

# Airborne Particulate Matter and Human Health: Toxicological Assessment and Importance of Size and Composition of Particles for Oxidative Damage and Carcinogenic Mechanisms

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Air pollution has been considered a hazard to human health. In the past decades, many studies highlighted the role of ambient airborne particulate matter (PM) as an important environmental pollutant for many different cardiopulmonary diseases and lung cancer. Numerous epidemiological studies in the past 30 years found a strong exposure-response relationship between PM for short-term effects (premature mortality, hospital admissions) and long-term or cumulative health effects (morbidity, lung cancer, cardiovascular and cardiopulmonary diseases, etc). Current research on airborne particle-induced health effects investigates the critical characteristics of particulate matter that determine their biological effects. Several independent groups of investigators have shown that the size of the airborne particles and their surface area determine the potential to elicit inflammatory injury, oxidative damage, and other biological effects. These effects are stronger for fine and ultrafine particles because they can penetrate deeper into the airways of the respiratory tract and can reach the alveoli in which 50% are retained in the lung parenchyma. Composition of the PM varies greatly and depends on many factors. The major components of PM are transition metals, ions (sulfate, nitrate), organic compound, quinoid stable radicals of carbonaceous material, minerals, reactive gases, and materials of biologic origin. Results from toxicological research have shown that PM have several mechanisms of adverse

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cellular effects, such as cytotoxicity through oxidative stress mechanisms, oxygen-free radical-generating activity, DNA oxidative damage, mutagenicity, and stimulation of proinflammatory factors. In this review, the results of the most recent epidemiological and toxicological studies are summarized. In general, the evaluation of most of these studies shows that the smaller the size of PM the higher the toxicity through mechanisms of oxidative stress and inflammation. Some studies showed that the extractable organic compounds (a variety of chemicals with mutagenic and cytotoxic properties) contribute to various mechanisms of cytotoxicity; in addition, the water-soluble fraction (mainly transition metals with redox potential) play an important role in the initiation of oxidative DNA damage and membrane lipid peroxidation. Associations between chemical compositions and particle toxicity tend to be stronger for the fine and ultrafine PM size fractions. Vehicular exhaust particles are found to be most responsible for small-sized airborne PM air pollution in urban areas. With these aspects in mind, future research should aim at establishing a cleared picture of the cytotoxic and carcinogenic mechanisms of PM in the lungs, as well as mechanisms of formation during internal engine combustion processes and other sources of airborne fine particles of air pollution.

**Keywords** air pollution; particulate matter (PM); particle size; particle composition; cytotoxic activity; genotoxicity; lung cancer; cardiovascular diseases

## INTRODUCTION

Air pollution in urban and industrial areas was considered dangerous to human health for centuries. Numerous studies in the past decades have observed associations between elevated air pollution levels and various health outcomes, including mortality, hospitalization for respiratory and cardiovascular diseases, aggravation of asthma attacks, and adverse lung functions. In recent years, most epidemiological and toxicological studies focused on the inhalation of airborne particulate matter (mainly vehicular exhaust particles) because they found a stronger correlation of mortality and adverse respiratory health effects with fine respirable particles than with other atmospheric gas pollutants. Current attention has been focused on the size and composition characteristics of these particles, as well as on toxicological mechanisms in laboratory animals, controlled human exposures, and population-based epidemiology.

### Air Pollution: Historical Considerations

The burning of coal in the 17th century near the palace at Westminster was forbidden by Queen Elizabeth I because of the offensive nature of smoke, and John Evelyn in 1661 in his diatribe proposed that factories should be moved away from London (1). By the 19th century, clinicians in England attributed lung diseases to air pollution, but in recent decades there was systematic research for concrete evidence of adverse health effect in humans (2, 3).

Despite the ill effects of air pollution in cities and industrial areas, legislation to control air pollution was lacking in many industrial countries. Then, major industrial air pollution episodes occurred in the Meuse Valley (Belgium, 1930) (4, 5), and in Donora Valley (Pennsylvania, United States, 1948) (6) and in urban London (December 1952). In the first two occasions, air pollution was caused by excessive smoke from coal-burning domestic appliances and industrial furnaces, whereas in London a dense fog occurred during inversion weather and lasted for four days. Measured concentrations of smoke particles and sulfur dioxide (SO<sub>2</sub>) rose to more than 10 times normal levels. It was estimated that 4,000 excess deaths occurred during or after the episode, mostly elderly persons with chronic heart and lung diseases (7).

