Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 279371, 22 pages
http://dx.doi.org/10.1155/2013/279371

Review Article

Recent Advances in Particulate Matter and Nanoparticle Toxicology: A Review of the *In Vivo* and *In Vitro* Studies

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Received 7 March 2013; Revised 8 May 2013; Accepted 22 May 2013

Academic Editor: Tim Nawrot

Epidemiological and clinical studies have linked exposure to particulate matter (PM) to adverse health effects, which may be registered as increased mortality and morbidity from various cardiopulmonary diseases. Despite the evidence relating PM to health effects, the physiological, cellular, and molecular mechanisms causing such effects are still not fully characterized. Two main approaches are used to elucidate the mechanisms of toxicity. One is the use of *in vivo* experimental models, where various effects of PM on respiratory, cardiovascular, and nervous systems can be evaluated. To more closely examine the molecular and cellular mechanisms behind the different physiological effects, the use of various *in vitro* models has proven to be valuable. In the present review, we discuss the current advances on the toxicology of particulate matter and nanoparticles based on these techniques.

1. Introduction

Exposure to particulate matter (PM) is associated with increases in visits to emergency rooms and mortality [1]. The Meuse valley fog of 1930 [2], the Donora smog incident of 1948 [3], and the London great smog event of 1952 [4] triggered the studies of health effects related to the exposure to PM in large cities and later on the legislation regarding the level limits of PM. For instance, in the US, the Clean Air Act was enacted in 1972.

Inhaled particles penetrate into the respiratory tract where they target different anatomical sites, depending among other properties on the aerodynamic size. Particles are categorized according to aerodynamic size, PM_{10}, thoracic particles, ($\leq 10\,\mu m$) and $PM_{2.5}$ ($\leq 2.5\,\mu m$), or fine fraction. The particles with a range of aerodynamic sizes between 10 and $2.5\,\mu m$ ($PM_{10\text{-}2.5}$) are know as coarse fraction. If the aerodynamic size is equal or less than $0.1\,\mu m$, the particles are called ultrafine particles (UFP), and one of the main sources of this type of primary particles is diesel exhaust (DEP). Engineered particles, measured by their geometric size and with at least one dimension smaller than $0.1\,\mu m$, are known as nanoparticles (NP) [5]. The primary anatomical target of particles with different sizes is summarized on Figure 1.

Air Quality Standards have been adopted by many countries around the world to protect public health and welfare against the adverse effects of air pollution. In fact, member countries of the World Health Organization (WHO) have adopted a constitution that sets guidelines on air pollutants. The WHO, which has representation from nearly 200 countries, recommends daily PM_{10} concentrations not to exceed $50\,\mu g/m^3$ [6]. Many countries, however, have chosen to set Air Quality Standards that are more relaxed or more stringent than the WHO Standard. Air Quality Standards

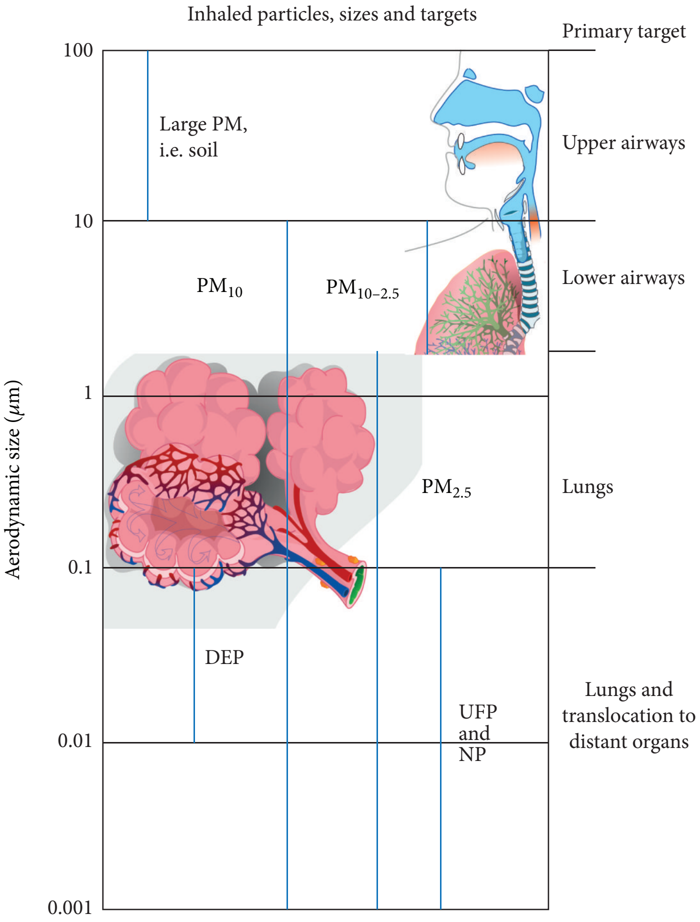

Figure 1: Schematization of the size and main target for particulate matter and nanoparticles.

are generally created or revised according to national policy and scientific information that demonstrates a plausible association between health-related illnesses and exposure to pollutants. The limits for PM_{10} and $PM_{2.5}$ that are used in different countries and regions are shown in Table 1 (Modified from [6]).

Despite all the efforts for measuring the health impact of inhaled particulate matter, we are still far from fully understanding all the effects and mechanisms related to those effects, and also, we still do not understand what is the role of the length (size), shape, and composition of particles in their biological effects. In the present paper, we reviewed the relevant information related to three main aspects of the problem: (1) the determination and role of the chemical and biological components of particles, (2) the evaluation of the *in vivo* effects, both at pulmonary and systemic targets, and (3) the evaluation of the mechanisms of the cellular effects of particles with different sizes, shapes, and composition. Among the large amount of articles that are published in these fields, we choose those that we consider are helping to understand the different problems and also those articles that are opening new questions, pushing the limits of our knowledge forward.

2. Characterization of Particles

Combustion particles from traditional fuels (biomass, coal, wood, crude oil, and diesel with high content in sulfur) are now found in much lower concentrations in air than 30–40 years ago due to improved and cleaner technology. The relative size distribution has changed, and other pollutants have gained prominence, such as ultrafine PM (UFP) [7]. These new and lighter airborne PM is found not only in big cities but also in large and small towns. Interestingly, they differ in composition with regard to various heavy metals and polycyclic aromatic hydrocarbons (PAHs) and are often found to have a higher oxidative and toxic potentials.

Depending on the source and composition of the PM different subsets of components may be found on different fractions. $PM_{2.5}$ comprises the soot fraction and particles grown from the gas phase with subsequent agglomeration. $PM_{2.5}$ includes inorganic ions such as sulfate, nitrate, and ammonia, as well as combustion-form carbon, organic aerosols, metals, and other combustion products. $PM_{10\text{-}2.5}$ is dominated by mechanically abraded or ground particles including finely divided minerals such as oxides of aluminum silicate, iron, calcium, and potassium [8].

Table 1: Comparison of the WHO guidelines and standards from different countries. Modified from WHO air quality guidelines, global update, 2005, a report on a Working Group meeting, Bonn, Germany, 18–20 October 2005 [5].

	Selected air quality guidelines and standards			
Source	PM$_{10}$ (μg/m^3)		PM$_{2.5}$ (μg/m^3)	
	1 year	24 hours	1 year	24 hours
WHO [2]	20	50	10	25
European Union	40	50	25	
United States	50	150	12	35
California	20	50	15	65
Japan		100	12	65
Brazil	50	150		
Mexico	50	120	15	65
South Africa	60	180	15	65
India (sensitive populations/ residential/industrial)	50/60/120			
China (Classes I/II/III)	40/100/150	50/150/250		35

UFPs are composed of both primary and secondary particulate matters [9]. The primary fraction is the one that is emitted directly from the emission sources and often includes agglomerate/aggregates of smaller particles [9]. Their size is generally in the range between 30 and 100 nm [10, 11]. The primary fraction is generally associated with diesel engines and automobiles and biomass combustion which are thought to initially have been emitted at around the 30 nm diameter size (the so called nucleation mode) and later coagulate into the larger fraction of the ultrafine mode. The secondary fraction is composed of particulate matter formed in the atmosphere and includes sulfuric acid and sulfates and organic reaction products of low volatility [9]. Photoreactions of oxides of nitrogen (NOx) and sulfur dioxide (SO_2) are involved in this process; both of which are products of combustion. This fraction size is generally in the range between 100 and 200 nm, which is partially distinguishable from other directly anthropogenic sources.

The role of composition on toxic effects has been explored during the last decade by different authors. The dogma during the 1990's was that the size of the particle was the predominant factor of toxicity, the smaller particles being the more toxic [12]. Nevertheless, during the last 15 years, evidence has linked surface area, reactivity, and different components of the particles with their toxicity [13–15]. The first efforts were done by collecting samples from associated to different sources such as indoor and outdoor [16], different cities [17], or regions within a large city [18] and comparing their *in vitro* toxic effects. In some cases, partial chemical characterizations or determinations of the presence of some components were empirically related with differences in the intensity of toxic effects [19–21]. Later on, comprehensive characterizations were correlated using different statistical models [13–15]. Currently, the characterization of size, physicochemical, and chemical composition is necessary to understand the toxicology of particles. For instance, for nanoparticles (NP), the determination of particle size, the dynamics of agglomeration and aggregation, the area, and the charge are mandatory for any toxicological evaluation [22]. In the field of urban particles, considering that they are complex mixtures, there are no standard measurements of physicochemical and chemical components, but the determination of total carbon, black carbon, transitional metals, nitrates, sulfates, oxidative potential, and polycyclic aromatic hydrocarbons is among the most evaluated components [23, 24]. A recent report of a meta-analysis and multisite time series evaluating elemental carbon, organic carbon matter, sulfate, and nitrate on PM$_{2.5}$ and in its relation to hospital admissions demonstrates that changes in elemental carbon content are associated with cardiovascular hospital admissions [25]. The authors conclude that a stronger communication between risk assessors and epidemiologist would help to better understand the role of the components of air pollutants on population effects.

Among the many components that are present in PM, the biological components seem to play a central role in the biological effects. There is increasing evidence that when PM is inhaled the biological component is responsible for stimulating alveolar macrophages and respiratory epithelial tissue to release proinflammatory cytokines and chemokines. The biological components may also have synergetic effects with other components of the PM, such as diesel exhaust enhancing IgE production and thus facilitating allergic sensitization [26].

These biological components may be released by passive or active mechanisms from plants, soil, biofilms, solid, or liquid sources to become suspended in the air. The measurement of protein associated with PM is considered as a general indicator of how much of the PM comes from a biological source. It represents about 1–4% of the total mass of PM$_{10}$ for urban and rural areas [27, 28].

Airborne biological particles or dust containing biological agents and/or substances of biological origin are important components of the coarse and fine PM. These components are represented by different types of primary or secondary (fragmented biological cells) biological aerosols [29]. The biological matter is predominantly comprised by plant pollen, spores, and microorganisms (mold and bacteria) or microbial metabolites [30, 31] and is related to allergic, toxic, and infectious responses in exposed individuals.

