortality or morbidity from air pollution exposures. Human clinical studies (some of which were conducted with sulfuric acid aerosols and SO<sub>2</sub> in the early 1950's by Amdur *et al.*, 1952, 1953) showed irritancy from acid aerosols and SO<sub>2</sub> together or alone but only at high concentrations or, in the case of asthmatics, at rarely encountered levels (U.S. EPA, 1982). It was in the early 1990's that the sensitivity of epidemiologic methods was revolutionized and associations between air pollution and health outcomes at ambient concentrations became apparent. Those low-level effects revealed by the early epidemiology brought about skepticism from many, and it was the toxicology that provided the biological plausibility for those epidemiologic findings and subsequent regulatory decisions.

In the meantime, the novel air pollution scenario of autocentric Los Angeles had begun to inspire investigations into the lung toxicity of O<sub>3</sub>, the primary oxidant of modern day smog. Early studies were conducted in Los Angeles on sentinel animals exposed to the ambient pollutant mixture (Catcott *et al.*, 1958) or by drawing air from above a busy roadway in midday and pumping it into chambers containing rats (Kotin and Falk, 1955). These studies showed a number of effects ranging from lung lesions to nonspecific effects like body weight loss, sensitivity to infection, or elevation of indicators of oxidant challenge. Clearly, these studies score high on the relevancy of exposure to the human scenario, but they have many uncontrolled variables.

The bulk of the early toxicological studies of O<sub>3</sub> were conducted in the laboratories of Herb Stockinger, along with his colleagues at the Air Pollution Control Association (forerunner to the Environmental Protection Agency [EPA]) in Cincinnati, OH. Their studies encompassed a panoply of endpoints that included lung pathology, biochemistry, and physiology (Scheel *et al.*, 1959). However, what was unusual about these early studies was their variety of outcome assessments linked to a range of systemic organ function and biochemical measurements, neurobehavioral changes, and infectivity indices. Moreover, these outcomes were also assessed in light of host (susceptibility) factors such as exercise, disease state, and other organ system interactions (e.g., modeled by thyroidectomy) (Stokinger, 1957; Stokinger and Scheel, 1962; Stokinger *et al.*, 1956). These studies demonstrated, rather convincingly, that although O<sub>3</sub> had clear effects on the lung, it could also affect other organ systems indirectly—and vice versa. The fact that most of the studies were done at very high concentrations led to their diminished consideration in the 1970's, at which time human clinical exposure studies showed effects at relatively low concentrations augmented with exercise; there were no apparent systemic impacts observed in these studies. Nevertheless, it was these early animal toxicology studies that convinced many California residents of the potential toxicity of O<sub>3</sub> beyond that they felt as an irritant nuisance each day.

Collectively, these studies of classical sulfurous and sooty industrial air pollution and that characteristic of the automobile-associated oxidant smog fed the perceptions in a public already activated by the environmental movement of the 1960's. Public demand forced politicians to take action, resulting in the initial Clean Air Act (CAA) legislation of 1967, famously amended in 1970, that established the authority under the newly formed EPA to develop and enforce air pollution standards to protect human and environmental health (National Ambient Air Quality Standards; NAAQS). Revisions to the CAA in 1990 targeted the way in which HAPs were regulated, from a risk-based approach to a technology-based approach. Animal toxicology plays the major role in the review of health effects related to air toxics. For NAAQS reviews, animal toxicology, together with population (epidemiology) and controlled human exposure studies, continues to provide the credible foundation of biological science upon which regulatory decisions can be made.

The following sections highlight more recent developments in experimental studies for several key air pollutants, including NAAQS air pollutants and HAPs. Pollutants reviewed under the NAAQS (also referred to as "criteria pollutants") include those that are widespread, derived from numerous sources, and endanger public health and welfare. There are currently six criteria air pollutants—PM, O<sub>3</sub>, sulfur oxides, nitrogen oxides, carbon monoxide, and lead. Under the 1970 CAA amendments, the NAAQS pollutants are required to undergo scientific review every 5 years.

## PARTICULATE MATTER

PM is comprised of a complex range of chemically and physically diverse substances that exist in the atmosphere as discrete, suspended liquid, or solid particles. PM has contributions from both primary sources (i.e., emitted directly into the atmosphere) and secondary processes (i.e., formed in the atmosphere from precursor emissions). Both primary emissions and secondary precursor emissions can originate from either anthropogenic or natural sources. PM is typically classified by size according to its nominal median aerodynamic diameter (measured in micrometers [ $\mu\text{m}$ ]). The most common approach for differentiating PM is by size classification—coarse particles or  $\text{PM}_{10}$ , fine particles or  $\text{PM}_{2.5}$ , thoracic coarse PM or  $\text{PM}_{10-2.5}$ , and ultrafine particles (UFPs) or  $\text{PM}_{0.1}$ .

### Sulfate and Sulfuric Acid

Beginning in the late 1940's, there was general concern regarding potential health effects among air pollution scientists of sulfuric acid that was emitted by industry, including the zinc plant involved in the 1948 historical air pollution episode in Donora, PA (Amdur, 1989). During oil and coal combustion or the smelting of metal ores, sulfuric acid condenses with available metal ions and water vapor to form submicron sulfuric acid fume and sulfated fly ash. Once in the atmosphere, sunlight-driven chemistry can transform fine sulfuric acid to its neutralized form (i.e., sulfate). In the 1950's, early toxicologists studying PM focused on sulfuric acid and sulfate as "obvious" potential irritants.

Consistent with other pulmonary irritants, sulfuric acid can produce alterations in the mechanical function of the lungs. In guinea pigs, sulfuric acid concentrations ranging from 100 to 1000  $\mu\text{g}/\text{m}^3$  caused a dose-dependent increase in airway resistance (Amdur *et al.*, 1978) because of reflex airway narrowing. The magnitude of bronchoconstriction observed was not only related to acid concentration but also to particle size (Amdur, 1958; Amdur *et al.*, 1978). As particle size was reduced from 7  $\mu\text{m}$  to the submicron range, the concentration of sulfuric acid necessary to induce a response and the time to the onset of the response fell significantly (Amdur *et al.*, 1978). The PM size-based differential response was likely due to filtration of large particles in the nose, with smaller particles able to penetrate deep into the lung.

Amdur and associates also showed early on that generic binary interactions between particles and gases in the absence of light altered the toxicity of either the particle or the gas acting alone. Bronchoconstriction was potentiated in guinea pigs beyond that of particles alone with co-exposure to hydrated metal salts and  $\text{SO}_2$  (Amdur, 1957). The mechanisms behind this interaction has yet to be fully discerned, but it appears to involve either (1) increased solubility of  $\text{SO}_2$  in a hydrated aerosol and subsequent enhanced respiratory penetration or (2) the ability of the metal to catalyze the

oxidation of the dissolved  $\text{SO}_2$  to sulfate. The degree of response to the mixture was determined to be dictated by the aerosol concentration, indicating that the PM inhaled was the proximate irritant. Studies in humans, however, have been less revealing about such interactions, but this animal toxicological database affirms the need to consider the complexity of the atmospheric challenge in estimating biological outcomes.

More complex research into interactions that might impact respiratory irritancy involved the emission mix of sulfuric acid and metal oxide particles common to metal smelting and coal combustion (Amdur *et al.*, 1986). These emitted metal oxide particles, once aged and cooled, were a mixture of singlet and agglomerated UFPs that would be expected to distribute throughout the lung upon inhalation. Guinea pigs exposed to sulfuric acid combined with ultrafine zinc oxide at concentrations of 30–60  $\mu\text{g}/\text{m}^3$  had progressive decreases in lung function (diffusing capacity, total lung capacity, and vital capacity) and increases in lavage fluid cells, protein, and a variety of enzymes that were not completely resolved 96 h after exposure (Amdur, 1989). It is unclear whether the acid was on the surface of the particles or dissolved the particles, but the combination was clearly more toxic than acid alone. These effects greatly exceeded the changes in airway resistance found with relatively simple, binary mixture of  $\text{SO}_2$  and water soluble metal salts.

Since these studies of sulfuric acid,  $\text{SO}_2$ , and various particle mixtures, there have been a number of animal and human exposures to the putative penultimate irritant, sulfuric acid. However, these studies have generally shown little acute irritancy to this acid at near ambient concentrations. Yet, sulfate remains a major portion of today's PM, and one wonders if the focus on the singular toxicant misdirected the science and that the renewed interest in mixtures may reveal a "necessary, but not sufficient" role for particle acidity in health responses.

### Particle Deposition

Early on, differentiation between total respiratory deposition of various sized particles was recognized by Brown *et al.* (1950) and Van Wijk and Patterson (1940). Around this same time, other aerosol researchers developed deposition efficiency curves for different particle sizes by region of the respiratory tract, including the upper respiratory tract and pulmonary air spaces (Hatch and Hameon, 1948). These experimental data, together with additional data that led to further refinement of particle deposition predictions, led to the creation of anatomical models for determining particle deposition in humans. The TGLD (1966) used the conventional division of the respiratory tract into three compartments (extrathoracic, tracheobronchial, and pulmonary regions) in their development in one of the first theoretical models to simulate particle deposition behavior by aerodynamic size (Fig. 1). From this work, the concept of "respirable" dust was proposed for particle sampling, in which

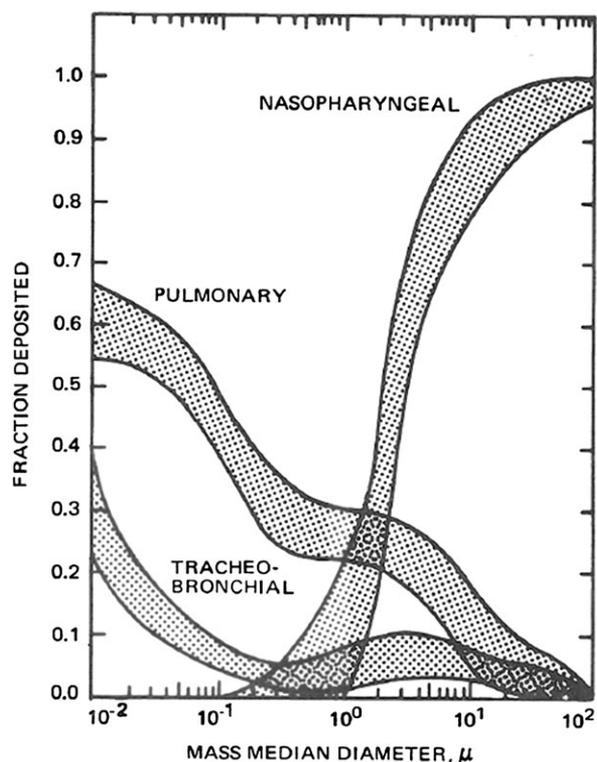


FIG. 1. Fraction of particles deposited in the three respiratory tract compartments as a function of particle diameter. Source: U.S. Department of Health, Education, and Welfare (1969).

particle collection was by aerodynamic diameter similar to the way fractionation occurs in the respiratory tract.

In general, given equivalent nasal breathing conditions, larger particles are preferentially deposited in the extrathoracic region of the respiratory tract, with smaller particles preferentially deposited in the tracheobronchial and alveolar regions. Based on early studies by Giacomelli-Maltoni *et al.* (1972), Hounam *et al.* (1969), Lippmann (1970), Martens and Jacobi (1973), and Rudolf and Heyder (1974), particles about 10  $\mu\text{m}$  or larger are effectively deposited in the extrathoracic region during nasal breathing (U.S. EPA, 1982). The potential health effects of greatest concern are associated with particles that can penetrate to the tracheobronchial and alveolar regions. For nasal breathing, the particle size associated with maximum deposition in the pulmonary region is about 2.5  $\mu\text{m}$ , as first estimated by Lippmann (1977).

In more recent years, advanced particle dosimetry models that incorporate deposition as well as clearance are more representative of respiratory tract anatomy and airflow characteristics and account for differences in breathing patterns and particle properties. The outputs from these advanced models have generally supported the early work conducted to measure regional deposition based on particle size. Of late, research efforts are focused on factors that could modify deposition behavior, such as age and disease state. For example, human volunteers with chronic obstructive pulmo-

nary disease (COPD) have an average deposition rate of about 2.5 times that of healthy adults (Bennett *et al.*, 1997). This research will continue to help identify people who may be at greater risk for PM-related health effects because of greater particle deposition or decreased clearance mechanisms.

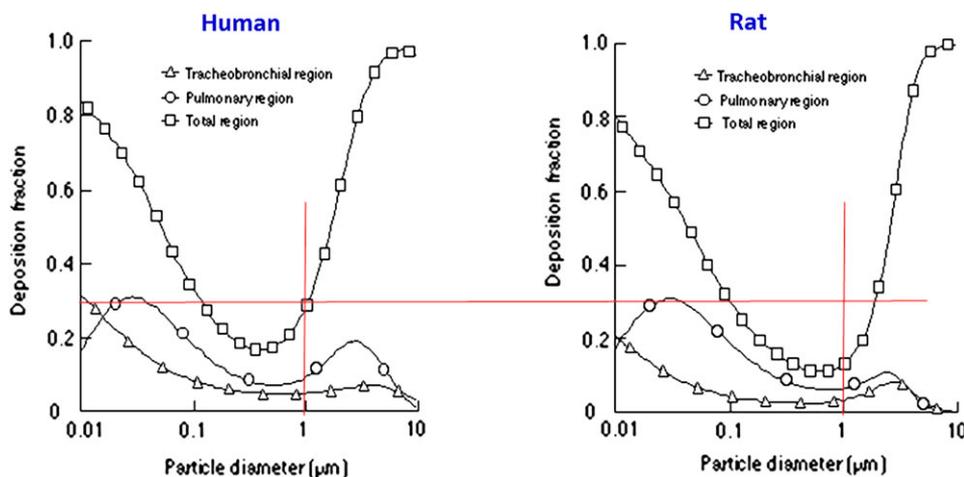
Animals used in toxicology are clearly much smaller than are humans and have respiratory tracts that are comparably smaller (following defined allometric patterns) with some differences in branching patterns and therefore particle deposition (Schlesinger *et al.*, 1997). There are many publications relating rodent and human lungs along these lines, but for the purposes of this brief review, it can be said that the essential qualitative profile of particle deposition by size between the rat and the human are quite similar (Fig. 2). There are, of course, some differences between species in size-dependent deposition, but generally, particles less than 2  $\mu\text{m}$  are considered respirable in most rodents. Hence, the utility of the animal model can be substantiated with some adjustment for delivered dose and generally homologous biology.