The effect of a severe pollution episode on health was demonstrated in the Los Angeles basin. The notorious Los Angeles “smog” has been attributed to photochemical oxidants that result from bright sunlight acting on the exhaust fumes of vehicles (8). Air pollution episodes were serious in some of the rapidly expanding cities, such as Chicago, Mexico City, Lagos, Cairo, Tokyo, and Athens, during the 1970s and 1980s, which raised public concern for air quality and environmental problems. As a consequence, many developed countries introduced environmental legislation and created environmental protection agencies (9).

## Health Effects of Gaseous Pollutants

The adverse health effects of air pollution have been extensively studied in the past decades, and despite the differences of air pollutants and variations in local atmospheres, the basic features of acute and long-term health effects are well known. Epidemiological studies, data of concentrations of the main pollutants, exposure, and dose for the exposed population are at present sufficient to make definitive judgment as to the specific cause of observed health effects (10, 11). The basic health effects are (a) excess mortality, mainly among the elderly and chronically ill persons; (b) effects on elderly people with preexisting cardiopulmonary diseases; (c) exacerbation of symptoms among people with acute and chronic pulmonary disease (bronchitis and emphysema) (hospital admissions); and (d) increased irritation of eye and respiratory system, especially asthma attacks, respiratory infections, and so on (12–14).

In the course of investigating and regulating air pollutants, most studies focused on the important air pollutants: sulfur dioxide (SO<sub>2</sub>), nitrogen oxides (NO<sub>x</sub>), acid aerosols (sulfates and nitrate), carbon monoxide (CO), ozone (O<sub>3</sub>), and smoke and fine particulate matter (12). The biological effects and the importance of air pollutant for long-term adverse health effects to people in urban

areas were investigated extensively by both toxicological and epidemiological studies (15, 16).

Studies found an association between increased concentrations of SO<sub>2</sub> and daily mortality (with coexisting particulate matter), as well as morbidity of bronchial asthma, persistent cough and phlegm, bronchoconstriction, and irritability of the respiratory system (17–19). CO is an air pollutant that shows a distinct diurnal pattern with peaks corresponding with the morning and evening traffic rush hours. It is absorbed through the lungs and reacts with hemoglobin of the blood, resulting in a reduction of oxygen-carrying capacity. As levels of CO increase, the adverse health effects include headaches, drowsiness, coma, respiratory failure, and ultimately death at very high concentrations (20). Epidemiological studies found an association between increased incidence of acute respiratory diseases and increasing levels of NO<sub>2</sub> (21) and decreased pulmonary function (22). Photochemical oxidants are secondary pollutants formed by the action of sunlight on an atmosphere that contains reactive hydrocarbons and NO<sub>x</sub>. The most important photochemical oxidants are ozone and peroxyacetyl nitrate (PAN). The effect of ozone was clearly demonstrated in the Los Angeles basin (air pollution episode, September 1979, lasting ten consecutive days), with concentrations exceeded 0.35 ppm. A health survey found burning or irritation of the eyes, headaches, breathing irritation, and sore throats. Hospitals reported increases of up to 50% in the number of patients being admitted with chronic lung diseases such as emphysema and asthma (23). Ozone is a strong oxidant and affects lung function (24, 25).

Suspended particulate matter (SPM) refers to the wide range of fine and ultrafine particles dispersed into the air from combustion processes, industrial activities, traffic-related particles, and natural sources (soil, plant materials, etc.). PM range in size from 0.1 to about 25 μm in aerodynamic diameter, and the constituents vary over time and space. PM and SO<sub>2</sub> are often regarded as “traditional” pollutants of urban areas. Total suspended particulates (TSP) and smoke (measured on filter paper by the reflectance method), which includes particles of approximately 10 μm or less, were traditionally measured for decades in urban areas. Studies indicated that PM were not at high levels in urban areas and their adverse health effects relatively small (26). However, in recent years the significance of increasing concentrations of airborne traffic-related particles in urban areas and their adverse effects on the cardiovascular and respiratory systems became obvious from numerous toxicological and epidemiological studies.

## Health Effects of Airborne Particulate Matter

The introduction of new clean air regulations and the successful abatement of traditional air pollutants in the 1960s and 1970s in many industrialized

countries led to the reduction of pollution episodes in urban areas, elimination of winter smog (London-type pollution), and significantly improved levels of photochemical pollutants (California-type pollution) because of aggressive controls on vehicle exhausts and industrial fumes. But the problem of adverse health effects and excess deaths by airborne particulate matter (PM) in urban areas had not been eliminated, as it was believed by an extensive review of Holland et al (27). It was argued that high levels of PM posed health problems, but at lower concentrations their effects were confounded by other factors. Many scientists disagreed with the main conclusions and through the careful analysis of data supported that PM has adverse health effects even at relatively low concentrations (28, 29).