PM may be an efficient carrier of secondary allergens or proinflammatory compounds [32–34]. Recently, good correlation has been found for major allergens, mainly from pollen, and asthmatic patients. In fact, pollen from grasses, weeds and trees, among others were found onto different size of particles [35]. Most of the primary allergens (intact pollen, 10–100 μm) cannot reach the small airways; however, the secondary pollen allergens present in PM$_{2.5}$ can easily penetrate there [36].

Endotoxin lipopolysaccharides (LPSs) are other proinflammatory compounds from microbial origin present in PM. Endotoxin is a component of the cell wall of gram-negative bacteria, and its main source is debris deposited on urban or rural soil. When the LPS is resuspended and inhaled, it stimulates alveolar macrophages and respiratory epithelial

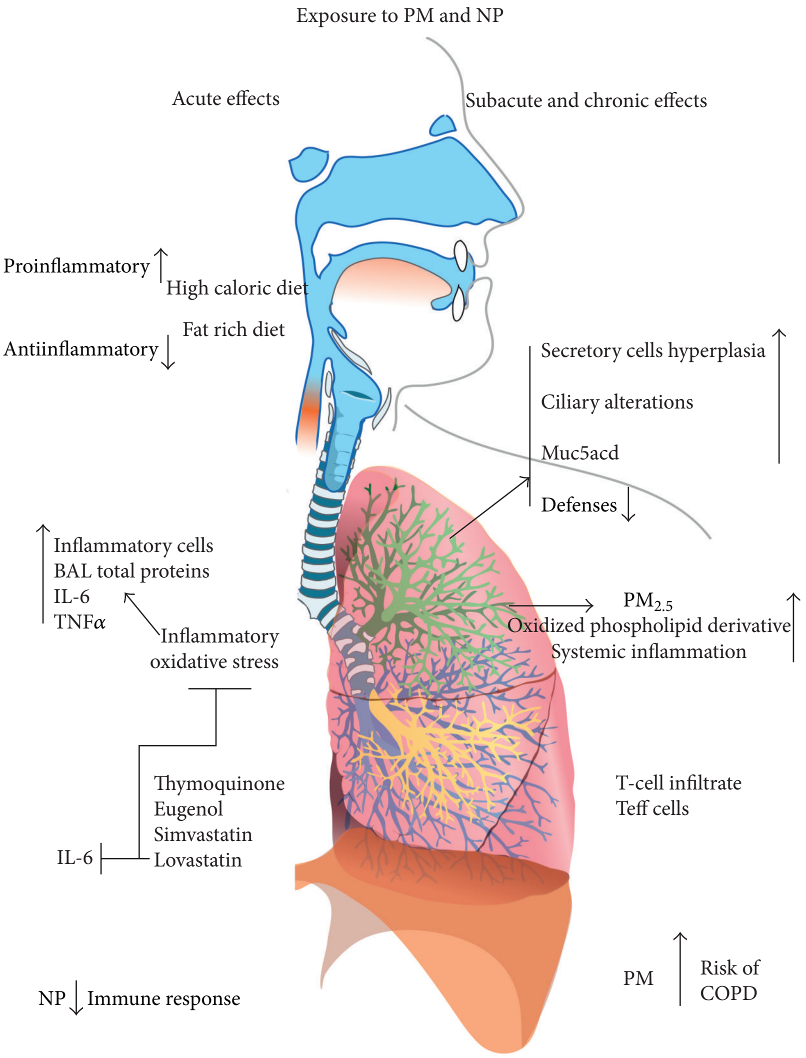

Figure 2: Schematization of the main acute, subacute, and chronic effects induced by inhaled particles and nanoparticles. In the acute side it is interesting to highlight that a high-caloric- and fat-rich diet provides a scenario facilitating proinflammatory effects of particles. Also, Tnymoquinone, eugenol, simstatin, and lovastin have a protective effect *in vivo*. In the subacute and chronic side, it is interesting to highlight the presence of tissue alterations, lung infiltration by T cells, and increases in the risk of COPD.

tissue to release cytokines/chemokines, initiating an inflammatory cascade [37, 38]. Another biological component with similar effects is the β-1,3-Glucan, a glucose polymer which is structural component of most fungal cell walls. β-1,3-Glucan has been used as an indicator of the presence of mold [39, 40].

3. *In Vivo* Studies

The main target for inhaled particles is the respiratory system, but there is strong evidence that systemic effects can be induced. We are presenting some of the most relevant studies regarding the local and systemic effects induced by inhaled particles. In Figure 2, we summarize some of the most relevant acute, subacute, and chronic effects induced *in vivo* by particles.

3.1. Respiratory Effect of Particles

3.1.1. Acute Effects. Several studies have investigated the respiratory effects of particulate air pollution and nanoparticles. While most of the studies have focused on the respiratory effects following inhalation, intratracheal or intranasal instillation, others have investigated the effects of intravenous, intraperitoneal, or oral administration.

It is well established that pulmonary exposure to particulate air pollution causes inflammation and oxidative stress [41–43]. It has been demonstrated that acute (within 24 h), single-dose intratracheal instillation of diesel exhaust particles (15–30 μg/mouse), a relevant type of $PM_{2.5}$, causes lung inflammation characterized by influx of inflammatory cells, increases total proteins, a marker of epithelial permeability, and oxidative stress [41, 42]. The release of interleukin-6 (IL-6) was found to increase in bronchoalveolar lavage (BAL) fluid at 18 h but not at 4 or 24 h [42]. Similarly, at 18 h time point, airway resistance to methacholine measured invasively using forced oscillation technique increased significantly and dose-dependently following exposure to DEP [42]. Pretreatment with thymoquinone, a constituent of *Nigella sativa*, ameliorated DEP-induced pulmonary effects [42].

Acute exposure (24 h) of healthy mice by intranasal instillation to $PM_{2.5}$ (5 or 15 μg/mouse) collected from the urban area of Sao Paulo caused lung inflammation and oxidative stress and worsened lung impedance in dose-dependent pattern [44]. The same research group has more recently reported that pretreatment of mice with eugenol, a methoxyphenol component of clove oil with anti-inflammatory and antioxidant properties, prevented the changes in lung mechanics, pulmonary inflammation, and alveolar collapse elicited by acute exposure to DEP [45].

The statins are hydroxy-methyl-glutaryl-CoA reductase inhibitors, broadly used in the treatment of hyperlipidemia. They play a key role in the primary and secondary prevention of atherosclerotic heart disease and stroke. Moreover, they have been reported to have potential benefits for a variety of other cardiovascular and noncardiovascular diseases, including cancer, respiratory and neurological disorders [46, 47]. Interestingly, Ferraro and coworkers [48] reported that residual oil fly ash (ROFA) and Urban Air Particle (UAP) from Buenos Aires produced an acute pulmonary injury in mice, characterized by a neutrophilic inflammation, a rise in O_2^- generation, and production of the proinflammatory tumour necrosis alpha (TNFα) cytokine. Simvastatin pretreatment had no significant effect per se on any of these biomarkers but prevented the pulmonary cytotoxicity and inflammation induced by ROFA and UAP. More recently, Miyata and coworkers [49] found that pulmonary exposure to PM_{10} in rabbits accelerated the turnover of polymorphonuclear leukocytes (PMNs) by shortening their transit time through the marrow. Interestingly, they found that lovastatin dampens these systemic responses by decreasing the levels of PM_{10}-induced circulating mediators (IL-6), thereby suppressing the bone marrow stimulation. The effect of statins was predominant on PMNs in the postmitotic pool as evident by the use of $5'$-bromo-$2'$-deoxyuridine (BrdU/G3). Interestingly, statins also reduced the retention of these newly released PMNs in the lung tissues. These results corroborate the previous finding from the same research group reporting that PM_{10} particles induced systemic inflammatory responses characterized by an increase in systemic proinflammatory mediators such as IL-6 [50].

The metabolism of L-arginine plays an important homeostatic role in the airways, through synthesis of the bronchodilating molecule, nitric oxide (NO), from L-arginine, by the nitric oxide synthase (NOS) isozymes. The arginase isozymes (arginases 1 and 2) convert L-arginine into L-ornithine and urea and thus compete with the NOS isozymes for substrate. Arginase overexpression contributes to airways hyperresponsiveness in asthma, and its expression is further augmented in cigarette smoking asthmatics [51]. It has been recently reported that arginase is upregulated following exposure to O_3 and concentrated ambient particles in murine models of asthma and contributes to the pollution-induced exacerbation of airways responsiveness [52].

The question, whether a diet challenge increases the inflammatory response in the alveolar and the blood compartment in response to carbon nanoparticles (CNP) was investigated by Götz and coworkers [53]. In their study, mice were fed a high-caloric carbohydrate-rich or a fat-rich diet for six weeks and were compared to mice kept on a purified low fat diet, respectively. Bronchoalveolar lavage (BAL) and blood samples were taken 24 h after intratracheal CNP instillation and checked for cellular and molecular markers of inflammation. The authors reported an increase in BAL proinflammatory factors in high-caloric groups and reductions in serum concentrations of anti-inflammatory factors in fat-rich group. They concluded that extended feeding periods, leading to manifest obesity, are necessary to generate an increased susceptibility to particle-induced lung inflammation, although the diet challenge already was efficient in driving proinflammatory systemic events.

Barlow and coworkers [54] assessed the effects of intratracheally instilled PM_{10} collected from London on macrophage clearance in rats *ex vivo*. These authors concluded that acute PM_{10} exposure has an effect on macrophage phagocytosis and chemotaxis that may be deleterious to particle clearance within the alveolar region of the lung. The decrease in chemotactic ability may represent one mechanism that promotes inflammation after increases in ambient PM levels. They also concluded that further investigation is warranted to determine the effects of chronic PM_{10} exposure on macrophage clearance mechanisms.

NPs induce inflammatory responses and oxidative stress but may also have immune-suppressive effects, impairing macrophage function and altering epithelial barrier functions. The question related to whether exposure to NP may increase the risk of pulmonary infection has been recently investigated [55]. It has been demonstrated that Cu NP exposure impaired host defense against bacterial lung infections and induced a dose-dependent decrease in bacterial clearance [55]. Moreover, it has been demonstrated that acute exposure to DEP by inhalation exacerbates lung inflammation induced by lipopolysaccharide [56].

In an interesting study, the impact of pulmonary exposure to carbon black NP on emphysematous lung injury induced by porcine pancreatic elastase (PPE) was investigated in mice [57]. It has been demonstrated that carbon black NP exacerbates PPE-induced pulmonary inflammation and emphysema. This enhancement may be mediated, at least partly, via the increased local expression of proinflammatory molecules.