### Particle Translocation

Since the early work of Amdur showing greater irritant potency with decreasing particle size (Amdur and Corn, 1963; Amdur and Creasia, 1966), it has been recognized that smaller particles (< 0.1  $\mu\text{m}$ ) may be more toxic than larger particles. UFPs in the environment exist in extremely high numbers but contribute negligibly to mass. When inhaled at the same mass concentration, UFPs with a diameter of 20 nm have a number concentration that is approximately six orders of magnitude higher than for a 2.5- $\mu\text{m}$  particle; the collective particle surface area is also greatly increased. It is this large particle number and large surface area that are of particular concern, as UFPs deposit with high efficiency in the lungs.

Because of their small size, UFPs are inefficiently removed from the lungs with the dominant clearance mechanism of phagocytosis by alveolar macrophages. The increased retention time of UFPs allows these particles to evade macrophage phagocytosis and penetrate into the interstitium more easily than larger sized particles (Ferin *et al.*, 1992; Takenaka *et al.*, 1986). These data demonstrate that UFP interactions with resident respiratory tract cells differ from larger particles, raising the possibility of particle translocation out of the lungs. Migration into the circulation is a hypothesized pathway for UFP effects, although the evidence shows that only a very small amount of UFPs leave the lungs via this mechanism (Brown *et al.*, 2002; Burch, 2002; Mills *et al.*, 2006; Möller *et al.*, 2008; Wiebert *et al.*, 2006a,b). However, UFP translocation may be increased in individuals with compromised alveolar epithelial membrane and/or endothelial integrity (i.e., airway diseases), which could contribute to PM-mediated cardiovascular effects.

Recently, toxicological studies have also demonstrated the translocation of soluble and poorly soluble particles from



**FIG. 2.** Fraction of particle deposition in the three respiratory tract compartments as a function of particle diameter for humans and rats. Data were derived from the multiple path particle dosimetry model (MPPD) (Ashagarian).

the olfactory mucosa via the axons to the olfactory bulb of the brain (Dorman *et al.*, 2001; Elder *et al.*, 2006; Oberdörster *et al.*, 2004; Persson *et al.*, 2003; Wang *et al.*, 2007). Rapid translocation via the axon to the olfactory bulb has been observed for numerous compounds of varying composition, particle size, and solubility. However, the magnitude of particle transport out of the nose remains poorly characterized, and it is unclear how these particles that reach the olfactory bulb affect the brain.

### Toxicology Establishes Biological Plausibility for Epidemiologic Findings

By the mid-1990's, the epidemiologic data provided strong support for the associations between ambient PM concentrations and mortality, but no credible supporting toxicological data were available to support these findings. One example of burgeoning experimental evidence is research on the metal components of PM. It was the production of a biologically coherent toxicology database via analogous mortality observations in laboratory animals with PM surrogates that established a platform of biological plausibility upon which to continue and expand the epidemiologic investigations.

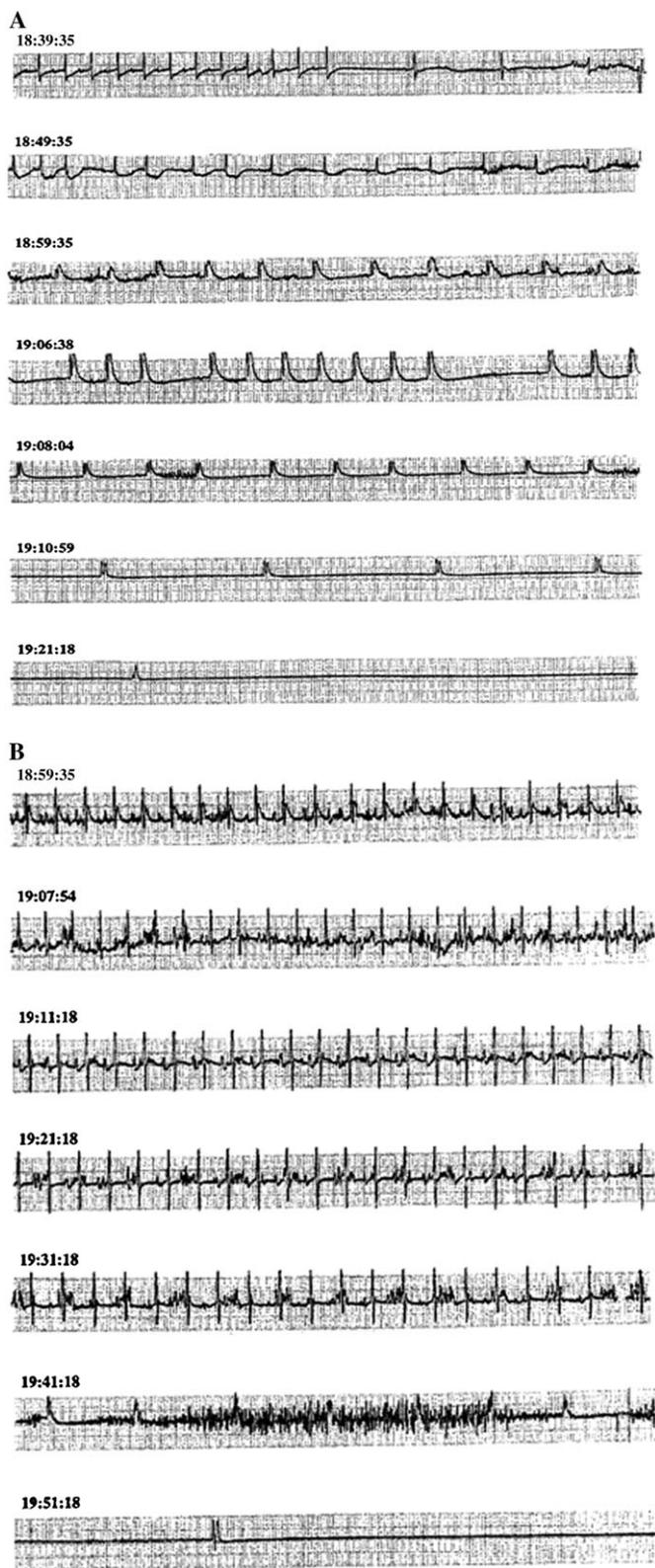
This concept of “biological plausibility” for PM-related mortality was first provided by rats with underlying pulmonary hypertension exposed to residual oil fly ash (ROFA), a particle containing relatively high levels of bioavailable metals. Both intratracheal instillation (0.25–2.5 mg) and inhalation (580  $\mu\text{g}/\text{m}^3$ ) exposure resulted in lethality, thus providing support for the epidemiologic findings (Costa and Dreher, 1997; Killingsworth *et al.*, 1997). Closer examination of the electrocardiogram (ECG) provided the initial toxicological evidence that the heart was a target organ for PM. In diseased animals exposed to ROFA that subsequently died, two possible scenarios were proposed (Fig. 3): a relatively slow failure of the myocardium related to

inflammatory-induced pulmonary edema or a rapid failure of the heart due to conduction-related arrhythmia (Watkinson *et al.*, 1998). Collectively, these studies provided support for the mortality associations in the epidemiologic literature and resulted in the hypothesis that PM-related health effects may be induced by metals.

Further support for the metal hypothesis was provided by a toxicological study that utilized PM filter samples when the steel mill was open (1986), closed (1987), and reopened (1988) in the Utah Valley (Dye *et al.*, 2001) during the same period that the epidemiology demonstrated increased respiratory hospital admissions (Pope, 1989). The extracts containing the greatest amounts of metals (1986 and 1988) caused acute pulmonary injury and inflammation and airway responsiveness in rats, whereas the 1987 extract did not cause significant biochemical or cellular lavage fluid changes. These same Utah Valley filter extracts in human volunteers resulted in a similar pattern of response (Ghio and Devlin, 2001). The chemical analysis of the Utah Valley PM samples demonstrated much greater metal content in the 1986 and 1988 extracts compared with the 1987 extract, providing more support that the metals in PM appeared to be the causative agent. Thus, metals have garnered considerable interest regarding their role in PM toxicity (Costa and Dreher, 1999).

### Cardiovascular Effects

It had been long recognized that PM affected the respiratory system, with the early efforts of Amdur and others. With the findings of Costa and Dreher (1997), Killingsworth *et al.* (1997), and Watkinson *et al.* (1998), more researchers began to investigate the effects of PM on the cardiovascular system. Although the heart, as part of the cardiopulmonary system, has always held an indirect role in health impacts or disease from



**FIG. 3.** ECG waveforms of individual rats exposed to ROFA particles. (A) Progressive development of hypoxia-like changes in ECG waveform features and gradual cardiac failure culminating in asystole. (B) Essentially normal ECG waveforms with superimposed “noise” (possibly due to ectopic stimulation) followed by abrupt cardiac failure and asystole. Source: Watkinson *et al.* (1998).

air pollution, both epidemiologic and toxicological studies pointed to major cardiac involvement with PM exposure.

Subsequent toxicological studies examined ECGs during exposure to PM and suggested disruption of the neural control of the heart as a possible mode of action. PM may target the autonomic nervous system (ANS) to cause downstream effects including alterations in heart rate variability (Godleski *et al.*, 2000; Tankersly *et al.*, 2004; Wellenius *et al.*, 2002), changes in conductivity and repolarization (Campen *et al.*, 2003; Godleski, *et al.*, 2000), and arrhythmia incidence (Watkinson *et al.*, 1998; Wellenius *et al.*, 2002; Wichers *et al.*, 2004). More recently, the ANS has been linked to cardiac oxidative stress following PM<sub>2.5</sub> exposure (Ghelfi *et al.*, 2008; Rhoden *et al.*, 2005) through the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) irritant receptor located on the sensory C-fibers of the lungs.

Godleski *et al.* (2000) first showed that PM exposure can lead to myocardial ischemia in dogs with coronary occlusion by identifying changes in the ST-segment of the ECG. Following these findings, associations between ambient PM concentrations and ST-segment depression were reported in epidemiologic studies (Gold *et al.*, 2005; Pekkanen *et al.*, 2002; Zhang *et al.*, 2009). The mechanism responsible for the exacerbation of myocardial ischemia recently has been explored and may be attributable to reduced myocardial blood flow, perhaps via dysfunctional collateral vessels in the heart (Bartoli *et al.*, 2009).

The effects of PM (plus O<sub>3</sub>) on the systemic vasculature were first demonstrated as acute conduit artery vasoconstriction in human volunteers (Brook *et al.*, 2002). This work led to a closer examination by toxicologists of vascular responses following PM exposure and found impairment of endothelium-dependent dilation (Nurkiewicz *et al.*, 2004, 2006, 2008; Tamagawa *et al.*, 2008), which may be attributable to increased systemic inflammation and/or vascular oxidative stress.

Moreover, the systemic inflammation and oxidative stress associated with endothelial dysfunction can contribute to the development or progression of atherosclerosis with longer PM exposure durations, as demonstrated in recent studies. With subchronic PM exposure in mice prone to developing atherosclerosis, atherosclerotic plaque lesion area in aortas was increased (Araujo *et al.*, 2008; Chen and Nadziejko, 2005; Sun *et al.*, 2005; Ying *et al.*, 2009). Additional responses to support this progression include changes in the composition of the plaque to a more advanced stage (Ying *et al.*, 2009) and elevated expression of tissue factor (Sun *et al.*, 2008), a major regulator of hemostasis and thrombosis following vascular injury or plaque erosion.

#### Potential Systemic Effects Revealed by Toxicological Research

As the cardiovascular effects from exposure to PM became recognized, researchers hypothesized that other extrapulmonary organs could also be targeted. Recent toxicological studies

provide evidence of PM affecting the central nervous system (CNS) and reproductive and developmental outcomes, although the body of literature is severely limited compared what is known about the pulmonary and cardiovascular effects of PM.

### *CNS Effects*

The translocation of soluble and poorly soluble particles from the nose as described above has been proposed as a possible mechanism by which PM or its components may access the CNS directly. Alternatively, PM may act indirectly to affect the CNS via systemic inflammation or autonomic responses through respiratory tract receptors. This is an emerging field, and the available literature is sparse.

The first studies to report neuropathological effects of air pollutants were in mongrel dogs living in Mexico City (Calderón-Garcidueñas *et al.*, 2002, 2003). Histological results from these animals indicated chronic brain inflammation. Additional studies from this group using autopsy brain samples from Mexico City residents indicated brain inflammation and amyloid deposits, causes of neuronal dysfunction that precede the appearance of neuritic plaque formation, and neurofibrillary tangles that are hallmarks of Alzheimer's disease (Peters *et al.*, 2006).

More recent studies have specifically examined CNS effect with exposure to PM. Similar to the studies above, proinflammatory responses in the brain as measured by cytokine expression and production were observed following acute and subchronic PM exposures (Campbell *et al.*, 2005; Kleinman *et al.*, 2008). Loss of dopaminergic neurons in the substantia nigra has also been observed, which is consistent with Parkinson's disease (Veronesi *et al.*, 2005). Though the effect of ambient air pollution on CNS outcomes has recently begun to draw more attention, the evidence for a PM-induced CNS effect is limited.

### *Reproductive and Developmental Effects*

Infants and fetal development processes may be particularly vulnerable to PM exposure. Epidemiologic studies have reported increased risk of low birth weight, preterm birth, growth restriction, and infant mortality associated with PM exposure, but there is little toxicological evidence to provide biological plausibility. Several hypotheses have been proposed involving direct effects on fetal health, altered placenta function, or indirect effects on the mother's health (Bracken *et al.*, 2003; Clifton *et al.*, 2001; Maisonet *et al.*, 2004; Schatz *et al.*, 1990; Sram *et al.*, 2005).