The reason that PM became very important air pollutants in recent decades and their adverse health effects became more hazardous is that air pollution in urban areas has changed. Air pollution from combustion of traditional fossil fuels (biomass, coal, wood, crude oil, diesel with high content in sulfur) is now in much lower concentrations than 30 to 40 years ago because better and cleaner technology, but other pollutants have gained prominence, such as fine and ultrafine PM, because of a dramatic increase in motor vehicle use worldwide with the consequent rise in exhaust emissions in urban areas. Airborne PM is found not only in big cities but also in small and large towns, and their size distribution and composition (heavy metals, PAHs, etc.) has changed, resulting in higher oxidative cellular damage and toxicological effects (30, 31).

In the 1990s, epidemiologists focused on day-to-day variations in air pollution over long periods as determinants of day-to-day variations in mortality, hospital admissions, and lung function changes. These time-series studies have several advantages, with large differences in exposure over time and lots of collected data, leading to greater statistical power to detect small increases in adverse health effects of air pollution. Health effects from exposure to airborne fine particles were revealed by two very important cohort studies in the United States. Both studies were based on observations from the late 1970s through the late 1980s (32, 33). Then, another study found significant health effects (increased mortality from non-malignant respiratory diseases and lung cancer in male non-smokers) for high concentrations of PM with aerodynamic diameter less than 10  $\mu\text{m}$  (PM<sub>10</sub>) (34).

Additional epidemiological studies have provided more quantification of subtle health effects associated with fine PM, which is common in the contemporary urban areas and big cities in the developed countries, by better definitions and measures of air pollution exposures and health endpoints. In addition, advanced biostatistical and econometric techniques for longitudinal or cross-sectional analysis have greatly expanded the evaluation of health effects (35).

## EPIDEMIOLOGICAL EVIDENCE

### Short-Term Health Effects

Numerous studies from the 1980s onwards evaluated the short-term effect of air pollution on health, with emphasis on mortality and hospital admissions. Some of the most interesting studies were those that observed changes in daily death counts associated with short-term changes in PM air pollution. Although precise comparison between studies is difficult, due to differences in measurements, most of the results suggest that a  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$  was associated with a 0.5% to 1.5% increase in daily mortality. A great number of these studies came from various areas of the United States (36–40).

In European countries, scientists combined their initial studies to cover populations of approximately 43 million people living in 29 countries. The APHEA-1 project (Air Pollution and Health: A European Approach) used older data of PM (mostly for TSP and smoke measurements) air pollution, and in the late 1990s a new series of studies (APHEA-2) was performed with data concerning finer particulates of  $10 \mu\text{m}$  (41, 42). The results of the data collected showed that all-cause daily mortality increased by 0.6% for each  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$ . The APHEA-2 hospital admission studies, which covered 38 million people living in 8 European cities, showed the following results: (a) asthma and chronic obstruction pulmonary disease (COPD) in people aged older than 65 years increased by 1.0% for each  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$ ; and (b) admissions for cardiovascular diseases (CVD) increased by about 0.5% for each  $10 \mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{10}$  and by about 1.1% for each  $10 \mu\text{g}/\text{m}^3$  increase of black smoke (particles smaller than  $10 \mu\text{m}$ ). The last finding is considered a good indication for the important role of diesel exhaust smoke in CVD (43).

Additional studies on short-term effects of particulate air pollution were focused on the 20 largest metropolitan areas of the United States (during the 1987–1994 period). It was found that all-cause mortality increased by 0.5% for each  $10 \mu\text{g}/\text{m}^3$  of  $\text{PM}_{10}$  (44, 45). Hospital admissions were studied in 10 major US cities. The results showed that individuals aged older than 65 were admitted to hospitals in increasing numbers for days with high PM concentrations: (a) COPD admission increased by 1.5% and (b) CVD increased by 1.1% for each  $10 \mu\text{g}/\text{m}^3$  of  $\text{PM}_{10}$  (46).

In recent years, numerous studies and reviews provided additional evidence on the association between short-term health effects and particulate air pollution, especially in large cities affected by vehicular exhaust pollution (47–51).

### Long-Term Health Effects

Short-term studies are based on air pollution episodes or on days with severe or very high concentrations of air pollutants. But, what happens with

long-term cumulative exposures at low or very low concentrations of particulates? Are standards and health guidelines sufficient to protect human health? Epidemiological studies were designed to study the associations between cumulative exposures (months, years, decades) to suspended particulate air pollution and long-term effects on morbidity endpoints. Studies that measured deaths in short-term PM exposure capture only the very frail people who would have died in a few days anyway, whereas studies on long-term exposures to PM measure the overall health effects for varied concentrations and length of exposure. Most of these studies have been cross-sectional, assuming that current air pollution exposures are representative of long-term previous exposures (11).