TiO_2 nanoparticles have several industrial applications, and, as such, also have different sizes, shapes, chemistry, and crystalline structures [58, 59]. TiO_2 occurs in four crystalline polymorphs of which rutile and anatase are most common [60]. Rutile is considered as a more inert form, whereas anatase is an active form of TiO_2. Anatase and rutile TiO_2 particles, delivered at similar surface area doses, increased release of lactate dehydrogenase, interleukin-8, and reactive oxygen species, as well as depressed mitochondrial activity in dissimilar patterns in cultured human epithelial cells [61].

In vivo, it was observed that ultrafine anatase TiO_2 particles produced increases in bronchoalveolar lavage inflammatory indicators, cell proliferation, and histopathology compared to ultrafine rutile TiO_2 particles [62]. However, with both crystalline forms of TiO_2 particles, pulmonary effects were observed at 24 h and resolved by one week after exposure [62]. Furthermore, it has also been demonstrated that the intratracheal instillation of rutile TiO_2 nanorods caused upregulation of lung and systemic inflammation and triggered platelet aggregation [63].

TiO_2-based photocatalysis has attracted extensive interest because of its great advantages in the complete mineralization of organic pollutants in waste water and air [64, 65]. As a popular photocatalyst, TiO_2 has been widely studied because of its various merits, such as optical-electronic properties, low cost, and chemical stability. Characteristics of TiO_2 nanoparticles can be modified by several methods to improve their performance. In this context, TiO_2 nanorods are doped with iron in order to increase their photocatalytic activity [63, 64]. It has been recently demonstrated that exposure to SiO_2-coated rutile TiO_2 nanoparticles ($cnTiO_2$) caused pulmonary neutrophilia, increased expression of tumor necrosis factor-alpha (TNFα) and neutrophil-attracting chemokine CXCL1 in the lung tissue. Uncoated rutile and anatase as well as nanosized SiO_2 did not induce significant inflammation [66]. More recently, pulmonary exposure to well-characterized rutile Fe-TiO_2 promotes pulmonary and systemic inflammation and oxidative stress. It also enhances thrombotic potential, heart rate, and systolic blood pressure (SBP) and induces hepatotoxicity. Moreover, rutile Fe-TiO_2 showed direct toxicity on human lung cancer cells NCI-H460-Luc2 and human hepatoma cells HepG2 [67].

3.1.2. Subacute and Chronic Effects. It has been demonstrated that rats exposed for 6 months to urban air pollution developed secretory cell hyperplasia in the airways and ultrastructural ciliary alterations of the epithelium of the airways, suggesting that chronic exposure to urban levels of air pollution may cause respiratory alterations [68]. Moreover, rats submitted to prolonged exposure to low levels of air pollution experienced deteriorated respiratory defenses against infectious agents [69]. Recently, it has been reported that intranasal instillation of DEP over 60 days increased the expression of Muc5ac in the lungs and the acid mucus content in the nose compared with the 30-day treatment. Moreover, DEP exposure enhanced the total leukocytes in the BAL and the nasal epithelium thickness compared to saline-treated control group [70].

Chronic exposure to $PM_{2.5}$ resulted in prominent inflammatory responses in the lung typified by increased levels of oxidized phospholipid derivatives as well as a systemic inflammatory response [71]. In a subsequent study, the same group has extended some of their observations and reported that exposure to $PM_{2.5}$ resulted in increased T-cell infiltration and increased activation of T-effector cells (evidenced by an increase in $CD4^+CD44^+CD62L^-$ and $CXCR3^+$ T cells in the lungs) and suggests a phenotypic switch to a Th1/Th17 phenotype in lung Teff cells. These results have important implications for how $PM_{2.5}$ may detrimentally modulate pulmonary and systemic immune responses [72].

Chronic obstructive pulmonary disease (COPD) is characterized by not fully reversible airflow obstruction that is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. The major etiological factor for COPD is chronic oxidative stress as a result of long-term smoking, use of biomass fuels, and air pollution exposure [73]. Lopes and coworkers recently reported a study in which the effects of chronic exposure (2 months) to ambient levels of PM on development of protease (papain) induced emphysema and pulmonary remodeling were investigated in mice [74]. They found that mean linear intercept and the total amount of collagen fibers in parenchyma were significantly greater in the lungs of mice that were treated with papain and exposed to ambient particles compared to those mice treated with papain and exposed to filtered air for 2 months. These increases in destruction of lung parenchyma and in lung collagen content observed only in the group of mice treated with papain and exposed to ambient particles were associated with an increase in the amount of 8-isoprostane expression in lung tissue, suggesting that the increase in oxidative stress is a possible mechanism to explain these alterations [74].

Different types of NPs can cause various inflammatory reactions in the lung. In mice lungs, the toxicity of single-wall carbon nanotube (SWCNT) in causing epithelioid granulomas and interstitial inflammation 7 and 90 days after intratracheal instillation has been shown to be greater as compared with other NPs, like carbon black or quartz particles [75]. However, the significance of the SWCNT-induced inflammation has been a matter of scientific debate. Initially it has been reported that intratracheally instilled SWCNT in rats causes discrete granulomas that were not dose-responsive, and an absence of signs of inflammation in BAL suggested the possibility that large agglomerates of SWCNT caused the granulomas [76]. A second study in rats, using SWCNT aspiration, also reported slight change in the differentials of BAL and a relative lack of histopathologic evidence of inflammation [77]. Studies in mice demonstrated significant inflammation, confirmed that SWCNT-induced inflammation was often granulomatous, and, most importantly, demonstrated that inflammation was present whether the SWCNTs were inhaled or aspirated [75, 78]. It was concluded that SWCNT inhalation was more effective than aspiration in causing inflammatory response, oxidative stress, collagen deposition, and fibrosis as well as mutations of K-ras gene locus in the lung of C57BL/6 mice [79]. Similarly to SWCNT, multiple wall carbon nanotube (MWCNT) exposures by inhalation at concentrations of 5 mg/m^3 or less for 14 days produced slight evidence of pulmonary inflammation but suppressed T-cell-dependent immune functions [80]. However, the intratracheal instillation of shorter MWCNT failed to show the occurrence of inflammation or fibrosis [81]. Recently, it has been demonstrated that inhalation of MWCNTs for up to 13 weeks caused granulomatous inflammation and pleural thickening at exposure concentrations greater than 6 mg/m^3. However, influx of inflammatory cells in BAL fluid and interstitial fibrosis wereas demonstrated at exposures above 0.4 mg/m^3 [82].

Pulmonary inflammation caused by NP may also result in changes in membrane permeability, which in turn can result in particle translocation beyond the lung and affecting cardiovascular system [83]. Indeed, it has been shown that NPs have the potential to enter the brain [84] and blood circulation [85, 86] and subsequently other major organs causing inflammation and oxidative stress in these organs.

3.2. Extrapulmonary Effects of Particles

3.2.1. Possible Mechanisms Involved. Despite the consistency of the epidemiologic observations, the pathophysiological mechanisms linking air pollution to adverse cardiovascular events remain unclear. There are three primary hypotheses that are being investigated to explain the extrapulmonary effect of NP [87, 88], and in Figure 3, we summarize the main mechanisms proposed for the systemic effects. The first one relates the effect of particles to their ability to impact the autonomic nervous system. Studies showed that particulate air pollution exposure is associated with rapid changes in autonomic nervous system balance, favoring sympathetic nervous system activation and parasympathetic withdrawal leading to changes in the pattern of breathing, heart rate, and heart rate variability. Decreased heart rate variability indicates the existence of a state of cardiac autonomy dysfunction and is a risk factor for sudden cardiac death because of arrhythmias [89]. The mechanisms responsible for the increase of the sympathetic drive remain unclear but may involve activations of pulmonary neural reflex arcs and direct effects of pollutants on cardiac ion channels [89]. Inhaled particles may affect the cardiovascular system through inflammatory mediators produced in the lungs and released into the circulation [87, 88]. It has been suggested that inhaled particles may lead to systemic inflammatory response through the release of IL-6, TNFα or histamine, and oxidative stress within the lungs and/or systemically [87, 88].

Moreover, several studies have shown that nanoparticles, owing to their small size, could avoid normal phagocytic defenses in the respiratory system and gain access to the systemic circulation and therefore to different extrapulmonary sites [85, 86, 90–93]. The UFP can pass from the lungs into the blood circulation in hamsters [86]. Others [91–94] have also reported extrapulmonary translocation of UFPs after intratracheal instillation or inhalation in other animal species. However, the amount of UFPs that translocated into blood and extrapulmonary organs differed amongst these studies. It has also been shown that, following intranasal delivery, polystyrene microparticles (1.1 μm) can translocate to tissues in the systemic compartment [95]. Recent studies [96–98] have provided morphological data illustrating that inhaled particles are transported into the pulmonary capillary space, presumably by transcytosis. Recently, Elder et al. [91] demonstrated that the olfactory neuronal pathway represents a significant exposure route of central nervous system (CNS) tissue to inhaled UFPs. These authors showed that, in rats, which are obligatory nose breathers, translocation of inhaled nanosized particles along neurons is a more efficient pathway to the CNS than via the blood circulation across the blood-brain barrier. They speculated that given that this neuronal translocation pathway was also demonstrated in nonhuman primates, it is likely to be operative in humans as well [84, 91]. In humans, the literature on the translocation of UFPs from the lungs into the blood circulation is still conflicting [85, 99, 100]. However, given the deep penetration of nanoparticulate matter into the alveoli and close apposition of the alveolar wall and capillary network, such particle translocation seems plausible either as a naked particle or after ingestion by alveolar macrophages [98]. Naked particles have been reported to be taken up (and/or adsorbed) by erythrocytes [101] and can presumably be distributed to various organs. The distribution of radiolabelled ultrafine carbon particles, commonly known as "Technegas", has been investigated after their inhalation by nonsmoking healthy human volunteers [85]. The size of the individualized particles was in the order of 5 to 10 nm, as we confirmed by electron microscopy of the particles. Radioactivity, which was largely particle-bound, as assessed by thin layer chromatography, was detected in blood already after 1 minute, reaching a maximum between 10 and 20 min-, and remaining at this level up to 60 min. Gamma camera images showed substantial radioactivity over the liver and other areas of the body. The presence of radioactivity in the liver is compatible with an accumulation of particles in Kupffer cells, as is known to occur with colloidal particles [102]. More recently, Péry and coworkers [103] developed a physiologically based kinetic model for (99 m) technetium-labelled carbon nanoparticles (Technegas). The model was designed to analyze imaging data obtained from the study of Nemmar and coworkers [85]. It included different translocation rates and kinetics for free technetium and small and large technetium-labelled particles. The authors concluded that the percentage of small particles able to translocate was estimated at 12.7% of total particles, whereas the percentage of unbound technetium was estimated at 6.7% of total technetium [103].