The majority of toxicological research to date for reproductive and developmental effects has used diesel exhaust as the test challenge. The few studies that report on exposure of animals to ambient air in Sao Paulo have demonstrated decreased fertility (Veras *et al.*, 2009), reduced fetal weight (Rocha *et al.*, 2008; Veras *et al.*, 2008), and altered placental

morphology (Veras *et al.*, 2008) as the primary outcomes. Other recent research has shown increased frequency of heritable DNA mutations and epigenetic modifications (Somers *et al.*, 2004; Yauk *et al.*, 2008). More directed toxicological studies will be needed to dissect the many variables and factors that might be impacted directly or indirectly by PM exposures.

The collective database of PM toxicology provides a fabric of evidence that is essential to the plausibility of epidemiologic evidence of human health impacts while providing a more detailed understanding of how it is that PM imparts its effects. It is this mutually supportive fabric of data that makes the PM story so compelling and of such public health significance.

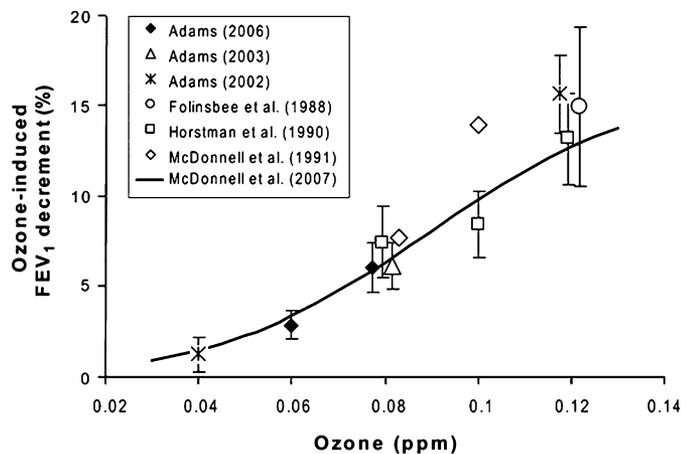
## OZONE

Ground-level O<sub>3</sub> is a secondary pollutant formed by photochemical reactions involving oxygen, volatile organic compounds, and nitrogen oxides. O<sub>3</sub> levels have distinct temporal and spatial differences dependent on the concentrations of precursors, weather patterns, the intensity and spectral distribution of sunlight, and long-range transport processes. Typically, O<sub>3</sub> concentrations peak in early to midafternoon in the summertime and later in the day in areas where O<sub>3</sub> abundance is dependent upon transport from upwind sites.

### Effects of Acute O<sub>3</sub> Exposures

Because the typical O<sub>3</sub> exposure in the ambient environment is at levels where effects can be measured, controlled human exposure studies have come to dominate the toxicology database used for health risk assessment. Animal toxicology is used mainly to define mechanisms and ascertain underlying remodeling and inflammation that have implications for long-term disease.

Controlled human exposure studies have provided strong and well-quantified exposure-response data on the human health effects of O<sub>3</sub> and have been conducted for over 50 years (Chambers *et al.*, 1957; Hallett, 1965; Young *et al.*, 1964). Early controlled human studies investigated the effects of exposure to O<sub>3</sub> in young, healthy, and nonsmoking adults at rest, with forced expiratory volume in 1 second (FEV<sub>1</sub>) decrements observed (Folinsbee *et al.*, 1978; Horvath *et al.*, 1979). Later, symptomatic responses and FEV<sub>1</sub> decrements were induced with lower O<sub>3</sub> exposure concentrations (120–240 ppb) when minute ventilation was increased by heavy exercise (Adams and Schelegle, 1983; Avol *et al.*, 1984; Folinsbee *et al.*, 1984; Gong *et al.*, 1986; Kulle *et al.*, 1985; Linn *et al.*, 1986; McDonnell *et al.*, 1983). Most recently, prolonged 6.6-h exposures are routinely done with moderate exercise at even lower concentrations, and it is these studies that have been used in the latest NAAQS for O<sub>3</sub> (U.S. EPA, 2007). Figure 4 shows a smooth dose-response curve for 6.6-h exposure data for



**FIG. 4.** Comparison of mean O<sub>3</sub>-induced FEV<sub>1</sub> decrements following 6.6 h of constant concentration exposure to O<sub>3</sub>. During each hour of the exposures, subjects were engaged in moderate exercise for 50 min and rest for 10 min. Following the third hour, subjects had an additional 35-min rest period for lunch. The McDonnell *et al.* (2007) curve illustrates the predicted FEV<sub>1</sub> decrement at 6.6 h as a function of O<sub>3</sub> concentration for a 23-year old (the average age of subjects that participated in the illustrated studies). Error bars (where available) are the SE of responses. The data at 0.08 and 0.12 ppm have been offset for illustrative purposes. Source: Reproduced from Brown *et al.* (2008), with permission from Environmental Health Perspectives.

exposures (Adams, 2002, 2003, 2006; Brown *et al.*, 2008; Folinsbee *et al.*, 1988; Horstman *et al.*, 1990; McDonnell *et al.*, 1991).

The alteration in breathing pattern (rapid shallow breaths) observed in the controlled human exposure studies is paralleled in the response of many animal species exposed to O<sub>3</sub> and other lower airway irritants (Tepper *et al.*, 1990). Bronchial C-fibers and rapidly adapting receptors appear to be the primary vagal afferents responsible for O<sub>3</sub>-induced changes in ventilatory rate in both humans (Folinsbee and Hazucha, 2000) and animals (Coleridge *et al.*, 1993; Schelegle *et al.*, 1993, 2001). Stimulation of vagal afferents by O<sub>3</sub> and reactive products is enhanced and sustained by secondary mechanisms activated at a cellular and molecular level (Passannante *et al.*, 1998). Mudway and Kelly (2000) provide a comprehensive review of mechanisms for O<sub>3</sub>-induced pulmonary response.

Stimulation of bronchial C-fibers by O<sub>3</sub> not only inhibits maximal inspiration but also, through local axon reflexes, induces neurogenic inflammation. This pathophysiologic process is characterized by release of tachykinins and other proinflammatory neuropeptides. O<sub>3</sub> exposure has been shown to elevate the C-fiber-associated tachykinin, substance P, in human bronchial lavage fluid (Hazbun *et al.*, 1993) and to deplete neuropeptides synthesized and released from C-fibers in human airway epithelium rich in substance P-immunoreactive axons. Substance P and other transmitters are known to induce granulocyte adhesion and subsequent transposition into the airways, increase vascular permeability and plasma protein extravasation, cause bronchoconstriction, and promote mucus secretion (Solway and Leff, 1991). In

addition, O<sub>3</sub> has been found to activate the airway nerves via stimulation of TRP subfamily A, member 1 channels on C-fibers (Taylor-Clark and Udem, 2010).

The O<sub>3</sub>-induced pulmonary function changes are rapidly reversed during exposure under certain circumstances (Folinsbee *et al.*, 1977; Hazucha *et al.*, 1992) and within about 2 h following exposure (Folinsbee and Hazucha, 1989). A slower recovery phase, especially at higher O<sub>3</sub> concentrations, may take at least 24 h to complete (Folinsbee and Hazucha, 2000). The prolonged recovery could be due to slowly resolving airway inflammation, as indicated by a decrease in immunoreactivity to substance P in the submucosa (Krishna *et al.*, 1997). The release of substance P may also be an important contributing mechanism to persistent post-O<sub>3</sub> bronchoconstriction (Krishna *et al.*, 1997). Additionally, the prolonged dysfunction of small airways (Frank *et al.*, 2001; Weinmann *et al.*, 1995) that likely contributes to the lingering response may be due to both neurogenic and inflammatory mediators in the small than large airways. Many of these inflammatory mediators have bronchoconstrictive properties (Blomberg *et al.*, 1999).

A single exposure to O<sub>3</sub> during moderate or heavy exercise results in a number of cellular and biochemical changes in the lung, including an inflammatory response characterized by increased numbers of polymorphonuclear neutrophils (PMNs), increased permeability of the epithelial cells lining the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins (Alexis *et al.*, 2010; Devlin *et al.*, 1991; Peden *et al.*, 1997; U.S. EPA, 2006). Inflammatory responses do not appear to be correlated with lung function responses (Balmes *et al.*, 1996, 1997; Devlin *et al.*, 1991). However, Vagaggini *et al.* (2010) recently reported a significant ( $r = 0.61$ ,  $p = 0.015$ ) correlation between changes in FEV<sub>1</sub> and changes in sputum neutrophils in mild-to-moderate asthmatics. Similar to lung function responses (Horstman *et al.*, 1995), asthmatics also have greater O<sub>3</sub> inflammatory responses than similarly exposed healthy individuals (Basha *et al.*, 1994; Peden *et al.*, 1997; Scannell *et al.*, 1996). In addition to demonstrating increased neutrophilic inflammation, allergic asthmatics also show eosinophilic inflammation (Peden *et al.*, 1997).

The interaction of O<sub>3</sub> with airway epithelial cell membranes and lining fluid has been well documented to cause lipid ozonation products and reactive oxygen species (U.S. EPA, 2006). Antioxidants and antioxidant enzymes in airway surface liquids protect the underlying epithelial cell layer against oxidative stress, including uric acid, ascorbic acid, tocopherols, and glutathione (GSH). In monkeys, a close association between site-specific O<sub>3</sub> dose, the degree of epithelial injury, and reduced GSH depletion was observed (Plopper *et al.*, 1998). However, Johansson *et al.* (2010) reported that GSH-deficient mice, with 70% depletion of lung GSH, were more resistant than GSH-sufficient mice to O<sub>3</sub>-induced increases in

epithelial permeability. In addition, the GSH-deficient mice tended to have lower inflammatory responses than the GSH-sufficient animals. Thus, differences in the levels of antioxidants between species and regions of the lung do not appear to be the primary factor determining susceptibility to tissue injury from O<sub>3</sub> (Duan *et al.*, 1993, 1996).

O<sub>3</sub> exposure causes an increase in specific and nonspecific airway responsiveness as indicated by a reduction in the concentration of a challenge agent required to produce a given reduction in FEV<sub>1</sub> or increase in specific airway resistance. Increased airway responsiveness is an important consequence of exposure to O<sub>3</sub> because its presence means that the airways are predisposed to narrowing on inhalation of a variety of stimuli (e.g., specific allergens, methacholine, histamine SO<sub>2</sub>, and cold air). Mechanisms underlying O<sub>3</sub>-induced increases in airway responsiveness are only partially understood, but such increases appear to be associated with a number of cellular and biochemical changes in airway tissue. Animal studies suggest that the early post-O<sub>3</sub> responsiveness is, at least in part, vagally mediated (Freed *et al.*, 1996) and that stimulation of C-fibers can lead to increased responsiveness of bronchial smooth muscle independently of systemic and inflammatory changes (Joad *et al.*, 1996). Characteristic O<sub>3</sub>-induced inflammatory airway neutrophilia, which at one time was considered a leading mechanism of airway responsiveness, has been found in a murine model to be only coincidentally associated, i.e., there was no cause and effect relationship (Zhang *et al.*, 1995). This observation does not rule out possible involvement of other cells (such as eosinophils or T-helper cells) in modulation of airway responsiveness.

Asthmatic subjects exposed to O<sub>3</sub>, who characteristically have increased airway responsiveness at baseline, have further increases in responsiveness (Kreit *et al.*, 1989). Several studies (Jörres *et al.*, 1996; Kehrl *et al.*, 1999; Molfino *et al.*, 1991) suggest an increase in specific (i.e., allergen induced) airway reactivity.

Changes in airway responsiveness after O<sub>3</sub> exposure appear to be resolved more slowly than changes in FEV<sub>1</sub> or respiratory symptoms (Folinsbee and Hazucha, 2000). Furthermore, in studies of repeated exposure to O<sub>3</sub>, unlike FEV<sub>1</sub>, the change in airway responsiveness is not attenuated with consecutive exposures (Dimeo *et al.*, 1981; Folinsbee *et al.*, 1994; Gong *et al.*, 1997; Kulle *et al.*, 1982). Increases in airway responsiveness do not appear to be strongly associated with decrements in lung function or increases in symptoms (Aris *et al.*, 1995).

### Effects of Subchronic or Chronic O<sub>3</sub> Exposures

Lung morphology changes in animals have been observed following long-term exposure to O<sub>3</sub>. In infant rhesus monkeys cyclically exposed to O<sub>3</sub>, the structure of the lung was altered with a narrowing of distal airways and entrances to alveolar spaces occurring in more proximal airways than normally

observed (Fanucchi *et al.*, 2006; Plopper *et al.*, 2007). Exposure of these nonhuman primates to allergen and O<sub>3</sub> caused increased airway resistance, increased eosinophils and goblet cells, and increased bronchial smooth muscle density and organization (Plopper *et al.*, 2007). These effects are similar to hallmarks observed with asthma development. Taken together with epidemiologic findings of impaired lung function growth (Avol *et al.*, 2001; Gauderman *et al.*, 2002) and asthma induction (McConnell *et al.*, 2002; McDonnell *et al.*, 1999), these studies provide biological plausibility that long-term O<sub>3</sub> exposure is associated with diminished lung reserves and disease promotion.

Older studies of O<sub>3</sub>-containing mixtures have also demonstrated subchronic or chronic effects on lung function and morphology. Dogs exposed on a daily basis for 68 months to irradiated auto exhaust plus NO<sub>x</sub> and SO<sub>x</sub>, followed by a 3-year recovery period, had abnormalities in lung function, most of which persisted or worsened over the recovery period (Lewis *et al.*, 1974). Enlargement of airspaces and loss of interalveolar septa in proximal acinar regions were most severe in dogs that were exposed to NO<sub>x</sub> and SO<sub>x</sub> with irradiated exhaust were consistent with COPD found in older humans—typically smokers (Hyde *et al.*, 1978). A similar study that examined the effects of relatively low concentrations of O<sub>3</sub> and NO<sub>2</sub> on host defense reported shortened survival times in mice exposed to the mixture with streptococcal pneumonia challenge (Ehrlich *et al.*, 1979). In mice that survived the entire 3-month exposure duration, marked morphological changes in lung tissue including thickening of the alveolar walls and fusion of individual alveoli, consistent with that observed in the dog study (Hyde *et al.*, 1978).