Epidemiological research has found consistent and coherent associations between long-term exposure and various health outcomes, such as reduced lung function, respiratory symptoms, chronic bronchitis, relative increase of lung cancer risk, and cardiopulmonary mortality (52). Scientists used the respiratory health effects in adults and in children as an example for long-term effects of particulate air pollution (53, 54).

There are now several studies on potential mechanisms by which long-term exposure to particulate air pollution affects the cardiovascular system (55). Fine and ultrafine particles from vehicular exhausts are affecting heart rate variability, blood viscosity and blood coagulability, cardiac arrhythmia, deep vein thrombosis, atherogenesis, and destabilization or rupture of atherosclerotic plaques (56–58).

Lung cancer risk and particulate air pollution were the subject of many recent studies. Large epidemiological cohort studies in the United States and in Europe assessed the relationship between long-term exposure to fine particulate air pollution ( $PM_{10}$  and  $PM_{2.5}$ ) and increased mortality from lung cancer, especially in combination with other known risk factors, such as smoking, passive smoking, and occupational exposures (59, 60).

One of the most important studies on long-term effects of fine particulate air pollution ( $PM_{2.5}$ ) and its relationship with all-cause, lung cancer, and cardiopulmonary mortality was conducted in the United States by Pope et al. (61). The study enrolled 1.2 million adults in 1982 (data from the American Cancer Society, Cancer Prevention II study). Participants completed a questionnaire that detailed individual risk factor data (age, weight, smoking, diet, occupational exposure, etc.). The risk factor data for 500,000 adults were linked with air pollution data from metropolitan areas throughout United States and were combined with data of causes of death up to 1998. Fine particulate and  $SO_2$  air pollution (the integrated average of  $PM_{2.5}$  concentrations was estimated, first by site and then by metropolitan area) were associated with increased mortality. Each  $10 \mu g/m^3$  elevation in fine particulate matter was associated with approximately a 4%, 6%, and 8% increased risk of all-cause, cardiopulmonary, and lung cancer mortality, respectively. It is interesting that data of

coarse particles and TSP were not consistently associated with mortality (61). A recent review of epidemiological evidence for long-term exposures suggests the existence of a dynamic exposure-response relationship between PM and mortality risk. Data showed that adverse health effects are dependent on both exposure concentrations and length of exposure, and that long-term exposures in PM have larger, more persistent, and cumulative effects than short-term exposures (62).

Research data collected during the past 20 years in many industrialized countries on air pollution, especially particulate matter (PM), contributed to various estimates of public-health impact on mortality and morbidity. One of the first studies estimated the contribution of air pollution and traffic-related air pollution in Austria, France, and Switzerland (63). A  $10 \mu\text{g}/\text{m}^3$  increase in PM was used to quantify the effects of air pollution. The findings of the study showed that air pollution caused 6% of total mortality (or more than 40,000 attributable cases per year) and about half of all mortality was attributed to motorized traffic. In addition, air pollution caused more than 25,000 new cases of chronic bronchitis (adults); more than 290,000 episodes of bronchitis (children); more than 500,000 cases of asthma attacks; and more than 16 million person-days of restricted activities. Similar results of increases in mortality (all-causes and cardiovascular/cardiopulmonary) from long-term exposures in particulate air pollution were estimated in the United States (64–67).

Therefore, the next appropriate step in the scientific research was to investigate the role of the size and composition of PM and especially the traffic-related fine PM.

## PHYSICAL AND CHEMICAL PROPERTIES

### Size of Airborne Particulate Matter

The size of suspended particulate matter ranges over a wide scale. The different-sized fractions of PM are mixtures of several components and can consist of solid particles or liquid droplets as well as semivolatile components. The aerodynamic diameter (a.d.) of airborne PM can range from approximately 0.005 to  $100 \mu\text{m}$ . The size categories are defined as: *TSP* particles less than  $100 \mu\text{m}$ ; *fine* particles refer to particles less than  $2.5 \mu\text{m}$ ; and *ultrafine* particles typically refer to particles less than  $100 \text{ nm}$ . There are also other definitions: *ultrafine* (or *nuclei* mode) particles, smaller than about  $0.1 \mu\text{m}$  in a.d.; *fine* particles (*accumulation* mode) smaller than  $1 \mu\text{m}$  a.d.; and *coarse* particles larger than  $1 \mu\text{m}$  a.d. (68). The US Environmental Protection Agency and other agencies in the air pollution regulation of PM have two main categories:  $\text{PM}_{2.5}$  and  $\text{PM}_{10}$ , which refer to particles with a.d. smaller than  $2.5 \mu\text{m}$  and  $10 \mu\text{m}$ , respectively (69). For modeling purposes of particle deposition, the human

respiratory system is divided into an extrathoracic (oro- or nasopharyngeal) region, the tracheobronchial tree (cylindrical airways), and the acinar (alveoli, gas-exchange section) region. The acinar region is the most important for PM retention and for pulmonary diseases (70).