Nurkiewicz and coworkers have studied the effects of inhaled particles and nanoparticles on systemic microvascular endothelium. First, they demonstrated that rats exposed to ROFA or TiO_2 presented a reduction in their capacity to respond to the Ca^{2+} ionophore A23187, which induce arteriolar dilatation [104]. In other studies, the same group has shown that exposure to ROFA or TiO_2 NP, by instillation or inhalation, induce systemic microvascular dysfunction [105, 106]. They also found that the nitric oxide (NO) signaling seems to be involved in the endothelial systemic effects of the particles [107].

3.2.2. Acute Effects. Several studies demonstrated that exposure to UFP or DEP caused pulmonary inflammation and prothrombotic events in ear vein of rats or femoral vein and artery of hamsters [108–112]. Mutlu and coworkers [113], showed that exposure to PM triggers IL-6 production by alveolar macrophages, resulting in reduced clotting times, intravascular thrombin formation, and accelerated carotid artery thrombosis [113]. The occurrence of oxidative stress in

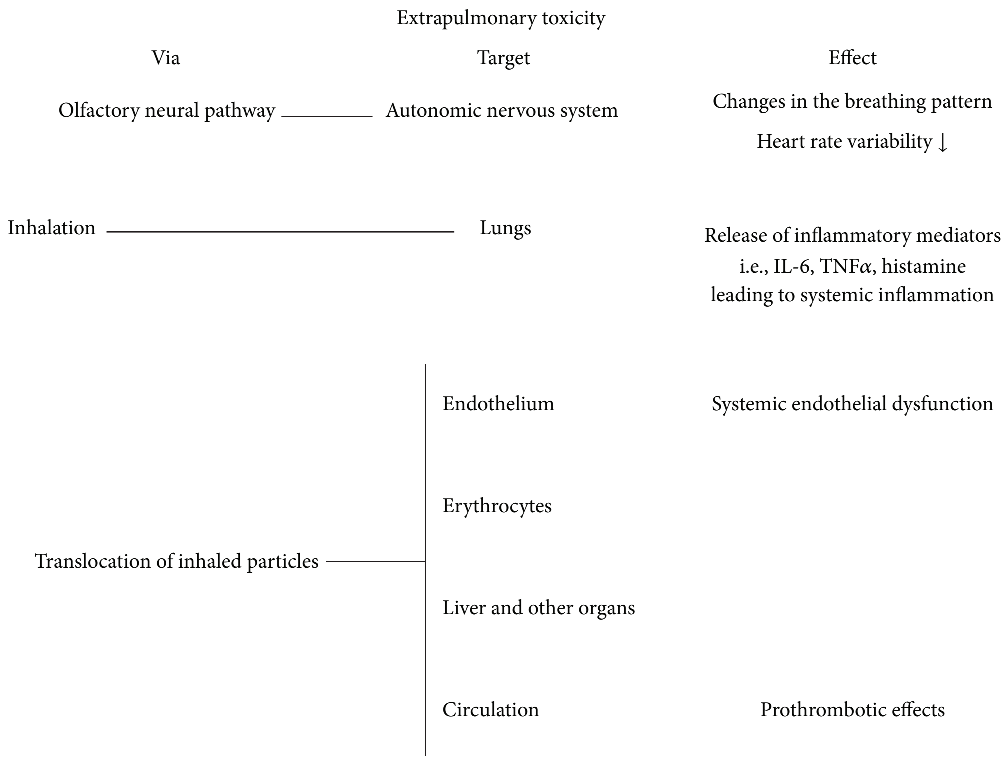

Figure 3: Summary of the main systemic effects associated with particle exposure and the possible mechanisms related to those effects.

the DEP-induced acute thrombotic tendency in pial cerebral venules, activation of circulating blood platelets, and lung inflammation have been reported in mice [25]. Moreover, the same authors showed that the antioxidant pretreatment with cysteine prodrug L-2-oxothiazolidine-4-carboxylic acid prevented DEP-induced inflammatory and the resulting thrombotic complications [25]. More recently, the acute (4 and 18 h) effects of DEP on pulmonary and cardiovascular parameters and the protective effect of thymoquinone were investigated in mice [41]. Four h after DEP administration, there were no significant changes in the cells in BAL, lung histology, or pulmonary function test. However, at 18 h after exposure, both lung inflammation and pulmonary function were significantly affected. Conversely, at both 4 h and 18 h, DEP caused systemic inflammation characterized by leukocytosis, increased IL-6 concentration, and reduced SBP. DEP reduced platelets number and aggravated pial arteriole thrombosis. The addition of DEP (0.1–1 μg/mL) to untreated blood induced platelet aggregation. The cardiovascular effects observed at 4 h after DEP exposure did not appear to result from pulmonary inflammation but possibly from the blood translocation of DEP and/or their associated components [41]. However, at 18 h, DEP-induced significant changes in pulmonary and cardiovascular functions and caused lung inflammation. Pretreatment with thymoquinone effectively prevented DEP-induced cardiorespiratory toxicity [41].

It has been reported that TNFα is a strong agonist for plasminogen activator inhibitor 1 (PAI-1) expression and has been found to play an important role in PAI-1 regulation in a variety of diseases. In a mouse endotoxemia model, TNFα has been found to contribute to the lipopolysaccharide-induced PAI-1 expression [114]. Budinger et al. [115] demonstrated that ambient PM-induced upregulation of PAI-1 disappeared upon treatment of mice with a TNFα inhibitor [115]. In line with the later findings, it has been recently demonstrated that repeated exposure to DEP-induced airway inflammation and hyper-reactivity, systemic inflammation, increased SBP, and accelerated coagulation. TNFα production was increased both in BAL and plasma. Pretreatment with curcumin significantly inhibited the release of TNFα and prevented the respiratory and cardiovascular effects of DEP [116].

An important aspect of the epidemiological associations between air pollution and either morbidity or mortality is that the acute adverse effects appear to be most marked in people with preexisting compromised cardiovascular function, such as hypertension or diabetes [89]. To give credibility for these observations, several experimental studies have been designed to test whether and to what extent the effects of particulate air pollution are aggravated, using an animal model of angiotensin II-induced hypertension. Indeed, exposure particulate matter with diameter $\leq 2.5\,\mu$m ($PM_{2.5}$) was found to potentiate angiotensin II-induced hypertension [117, 118]. In addition, $PM_{2.5}$ increased angiotensin II-induced cardiac hypertrophy, collagen deposition, and cardiac and vascular RhoA activation, suggesting that cardiovascular health effects are indeed the results of particulate air pollution exposure [118]. Evidence for exacerbation of thrombotic but not respiratory events was also reported in angiotensin II-induced hypertension in mice [119, 120]. With respect to diabetes mellitus, it has been shown that DEP equally increased airway resistance and caused infiltration of inflammatory cells in the lung of both diabetic and nondiabetic mice. However, the occurrence of oxidative stress, the presence of lung apoptotic cells, and the increase of total proteins, albumin and TNFα in BAL fluid were only seen in DEP-exposed diabetic mice suggesting an increased respiratory susceptibility to particulate air pollution [121]. Moreover, the same research group has shown that systemic and coagulation events are aggravated by diabetes in mice acutely exposed to DEP [122]. These authors stated that they may be relevant to the exacerbation of cardiovascular morbidity accompanying particulate air pollution in diabetic patients.

Novel evidence that pulmonary deposition of DEP potentiates the renal, systemic, and pulmonary effects of cisplatin-induced acute renal failure (ARF) has been recently reported by Nemmar and colleagues [123]. These findings highlight the importance of environmental factors such as particulate air pollution in aggravating ARF.

Several studies have showed that nanoparticles, owing to their small size, could avoid normal phagocytic defenses in the respiratory system and gain access to the systemic circulation and therefore to different extrapulmonary sites [83, 84, 90–93, 103, 123]. To specifically determine the effect of translocated particles, it has been recently demonstrated in both normotensive and spontaneously hypertensive rats that 24 h following their systemic administration, DEP affected blood pressure and caused pulmonary inflammation assessed by BAL [124, 125]. In a subsequent study in rats, it has been reported that i.v. administration of DEP (0.02 mg/kg) caused acute systemic effects mainly at 6 h and 18 h but not at 48 h or 168 h following particle exposure. While DEPs were found in lungs, heart, liver and kidneys, the histopathological changes were only seen in the lung. This implies that, at the dose and time-points investigated, DEP can cause inflammation in the lungs but not in other organs, suggesting that pulmonary tissue is the predominant site for inflammation based on the mode of delivery of DEP in this study [126]. Furthermore, it has been shown that ultrafine TiO_2 induces acute lung inflammation after i.p. administration and exhibits additive or synergistic effects with LPS, at least partly, via activation of oxidant-dependent inflammatory signaling and the NF-kappaB pathway, leading to increased production of proinflammatory mediators [127]. Geys et al. [128] have investigated the toxicity of quantum dots which have numerous possible applications for *in vivo* imaging. QDs caused marked vascular thrombosis in the pulmonary circulation, especially with carboxyl QDs. QDs were mainly found in lung, liver, and blood. Thrombotic complications were abolished, and P-selectin was not affected by pretreatment of the animals with heparin.

3.2.3. Subacute and Chronic Effect. Akinaga and coworkers [129] reported a study in which mice were continuously exposed, since birth, in two open-top chambers (filtered and nonfiltered for airborne particles $\leq 0.3\,\mu$m) placed 20 m from a street with heavy traffic in downtown Sao Paulo, 24 h per day for 4 months. They found that air pollution induced mild but significant vascular structural alterations in normal individuals, presented as coronary arteriolar fibrosis and elastosis.

PM has been shown to cause significant decreasing patterns of heart rate, body temperature, and physical activity in mice lacking apolipoprotein (ApoE$^{-/-}$) over 5 months of exposure to concentrated ambient PM, with smaller and nonsignificant change for C57 mice [130].

Sun and coworkers demonstrated that ApoE$^{-/-}$ mice exposed to concentrated regional northeastern $PM_{2.5}$ for 6 months (6 h/day for 5 day/week) in conjunction with high-fat chow potentiated plaque development markedly increased vascular inflammation (CD68$^+$ macrophage infiltration and inflammatory nitric oxide synthase (iNOS) expression) and vessel wall markers of oxidative stress [131]. Plaque progression was accompanied by alterations in vasomotor tone, including decreased endothelial-dependent vasodilatory function and heightened vasoconstriction to adrenergic stimuli. The same research group confirmed their findings by another set of experiments which was performed using an identical protocol of exposure but involving an apoE$^{-/-}$ model and a double-knockout (DK) model of ApoE$^{-/-}$ and low-density lipoprotein (LDL) receptor deficient mice (DK mice) to concentrated ambient $PM_{2.5}$ for 6 h a day for 5 days/week for up to 5 months. Although quantitative measurements showed that $PM_{2.5}$ exposure increased atherosclerotic lesions in the apoE$^{-/-}$ mice, changes produced by $PM_{2.5}$ in DK mice were not statistically significant [132]. In subsequent set of experiments, it has been shown that chronic ambient exposure to $PM_{2.5}$ increased tissue factor expression in macrophages and smooth muscle cells in atherosclerosis [133]. They also reported specific recruitment of monocytes into microcirculatory tissue niches (i.e., adipocytes) in response to long-term $PM_{2.5}$ exposure [134]. These experiments suggest a key role for $PM_{2.5}$ in the activation and mobilization of innate immune cell populations.