In another study of a complex mixture containing O<sub>3</sub>, rats exposed for 6 months to the polluted air of São Paulo had considerable airway damage, lung function alteration, and altered mucus rheology (Saldiva *et al.*, 1992). This collage of effects is not unlike a composite of injury one might suspect from a mixed atmosphere of oxidants and acid PM in controlled laboratory animal studies. Strangely enough, however, a 7-week study in rats exposed to the polluted Mexico City air, which had induced significant lesions in children, did not reveal in F-344 rats any nasal or lung histopathology (Moss *et al.*, 2001). Although there is no clear reason for the apparent differences in the findings, it is important to appreciate that all sentinel studies have elements of exposure that may be uncontrolled and hence at times can yield conflicting findings.

### HAZARDOUS AIR POLLUTANTS

HAPs represent an inclusive classification for air pollutants that are of anthropogenic origin that are generally of measurable quantity in the air and are not covered in the criteria pollutant list. Likely, ambient concentrations may be very low

if dispersed widely and pose potential cancer risk or may be concentrated in “hot spots” and pose a very different type of risk—that being noncancer irritancy or perhaps acute lung injury. The focus to date has been on the potential carcinogenicity of HAPs, as shown in chronic bioassays, mutagenicity tests in bacterial systems, structure-activity relationships, or—in a few special cases (e.g., benzene and asbestos)—their known carcinogenicity in humans. Noncancer issues frequently relate to direct lung toxicants, which, upon fugitive emissions or accidental release, might risk those with preexisting diseases (e.g., asthma) or which might lead to chronic lung disease. As such, assessments are generally specific to the chemical or class of chemical if mode of action is common. However, the reality of mixed chemical exposures points to the need to develop multipollutant approaches to assessing risk. The assessment of noncancer risk by air toxics to any organ system is based on the computation of long-term inhalation reference concentrations (RfCs) to which individuals may be exposed to daily over a lifetime without adverse, irreversible injury.

#### FORMALDEHYDE

Formaldehyde is a highly reactive and water soluble respiratory tract irritant and known animal carcinogen. The major sources of anthropogenic emissions of formaldehyde are motor vehicle exhaust, power plants, manufacturing plants that produce or use formaldehyde or substances that contain formaldehyde (i.e., adhesives), petroleum refineries, coking operations, incineration, wood burning, and tobacco smoke (Agency for Toxic Substances and Disease Registry (ATSDR), 1999; International Programme on Chemical Safety, 2002). Ambient levels of formaldehyde in outdoor air are often significantly lower than those measured in the indoor air of workplaces or residences (ATSDR, 1999; IARC, 1995). Because of its widespread use and toxicity, it poses a significant concern to human health. Formaldehyde is the first of the series of related aliphatic aldehydes that include acrolein and acetaldehyde.

The recent history of formaldehyde animal research has significantly advanced our knowledge of inhalation toxicology, particularly with regard to the nasal cavity as a portal-of-entry target tissue and nasal dosimetry. From this perspective, formaldehyde research has provided insight into the patterns of nasal lesion distribution and the correlation of dose with tissue effect and has led to the development of more refined nasal dosimetry models. Although there is a wealth of formaldehyde studies available in the literature, these contributions are exemplified in the series of related studies discussed below.

In the early 1980's, a thorough examination of the long-term inhalation toxicity and carcinogenicity of formaldehyde was conducted in two related studies (Kerns *et al.*, 1983; Swenberg

*et al.*, 1980). Significant concentration-related effects in the nasal cavities of rats and mice were shown that included rhinitis, epithelial dysplasia, and squamous metaplasia. In addition, rats exposed to the highest concentration had a high incidence of squamous cell carcinomas. These nasal lesions were observed primarily in the anterior regions of the nasal cavity. Most notably, the findings from these initial studies stimulated the concern for formaldehyde as a risk to human health and led to the beginning of extensive research on the toxicity, mechanisms of carcinogenicity, and the inhalation dosimetry of formaldehyde.

This work led Morgan *et al.* (1986, 1991) to study the relationship between the distribution of formaldehyde-induced nasal lesions and “delivered dose” to the underlying tissue. Morgan *et al.* (1986) examined histology data from Kerns *et al.* (1983) and Swenberg *et al.* (1980) to more precisely determine the sites of tumor origin and confirmed the earlier findings that the majority of carcinomas occurred in the anterior portion and septum of the nasal cavity. Morgan *et al.* (1991) then laid the foundation for studying airflow distribution patterns in the nasal cavity in order to test the hypothesis that the distribution of formaldehyde-induced nasal lesions may be partially attributable to regional uptake patterns influenced by airflow. Nonuniform and complex flow patterns were observed in nasal casts of both F344 rats and rhesus monkeys. The flow patterns in the monkey cast differed significantly and were far more complex and varied than those in rats. This study also found a good correlation between routes of flow and impaction on the airway wall with the reported distribution of formaldehyde-induced nasal lesions in rats and rhesus monkeys.

These observations are significant on several levels. First, the complex but generally consistent and orderly streamlines show a dependence of nasal airflow patterns on nasal geometry. Second, all observations indicate that flow into the nasal region is nonuniform, indicating that deposition onto tissue surfaces should be nonuniform. Third, airflow patterns play a role in the distinct distribution of nasal lesions known to be induced by agents such as formaldehyde.

However, use of this approach for dosimetric comparisons among different species was limited from a quantitative standpoint. Importantly, the limitations in the accuracy and resolution of this technique were subsequently addressed by the development of quantitative mathematical airflow models (i.e., computational fluid dynamics or CFD) capable of characterizing air-phase toxicant transport.

Expanding upon the work and observations of Morgan *et al.* (1986, 1991), Kimbell *et al.* (1993) used CFD modeling of airflow in the nasal cavity of the rat to describe disposition of inhaled gases using formaldehyde as an example. The results were among the first to demonstrate the application of CFD to regional dosimetry of inhaled gases in predicting quantitative mass flux (mass flow rates across a unit area; i.e., dose) patterns to the surface of the nasal airway walls. Consistent with earlier observations on airflow, these results also indicated that there

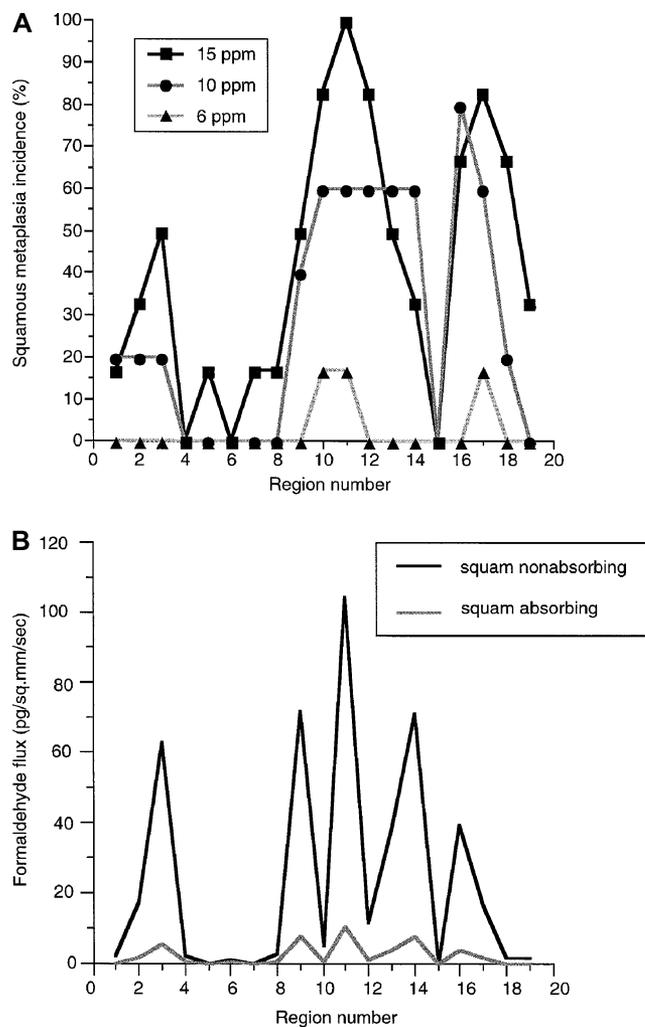
are considerable levels of nonuniformity across the surfaces of the nasal cavity. For example, high mass flux regions or “hot spots” were predicted in the anterior regions of the nasal cavity where airflow was the greatest. Conversely, low flux regions were predicted in regions more posterior to the nares that received the least airflow. In addition, the CFD-generated airflow streams corresponded well with those visualized in nasal casts by *Morgan et al. (1991)*.

To better assess the effect that species differences in airflow might have on interspecies extrapolation, especially for humans, and to provide a basis for development of more comprehensive dosimetry models, *Kimbell et al. (1997a)* expanded their rat nasal 3-D CFD model to include the olfactory region. Simulations revealed marked differences in flow rates among five major airflow streams and in localized areas. This research further demonstrated that airflow in the nasal cavity is complex and nonuniform, that both airflow patterns and predicted flux correspond with the distribution of formaldehyde-induced lesions, and that model-simulated airflow corresponds well with that observed in nasal casts.

To further validate these observations, *Kimbell et al. (1997b)* examined the extent of correlation between CFD-predicted flux and the pattern of nasal lesions. Specifically, these authors investigated the relationship between squamous metaplasia and areas of high formaldehyde flux in the nasal tissues of adult rats. For each region with high flux levels of formaldehyde into airway walls, the distribution of squamous metaplasia in that region was also high (*Fig. 5*).

The preceding studies briefly described basic aspects and results of CFD methodology applied to inspiratory airflow, correlation of airflow patterns with formaldehyde-induced nasal lesion, and predicted flux in the rat. The results of these investigations culminated in a vanguard study from *Kimbell et al. (2001)*. In this study, the authors applied CFD modeling to characterize the fate of inspired formaldehyde in the nasal cavities of the rat, rhesus monkey, and human. Despite the absolute difference in size of the nasal regions, comparative aspects regarding flux are apparent (*Fig. 6*). The highest formaldehyde flux is in the rat, with the maximum predicted flux being greatest for the monkey. Predicted flux values in the region of high tumor incidence in the rat nose and anterior portion of human nose were noted to be similar.

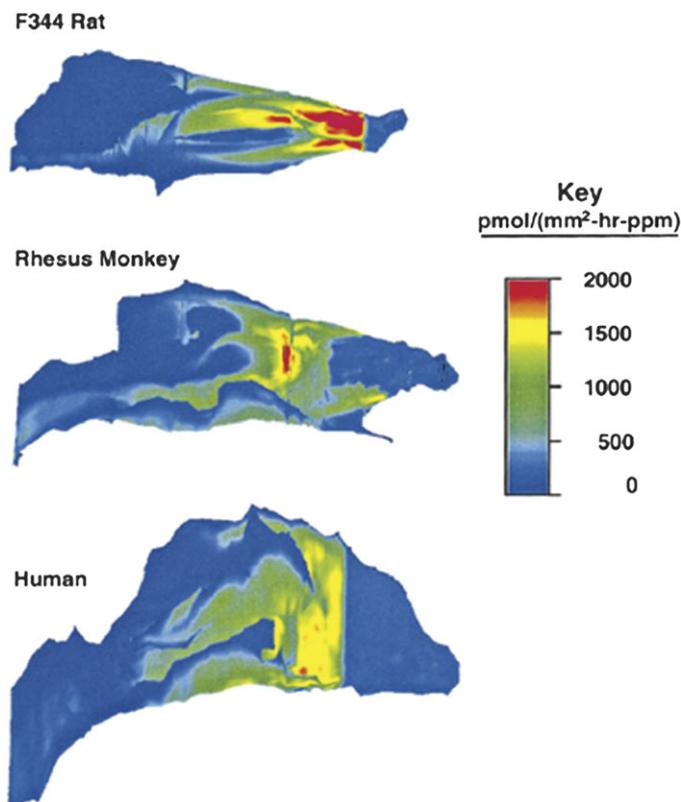
Although narrow in its focus, the formaldehyde research described above was selected because each study represents a fundamental and important step in enhancing our understanding of inhalation toxicology and dosimetry. The initial studies on airflow patterns in the nose and their correlation to target site injury greatly improved our perspective on inhalation dosimetry. In addition, this early research led to the development of advanced dosimetry models such as CFD. Just as important, this research has also greatly improved our understanding of interspecies extrapolation for the purposes of risk assessment in that these methods and modeling approaches have been expanded and applied to a variety of other chemicals.



**FIG. 5.** Graphs showing (A) the incidence of formaldehyde-induced squamous metaplasias and (B) modeled formaldehyde flux values along regions assigned to the perimeter of a transected nasal airway of rats. Source: Reprinted from *Kimbell et al. (1997b)*, with permission from Elsevier.

## PHOSGENE

Phosgene was used extensively as a chemical weapon in World War I. Not surprisingly, much of the early toxicology research on phosgene focused on the effects of acute, high-level exposures. With chemical warfare expressly condemned by the Geneva Convention in 1925, phosgene has since grown in use for the production of other chemicals, including isocyanates and polycarbonates. Suspected sources of phosgene today are fugitive emissions, thermal decomposition of chlorinated hydrocarbons, and photooxidation of chloroethylenes (*U.S. EPA, 2005*). With the cessation of its use as a war gas and the advent of improved safety procedures, the focus of phosgene toxicology research has shifted toward repeat exposure studies and the discovery of sensitive endpoints for measuring the health consequences of chronic, low-level exposures.



**FIG. 6.** Nasal wall flux spectra of inhaled formaldehyde simulated in rats, monkeys, and humans at normal inspiratory flow rates. Nostrils are to the right. Source: Kimbell *et al.* (2001).

### Acute Exposure Studies

Human data are limited to case studies following accidental exposures and are of limited value for the derivation of health benchmarks (AEGL, 2004; U.S. EPA, 2005). Current laboratory animal data suggest that inhaled phosgene is dispersed deep into the lungs and eliminated by rapid reactions with nucleophilic constituents of the alveolar region (Pauluhn *et al.*, 2007). Thus, it is not expected to leave the pulmonary circulation or cause substantial systemic effects. Phosgene inhalation induces abnormalities in the surfactant system of lipids and proteins at the air-liquid interface of the alveolus that are presumed to be key to the development of pulmonary edema that can lead to (1) acute respiratory failure or (2) chronic cellular inflammation that can result in pulmonary fibrosis (Gift *et al.*, 2008; Pauluhn *et al.*, 2007).