The ultrafine particles are generated directly by combustion and photochemical activity. The particles are unstable and persist briefly, aggregating to form larger accumulation particles. Fine and ultrafine particles are formed mostly by vehicular exhausts emissions. By contrast, part of coarse particles can be generated by combustion but mostly by mechanical processes that break down material from a variety of non-combustible sources into dust. Most of the suspended PM consists of 90% to 95% of coarse particles, whereas smaller particles are only 1% to 8% of the total mass. However, ultrafine and fine particles are very high in numbers, have greater total surface area than larger particles, and because of their porous surface, can adsorb and retain toxic substances. Examinations of the lungs of urban dwellers with high levels of air pollution showed large quantities of fine and ultrafine carbonaceous particle aggregates, which are mainly combustion products (71). During a pollution episode, each lung acinus could receive on average 30-million particles and each alveolus about 1500 particles (for 24 h exposure) of which 50% are being deposited (72). Lung airways and alveoli retain mostly PM<sub>2.5</sub> rather than PM<sub>10</sub>, a finding supported by various observations (73). Analytical electron microscopy measurements showed that 96% of effectively retained particles in the lung parenchyma were PM<sub>2.5</sub> and only 5% were ultrafine particles (0.1 μm) (74). Therefore, the size of PM and their retention play an important role in the PM cytotoxic effects.

## Chemical Composition of Airborne Particulate Matter

The chemical composition of PM varies greatly and depends on many factors, such as combustion sources, climate, season, and type of urban or industrial pollution. The major components of PM are organic compounds adsorbed onto particles, which can be volatile or semivolatile organic species (e.g., PAHs, nitro-PAHs, quinones), transition metals (iron, nickel, vanadium, copper, etc.), ions (sulfate, nitrate, acidity), reactive gases (ozone, peroxides, aldehydes), particle core of carbonaceous material (mainly from combustion processes and vehicular exhaust particles), materials of biologic origin (endotoxins, bacteria, viruses, animal and plant debris), and minerals (quartz, asbestos, soil dust). The composition of coarse particles consists mainly of insoluble crust-derived minerals, sea salt, material of biologic origin, and so on. By contrast, the fine and ultrafine particles are mainly carbonaceous aggregates with metals and organic species adsorbed on their surface cavities (75). Chemical composition of PM varies according to sources and combustion factors that govern their size and chemical components (76, 77).

Transition metals are thought to be very important in PM cellular toxicity. The bioavailability of transition metals in PM and their redox properties, which are considered very important for the toxic effects and the oxidative damage in the cardiopulmonary system (78–80). Experiments with metals and metal complexes that are associated with PM surfaces were found to cause acute injury in rats (81). Iron release from airborne PM or other redox metals can stimulate the generation of hydroxyl radicals (HO•) by Fenton-type reactions, causing extensive oxidative damage to cellular macromolecules (82, 83).

Experimental observations showed that most of the airborne particles from combustion are composed of chain-aggregated masses of fine carbonaceous spheres, which contain persistent-free radicals of quinoid and semiquinoid structure bound in a polymeric tarry matrix (84–86). These persistent radicals, found also in cigarette tar, can initiate a series of redox reactions with oxidative potential and cause cellular damage (87). Polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs are products of incomplete combustion processes and vehicular exhaust found in high concentrations in PM (88, 89) PAHs require metabolic activation to become electrophiles in order to exert their carcinogenic potential. In addition, PAHs are highly mutagenic, with tumor promoter activity, and are responsible for increased risk to malignant neoplasms, especially lung cancer (90, 91).

Airborne particles also contain a wide spectrum of organic chemical substances (olefins, aldehydes, ketones, nitro-compounds, quinones, etc.). Quinones (1,2- and 1,4-naphthoquinones, 9,10-anthraquinone, etc.) are considered of toxicological importance because they can generate reactive oxygen species (ROS) through redox-cycling. In addition to redox potential, quinines can react with –SH compounds and could deplete protective nucleophiles (92–94).

## **BIOLOGICAL MECHANISMS OF TOXICITY**

### **Free-Radical Generating Activity and Oxidative Stress**

In the past 30 years, vehicular traffic and other combustion processes in large cities have resulted in a significant increase of suspended particulate pollution. PM pollution is considered the most important factor in urban areas, compared with gas pollutants, and several mechanisms have been proposed to explain the adverse health effects in humans, especially the cardiopulmonary system (95).