Long-term cardiovascular effects of inhaled nickel hydroxide NPs (nano-NH) in hyperlipidemic, ApoE$^{-/-}$ mice were investigated by Kang and coworkers [135]. Mice were exposed to nano-NH at either 0 or 79 μg Ni/m^3, via a whole-body inhalation system, for 5 h/day, 5 days/week, for either 1 week or 5 months. Inhaled nano-NH induced significant oxidative stress and inflammation in the pulmonary and extrapulmonary organs, indicated by upregulated mRNA levels of antioxidant enzyme and inflammatory cytokine genes; increased mitochondrial DNA damage in the aorta; significant signs of inflammation in BAL fluid; changes in lung histopathology; and induction of acute-phase response. In addition, after 5-month exposures, nano-NH exacerbated the progression of atherosclerosis in ApoE$^{-/-}$ mice [135].

Emmerechts and coworkers have investigated how continuous traffic-related air pollution exposure affects haemostasis parameters in young and old mice. Young (10 weeks) and old (20 months) mice were placed in an urban roadside tunnel or in a clean environment for 25 or 26 days. They found in old mice that subchronic exposure to polluted air raised platelet numbers, von Willebrand factor, soluble P-selectin, and microvesicles, collectively substantiating a further elevation of thrombogenicity, already high at old age [136].

There is a potential for neurodegenerative consequence of particle entry to the brain. Histological evidence of neurodegeneration has been reported in both canine and human brains exposed to high ambient PM levels, suggesting the potential for neurotoxic consequences of PM entry [137, 138]. PM-mediated damage may be caused by the oxidative stress pathway which can enhance the susceptibility for neurodegenerative diseases. The relationship between PM exposure and central nervous system degeneration can also be detected under controlled experimental conditions [137, 138]. Morphometric analysis of the central nervous system of ApoE$^{-/-}$ mice exposed to concentrated ambient air pollution showed that the brain is a critical target for particulate air pollution exposure and implicated oxidative stress as affecting factor that links PM exposure and susceptibility to neurodegeneration [137, 138]. Further experimental studies are needed to clarify the effect and mechanisms underlying the neurotoxicity of particulate air pollution.

4. *In Vitro* Studies

In vivo models give a good insight of the toxic effects of particles, and considering the multiple interactions of different cell types in the lung, the complex responses are well documented, but the cellular mechanisms related to the specific responses become very difficult to clarify. In this regard, the *in vitro* models are used as a main tool to evaluate the cellular mechanisms related to the exposure to particles.

There are several approaches to evaluate the toxic effects of particles on cells that have been suggested or pointed as targets of PM and NP. Single cell cultures, cocultures, multiple cocultures, exposure under submerged conditions, and exposure under air-liquid interface are among the main strategies. We are discussing some of the most significant advances on the evaluation of PM *in vitro* toxicology. In Table 2, we summarize the most relevant *in vitro* evidence supporting the observed *in vivo* effects.

4.1. Particle Properties Linked to Primary Cell Interaction. In the lung, the particles may interact with the lung lining fluid and the epithelial cells. In addition the particles may be taken up by macrophages and other immune cells by phagocytosis or pinocytosis. The interaction of particles with the cellular plasma membrane and its receptors and ion channels may directly trigger a biological response. The important DEP-induced reactions often start from constituents leaking from the particles including metals and various PAHs, including derivatives like nitro-PAHs and various oxo-PAH (quinones). The relative position of such components on the particle is most likely of importance since just adding back extracted components may result in less effects than the native particle exerts [139]. Furthermore, the combination of particle constituents like endotoxins and chemicals in organic fraction may elicit more than additive cytokine response effects [15]. On the other hand, with regard to genotoxic effect, the response will be higher in the extracts as more of the carcinogenic PAH will be available to the cells [140, 141].

Table 2: *In vitro* evidence that supports and provides plausible mechanisms for the *in vivo* observed effects induced by PM and NP.

In vitro evidence supporting the observed *in vivo* effects	
In vivo observed effect	*In vitro* evidence
Oxidative stress	ROS increases via NADPH-oxidase in lung epithelial cell exposed to PM.
Local and systemic inflammation	Secretion of IL-1b, IL-6, IL-8, TNFa, MCP-1, and so forth, by lung cells, macrophages, and cocultures.
Hyperplasia	Proliferative stimuli induced by extracts of DEP components.
COPD	Increased cytotoxicity on exposed cell cultures.
Systemic and endothelial dysfunction	Endothelial cell activation by direct contact with particles or indirectly induced in cocultures where pneumocytes, macrophages, and other cell are exposed.
Particle translocation	Changes in the TEER values related to tight junctions Macrophage-dendritic transepithelial cells network alterations in the GJIC.

Although particle uptake in epithelial cells has also been reported to occur [142], most biological responses triggered by particles in these cells do not seem to depend on particle uptake [143]. Particles as such have been reported to trigger biological effects via acellular reactive oxygen species (ROS) formation. However, DEP-induced immune responses in A549 cells were reported to depend on activation of cellular ROS-formation via the NADPH oxidase [144]. Furthermore, emerging evidence suggests that particle constituents are able to bind to or otherwise activate various membrane and cytosolic receptors. Obviously, both AhR-ligand binding as well as reactive electrophilic PAH metabolites covalently binding to DNA are caused by chemical constituents released from the particle [143, 145].

4.2. In Vitro Studies with Implications to Various PM-Induced Cardiovascular Effects and Various Lung Diseases Including Cancer. As we have seen in previous sections, damage of the lung epithelial lining may have important implications with regard to pathogen diseases, asthma, and allergy. Direct or indirect induced chronic inflammation is also considered to be central element in various cardiovascular diseases, COPD, and a likely part of cancer development.

Regarding the latter, there is growing evidence suggesting that air pollution exposure increases risk of lung cancer [146, 147]. The components generally considered being of most interest for such effects are particles in the ultrafine ($PM_{0.1}$) and fine fraction ($PM_{2.5}$) including DEP and wood smoke particles (WSP) [148]. However, more recent *in vitro* evidence indicates that also the larger PM_{10} particles might play a role in cancer development through mechanisms such as damage to the lung epithelial cells, disruption of tight junction and gap junction, effects of cell proliferation including cytotoxicity, release of inflammatory mediators like chemokines and cytokines, changes in gene expression via receptor binding, and various forms of cellular DNA damage, including epigenetic changes. It is also possible to study *in vitro* effects of particle exposure on the later stages of cancer development like chromosomal instability and cell migration, which are important parts of tumor promotion and metastasis. However, we are not aware of that such studies have been published.

4.2.1. Tight Junction.

4.2.1. Tight Junction. Tight junctions between the epithelial cells represent an important barrier for the body protecting the rest of tissue and organs from exposure to various pathogenic intruders like virus, bacteria, fungi, air pollution PM, and various particle-bound allergens. Exposure to such components can result in infections and allergic/asthmatic reactions. If combined with PM exposure, the end result may be more chronic inflammatory reactions, which is considered to be an important part of many pulmonary diseases including COPD and cancer development. Geys and coworkers showed that the transepithelial electrical resistance (TEER) is linked to the tight junctions and the correlation between the TEER value and the translocation of particles through cellular monolayers [149]. Using an *in vitro* triple cell culture model consisting of human epithelial cells (16HBE14), monocyte-derived macrophages and dendritic cells, it was recently demonstrated that macrophages, and dendritic cells create a transepithelial network between epithelial cells to capture antigens without disrupting the epithelial tightness [150]. Using a similar model, Lehmann and coworkers [151] observed that a high concentration of DEP (NIST 2975, 125 μg/mL) can modulate the tight junction occluding mRNA in the cells of the epithelial defense system. In this connection, it is also interesting to note that NIST 2975 DEPs have been reported to increase the release of metalloproteinase MMP-1 from human lung epithelial cells (A549 and NCI-H292). MMP-1 is involved in the degradation of collagen and can thus damage the lung epithelial barrier [144]. These findings suggest that DEP can contribute to structural changes in the epithelial lining with inflammatory and possible carcinogenic implications.

4.2.2. Gap Junction Intercellular Communication (GJIC). GJIC is one way of intercellular exchange of low molecular weight molecules between adjacent cells. Chemically induced alterations in this type of communication have been found to result in abnormal cell growth and behavior and is considered to be an interesting assay for *in vitro* studies of chemicals that may act as tumor promoters [152]. Bay/bay-like regions of PAH have been reported to be potent inhibitors of GJIC [153]. Interestingly several high molecular weight PAHs with known strong carcinogenic properties possessed only weak (dibenzopyrenes) or no inhibition potency (dibenzofluoranthenes, naphtho[2,3-a]pyrene, and benzo[a]perylene) [154]. Furthermore, the PAH-induced inhibition of GJIC occurs in the absence of PAH metabolism and aryl hydrocarbon receptor (AhR) binding [155]. More probably, unmetabolized PAH changes GJIC through direct interaction with unknown factor(s) in the cellular membrane. In line with this, DEP has been reported to inhibit GJIC [156–158]. The GJIC-effects of a fractionated DEP extract were found to be due to components in the polar fraction, while the less polar nitro-PAH fraction showed the strongest mutagenic potential (Ames test) [158].

4.2.3. Cell Proliferation and Cytotoxicity. Measuring cellular proliferation and cytotoxicity has been used as one of the primary toxicity tests for particulate matter [15, 16, 159]. With relatively simple methods, differences in the intensity of cytotoxicity have been demonstrated. Equal masses of urban PM collected in different cities, or within a large city, associated with different sources presented differences in cellular proliferation and cytotoxicity [16, 17]. These results were of main interest to evaluate the role of different components of the toxic effects of particles and therefore identifying components such as endotoxin, organic carbon, and some elements, as the components associated to the cytotoxicity [14, 16, 17].

Increased cytotoxicity is often followed by proliferative stimuli considered to be of great importance for both fixation of the primarily DNA lesion as well as for tumor promotion phase. A number of compounds in DEP are cytotoxic; other compounds are known to be DNA damaged thus resulting in G1-arrest and/or accumulation in S-phase due to reduced DNA synthesis [160, 161]. However, DEPs also include compounds which may affect cell proliferation in other ways. Two nitrophenols isolated from DEP 3-methyl-4-nitrophenol (4-nitro-m-cresol, PNMC) and 4-nitro-3-phenylphenol (PNMPP) have been reported to have estrogenic and antiandrogenic activities. Most interestingly, proliferation of MCF-7 cells was stimulated by PNMC, PNMPP, and estradiol-17beta and the antiestrogens 4-hydroxytamoxifen and ICI 182,780 inhibited the proliferation [162]. Crude extract of DEP exhibited both estrogenic and antiestrogenic activities. Estrogenic activity of crude extract and some fractions was induced through estrogen-receptor- (ER-) mediated pathways. In particular, the acid polar fraction of DEPs, which contains phenols, induced high levels of estrogenic activity compared to other fractions [163].