Phosgene-induced pulmonary edema can be measured by lung lavage protein following acute inhalation exposure at concentrations as low as 0.1 ppm (Diller, 1985; Hatch *et al.*, 1986). Consistent with Habers rule, the concentration-duration ( $C \times T$ ) relationship for lung lavage protein is constant over a wide range of phosgene concentrations and single exposure durations, as is the  $C \times T$  relationship for the effects of phosgene on pulmonary gas exchange (Rinehart and Hatch, 1964).

Acute exposure to phosgene has produced a decreased immune response in rodents, as evidenced by an increased susceptibility to *in vivo* bacterial and tumor cell infections (Selgrade *et al.*, 1989; Yang *et al.*, 1995) and viral infection (Ehrlich and Burleson, 1991). This effect has been observed in rats at exposure concentrations as low as 0.01 ppm for 8 h.

### Repeat Exposure Studies

The large majority of studies of phosgene are of acute duration, spanning from minutes to several hours. However, several studies (Clay and Rossing, 1964; Franch and Hatch, 1986; Hatch *et al.*, 2001; Kodavanti *et al.*, 1997; Rossing, 1964; Selgrade *et al.*, 1995b) examined the effects of repeated short-term exposures over 2–12 weeks.

With continuous, low-level phosgene exposure, edema can transition to persistent cellular inflammation leading to the synthesis of type I collagen and pulmonary fibrosis (Pauluhn, *et al.*, 2007). Kodavanti *et al.* (1997) observed increases in lung collagen in rats exposed to various phosgene levels for various exposure durations to provide equal products of  $C \times T$ . Several effects indicative of pulmonary edema were observed, but collagen staining was the only response that increased from the 4th to 12th week of exposure and persisted to the end of a 4-week recovery period, indicating development of pulmonary fibrosis.

In a follow-up to the study of Kodavanti *et al.* (1997), Hatch *et al.* (2001) further evaluated the adaptive response that occurs in rats that receive repeat exposures to phosgene. Decreased lung lavage protein was observed following reexposure to phosgene after 1 recovery week. They hypothesized that this type of adaption may be the result of antioxidant changes, alveolar duct wall thickening leading to reduced cellular dose, increased scavenging of reactive species by mucus secretion, or changes in breathing that result in limitation of the alveolar dose. Others have suggested that this type of “self-tolerance” is common for lower respiratory tract irritants (Pauluhn, 2002) and related to an adaptively increased capacity and rate to store and synthesize lung surfactant (Pauluhn *et al.*, 2007).

Repeat exposure to phosgene can also chronically impair resistance to bacterial infection, likely through defects in alveolar macrophage phagocytosis (Selgrade *et al.*, 1995b). Pauluhn *et al.* (2007) have suggested that these host defense deficiencies might have been the result of phosgene-induced pulmonary effects, including alterations in the lung surfactant system. However, the immune cell effects reported by Selgrade *et al.* (1995b) occurred at slightly lower exposure levels than effects indicative of pulmonary edema (Kodavanti *et al.*, 1997), suggesting that the observed decrease in bacterial resistance may not be a secondary response to pulmonary effects (Gift *et al.*, 2008).

Though immunotoxicity in rats has been observed at slightly lower concentrations, phosgene’s pulmonary effects are better characterized, with less uncertainty on the mode of action and

relevance to humans. For these reasons and because benchmark dose analyses could be performed on the data of Kodavanti *et al.* (1997), collagen deposition serves as the basis of the EPA chronic inhalation RfC for phosgene of 0.0003 mg/m<sup>3</sup> (U.S. EPA, 2005).

Phosgene studies have provided valuable information for the design of future studies of phosgene and other irritants, such as O<sub>3</sub> and NO<sub>2</sub>, that can cause both pulmonary toxicity (Hatch *et al.*, 1986; Slade *et al.*, 1989) and immunotoxicity (Selgrade and Gilmour, 1994; Selgrade *et al.*, 1995a). It has been suggested that small animals, particularly obligate nasal breathers such as rats, are not good human models for the edematous effects of phosgene (Pauluhn *et al.*, 2007). However, this may not be true for other effects such as immunotoxicity and pulmonary fibrosis. As existing data gaps and uncertainties are addressed, future risk assessments will be able to better characterize the inhalation toxicity from chronic, low-level exposure to phosgene and similar irritants.

### MOVING FORWARD

To date, no thresholds have been found where air pollutants (notably PM and O<sub>3</sub>) are without some effect. This non-threshold concept arises from epidemiologic studies that involve large populations that encompass a wide range of sensitivities for a spectrum of potentially adverse health outcomes. Toxicology studies generally cannot address this kind of threshold but rather can delve into the mechanisms that may underlie these varied outcomes and account for their relative concentration (dose)-response patterns. By targeting the outcomes of most public health concern, one can begin to construct a risk paradigm that provides a sound biological basis for risk estimation. The advent of new gene-based technologies, involving high-throughput genomic or proteomic systems, may bring better hypothesis testing for the outcomes or allow an assessment of variability among potential pathways leading to responses. However, for the foreseeable future, most air pollutant assessments will rely heavily on human clinical or epidemiologic data either guided or elucidated by animal toxicology to establish plausibility.

Simultaneously, we are observing an evolving challenge with regard to air pollution. Ironically, the early days of air pollution toxicology tried to address the complexities of the phenomenon but realized the difficulty in defining the exposures being assessed. Reductionism by early toxicologists noted the individual toxicities of the major pollutant components or classes and, indeed, provided the rationale for the CAA regulation of individual pollutants. It took nearly 40 years for toxicologists and other air health scientists to accept that which they have known but have been unable to adequately address—mixtures. The issue of air pollutant mixtures has finally come to the forefront and been accepted as the challenge of the 21st century if we are to comprehensively and efficiently

grapple with air pollution. On the one hand, air pollutants, in all likelihood, interact to alter health outcomes. There are historic data from early studies, which strongly suggest such interactions, and certainly, the atmospheric chemists have known of the complex component chemistries for many years. What we have not been able to assess is whether these interactions really do make a difference in health outcomes and, if so, how much. The infinite number of mixture types must be systematically compartmentalized if there is going to be progress in this area. Whether this systematic approach is by physical and chemical attributes, temporal or spatial patterns, or perhaps conversely starting with types of health responses and working backward, it is unclear, but not every pollutant combination can be assessed separately—even with the best high-throughput systems. For this reason, many initial forays into the mixture issue have involved the study of primary sources of air pollution. This approach may open the study of source mixtures to simplify systematic assessments while at the same time providing useful data for control strategies. The regulatory community is moving ahead with pilot plans for examining controls of various source components and their impacts on the relative copollutants not directly being controlled and then computing a composite benefit. The advantage is more expeditious, cost-efficient controls, and a framework for assessing health benefits. Obviously, the role of atmospheric transformation of source components will be a necessary aspect of a full assessment, but the direction of this strategy is clear. Toxicology will be invaluable in these evaluations.

This multipollutant future is nowhere greater, however, than in its integral role in climate change. Obvious perhaps, but only recently appreciated, is the intimate relationship between air pollution and climate change. The gains in air pollution and its public health benefits may potentially be at risk as climate threatens to change the profile of air pollution and perhaps accelerate the complex atmospheric chemistries that have been tempered by existing regulations. New fuels and fuel additives, changing emissions from both mobile and stationary sources, and altered atmospheric conditions must be investigated if we are to be predictive in potential health and environmental impacts.

The toxicology of air pollution has already evolved substantially over its ~60 years of attention. It has gone from crude mixture studies with crude outcomes of mortality and pathological measures to sophisticated coupled chemical and biological systems. The tools for study are exquisite and continue to improve in sensitivity and diversity. But it is the toxicologist who is the key to the future of the science. We traditionally think of exposure—dose—effect as the optimal paradigm, but the new science is suggesting that the complexities of interactions can also happen at the biological domain, for instance, the loss of compensation or sensitization to subsequent challenges with the same or another toxicant or perhaps just a nonspecific stressor whose threshold of response

may have been lowered. Enhanced airway allergen sensitivity in asthmatics exposed to air pollution is the prototypic model, but this paradigm may well exist for other outcomes. Recently, studies of cardiac sensitivity to arrhythmic inducing stimuli have been found in rats exposed to very low concentrations of diesel exhaust (Hazari *et al.*, 2009). In such scenarios, the concentration or dose needed for an outcome may be substantially less than what we might envision in a classical toxicological sense because the system is being “primed” for a subsequent challenge. This approach may bring the oft-criticized dose-relevance concerns of toxicological studies into a clearer light and indeed may explain, in part, the individual variability at low concentrations often observed in population studies. Regardless, the air pollution toxicologist of the future will need to use conventional and cutting-edge technology as tools to introduce novel study designs capable of dissecting the complexity of the biological system itself as well, as that of air pollution in all its varied forms.

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#### REFERENCES

- Adams, W. C. (2002). Comparison of chamber and face mask 6.6 hour exposures to ozone on pulmonary function and symptoms responses. *Inhal. Toxicol.* **14**, 745–764.
- Adams, W. C. (2003). Comparison of chamber and face mask 6.6 hour exposure to 0.08 ppm ozone via square wave and triangular profiles on pulmonary responses. *Inhal. Toxicol.* **15**, 265–281.
- Adams, W. C. (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**, 127–136.
- Adams, W. C., and Schelegle, E. S. (1983). Ozone and high ventilation effects on pulmonary function and endurance performance. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **55**, 805–812.
- Acute Exposure Guideline Levels (AEGL) (2004). In *AEGL (Acute Exposure Guideline Levels) for Selected Airborne Chemicals, Vol. 2. Phosgene*. National Research Council of the National Academies, Washington, DC.
- Alexis, N. E., Lay, J. C., Hazucha, M., Harris, B., Hernandez, M. L., Bromberg, P. A., Kehrl, H., Diaz-Sanchez, D., Kim, C., Devlin, R. B., *et al.* (2010). Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. *Inhal. Toxicol.* **22**, 593–600.
- Amdur, M. (1958). The respiratory response of guinea pigs to sulfuric acid mist. *Arch. Environ. Occup. Health* **18**, 407–414.
- Amdur, M. O. (1954). Effect of a combination of SO<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub> on guinea pigs. *Public Health Rep.* **69**, 503–506.
- Amdur, M. O. (1957). The influence of aerosols upon the respiratory response of guinea pigs to sulfur dioxide. *Am. Ind. Hyg. Assoc. Q.* **18**, 149–155.
- Amdur, M. O. (1961). In *The Effect of Aerosols on the Response of Irritant Gases*, p. 281. Pergamon Press, New York, NY.
- Amdur, M. O. (1989). Health effects of air pollutants: sulfuric acid, the old and the new. *Environ. Health Perspect.* **81**, 109–113.
- Amdur, M. O., and Corn, M. (1963). The irritant potency of zinc ammonium sulfate of different particle sizes. *Am. Ind. Hyg. Assoc. J.* **24**, 326–333.
- Amdur, M. O., and Creasia, D. A. (1966). The irritant potency of m-terphenyl of different particle sizes. *Am. Ind. Hyg. Assoc. J.* **27**, 349–352.
- Amdur, M. O., Dubriel, M., and Creasia, D. A. (1978). Respiratory response of guinea pigs to low levels of sulfuric acid. *Environ. Res.* **15**, 418–423.
- Amdur, M. O., Melvin, W. W., Jr, and Drinker, P. (1953). Effects of inhalation of sulfur dioxide by man. *Lancet* **2**, 758–759.
- Amdur, M. O., Sarofim, A. F., Neville, M., Quann, R. J., McCarthy, J. F., Elliott, J. F., Lam, H. F., Rogers, A. E., and Conner, M. W. (1986). Coal combustion aerosols and SO<sub>2</sub>: an interdisciplinary analysis. *Environ. Sci. Technol.* **20**, 138–145.
- Amdur, M. O., Silverman, L., and Drinker, P. (1952). Inhalation of sulfuric acid mist by human subjects. *AMA Arch. Ind. Hug. Occup. Med.* **6**, 305–313.
- Araujo, J., Barajas, B., Kleinman, M., Wang, X., Bennett, B., Gong, K., Navab, M., Harkema, J., Sioutas, C., Lusic, A., *et al.* (2008). Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. *Circ. Res.* **102**, 589–596.
- Aris, R. M., Tager, I., Christian, D., Kelly, T., and Balmes, J. R. (1995). Methacholine responsiveness is not associated with O<sub>3</sub> induced decreases in FEV<sub>1</sub>. *Chest* **107**, 621–628.
- Agency for Toxic Substances and Disease Registry (ATSDR). (1999). *Toxicological Profile for Formaldehyde*. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.
- Avol, E. L., Gauderman, W. J., Tan, S. M., London, S. J., and Peters, J. M. (2001). Respiratory effects of relocating to areas of differing air pollution levels. *Am. J. Respir. Crit. Care Med.* **164**, 2067–2072.
- Avol, E. L., Linn, W. S., Venet, T. G., Shamoo, D. A., and Hackney, J. D. (1984). Comparative respiratory effects of ozone and ambient oxidant pollution exposure during heavy exercise. *J. Air Pollut. Control Assoc.* **34**, 804–809.
- 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**, 652–697.
- Balmes, J. R., Aris, R. M., Chen, L. L., Scannell, C., Tager, I. B., Finkbeiner, W., Christian, D., Kelly, T., Hearne, P. Q., Ferrando, R., *et al.* (1997). In *Effects of Ozone on Normal and Potentially Sensitive Human Subjects. Part I: Airway Inflammation and Responsiveness to Ozone in Normal and Asthmatic Subjects*. pp. 1–37, 81–99. Health Effects Institute, Cambridge, MA. Research report no. 78.
- Balmes, J. R., Chen, L. L., Scannell, C., Tager, I., Christian, D., Hearne, P. Q., Kelly, T., and Aris, R. M. (1996). Ozone induced decrements in FEV<sub>1</sub> and FVC do not correlate with measures of inflammation. *Am. J. Respir. Crit. Care Med.* **153**, 904–909.
- Bartoli, C. R., Wellenius, G. A., Coull, B. A., Akiyama, I., Diaz, E. A., Lawrence, J., Okabe, K., Verrier, R. L., and Godleski, J. J. (2009). Concentrated ambient particles alter myocardial blood flow during acute ischemia in conscious canines. *Environ. Health Perspect.* **117**, 333–337.
- Basha, M. A., Gross, K. B., Gwizdala, C. J., Haidar, A. H., and 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.
- Bennett, W. D., Zeman, K. L., Kim, C., and Mascarella, J. (1997). Enhanced deposition of fine particles in COPD patients spontaneously breathing at rest. *Inhal. Toxicol.* **9**, 1–14.
- Blomberg, A., Mudway, I. S., Nordenhall, C., Hedenstrom, H., Kelly, F. J., Frew, A. J., Holgate, S. T., and Sandstrom, T. (1999). Ozone induced lung