The most important pathophysiological mechanism that has been proposed to explain the association of PM exposure and occurrence of respiratory infections, lung cancer, and chronic cardiopulmonary diseases are oxidative stress through the generation of ROS. In addition, PM initiate inflammatory damage

and upregulation of proinflammatory mediators (cytokines and chemokines), endotoxin effects, stimulation of capsaicin/irritant receptors, procoagulant effects, modification of cellular components, cellular mutagenicity, and DNA damage (96).

All aerobic organisms contain elaborate antioxidant defenses (enzymatic and non-enzymatic) for cellular redox homeostasis and avoidance of oxidative damage to important biological macromolecules (proteins, carbohydrates, membrane lipids, and mitochondrial and cellular DNA). Endogenous metabolic factors can be responsible for excessive ROS production or weakening of antioxidant defenses but are regulated to a great extent by intracellular enzymatic mechanisms. High levels of ROS from exogenous factors (cigarette smoke, air pollution, etc.) can change the redox status of the cell, thereby triggering a cascade of events associated with inflammation (97, 98). Ambient air pollution is known from many studies to produce ROS in the cellular environment. Many recent observations showed that diesel exhaust particles (DEP), because of their fine and ultrafine composition, play an important role of oxidative cellular damage through the generation of oxygen-free radicals (e.g., hydroxyl, HO•, and superoxide anion, O<sub>2</sub><sup>•-</sup>) and ROS (e.g., H<sub>2</sub>O<sub>2</sub>), which take part in a series of mechanisms that cause membrane lipid peroxidation and oxidative DNA damage (99–101).

A three-phase response model has been developed to explain the process of oxidative stress in inducing cellular damage and subsequently adverse health effects. Initially, when oxidative stress is relatively low, various transcription factors (such as the nuclear factor erythroid-2, Nrf2) induce a series of antioxidant and detoxification enzymes (including catalase, superoxide dismutase, glutathione S-transferase, and so on; known collectively as phase 2 response) in which ameliorated (scavenge or destroy ROS) and the PM exposure do not lead to adverse biological outcomes (102, 103). In the second phase, if the protective antioxidant response fails or is inadequate to deal with increasing ROS production, the result is a proinflammatory situation and various cytotoxic effects. These effects are mediated by the redox-sensitive mitogen-activated protein (MAP) kinase and NF-κB cascades that are responsible for the expression of cytokines, chemokines, and adhesion molecules, which are involved in the inflammatory processes of the lungs (104, 105). At high levels of oxidative stress (in the third phase), the antioxidant defenses have been overwhelmed and cytotoxic effects ensue. Mitochondria may be involved, which release proapoptotic factors and induce apoptosis of lung cells (106).

Results from various studies have demonstrated that oxidative potential of fine and ultrafine particles is the result of significant amounts of organic carbon compounds, such as quinones and PAHs. A strong correlation has been found between PM concentration of redox-active compounds and damage in macrophages and bronchial epithelial cells. Electron microscopy showed that ultrafine particles are localized inside damaged mitochondria (107).

The role of quinones in toxicology through their redox potential and the formation of ROS has been studied extensively (108). Studies showed that the carcinogenicity of quinones results from the formation of ROS, through redox-cycling reactions and with the involvement of metal ions and NADPH (109, 110).

The principal pathways of metabolic activation of PAHs are (1) generation of diol epoxides catalyzed by cytochrome P450, leading to DNA adduct formation, considered to be essential to PAH mechanism of carcinogenesis; (2) formation of radical cations catalyzed by cytochrome P450 peroxidases; and (3) formation of redox-active quinones catalyzed by dihydrodiol dehydrogenases, contributing to PAH carcinogenesis and tumor promotion (111–114).

In addition, transition metal ions of PM (found adsorbed at high concentrations in particle cavities) with redox potential can contribute to ROS overproduction and play an important role in oxidative DNA and protein damage (115). Soluble metals on inhaled particles, such as Fe, Ni, V, Co, Cu and Cr, were associated with increased ROS production, followed by cellular oxidative stress in airway epithelial cells (116–118). Studies have identified certain metals by using metal chelators (such as EDTA, which increase the redox reactivity of some metals) and antioxidants (which scavenge oxygen free radicals) as responsible for oxidant effects and inflammation in experimental animals (119–121).