An important part of the known carcinogens found on air pollution particles is various PAHs. Some of these have also been reported to have mitogenic potency. More specifically, weak mitogenic effects have been reported, suggested to occur via increased Ca^{2+}, activation of epidermal growth factor receptor (EGFR) and insulin receptor [164–167]. Furthermore, disruptions of contact inhibition via AhR-dependent induction of JUN-D/cyclin [168] have been observed. This type of effect obviously would also result in increased cell proliferation. Most interestingly, it is known for a while that several of these have so-called "stealth properties" [169–171]. This is a property by which reactive metabolites are able to covalently bind to the DNA without easily being detected by the cells defense system. More specifically, some reactive PAH metabolites bind to DNA without triggering a G1-arrest. An increase in p53 seems to be induced but not a p21waf1/cip1-inhibition of p53 transcriptional activity. Furthermore, some PAH seems to induce mdm2 which may reduce the p53 activation [172, 173]. AhR-dependent inhibition of E2F1-dependent apoptosis [174] reduced p53 nuclear translocation, stimulation of cell survival signals, and inhibition of DNA damage induced apoptosis have been reported after exposure to certain PAH [175, 176]. Most importantly, such chemicals would change the balance between cell death and cell survival and cell proliferation following a DNA damaging event. If not compensated with increased DNA repair, the end result would necessarily be increased formation of mutation. Furthermore, reactive metabolites that react to a larger degree with DNA than other macromolecules in the cells will have a higher mutagenic potential [177, 178]. In line with this, it has recently been reported that several environmental pollutants including the carcinogenic PAH benzo[a]pyrene may change plasma membrane characteristics, thereby altering cell physiology and the balance between life or death of a cell [179].

4.2.4. Inflammatory Mediators. Several cytokines have been found to function as proliferation and/or survival factors, for example, IL-6, IL-8, and IL-1β [180] and which may have implications for several lung diseases including cancer development. Thus, a number of studies *in vitro* have elucidated the inflammatory potential of various air pollution particles [181]. In studies with BEAS-2B bronchial epithelial lung cells DEP from a pre year 2000 engine increased the release of chemokines such as IL-8 [182]; whereas EURO-4 DEP-induced typically IL-6 and IL-8, but also to a certain degree CCL5, CXCL10, and IL1β [139]. Increased CCL5 (RANTES) after DEP exposure (pre year 2000 engine) has also been reported by Hashimoto et al. [183]. Often the induced expression and release of pro-IL-1β found to be due to a combination of endotoxins and other particle components [184]. In general, oxidative stress is considered an important mechanism of particle-induced toxicity and inflammation [181] in addition to other pathways of particle effects. Direct ROS-formation by DEP may arise from enzymatic metabolism of organic compounds such as PAHs or directly [185, 186]. Possible mechanisms also include a direct activation of the membrane bound NADPH oxidase enzyme, inducing the formation of ROS near the plasma membrane [187]. A correlation between NADPH oxidase activation and proinflammatory response has been reported using both *in vitro* and *in vivo* systems exposed to air particles [188]. As seen typically in studies of air pollution collected from cities, there seems to be large seasonal differences in PM_{10} and $\mathrm{PM}_{2.5}$ both with regard to chemical composition and their biological effects as measured as proinflammatory cytokine release and cytotoxicity [184]. The summer PM_{10} exhibited a higher proinflammatory potential, partly due to biological components such as LPS as also previously reported by others [189, 190]. Typically induced cytokines reported include IL-6, IL-8, and IL-1β. However, it should be emphasized that no simple mechanism exists that explains all cellular effects, and in some cases contradictory results have been observed for IL-6 and IL-8 [191]. Furthermore, oxidative stress alone seems to be insufficient to induce proinflammatory responses in lung cells, pointing also to other mechanisms [192, 193]. Moreover, the mechanisms of particle-induced toxicity are likely to change with increasing concentrations.

Of particular interest, recent studies show that DEP may induce Ca^{2+} influx through proteinase-activated receptor-2 (PAR-2) mediated activation of TRPV4 channels in human bronchial epithelial cells. This effect is probably linked to IL-8 responses in bronchial epithelial cells induced by multiple compounds found in ambient air [194]. Studies also suggest that DEP exposure activates EGFR signaling [194]. The activation of EGFR signaling through cleavage and release of membrane bound transforming growth factor (TGF-α) by the metalloproteinase TNFα converting enzyme (TACE or ADAM17) seems to be a universal mechanisms of IL-8 regulation in airway epithelial cells by multiple endogenous and exogenous compounds, including DEP and various air pollution components [194]. It is also possible the particle/DEP-linked formation of reactive metabolites more directly could interfere with various cell signaling pathways or effect organelles, thereby initiating inflammatory reactions.

The vascular endothelium plays a central role in the inflammatory process and cytokine production, and various cellular signaling pathways trigger this response. Considering the evidence that particulate matter can translocate from the lungs within few minutes after exposure [85], the inflammatory signal could reach the vascular endothelium by direct exposure to particles. In this regard, several studies have shown that PM and NP induce endothelial dysfunction after exposure [16, 195–198]. The expression of early (E-Selectin, P-Selectin) and late adhesion molecules (ICAM-1, VCAM-1, PECAM-1) was associated with the presence of endotoxin [199], the size of the particles [200], and the oxidative stress induced by the particles and nanoparticles [201, 202]. Despite the evidence provided by these studies, there is no certainty of the amount of particles that can translocate, and therefore, the experimental conditions of exposure are always of concern.

In vivo, the epithelial cells or macrophages, or any cell that is interacting with a particle, have an interaction with other cell types, and those interactions may exacerbate or inhibit the inflammatory response. Therefore, the single cell cultures have the limitation of not evaluating those interactions. Cocultures of two or more cell types may help to improve the *in vitro* studies. A study using multiple cell cocultures of human lung epithelial cells, macrophages, mast cells, and endothelial cells demonstrated that when cocultures were exposed, a stronger cytokine production was observed in comparison to the responses obtained on single culture [203]. These types of models help to evaluate if the first contact of PM or NP with relevant cells is enough to induce an endothelial activation that may lead to systemic effects. In this regard, a modification of the model described by Alfaro-Moreno et al., using a coculture where the endothelial cells and epithelial cells are seeded on both sides of a membrane, demonstrated that by exposing the epithelial cells, an activation of the endothelial cells was evident within 24 h of exposure [204].

It is interesting to note that inflammatory diseases like asthma and COPD have been suggested to confer an increased risk of lung cancer, although this implication may not be straightforward [199, 200]. The hypothesis is based on that the release of inflammatory mediators (chemokines and cytokines) like IL-1β directly or via increased cytotoxicity (release of DAMP molecules) may result in an increased number of neutrophils/macrophages in the lung. Thus, several *in vivo* studies on other chemicals have reported that the recruitment of such cells will result in increased release of ROS molecules that might exacerbate the increased toxicity and thereby amplify the inflammatory process. Augmented inflammation in a tissue will increase the oxidative/nitrosative stress and lipid peroxidation (LPO), thereby further generating excess ROS, reactive nitrogen species, and DNA-reactive aldehydes. Miscoding etheno- and propano-modified DNA bases are generated interalia by reaction of DNA with these major LPO products [201]. The resulting highly cytotoxic environment will also create surroundings that favor selection of cells with mutations in p53, making them more resistant to cell death [202]. Additional putative mechanisms include impaired or imbalanced DNA repair pathways. In this way, persistent oxidative/nitrosative stress and excess LPO are induced by inflammatory processes in a self-perpetuating process and cause progressive accumulation of DNA damage in target organs including the lung [201].

However, the role of particle-induced inflammation in lung cancer development is very complex. During the latest years, it is becoming increasingly clear that cytokines and chemokines can have a profound role in not only progression, but also rejection of tumors [205].

4.2.5. Changes in Gene Expression via Receptor Binding. Certain changes in phenotypes might give increased probability to development into cancer cells. Regarding exposure to urban air particles, it is well known that some of these like DEP and wood initiate various AhR responses [145, 161, 206]. This is explained by the fact that potent AhR ligands such as PAH and dioxins are released from the particles. The activation of the AhR results in increased metabolism of xenobiotics, and changes in the balance between several metabolic and detoxification pathways are often seen [177]. These types of changes may have important implications for the cells, as more or less reactive metabolites are central for cancer initiation, promotion and for inflammatory reactions. Furthermore, this receptor has also very important physiological functions that extend beyond specific metabolism of xenobiotic, including effects on proliferation, contact inhibition and migration, and immune regulation [145]. All these process may have important implication for cancer development.

4.2.6. Epigenetic Changes. Gene transcription is activated when specific CpG sites are demethylated and histones are acetylated, and, conversely, silenced when sites are methylated and histones deacetylated. Furthermore, in addition to oncogenes, tumor suppressors and miRNAs are the major regulators of signaling in the cancer phenotype [207, 208]. Thus, possible implications of air pollution particle-induced epigenetic changes should clearly be explored *in vitro* systems as these endpoints may become important biological markers for epidemiological studies in the future.

4.2.7. Genotoxicity. It is well documented that different types of particles, their extracts as well as single components attached have genotoxic effects in human and animal studies *in vivo* [209] as well as *in vitro*. After exposure of cells in culture to different types of PM, several studies have shown that cells may be arrested in various parts of the cell cycle [160, 161, 210, 211]. Most often, such effects have been linked to DNA damage. Various forms of DNA damage have been reported after exposure to PM. The DNA damage includes DNA single-strand breaks, alkali-labile single-strand DNA breaks, and various forms of oxidative DNA damage including oxidized guanines measured as 8-oxo-7,8-dihydroguanine (8-oxoGua) and lesions detected as formamidopyrimidine DNA glycosylase (fpg) sites by the comet assay [161, 205]. Often this type of damage is associated with the formation of micronuclei and chromosomal damage. In line with this, positive effects of DEP on chromosome aberration and sister chromatid exchange have been reported in V79 cells without any additional activation system added [212]. The organic extract of $PM_{2.5}$ was reported to generate DNA breakage and micronucleus formation using BEAS-2B cells as a model system. Testing of various fractions in comet assay with fpg in this system suggested a possible role of ROS and that aliphatic/chlorinated hydrocarbons, PAH/alkylderivatives, and nitro-PAH/ketones/quinones may be important causative agents of the genotoxic effects [213]. Furthermore, it should be noted that DEP-extractable organic matter (EOM) has been reported to have a substantial higher capacity than the individual classic carcinogenic PAH with regard to induce oxidative damage to DNA in HepG2 cells [214].