- function decrements do not correlate with early airway inflammatory or antioxidant responses. *Eur. Respir. J.* **13**, 1418–1428.
- Boyland, E., McDonald, F. F., and Rumens, M. J. (1946). Variation in the toxicity of phosgene for small animals with the duration of exposure. *Br. J. Pharmacol. Chemother.* **1**, 81–89.
- Bracken, M., Triche, E., Belanger, K., Saftlas, A., Beckett, W., and Leaderer, B. (2003). Asthma symptoms, severity, and drug therapy: a prospective study of effects on 2205 pregnancies. *Obstet. Gynecol.* **102**, 739–753.
- Brook, R., Brook, J., Urch, B., Vincent, R., Rajagopalan, S., and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation* **105**, 1534–1536.
- Brown, J. H., Cook, K. M., Ney, F. G., and Hatch, T. F. (1950). Influence of particle size upon the retention of particulate matter in the human lung. *Am. J. Public Health* **40**, 450–458.
- Brown, J. S., Bateson, T. F., and McDonnell, W. F. (2008). Effects of exposure to 0.06 ppm ozone on FEV1 in humans: a secondary analysis of existing data. *Environ. Health Perspect.* **116**, 1023–1026.
- Brown, J. S., Zeman, K. L., and Bennett, W. D. (2002). Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am. J. Respir. Crit. Care Med.* **166**, 1240–1247.
- Burch, W. (2002). Comment on “Passage of inhaled particles into the blood circulation in humans”. *Circulation* **106**, e141–e142.
- Calderón-Garcidueñas, L., Azzarelli, B., Acuna, H., Garcia, R., Gambling, T., Osnaya, N., Monroy, S., Del Rosario, T. M., Carson, J., Villarreal-Calderon, A., et al. (2002). Air pollution and brain damage. *Toxicol. Pathol.* **30**, 373–389.
- Calderón-Garcidueñas, L., Maronpot, R., Torres-Jardon, R., Henriquez-Roldan, C., Schoonhoven, R., Acuna-Ayala, H., Villarreal-Calderon, A., Nakamura, J., Fernando, R., Reed, W., 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**, 524–538.
- Campbell, A., Oldham, M., Becaria, A., Bondy, S., Meacher, D., Sioutas, C., Misra, C., Mendez, L., and Kleinman, M. (2005). Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology* **26**, 133–140.
- Campen, M., McDonald, J., Gigliotti, A., Seilkop, S., Reed, M., and Benson, J. (2003). Cardiovascular effects of inhaled diesel exhaust in spontaneously hypertensive rats. *Cardiovasc. Toxicol.* **3**, 353–361.
- Catcott, E. J., Mc, C. C., and Kotin, P. (1958). Pulmonary pathology in dogs due to air pollution. *J. Am. Vet. Med. Assoc.* **133**, 331–335.
- Chambers, L. A., Griswold, S. S., and Motley, H. L. (1957). Report of a case of exposure to high ozone concentrations for two hours. *AMA Arch. Ind. Health* **15**, 108–110.
- Chen, L., and 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**, 217–224.
- Clay, J. R., and Rossing, R. G. (1964). Histopathology of exposure to phosgene: an attempt to produce pulmonary emphysema experimentally. *Arch. Pathol. Lab. Med.* **78**, 544–551.
- Clifton, V., Giles, W., Smith, R., Bisits, A., Hempenstall, P., Kessell, C., and Gibson, P. (2001). Alterations of placental vascular function in asthmatic pregnancies. *Am. J. Respir. Crit. Care Med.* **164**, 546–554.
- Coleridge, J. C. G., Coleridge, H. M., and Schelegle, E. S. G., and JF (1993). Acute inhalation of ozone stimulates bronchial C-fibers and rapidly adapting receptors in dogs. *J. Appl. Physiol.* **74**, 2345–2352.
- Costa, D., and Dreher, K. (1997). Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. *Environ. Health Perspect.* **105**(Suppl.), 1053–1060.
- Costa, D., and Dreher, K. (1999). What do we need to know about airborne particles to make effective risk management decisions? A toxicology perspective. *Hum. Ecol. Risk Assess.* **5**, 481–491.
- Costa, D. L., and Gordon, T. (2000). Profiles in Toxicology: Mary O. Amdur. *Toxicol. Sci.* **56**, 5–7.
- Devlin, R. B., McDonnell, W. F., Mann, R., Becker, S., House, D. E., Schreinemachers, D., and Koren, H. S. (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.
- Diller, W. (1985). Pathogenesis of phosgene poisoning. *Toxicol. Ind. Health* **1**, 7–15.
- Dimeo, M. J., Glenn, M. G., Holtzman, M. J., Sheller, J. R., Nadel, J. A., and Boushey, H. A. (1981). Threshold concentration of ozone causing an increase in bronchial reactivity in humans and adaptation with repeated exposures. *Am. Rev. Respir. Dis.* **124**, 245–248.
- Dorman, D., Struve, M., James, R., Marshall, M., Parkinson, C., and Wong, B. (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**, 79–87.
- Duan, X., Buckpitt, A. R., Pinkerton, K. E., Ji, C., and Plopper, C. G. (1996). Ozone-induced alterations in glutathione in lung subcompartments of rats and monkeys. *Am. J. Respir. Cell Mol. Biol.* **14**, 70–75.
- Duan, X., Buckpitt, A. R., and Plopper, C. G. (1993). Variation in antioxidant enzyme activities in anatomic subcompartments within rat and rhesus monkey lung. *Toxicol. Appl. Pharmacol.* **123**, 73–82.
- Dye, J., Lehmann, J., McGee, J., Winsett, D., Ledbetter, A., Everitt, J., Ghio, A., and Costa, D. (2001). Acute pulmonary toxicity of particulate matter filter extracts in rats: coherence with epidemiological studies in Utah Valley residents. *Environ. Health Perspect.* **109**, 395–403.
- Ehrlich, J., and Bursleson, G. (1991). Enhanced and prolonged pulmonary influenza virus infection following phosgene inhalation. *J. Toxicol. Environ. Health* **34**, 259–273.
- Ehrlich, R., Findlay, J. C., and Gardner, D. E. (1979). Effects of repeated exposures to peak concentrations of nitrogen dioxide and ozone on resistance to streptococcal pneumonia. *J. Toxicol. Environ. Health* **5**, 631–642.
- Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Ito, Y., Finkelstein, J., et al. (2006). Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ. Health Perspect.* **114**, 1172–1178.
- Fanucchi, M. V., Plopper, C. G., Evans, M. J., Hyde, D. M., Van Winkle, L. S., Gershwin, L. J., and Schelegle, E. S. (2006). Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. *Am. J. Physiol. Lung Cell Mol. Physiol.* **291**, 644–650.
- Ferin, J., Oberdorster, G., and Penney, D. (1992). Pulmonary retention of ultrafine and fine particles in rats. *Am. J. Respir. Cell Mol. Biol.* **6**, 535–542.
- Folinsbee, L. J., Bedi, J. F., and Horvath, S. M. (1984). Pulmonary function changes after 1 h continuous heavy exercise in 0.21 ppm ozone. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **57**, 984–988.
- Folinsbee, L. J., Drinkwater, B. L., Bedi, J. F., and Horvath, S. M. (1978). The influence of exercise on the pulmonary function changes due to exposure to low concentrations of ozone. In *Environmental Stress: Individual Human Adaptations* (L. J. Folinsbee, J. A. Wagner, J. F. Borgia, B. L. Drinkwater, J. A. Gliner, and J. F. Bedi, Eds.), pp. 125–145. Academic Press, New York.
- Folinsbee, L. J., and Hazucha, M. J. (1989). Persistence of ozone induced changes in lung function and airway responsiveness. In *Atmospheric Ozone Research and Its Policy Implications: Proceedings of the 3rd U.S. Dutch International Symposium*, May 1988 (T. Schneider, S. D. Lee, G. J. R. Wolters, and L. D. Grant, Eds.), pp. 483–492. Elsevier Science Publishers, Amsterdam, The Netherlands.

- Folinsbee, L. J., and Hazucha, M. J. (2000). Time course of response to ozone exposure in healthy adult females. *Inhal. Toxicol.* **12**, 151–167.
- Folinsbee, L. J., Horstman, D. H., Kehrl, H. R., Harder, S., Abdul Salaam, S., and Ives, P. J. (1994). Respiratory responses to repeated prolonged exposure to 0.12 ppm ozone. *Am. J. Respir. Crit. Care Med.* **149**, 98–105.
- Folinsbee, L. J., McDonnell, W. F., and Horstman, D. H. (1988). Pulmonary function and symptom responses after 6.6 hour exposure to 0.12 ppm ozone with moderate exercise. *J. Air Pollut. Control Assoc.* **38**, 28–35.
- Folinsbee, L. J., Silverman, F., and Shephard, R. J. (1977). Decrease of maximum work performance following ozone exposure. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **42**, 531–536.
- Franch, S., and Hatch, G. E. (1986). Pulmonary biochemical effects of inhaled phosgene in rats. *J. Toxicol. Environ. Health* **19**, 413–423.
- Frank, R., Liu, M. C., Spannhake, E. W., Mlynarek, S., Macri, K., and Weinmann, G. G. (2001). Repetitive ozone exposure of young adults: evidence of persistent small airway dysfunction. *Am. J. Respir. Crit. Care Med.* **164**, 1253–1260.
- Freed, A. N., Chou, C. L., Fuller, S. D., and Croxton, T. L. (1996). Ozone-induced vagal reflex modulates airways reactivity in rabbits. *Respir. Physiol.* **105**, 95–102.
- Gauderman, W. J., Gilliland, G. F., Vora, H., Avol, E., Stram, D., McConnell, R., Thomas, D., Lurmann, F., Margolis, H. G., Rappaport, E. B., et al. (2002). Association between air pollution and lung function growth in southern California children: results from a second cohort. *Am. J. Respir. Crit. Care Med.* **166**, 76–84.
- Ghelfi, E., Rhoden, C., Wellenius, G., Lawrence, J., and Gonzalez-Flecha, B. (2008). Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated air particles are mediated by TRP-dependent pulmonary reflexes. *Toxicol. Sci.* **102**, 328–336.
- Ghio, A., and Devlin, R. (2001). Inflammatory lung injury after bronchial instillation of air pollution particles. *Am. J. Respir. Crit. Care Med.* **164**, 704–708.
- Giacomelli-Maltoni, G., Melandri, C., Prodi, V., and Tarroni, G. (1972). Deposition efficiency of monodisperse particles in human respiratory tract. *Am. Ind. Hyg. Assoc. J.* **33**, 603–610.
- Gift, J. S., McGaughy, R., Singh, D. V., and Sonawane, B. (2008). Health assessment of phosgene: approaches for derivation of reference concentration. *Regul. Toxicol. Pharmacol.* **51**, 98–107.
- Godleski, J., Verrier, R., Koutrakis, P., Catalano, P., Coull, B., Reinisch, U., Lovett, E., Lawrence, J., Murthy, G., Wolfson, J., et al. (2000). Mechanisms of morbidity and mortality from exposure to ambient air particles. *Res. Rep. Health Eff. Inst.* **91**, 5–88; discussion 89–103.
- Gold, D., Litonjua, A., Zanobetti, A., Coull, B., Schwartz, J., MacCallum, G., Verrier, R., Nearing, B., Canner, M., Suh, H., et al. (2005). Air pollution and ST-segment depression in elderly subjects. *Environ. Health Perspect.* **113**, 883–887.
- Gong, H., Jr., Bradley, P. W., Simmons, M. S., and Tashkin, D. P. (1986). Impaired exercise performance and pulmonary function in elite cyclists during low level ozone exposure in a hot environment. *Am. Rev. Respir. Dis.* **134**, 726–733.
- Gong, H., Jr., McManus, M. S., and Linn, W. S. (1997). Attenuated response to repeated daily ozone exposures in asthmatic subjects. *Arch. Environ. Health* **52**, 34–41.
- Hallett, W. Y. (1965). Effect of ozone and cigarette smoke on lung function. *Arch. Environ. Health* **10**, 295–302.
- Hatch, G., Kodavanti, U., Crissman, K., Slade, R., and Costa, D. (2001). An “injury-time integral” model for extrapolating from acute to chronic effects of phosgene. *Toxicol. Ind. Health* **17**, 285–293.
- Hatch, G. E., Slade, R., Stead, A. G., and Graham, J. A. (1986). Species comparison of acute inhalation toxicity of ozone and phosgene. *J. Toxicol. Environ. Health* **19**, 43–53.
- Hatch, T. F., and Hameon, W. C. (1948). Influence of particle size in dust exposure. *J. Ind. Hyg.* **30**, 172–180.
- Hazari, M. S., Haykal-Coates, N., Winsett, D. W., Costa, D. L., and Farraj, A. K. (2009). A single exposure to particulate or gaseous air pollution increases the risk of aconitine-induced cardiac arrhythmia in hypertensive rats. *Toxicol. Sci.* **112**, 532–542.
- Hazbun, M. E., Hamilton, R., Holian, A., and Eschenbacher, W. L. (1993). Ozone-induced increases in substance P and 8-epi-prostaglandin F<sub>2</sub>α in the airways of human subjects. *Am. J. Respir. Cell Mol. Biol.* **9**, 568–572.
- Hazucha, M. J., Folinsbee, L. J., and Seal, E., Jr. (1992). Effects of steady state and variable ozone concentration profiles on pulmonary function. *Am. Rev. Respir. Dis.* **146**, 1487–1493.
- Horstman, D. H., Ball, B. A., Brown, J., Gerrity, T., and Folinsbee, L. J. (1995). Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. *Toxicol. Ind. Health* **11**, 369–385.
- Horstman, D. H., Folinsbee, L. J., Ives, P. J., Abdul Salaam, S., and McDonnell, W. F. (1990). Ozone concentration and pulmonary response relationships for 6.6 hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. *Am. Rev. Respir. Dis.* **142**, 1158–1163.
- Horvath, S. M., Gliner, J. A., and Matsen Twisdale, J. A. (1979). Pulmonary function and maximum exercise responses following acute ozone exposure. *Aviat. Space Environ. Med.* **50**, 901–905.
- Hounam, R., Black, A., and Walsh, M. (1969). Deposition of aerosol particles in the nasopharyngeal region of the human respiratory tract. *Nature* **221**, 1254–1255.
- Hyde, D., Orthoefer, J., Dungworth, D., Tyler, W., Carter, R., and Lum, H. (1978). Morphometric and morphologic evaluation of pulmonary lesions in beagle dogs chronically exposed to high ambient levels of air pollutants. *Lab. Invest.* **38**, 455–469.
- International Agency for Research on Cancer (IARC). (1995). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 62. Wood Dust and Formaldehyde*. International Agency for Research on Cancer, Lyon, France.
- International Programme on Chemical Safety (IPCS). (2002). *Formaldehyde. Concise International Chemical Assessment Document, Vol. 40*. World Health Organization, Geneva, Switzerland.
- Joad, J. P., Kott, K. S., and Bric, J. M. (1996). The local C-fiber contribution to ozone-induced effects on the isolated guinea pig lung. *Toxicol. Appl. Pharmacol.* **141**, 561–567.
- Johansson, E., Wesselkamper, S. C., Shertzer, H. G., Leikauf, G. D., Dalton, T. P., and Chen, Y. (2010). Glutathione deficient C57BL/6J mice are not sensitized to ozone-induced lung injury. *Biochem. Biophys. Res. Commun.* **396**, 407–412.
- Jörres, R., Nowak, D., Magnussen, H., Speckin, P., and Koschyk, S. (1996). The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. *Am. J. Respir. Crit. Care Med.* **153**, 56–64.
- Kehrl, H. R., Peden, D. B., Ball, B. A., Folinsbee, L. J., and Horstman, D. H. (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**, 1198–1204.
- Kerns, W. D., Pavkov, K. L., Donofrio, D. J., Gralla, E. J., and Swenberg, J. A. (1983). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res.* **43**, 4382–4392.
- Killingsworth, C. R., Alessandrini, F., Krishna Murthy, G. G., Catalano, P. J., Paulauskis, J. D., and Godleski, J. J. (1997). Inflammation, chemokine expression, and death in monocrotaline-treated rats following fuel oil fly ash inhalation. *Inhal. Toxicol.* **9**, 541–565.
- Kimbell, J. S., Godo, M. N., Gross, E. A., Joyner, D. R., Richardson, R. B., and Morgan, K. T. (1997a). Computer simulation of inspiratory airflow in all