## Lung Cancer Risk

Cancer risks in relation to airborne PM after long-term exposure in urban areas have been studied with different epidemiological methodologies, mainly ecologic, cohort, and case-control studies. Several studies in the 1990s collected adequate epidemiological data on air pollution and lung cancer, and their evidence was reviewed (122). The problem with most of the cohort investigations was that they did not contain information on smoking for all study subjects, and findings for non-smokers were too small for a meaningful interpretation of urban to rural differences. In most of the case-control studies, the increased lung cancer risks were seen primarily in smokers. Ecologic studies generally were not suitable for assessment of causal relationships. A number of studies have been performed on populations living near industries with heavy emissions of air pollutants. Most of the studies on lung cancer and air pollution gave somewhat inconsistent results and positive interactions with smoking (additive or multiplicative) (123).

The relationship among long-term exposure to ambient PM and lung cancer was established for the first time by three cohort studies in the United States. The first study was the AHSMOG study based on 6,338 non-smoker adults (Seventh Day Adventists) followed for 15 years. The investigators reported relative risk (RR) increases of lung cancer mortality among men in

relation to long-term exposure to PM<sub>10</sub> (measured initially from total suspended particulates, TSP, and as PM<sub>10</sub> in the past 5 years). The RR was 3.36 [(95% confidence interval, (CI): 1.57–7.19] associated with an interquartile range of PM 24  $\mu\text{g}/\text{m}^3$ ). Although the mean ozone concentration was not associated with lung cancer incidence, there was an association in males when exposure was formulated as the number of hours per year with elevated ozone concentrations (RR = 4.19, 95% CI: 1.81–9.69) (124).

The second study was based on 8,111 residents of six US cities followed in the period of 1974–1989. Differences in the long-term average PM concentrations, between the most and least polluted cities was  $\sim 20 \mu\text{g}/\text{m}^3$ . Relative risks after adjustment (age, smoking habits, education, body mass index, etc.) were to 1.37, which is estimated as a 19% increase per 10  $\mu\text{g}/\text{m}^3$  (32). The third and largest US investigation was published in 2002 by Pope et al. (61) This study was based on the mortality of approximately 500,000 adult men and women followed for 17 years. Personal information and other confounders or risk factors were collected with questionnaires (age, race, marital status, smoking, body mass, occupational exposures, diet, etc.). For each metropolitan area, PM<sub>2.5</sub> concentrations were compiled from several data sources. The investigation found a significant increase in mortality from lung cancer. Relative risk was 1.14 (95% CI: 1.04–1.23), or 14%, for a difference of 10  $\mu\text{g}/\text{m}^3$  of PM<sub>2.5</sub>. The importance of these three studies is that they avoided various limitations of previous geographic comparisons (case-control studies) and controlled the various confounders or risk factors, especially smoking habits (60, 125).

The European studies on the relationship of long-term exposure to PM and lung cancer risk are considered more valuable because the ambient levels of PM are more variable in European cities and usually higher than in the United States, and the European populations have a wide range of different exposures and living habits (especially smoking and diet), which might modify the final risk. The first study (case-control) did not control the various confounders. It was conducted among 755 men in Trieste (Italy) who died from lung cancer and 755 controls who died from other causes in the period of 1979–1981 and 1985–1986. The RRs were 1.1 (95% CI: 0.8–1.5) for PM exposure less than 30  $\mu\text{g}/\text{m}^3$  and 1.4 for more than 30  $\mu\text{g}/\text{m}^3$  (126). The second study in the Netherlands was a cohort study (on Diet and Cancer, NLCS) that examined the long-term exposure of 4,492 participants, and their deaths from lung cancer were recorded for the period of 1986–94. Risk of lung cancer was, after adjustment for several confounding factors, 1.06 (95% CI: 0.43–2.63) for an increase of 10  $\mu\text{g}/\text{m}^3$  (black smoke), but the number of lung cancer cases were too small ( $n = 60$ ) (127). The third study was a French cohort study and was based on 14,284 adults (in 24 areas of France), with 178 deaths recorded from lung cancer between 1974–2000. Relative risk for lung cancer associated with an increase in exposure to 10  $\mu\text{g}/\text{m}^3$  of TSP was 0.97 (95% CI: 0.94–1.01)

when all 24 areas were considered, and 1.0 when the analysis was restricted to 18 areas (128).

A nested case-controlled European study was conducted in seven countries with over half a million volunteers (35–74 years of age) between 1993 and 1998. The study investigated the association between lung cancer and long-term exposure to PM<sub>10</sub>. Cancer cases were recorded from cancer registries. Overall, 271 lung cancer cases and 737 controls were included. Data for PM<sub>10</sub> exposure were available for 113 cases and 312 controls only. Relative risks were 0.91 (95% CI: 0.70–1.18) for an increase in PM<sub>10</sub> of 10  $\mu\text{g}/\text{m}^3$  and 0.98 (95% CI: 0.66–1.45) for exposures over 27  $\mu\text{g}/\text{m}^3$  (129). Various other studies were conducted in Norway, Greece, and Sweden for the investigation of the association of lung cancer risk and long-term PM exposure, but their results were not consistent, or the age-adjusted RR was small and not statistically significant, or no association was observed (130–132). A recent review describes in detail all European studies and investigated the association of lung cancer risk and long-term exposure to ambient particulate matter (133).