While many reports focus on DNA breaks and/or oxidative-DNA damage with regard to cancer development [209], others link the PM-induced genotoxic and carcinogenic effect to the "classic" carcinogenic PAH giving rise to DNA adducts, often analysed by the ^{32}P postlabelling study [214]. Such PAH needs to be metabolically activated to reactive, electrophilic metabolites that covalently bind to DNA. Both acellular as well as various cells *in vitro* are used. The adduct levels formed are linked to PAH levels in extracts, fractionated extracts, or single PAH compound tested separately [214]. The results from such studies indicate that most DNA adducts detected in cells incubated with extractable organic matter (EOM) from ambient air have their origin in the low concentrations of carcinogenic PAH representing a very low part of EOM total mass (0.03–0.17%; [199]). In general, the bulky DNA adducts are more often associated with high potency to form gene mutations, considered to be of particular important for the initiation phase of cancer development.

An important point in evaluating genotoxic potential is the use of a metabolic activation system with sufficient ability and capacity to activate these carcinogenic PAHs. Certain types of lung epithelial cells (e.g., Clara and type II) *in vivo* have a relatively high level of CYP enzymes due to exposure to AhR ligands (various PAH, dioxins) linked to ambient air particles. Accordingly, several publications have shown DNA adducts, DNA breaks and oxidative DNA damage(s) after exposure to ambient particles [215–218]. Thus, lung epithelial cells will in the *in vivo* situation have a clear capacity to activate various carcinogenic compounds including PAH. However, in contrast, the various lung epithelial cell lines as well as primary lung cells from laboratory animals used *in vitro* have a much lower capacity to activate such compounds. Such cells are thus, not always, the best choice to use when testing for genotoxic effects of various ambient air particle types. Interestingly, some liver-derived cell lines seem to have a more interesting capacity to metabolic activated PAH somewhat more similar to human *in vivo* situation; although metabolic enzyme profile in liver will be different compared to lung. Such models have nevertheless been suggested to represent better *in vitro* models for investigating the genotoxic potential of complex mixtures containing PAH [214, 219]. Another important aspect is to use a test system that can detect the primary DNA damage of importance. This could include technique such as the ^{32}P postlabeling technique to detect the larger and bulky DNA adducts. In order to detect and evaluate DNA damaging constituents which causes smaller DNA adduct/and other DNA lesions, the comet assay with or without addition of fpg is a good supplement [220].

Although not presently in use, it is possible to test the capacity of particles and their extracts to transform epithelial cells *in vitro*, representing a test of both “initiating” as well as tumor “promoting” properties. In a transformation assay using BALB/c 3T3 cells, DEPs have reported to cause morphological transformation [212]. Similarly, it was reported that DEP and two related compounds, 1-nitropyrene (1-NP) and dibenzo(a,i)pyrene (DBP), are capable of transforming rat tracheal epithelial cells [221]. Various coculture systems also add important information to the problem of a “relevant metabolic activation model” when testing genotoxic effects of PM *in vitro*. In a recent study, results supporting the notion that highly reactive benzo[a]pyrene (B[a]P) derived metabolites produced within human alveolar macrophage could be transferred to a secondary target epithelial cell line were presented [222]. Such findings have in addition important *in vivo* implications when explaining possible mechanisms involved in ambient air induced lung cancer. By using DNA repair capacity *in vitro* many important aspects of the role of DNA repair in maintaining genetic stability and preventing carcinogenesis can be elucidated [223]. Furthermore, studies and analyses of polymorphisms of DNA repair genes involved in nucleotide excision repair (NER) may turn out to be useful biomarkers to identify individuals susceptible to DNA damage resulting from ambient air exposure [224]. Also the level of proteins involved in the DNA response like gamma-H2AX, p53, and p21 (WAF1) protein levels has been analyzed and linked to PM-induced genotoxic and cytotoxic effects [161, 225]. Most interestingly, it has been reported that ambient air PM greatly inhibits nucleotide excision repair (NER) for ultraviolet (UV) light and benzo[a]pyrene diol epoxide (BPDE) induced DNA damage in human lung cells. PM increased both spontaneous and UV-induced mutagenesis, suggesting that the carcinogenicity of PM may act through its combined effect on suppression of DNA repair and enhancement of DNA replication errors [226].

5. Conclusions

Urban air pollution consists of an extremely complex mixture of gaseous and particulate agents. The majority of published studies concur to the statement that whilst gaseous pollutants, such as ozone or SO_2, play a significant role, the unifying element of the adverse health effects of urban air pollution consists of respirable PM [1, 88]. Many studies using animal models have been performed to elucidate PM effects in different organs, in relation to different diseases. With respect to acute effects, most studies have focused on inflammatory responses, and relatively few studies have included more disease-specific responses, perhaps with the exception of studies on allergy-related responses. In contrast more studies on chronic effects have elucidated disease-related processes, such as DNA damage, lung parenchyma destruction, increased plaque volume in arteries, lung fibrosis, or granuloma formation. An increased focus on more direct disease-related parameters in models that closely resemble the human disease pattern would improve the usefulness of the *in vivo* models.

Since the *in vitro* models prove themselves to be most useful to study mechanistic responses, such as initiation events of inflammatory effects or genotoxicity, it would be of interest for the interpretation of results if the *in vivo* studies could also to a greater extent cover mechanistic effects, to discover a possible coherence of results with the *in vitro* studies. Whereas the relationship between some *in vitro* end points, particularly with respect to genotoxicity and indicators of cancer development and disease, has been established; with respect to other end points, this relationship has not been fully developed. Improved *in vitro* models that seek to cover this field need to be further developed.

The *in vitro* models have proven useful in studying the importance of a range of particle sizes and components. For example, evidence suggests that the ultrafine fraction of these particles shows more toxicity at equal mass concentrations compared to larger particles, because of their increased reactivity, surface area, and particle number on a mass basis. Furthermore, a coherence of certain *in vitro* cellular effects and responses in biopsies from human volunteers has been shown for the exposure to diesel exhaust particles [194, 227]. On the other hand, sometimes very high concentrations used in *in vitro* models suggest caution in the interpretation of *in vitro* results and again points to the development of more sensitive models.

Nanotechnology develops products with highly different physical and chemical properties, and they are also used in a variety of areas such as diagnosis, imaging, drug delivery, information, and communication technologies, and their extensive use in the consumer and industrial products is just beginning to emerge [87]. Thus, in order to cope with such a variation of type of material and use, structure activity and *in vitro* studies will be of help [87].

The increased risk of respiratory and cardiovascular diseases requires additional toxicological studies to be performed and specific measures to be taken for environmental PM and newly developed engineered NP.

Acknowledgment

We want to thank Victor Montalvo for his assistance on preparing and designing the figures.

References

[1] R. D. Brook, S. Rajagopalan, C. A. Pope et al., “Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the american heart association,” *Circulation*, vol. 121, no. 21, pp. 2331–2378, 2010.

[2] B. Nemery, P. H. M. Hoet, and A. Nemmar, “The Meuse Valley fog of 1930: an air pollution disaster,” *The Lancet*, vol. 357, no. 9257, pp. 704–708, 2001.

[3] J. G. Townsend, “Investigation of the smog incident in Donora, Pa., and vicinity,” *American Journal of Public Health*, vol. 40, no. 2, pp. 183–189, 1950.

[4] W. P. D. Logan, “Mortality in the London fog incident,” *The Lancet*, vol. 261, no. 6755, pp. 336–338, 1953.

[5] M. R. Gwinn and V. Vallyathan, “Nanoparticles: health effects—pros and cons,” *Environmental Health Perspectives*, vol. 114, no. 12, pp. 1818–1825, 2006.

[6] WHO air quality guidelines, global update. Report on a Working Group meeting, Bonn, Germany, WHO Regional Office for Europe, Copenhagen, Denmark, 2005, http://www.euro.who.int/document/e87950.pdf.

[7] A. Ibald-Mulli, H.-E. Wichmann, W. Kreyling, and A. Peters, “Epidemiological evidence on health effects of ultrafine particles,” *Journal of Aerosol Medicine*, vol. 15, no. 2, pp. 189–201, 2002.

[8] “U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards,” Revisions to the ambient monitoring regulations: relevancy for the AIRNOW community, 2007.

[9] C. Xiong and S. K. Friedlander, “Morphological properties of atmospheric aerosol aggregates,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 21, pp. 11851–11856, 2001.

[10] P. Wåhlin, F. Palmgren, and R. Van Dingenen, “Experimental studies of ultrafine particles in streets and the relationship to traffic,” *Atmospheric Environment*, vol. 35, no. 1, pp. S63–S69, 2001.

[11] A. Kocbach, Y. Li, K. E. Yttri, F. R. Cassee, P. E. Schwarze, and E. Namork, “Physicochemical characterisation of combustion particles from vehicle exhaust and residential wood smoke,” *Particle and Fibre Toxicology*, vol. 3, article 1, 2006.

[12] R. M. Harrison and J. Yin, “Particulate matter in the atmosphere: which particle properties are important for its effects on health?” *Science of the Total Environment*, vol. 249, no. 1–3, pp. 85–101, 2000.

[13] S. Becker, L. A. Dailey, J. M. Soukup, S. C. Grambow, R. B. Devlin, and Y.-C. T. Huang, “Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress,” *Environmental Health Perspectives*, vol. 113, no. 8, pp. 1032–1038, 2005.

[14] J. M. Veranth, T. A. Moss, J. C. Chow et al., “Correlation of *in vitro* cytokine responses with the chemical composition of soil-derived particulate matter,” *Environmental Health Perspectives*, vol. 114, no. 3, pp. 341–349, 2006.

[15] I. Rosas Pérez, J. Serrano, E. Alfaro-Moreno et al., “Relations between PM_{10} composition and cell toxicity: a multivariate and graphical approach,” *Chemosphere*, vol. 67, no. 6, pp. 1218–1228, 2007.

[16] C. Monn and S. Becker, “Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine ($PM_{2.5}$) and coarse particles ($PM_{10-2.5}$) in outdoor and indoor air,” *Toxicology and Applied Pharmacology*, vol. 155, no. 3, pp. 245–252, 1999.

[17] P. A. Steerenberg, L. Van Amelsvoort, M. Lovik et al., “Relation between sources of particulate air pollution and biological effect parameters in samples from four european cities: an exploratory study,” *Inhalation Toxicology*, vol. 18, no. 5, pp. 333–346, 2006.

[18] E. Alfaro-Moreno, L. Martínez, C. García-Cuellar et al., “Biologic effects induced *in vitro* by PM_{10} from three different zones of Mexico City,” *Environmental Health Perspectives*, vol. 110, no. 7, pp. 715–720, 2002.

[19] J. M. Soukup and S. Becker, “Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse material, including particulate endotoxin,” *Toxicology and Applied Pharmacology*, vol. 171, no. 1, pp. 20–26, 2001.