- regions of the F344 rat nasal passages. *Toxicol. Appl. Pharmacol.* **145**, 388–398.
- Kimbell, J. S., Gross, E. A., Joyner, D. R., Godo, M. N., and Morgan, K. T. (1993). Application of computational fluid dynamics to regional dosimetry of inhaled chemicals in the upper respiratory tract of the rat. *Toxicol. Appl. Pharmacol.* **121**, 253–263.
- Kimbell, J. S., Gross, E. A., Richardson, R. B., Conolly, R. B., and Morgan, K. T. (1997b). Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. *Mutat. Res.* **308**, 143–154.
- Kimbell, J. S., Subramaniam, R. P., Gross, E. A., Schlosser, P. M., and Morgan, K. T. (2001). Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. *Toxicol. Sci.* **64**, 100–110.
- Kleinman, M., Araujo, J., Nel, A., Sioutas, C., Campbell, A., Cong, P., Li, H., and Bondy, S. (2008). Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways. *Toxicol. Lett.* **178**, 127–130.
- Kodavanti, U. P., Costa, D. L., Giri, S. N., Starcher, B., and Hatch, G. E. (1997). Pulmonary structural and extracellular matrix alterations in Fischer 344 rats following subchronic phosgene exposure. *Fundam. Appl. Toxicol.* **37**, 54–63.
- Kotin, P., and Falk, H. L. (1955). Air pollution and its effect on health. *Calif. Med.* **82**, 19–24.
- Kreit, J. W., Gross, K. B., Moore, T. B., Lorenzen, T. J., D'Arcy, J., and Eschenbacher, W. L. (1989). Ozone induced changes in pulmonary function and bronchial responsiveness in asthmatics. *J. Appl. Physiol.* **66**, 217–222.
- Krishna, M. T., Springall, D., Meng, Q.-H., Withers, N., Macleod, D., Biscione, G., Frew, A., Polak, J., and Holgate, S. (1997). Effects of ozone on epithelium and sensory nerves in the bronchial mucosa of healthy humans. *Am. J. Respir. Crit. Care Med.* **156**, 943–950.
- Kulle, T. J., Sauder, L. R., Hebel, J. R., and Chatham, M. D. (1985). Ozone response relationships in healthy nonsmokers. *Am. Rev. Respir. Dis.* **132**, 36–41.
- Kulle, T. J., Sauder, L. R., Kerr, H. D., Farrell, B. P., Bermel, M. S., and Smith, D. M. (1982). Duration of pulmonary function adaptation to ozone in humans. *Am. Ind. Hyg. Assoc. J.* **43**, 832–837.
- Labelle, C. W., and Brieger, H. (1955). The vapor toxicity of a composite solvent and its principal components. *AMA Arch. Ind. Health* **12**, 623–627.
- Laskin, S., Kuschner, M., and Drew, R. T. (1970). In *Inhalation Exposure to Sulphur Dioxide and Benzo(a)pyrene*. pp. 322–351. Oak Ridge National Laboratory, Gatlinburg, TN.
- Lewis, T. R., Moorman, W. J., Yang, Y. Y., and Stara, J. F. (1974). Long-term exposure to auto exhaust and other pollutant mixtures: effects on pulmonary function in the beagle. *Arch. Environ. Health* **29**, 102–106.
- Linn, W. S., Avol, E. L., Shamoo, D. A., Spier, C. E., Valencia, L. M., Venet, T. G., Fischer, D. A., and Hackney, J. D. (1986). A dose response study of healthy, heavily exercising men exposed to ozone at concentrations near the ambient air quality standard. *Toxicol. Ind. Health* **2**, 99–112.
- Lippmann, M. (1970). Deposition and clearance of inhaled particles in the human nose. *Ann. Otol. Rhinol. Laryngol.* **79**, 519–528.
- Lippmann, M. (1977). Regional deposition of particles in the human respiratory tract. In *Handbook of Physiology, Section 9: Reactions to Physical Agents* (D. H. K. Lee, H. L. Falk, S. D. Murphy, and S. R. Geiger, Eds.), pp. 213–232. American Physiological Society, Bethesda, MD.
- Maisonet, M., Correa, A., Misra, D., and Jaakkola, J. (2004). A review of the literature on the effects of ambient air pollution on fetal growth. *Environ. Res.* **95**, 106–115.
- Martens, A., and Jacobi, W. (1973). Die In-Vivo Bestimmung der Aerosolteilchen-deposition im Atemtrakt bei Mund-Bzw Nasenatmung [In vivo determination of aerosol particle deposition in the total respiratory tract]. In *Aerosole in Physik, Medizin und Technik*, Proc. Ann. Conf. of the Assoc. for Aerosol Research, pp. 117–122. Gesellschaft für Aerosolforschung, Bad Soden, Federal Republic of Germany.
- McConnell, R., Berhane, K., Gilliland, F. D., London, S. J., Islam, T., Gauderman, W. J., Avol, E., Margolis, H. G., and Peters, J. M. (2002). Asthma in exercising children exposed to ozone. *Lancet* **359**, 386–391.
- McDonnell, W. F., Horstman, D. H., Hazucha, M. J., Seal, E., Jr., Haak, E. D., Salaam, S. A., and House, D. E. (1983). Pulmonary effects of ozone exposure during exercise: dose response characteristics. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **54**, 1345–1352.
- McDonnell, W. F., Kehrl, H. R., Abdul-Salaam, S., Ives, P. J., Folinsbee, L. J., Devlin, R. B., O'Neil, J. J., and Horstman, D. H. (1991). Respiratory response of humans exposed to low levels of ozone for 6.6 hours. *Arch. Environ. Health* **46**, 145–150.
- McDonnell, W. F., Stewart, P. W., and Smith, M. V. (2007). The temporal dynamics of ozone-induced FEV1 changes in humans: an exposure-response model. *Inhal. Toxicol.* **19**, 483–494.
- McDonnell, W. F., Stewart, P. W., Smith, M. V., Pan, W. K., and Pan, J. (1999). Ozone induced respiratory symptoms: exposure response models and association with lung function. *Eur. Respir. J.* **14**, 845–853.
- Miller, F. W., and Miller, R. M. (1993). In *Environmental Hazards: Air Pollution-A Reference Handbook*. ABC-CLIO, Santa Barbara, CA.
- Mills, N., Amin, N., Robinson, S., Anand, A., Davies, J., Patel, D., de la Fuente, J., Cassee, F., Boon, N., Macnee, W., et al. (2006). Do inhaled carbon nanoparticles translocate directly into the circulation in humans? *Am. J. Respir. Crit. Care Med.* **173**, 426–431.
- Molfino, N. A., Wright, S. C., Katz, I., Tarlo, S., Silverman, F., McClean, P. A., Szalai, J. P., Raizenne, M., Slutsky, A. S., and Zamel, N. (1991). Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. *Lancet* **338**, 199–203.
- Möller, W., Felten, K., Sommerer, K., Scheuch, G., Meyer, G., Meyer, P., Haussinger, K., and Kreyling, W. (2008). Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. *Am. J. Respir. Crit. Care Med.* **177**, 426–432.
- Morgan, K. T., Jiang, X. Z., Starr, T. B., and Kerns, W. D. (1986). More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicol. Appl. Pharmacol.* **82**, 264–271.
- Morgan, K. T., Kimbell, J. S., Monticello, T. M., Patra, A. L., and Fleishman, A. (1991). Studies of inspiratory airflow patterns in the nasal passages of the F344 rat and rhesus monkey using nasal molds: relevance to formaldehyde toxicity. *Toxicol. Appl. Pharmacol.* **110**, 223–240.
- Moss, O. R., Gross, E. A., James, R. A., Janszen, D. B., Ross, P. W., Roberts, K. C., Howard, A. M., Harkema, J. R., Calderon-Garciduenas, L., and Morgan, K. T. (2001). Respiratory tract toxicity in rats exposed to Mexico City air. Research report 100. Health Effects Institute, Cambridge, MA.
- Mudway, I. S., and Kelly, F. J. (2000). Ozone and the lung: a sensitive issue. *Mol. Aspects Med.* **21**, 1–48.
- Nurkiewicz, T., Porter, D., Barger, M., Castranova, V., and Boegehold, M. (2004). Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation. *Environ. Health Perspect.* **112**, 1299–1306.
- Nurkiewicz, T., Porter, D., Barger, M., Millecchia, L., Rao, K., Marvar, P., Hubbs, A., Castranova, V., and Boegehold, M. (2006). Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. *Environ. Health Perspect.* **114**, 412–419.
- Nurkiewicz, T., Porter, D., Hubbs, A., Cumpston, J., Chen, B., Frazer, D., and Castranova, V. (2008). Nanoparticle inhalation augments particle-dependent systemic microvascular dysfunction. *Part Fibre Toxicol.* **5**, 1.

- Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C. (2004). Translocation of inhaled ultrafine particles to the brain. *Inhal. Toxicol.* **16**, 437–445.
- Passannante, A. N., Hazucha, M. J., Bromberg, P. A., Seal, E., Folinsbee, L., and Koch, G. (1998). Nociceptive mechanisms modulate ozone induced human lung function decrements. *J. Appl. Physiol.* **85**, 1863–1870.
- Pattel, R. E., and Burgess, F. (1957). Toxic effects of mixtures of sulphur dioxide and smoke with air. *J. Med. Microbiol.* **73**, 411–419.
- Pauluhn, J. (2002). Critical analysis of biomonitoring endpoints for measuring exposure to polymeric diphenyl-methane-4,4-diisocyanate (MDI) in rats: a comparison of markers of exposure and markers of effect. *Arch. Toxicol.* **76**, 13–22.
- Pauluhn, J., Carson, A., Costa, D. L., Gordon, T., Kodavanti, U. P., Last, J. A., Matthey, M. A., Pinkerton, K. E., and Sciuoto, A. M. (2007). Workshop summary: phosgene induced pulmonary toxicity revisited: appraisal of early and late markers of pulmonary injury from animal models with emphasis on human significance. *Inhal. Toxicol.* **19**, 789–810.
- Peden, D. B., Boehlecke, B., Horstman, D. D., and 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.
- Pekkanen, J., Peters, A., Hoek, G., Tiittanen, P., Brunekreef, B., de Hartog, J., Heinrich, J., Ibaldo-Mullis, A., Kreyling, W. G., Lanki, T., et al. (2002). Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the exposure and risk assessment for fine and ultrafine particles in ambient air (ULTRA) study. *Circulation* **106**, 933–938.
- Persson, E., Henriksson, J., Tallkvist, J., Rouleau, C., and Tjalve, H. (2003). Transport and subcellular distribution of intranasally administered zinc in the olfactory system of rats and pikes. *Toxicology* **191**, 97–108.
- Peters, A., Veronesi, B., Calderon-Garciduenas, L., Gehr, P., Chen, L., Geiser, M., Reed, W., Rothen-Rutishauser, B., Schurch, S., and Schulz, H. (2006). Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Part Fibre Toxicol.* **3**, 13.
- Plopper, C. G., Hatch, G. E., Wong, V., Duan, X., Weir, A. J., Tarkington, B. K., Devlin, R. B., Becker, S., and Buckpitt, A. R. (1998). Relationship of inhaled ozone concentration to acute tracheobronchial epithelial injury, site-specific ozone dose and glutathione depletion in rhesus monkeys. *Am. J. Respir. Cell Mol. Biol.* **19**, 387–399.
- Plopper, C. G., Smiley-Jewell, S. M., Miller, L. A., Fanucchi, M. V., Evans, M. J., Buckpitt, A. R., Avdalovic, M., Gershwin, L. J., Joad, J. P., Kajekar, R., et al. (2007). Asthma/allergic airways disease: does postnatal exposure to environmental toxicants promote airway pathobiology. *Toxicol. Pathol.* **35**, 97–110.
- Pope, C. I. (1989). Respiratory disease associated with community air pollution and a steel mill, Utah Valley. *Am. J. Public Health* **79**, 623–628.
- Ray, S. C., King, E. J., and Harrison, C. V. (1951). The action of anthracite and bituminous coal dusts mixed with quartz on the lungs of rats. *Br. J. Ind. Med.* **8**, 74–76.
- Rhoden, C., Wellenius, G., Ghelfi, E., Lawrence, J., and Gonzalez-Flecha, B. (2005). PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. *Biochim. Biophys. Acta* **1725**, 305–313.
- Rinehart, W. E., and Hatch, T. (1964). Concentration-time product (Ct) as an expression of dose in sublethal exposures to phosgene. *Am. Ind. Hyg. Assoc. J.* **25**, 545–553.
- Rocha, E., Lichtenfels, A., Amador Pereira, L., and Saldiva, P. (2008). Effects of ambient levels of air pollution generated by traffic on birth and placental weights in mice. *Fertil. Steril.* **90**, 1921–1924.
- Rossing, R. G. (1964). Physiologic effects of chronic exposure to phosgene in dogs. *Am. J. Physiol.* **207**, 265–272.
- Rudolf, G., and Heyder, J. (1974). Deposition of aerosol particles in the human nose. In *Aerosole in Naturwissenschaft, Medizin und Technik: Kongressbericht der Aerosoltagung* (V. Bohlau and H. Straubel, Eds.), pp. 16–19. Gesellschaft für Aerosolforschung, Bad Soden, Federal Republic of Germany.
- Saldiva, P. H., King, M., Delmonte, V. L., Macchione, M., Parada, M. A., Daliberto, M. L., Sakae, R. S., Criado, P. M., Silveira, P. L., Zin, W. A., et al. (1992). Respiratory alterations due to urban air pollution: an experimental study in rats. *Environ. Res.* **57**, 19–33.
- Scannell, C., Chen, L., Aris, R. M., Tager, I., Christian, D., Ferrando, R., Welch, B., Kelly, T., and Balmes, J. R. (1996). Greater ozone-induced inflammatory responses in subjects with asthma. *Am. J. Respir. Crit. Care Med.* **154**, 24–29.
- Schatz, M., Zeiger, R., and Hoffman, C. (1990). Intrauterine growth is related to gestational pulmonary function in pregnant asthmatic women. Kaiser-Permanente Asthma and Pregnancy Study Group. *Chest* **98**, 389–392.
- Scheel, L. D., Dobrogorski, O. J., Mountain, J. T., Svrbely, J. L., and Stokinger, H. E. (1959). Physiologic, biochemical, immunologic and pathologic changes following ozone exposure. *J. Appl. Physiol.* **14**, 67–80.
- Schelegle, E. S., Carl, M. L., Coleridge, H. M., Coleridge, J. C. G., and Green, J. F. (1993). Contribution of vagal afferents to respiratory reflexes evoked by acute inhalation of ozone in dogs. *J. Appl. Physiol.* **74**, 2338–2344.
- Schelegle, E. S., Eldridge, M. W., Cross, C. E., Walby, W. F., and Adams, W. C. (2001). Differential effects of airway anesthesia on ozone-induced pulmonary responses in human subjects. *Am. J. Respir. Crit. Care Med.* **163**, 1121–1127.
- Schlesinger, R. B., Ben-Jebria, A., Dahl, A. R., Snipes, M. B., and Ultman, J. (1997). Disposition of Inhaled Toxicants. In *Handbook of Human Toxicology* (E. J. Massaro, Ed.), pp. 493–550. CRC Press, Boca Raton, FL.
- Selgrade, M. K., Cooper, K. D., Devlin, R. B., van Loveren, H., Biagini, R. E., and Luster, M. I. (1995a). Immunotoxicity—bridging the gap between animal research and human health effects. *Toxicol. Sci.* **24**, 13–21.
- Selgrade, M. K., Gilmore, M. I., and Yang, Y. G. (1995b). Pulmonary host defenses and resistance to infection following subchronic exposure to phosgene. *Inhal. Toxicol.* **7**, 1257–1268.
- Selgrade, M. K., and Gilmour, M. I. (1994). In *Effects of Gaseous Air Pollutants on Immune Responses and Susceptibility to Infectious and Allergic Disease* 2nd ed. Raven Press, Ltd., New York, NY.
- Selgrade, M. K., Starnes, D. M., Illing, J. W., Daniels, M. J., and Graham, J. A. (1989). Effects of phosgene exposure on bacterial, viral, and neoplastic lung disease susceptibility in mice. *Inhal. Toxicol.* **1**, 243–259.
- Slade, R., Highfill, J. W., and Hatch, G. E. (1989). Effects of depletion of ascorbic acid or nonprotein sulfhydryls on the acute inhalation toxicity of nitrogen dioxide, ozone, and phosgene. *Inhal. Toxicol.* **1**, 261–271.
- Solway, J., and Leff, A. R. (1991). Sensory neuropeptides and airway function. *J. Appl. Physiol.* **71**, 2077–2087.
- Somers, C. M., McCarty, B. E., Malek, F., and Quinn, J. S. (2004). Reduction of particulate air pollution lowers the risk of heritable mutations in mice. *Science* **304**, 1008–1010.
- Sram, R. J., Binkova, B., Dejmek, J., and Bobak, M. (2005). Ambient air pollution and pregnancy outcomes: a review of the literature. *Environ. Health Perspect.* **113**, 375–382.
- Stokinger, H. E. (1957). Evaluation of the hazards of ozone and oxides of nitrogen; factors modifying toxicity. *AMA Arch. Ind. Health* **15**, 181–190.
- Stokinger, H. E., and Scheel, L. D. (1962). Ozone toxicity. Immunochemical and tolerance-producing aspects. *Arch. Environ. Health* **4**, 327–334.
- Stokinger, H. E., Wagner, W. D., and Wright, P. G. (1956). Studies of ozone toxicity. I. Potentiating effects of exercise and tolerance development. *AMA Arch. Ind. Health* **14**, 158–162.
- Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R., Aguinaldo, J., Fayad, Z., Fuster, V., Lippmann, M., et al. (2005). Long-

- term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. *JAMA* **294**, 3003–3010.
- Sun, Q., Yue, P., Kirk, R., Wang, A., Moatti, D., Jin, X., Lu, B., Schecter, A., Lippmann, M., Gordon, T., *et al.* (2008). Ambient air particulate matter exposure and tissue factor expression in atherosclerosis. *Inhal. Toxicol.* **20**, 127–137.
- Swenberg, J. A., Kerns, W. D., Mitchell, R. I., Gralla, E. J., and Pavkov, K. L. (1980). Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. *Cancer Res.* **40**, 3398–3402.
- Takenaka, S., Dornhofer-Takenaka, H., and 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.
- Tamagawa, E., Bai, N., Morimoto, K., Gray, C., Mui, T., Yatera, K., Zhang, X., Xing, L., Li, Y., and Laher, I. (2008). Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction. *Am. J. Physiol. Lung Cell Mol. Physiol.* **295**, L79–L85.
- Tankersly, C., Campen, M., Bierman, A., Flanders, S., Broman, K., and Rabold, R. (2004). Particle effects on heart-rate regulation in senescent mice. *Inhal. Toxicol.* **16**, 381–390.
- Task Group on Lung Dynamics (TGLD). (1966). Deposition and retention models for internal dosimetry of the human respiratory tract. *Health Phys.* **12**, 173–207.
- Taylor-Clark, T. E., and Udem, B. J. (2010). Ozone activates airway nerves via the selective stimulation of TRPA1 ion channels. *J. Physiol.* **588**, 423–433.
- Tepper, J. S., Wiester, M. J., Weber, M. F., and Ménache, M. G. (1990). Measurements of cardiopulmonary response in awake rats during acute exposure to near-ambient concentrations of ozone. *J. Appl. Toxicol.* **10**, 7–15.
- U.S. Department of Health, Education, and Welfare. (1969). *Air Quality Criteria for Particulate Matter. National Air Pollution Control Administration*. Report no. AP-49. Washington, DC.
- U.S. Environmental Protection Agency (U.S. EPA) (1982). In *Air Quality Criteria for Particulate Matter and Sulfur Oxides*, Vol. 1, 2, 3. U.S. Environmental Protection Agency, Washington, DC.
- U.S. Environmental Protection Agency (U.S. EPA). (2005). *Toxicological Review of Phosgene: In Support of Summary Information on the Integrated Risk Information System (IRIS)*. Report no. EPA/635/R-06/001. U.S. EPA, Washington, DC.
- U.S. Environmental Protection Agency (U.S. EPA). (2006). *Air Quality Criteria for Ozone and Related Photochemical Oxidants* Report no. EPA/600/R-05/004aF. U.S. EPA, Research Triangle Park, NC.
- U.S. EPA. (2007). *Review of the National Ambient Air Quality Standards for Ozone: Policy Assessment of Scientific and Technical Information, Staff Paper*. U.S. EPA, Research Triangle Park, NC.
- Vagaggini, B., Bartoli, M. L., Cianchetti, S., Costa, F., Bacci, E., Dente, F. L., Di Franco, A., Malagrino, L., and Paggiaro, P. (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.* **19**, 1–5.
- Van Wijk, A. M., and Patterson, H. S. (1940). The percentage of particles of different size, removed from dust laden air by breathing. *J. Ind. Hyg. Toxicol.* **22**, 31–35.
- Veras, M., Damaceno-Rodrigues, N., Caldini, E., Maciel Ribeiro, A., Mayhew, T., Saldiva, P., and Dolnikoff, M. (2008). Particulate urban air pollution affects the functional morphology of mouse placenta. *Biol. Reprod.* **79**, 578–584.
- Veras, M., Damaceno-Rodrigues, N., Guimarães Silva, R., Scoriza, J., Saldiva, P., Caldini, E., and Dolnikoff, M. (2009). Chronic exposure to fine particulate matter emitted by traffic affects reproductive and fetal outcomes in mice. *Environ. Res.* **109**, 536–543.
- Veronesi, B., Makwana, O., Pooler, M., and Chen, L. (2005). Effects of subchronic exposures to concentrated ambient particles: VII. Degeneration of dopaminergic neurons in Apo E<sup>-/-</sup> mice. *Inhal. Toxicol.* **17**, 235–241.
- Wang, J., Chen, C., Yu, H., Sun, J., Li, B., Li, Y., Gao, Y., He, W., Huang, Y., and Chai, Z. (2007). Distribution of TiO<sub>2</sub> particles in the olfactory bulb of mice after nasal inhalation using microbeam SRXRF mapping techniques. *J. Radioanal. Chem.* **272**, 527–531.
- Watkinson, W., Campen, M., and Costa, D. (1998). Cardiac arrhythmia induction after exposure to residual oil fly ash particles in a rodent model of pulmonary hypertension. *Toxicol. Sci.* **41**, 209–216.
- Weinmann, G. G., Weidenbach-Gerbase, M., Foster, W. M., Zacur, H., and Frank, R. (1995). Evidence for ozone-induced small-airway dysfunction: lack of menstrual-cycle and gender effects. *Am. J. Respir. Crit. Care Med.* **152**, 988–996.
- Wellenius, G., Saldiva, P., Batalha, J., Murthy, G., Coull, B., Verrier, R., and Godleski, J. (2002). Electrocardiographic changes during exposure to residual oil fly ash (ROFA) particles in a rat model of myocardial infarction. *Toxicol. Sci.* **66**, 327–335.
- Wichers, L., Nolan, J., Winsett, D., Ledbetter, A., Kodavanti, U., Schladweiler, J., Costa, D., and Watkinson, W. (2004). Effects of instilled combustion-derived particles in spontaneously hypertensive rats. Part 1: cardiovascular responses. *Inhal. Toxicol.* **16**, 391–405.
- Wiebert, P., Sanchez-Crespo, A., Falk, R., Philipson, K., Lundin, A., Larsson, S., Möller, W., Kreyling, W., and Svartengren, M. (2006a). No significant translocation of inhaled 35-nm carbon particles to the circulation in humans. *Inhal. Toxicol.* **18**, 741–747.
- Wiebert, P., Sanchez-Crespo, A., Seitz, J., Falk, R., Philipson, K., Kreyling, W., Moller, W., Sommerer, K., Larsson, S., and Svartengren, M. (2006b). Negligible clearance of ultrafine particles retained in healthy and affected human lungs. *Eur. Respir. J.* **28**, 286–290.
- Yang, Y. G., Gilmour, M. I., Lange, R., Gurlison, G. R., and Selgrade, M. K. (1995). Effect of acute exposure to phosgene on pulmonary host defense and resistance infection. *Inhal. Toxicol.* **7**, 393–404.
- Yauk, C., Polyzos, A., Rowan-Carroll, A., Somers, C., Godschalk, R., Van Schooten, F., Berndt, M., Pogribny, I., Koturbash, I., Williams, A., *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. U.S.A.* **105**, 605–610.
- Ying, Z., Kampfrath, T., Thurston, G., Farrar, B., Lippmann, M., Wang, A., Sun, Q., Chen, L., and Rajagopalan, S. (2009). Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species. *Toxicol. Sci.* **111**, 80–88.
- Young, W. A., Shaw, D. B., and Bates, D. V. (1964). Effect of low concentrations of ozone on pulmonary function in man. *J. Appl. Physiol.* **19**, 765–768.
- Zhang, L.-Y., Levitt, R. C., and Kleeberger, S. R. (1995). Differential susceptibility to ozone-induced airways hyperreactivity in inbred strains of mice. *Exp. Lung Res.* **21**, 503–518.
- Zhang, Z., Whitsel, E., Quibrera, P., Smith, R., Liao, D., Anderson, G., and Prineas, R. (2009). Ambient fine particulate matter exposure and myocardial ischemia in the Environmental Epidemiology of Arrhythmogenesis in the Women's Health Initiative (EEAWHI) study. *Environ. Health Perspect.* **117**, 751–756.