## Genotoxicity

Particulate matter, especially traffic-related airborne particles, contains a large number of genotoxic/mutagenic chemical substances, which can cause DNA damage and promote malignant neoplasms. In recent decades, a number of experimental studies that use different short-term assays have provided evidence for the mutagenic potential of airborne PM. Most studies focused their observations on the genotoxicity of extractable organic compounds and mixtures but also on the water-soluble substances (such as metals) and volatile organic compounds (117, 134, 135).

The genotoxicity of PM was extensively studied with the *Salmonella typhimurium* assay (Ames test) by various research groups and reviewed by Claxton et al. (136). Studies showed that the mutagenicity of airborne PM is due to at least 500 identified organic compounds from varying chemical classes. Mutagenicity was associated with moderately polar/highly polar classes of substances that tend to contain nitroaromatics (nitro-PAHs), aromatic amines, and aromatic ketones. These compounds are produced in the atmosphere when organic compounds (even non-mutagenic) are exposed to NO<sub>x</sub> and sunlight. Combustion emissions were associated with much of the mutagenicity and carcinogenicity of urban PM (137).

Human-derived cell lines have been used to investigate DNA damage induced by extractable organic material (especially PAH-containing mixtures bound onto PM<sub>10</sub> particles using a variety of end-points). Target cells used were human leukocytes, human alveolar carcinoma, human myeloid leukemia, human trancheobronchial epithelia, human fibroblasts, and so on. Airborne PM used in these studies were TSP, PM<sub>10</sub>, PM<sub>2.5</sub>, PM<sub>2.5–10</sub>, residual oil fly

ash (ROFA), and other types of PM. All studies showed positive DNA damage, single-strand breaks, micronuclei sister chromatid exchange, and oxidative DNA damage. Measurements were analyzed by alkaline single-cell gel electrophoresis and comet assay. These studies suggest that the mechanisms of genotoxicity of PM is the result of adduct-forming compounds (through cell-particulate interactions) and oxidizing DNA damage (138–140).

Apart from organic-soluble fractions of PM, other studies focused on the water-soluble fractions (mainly transition metals) and compared their DNA damage potential. Results of these studies showed that the constituents of the water-soluble PM extracts are more likely to induce oxidative DNA damage than the organic compounds (141, 142). A series of studies in the past decade suggest that after inhalation and deposition of PM in the lung, alveoli are able to stimulate the formation of reactive oxygen species (ROS), especially hydroxyl ( $\text{HO}^\bullet$ ) and superoxide anion radicals ( $\text{O}_2^{\bullet-}$ ). These ROS, which can be generated by transition metals and/or quinoid redox cycling, initiate a cascade of reactions and can play an important role in oxidative damage to cellular membrane lipids, proteins-enzymes, and DNA. In addition, ROS can initiate pulmonary inflammation and, through complex mechanisms, might contribute to the impairment of excision repair mechanisms of DNA and activation of oncogenes (143–145). A recent review summarizes the toxicological assessment of various biological mechanisms behind the associations of ambient particulate matter and health risks to humans (146).

## CONCLUSIONS

There is emerging evidence from numerous scientific investigations for the relationship between fine airborne particulates of air pollution and health risks to humans. Studies focused on traffic-related airborne particles because their size is in the fraction of ultrafine particles (especially diesel engine emissions) and penetrate deep in the lung acinus. Epidemiological evidence has established the risk to human health in urban areas and quantified the degree of risks for various diseases. The current data provide sufficient evidence as to the importance of size and compositions of PM in cytotoxic and toxicological mechanisms. Organic compounds, such as PAHs, nitro-PAHs, and others organic constituents, play an important role in genotoxic and cytotoxic mechanisms. In addition, recent data emphasize the importance of water-soluble constituents, such as transition metals, quinoid substances, and stable semiquinone radicals in the carbonaceous section with redox potential. These substances are known to be adsorbed onto the surface cavities of the PM and can be released into the lung alveoli and deposited in the lung parenchyma. Toxicological studies *in vivo* and *in vitro* showed that PM have the ability to generate continuously high reactive ROS, which contribute to the genotoxic and cytotoxic mechanisms of PM.

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