[20] S. Becker, M. J. Fenton, and J. M. Soukup, “Involvement of microbial components and toll-like receptors 2 and 4 in cytokine responses to air pollution particles,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 27, no. 5, pp. 611–618, 2002.

[21] M. R. Wilson, J. H. Lightbody, K. Donaldson, J. Sales, and V. Stone, “Interactions between ultrafine particles and transition metals *in vivo* and *in vitro*,” *Toxicology and Applied Pharmacology*, vol. 184, no. 3, pp. 172–179, 2002.

[22] M. Hassellöv, J. W. Readman, J. F. Ranville, and K. Tiede, “Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles,” *Ecotoxicology*, vol. 17, no. 5, pp. 344–361, 2008.

[23] M. G. Perrone, M. Gualtieri, V. Consonni et al., “Particle size, chemical composition, seasons of the year and urban, rural or remote site origins as determinants of biological effects of particulate matter on pulmonary cells,” *Environmental Pollution*, vol. 176, pp. 215–227, 2013.

[24] R. Quintana, J. Serrano, V. Gómez et al., “The oxidative potential and biological effects induced by PM_{10} obtained in Mexico City and at a receptor site during the MILAGRO Campaign,” *Environmental Pollution*, vol. 159, no. 12, pp. 3446–3454, 2011.

[25] J. I. Levy, D. Diez, Y. Dou, C. D. Barr, and F. Dominici, “A meta-analysis and multisite time-series analysis of the differential toxicity of major fine particulate matter constituents,” *American Journal of Epidemiology*, vol. 175, no. 11, pp. 1091–1099, 2012.

[26] P. Schwarze, A. Totlandsdal, and J. I. Herseth, *Importance of Components and Sources for Health Effects of Particulate Air Pollution*, 2010.

[27] I. Rosas, A. Yela, E. Salinas, R. Arreguin, and A. Rodriguez-Romero, “Preliminary assessment of protein associated with airborne particles in Mexico City,” *Aerobiologia*, vol. 11, no. 2, pp. 81–86, 1995.

[28] M. Y. Menetrez, K. K. Foarde, R. K. Esch et al., "An evaluation of indoor and outdoor biological particulate matter," *Atmospheric Environment*, vol. 43, no. 34, pp. 5476–5483, 2009.

[29] S. Després, J. A. Huffman, and S. M. Burrows, "Primarybiological aerosol particles in theatmosphere: a review," *Tellus*, vol. 64, pp. 1–58, 2012.

[30] M. Y. Menetrez, K. K. Foarde, and D. S. Ensor, "Fine biological PM: understanding size fractiontransport and exposure potential (Extended Abstract)," in *Proceedings of the The Air and Waste Management Association Specialty Conference (PM '00)*, Particulate Matter and Health—The Scientific Basis for Regulatory Decision-making, Charlestown, SC, USA, 2000.

[31] M. Y. Menetrez, K. K. Foarde, and D. S. Ensor, "An analytical method for the measurement of nonviable bioaerosols," *Journal of the Air and Waste Management Association*, vol. 51, no. 10, pp. 1436–1442, 2001.

[32] R. B. Knox, C. Suphioglu, P. Taylor et al., "Major grass pollen allergen Lol p 1 binds to diesel axhaust particles: implications for asthma and air pollution," *Clinical and Experimental Allergy*, vol. 27, no. 3, pp. 246–251, 1997.

[33] H. Ormstad, "Suspended particulate matter in indoor air: adjuvants and allergen carriers," *Toxicology*, vol. 152, no. 1–3, pp. 53–68, 2000.

[34] A. Adhikari, T. Reponen, S. A. Grinshpun, D. Martuzevicius, and G. Lemasters, "Correlation of ambient inhalable bioaerosols with particulate matter and ozone: a two-year study," *Environmental Pollution*, vol. 140, no. 1, pp. 16–28, 2006.

[35] F. Ferreira, T. Hawranek, P. Gruber, N. Wopfner, and A. Mari, "Allergic cross-reactivity: from gene to the clinic," *Allergy*, vol. 59, no. 3, pp. 243–267, 2004.

[36] C. Monn, "Exposure assessment of air pollutants: a review on spatial heterogeneity and indoor/outdoor/personal exposure to suspended particulate matter, nitrogen dioxide and ozone," *Atmospheric Environment*, vol. 35, no. 1, pp. 1–32, 2001.

[37] P. S. Thorne, "Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation," *Toxicology*, vol. 152, no. 1–3, pp. 13–23, 2000.

[38] L. Mueller-Annelling, E. Avol, J. M. Peters, and P. S. Thorne, "Ambient endotoxin concentrations in PM_{10} from Southern California," *Environmental Health Perspectives*, vol. 112, no. 5, pp. 583–588, 2004.

[39] H. A. Burge and H. M. Ammann, *Fungal Toxins and (1,3)-b-D-glucan; Bioaerosols: Assessment and Control*, ACGIH, 1999.

[40] U. Singh, T. Reponen, K. J. Cho et al., "Airborne endotoxin and β-D-glucan in PM1 in agricultural and home environments," *Aerosol and Air Quality Research*, vol. 11, no. 4, pp. 376–386, 2011.

[41] A. Nemmar, S. Al-Salam, S. Dhanasekaran, M. Sudhadevi, and B. H. Ali, "Pulmonary exposure to diesel exhaust particles promotes cerebral microvessel thrombosis: protective effect of a cysteine prodrug l-2-oxothiazolidine-4-carboxylic acid," *Toxicology*, vol. 263, no. 2-3, pp. 84–92, 2009.

[42] A. Nemmar, S. Al-Salam, S. Zia et al., "Contrasting actions of diesel exhaust particles on the pulmonary and cardiovascular systems and the effects of thymoquinone," *British Journal of Pharmacology*, vol. 164, no. 7, pp. 1871–1882, 2011.

[43] K.-I. Inoue, H. Takano, M. Sakurai et al., "Pulmonary exposure to diesel exhaust particles enhances coagulatory disturbance with endothelial damage and systemic inflammation related to lung inflammation," *Experimental Biology and Medicine*, vol. 231, no. 10, pp. 1626–1632, 2006.

[44] D. R. Riva, C. B. Magalhães, A. A. Lopes et al., "Low dose of fine particulate matter ($PM_{2.5}$) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice," *Inhalation Toxicology*, vol. 23, no. 5, pp. 257–267, 2011.

[45] W. A. Zin, A. G. L. S. Silva, C. B. Magalhães et al., "Eugenol attenuates pulmonary damage induced by diesel exhaust particles," *Journal of Applied Physiology*, vol. 112, no. 5, pp. 911–917, 2012.

[46] K. I. Paraskevas, A. A. Tzovaras, D. D. Briana, and D. P. Mikhailidis, "Emerging indications for statins: a pluripotent family of agents with several potential applications," *Current Pharmaceutical Design*, vol. 13, no. 35, pp. 3622–3636, 2007.

[47] R. P. Young, R. Hopkins, and T. E. Eaton, "Pharmacological actions of statins: potential utility in COPD," *European Respiratory Review*, vol. 18, no. 114, pp. 222–232, 2009.

[48] S. A. Ferraro, J. S. Yakisich, F. T. Gallo, and D. R. Tasat, "Simvastatin pretreatment prevents ambient particle-induced lung injury in mice," *Inhalation Toxicology*, vol. 23, no. 14, pp. 889–896, 2011.

[49] R. Miyata, N. Bai, R. Vincent, D. D. Sin, and S. F. van Eeden, "Novel properties of statins: suppression of the systemic and bone marrow responses induced by exposure to ambient particulate matter (PM_{10}) air pollution," *American Journal of Physiology*, vol. 303, no. 6, pp. L492–L499, 2012.

[50] E. Tamagawa, N. Bai, K. Morimoto et al., "Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction," *American Journal of Physiology*, vol. 295, no. 1, pp. L79–L85, 2008.

[51] M. L. North, N. Khanna, P. A. Marsden, H. Grasemann, and J. A. Scott, "Functionally important role for arginase 1 in the airway hyperresponsiveness of asthma," *American Journal of Physiology*, vol. 296, no. 6, pp. L911–L920, 2009.

[52] M. L. North, H. Amatullah, N. Khanna et al., "Augmentation of arginase 1 expression by exposure to air pollution exacerbates the airways hyperresponsiveness in murine models of asthma," *Respiratory Research*, vol. 12, article 19, 2011.

[53] A. A. Götz, J. Rozman, H. G. Rödel et al., "Comparison of particle-exposure triggered pulmonary and systemic inflammation in mice fed with three different diets," *Particle and Fibre Toxicology*, vol. 8, article 30, 2011.

[54] P. G. Barlow, D. M. Brown, K. Donaldson, J. MacCallum, and V. Stone, "Reduced alveolar macrophage migration induced by acute ambient particle (PM_{10}) exposure," *Cell Biology and Toxicology*, vol. 24, no. 3, pp. 243–252, 2008.

[55] J. S. Kim, A. Adamcakova-Dodd, P. T. O'Shaughnessy, V. H. Grassian, and P. S. Thorne, "Effects of copper nanoparticle exposure on host defense in a murine pulmonary infection model," *Particle and Fibre Toxicology*, vol. 8, article 269, 2011.

[56] K.-I. Inoue, H. Takano, R. Yanagisawa et al., "Effects of inhaled nanoparticles on acute lung injury induced by lipopolysaccharide in mice," *Toxicology*, vol. 238, no. 2-3, pp. 99–110, 2007.

[57] K.-I. Inoue, R. Yanagisawa, E. Koike et al., "Effects of carbon black nanoparticles on elastase-induced emphysematous lung injury in mice," *Basic and Clinical Pharmacology and Toxicology*, vol. 108, no. 4, pp. 234–240, 2011.

[58] J. S. Tsuji, A. D. Maynard, P. C. Howard et al., "Research strategies for safety evaluation of nanomaterials—part 4: risk assessment of nanoparticles," *Toxicological Sciences*, vol. 89, no. 1, pp. 42–50, 2006.

[59] J. J. Li, S. Muralikrishnan, C.-T. Ng, L.-Y. L. Yung, and B.-H. Bay, "Nanoparticle-induced pulmonary toxicity," *Experimental Biology and Medicine*, vol. 235, no. 9, pp. 1025–1033, 2010.

[60] A. K. Madl and K. E. Pinkerton, "Health effects of inhaled engineered and incidental nanoparticles Health effects of inhaled nanoparticles," *Critical Reviews in Toxicology*, vol. 39, no. 8, pp. 629–658, 2009.

[61] C. M. Sayes, R. Wahi, P. A. Kurian et al., "Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells," *Toxicological Sciences*, vol. 92, no. 1, pp. 174–185, 2006.

[62] D. B. Warheit, T. R. Webb, K. L. Reed, S. Frerichs, and C. M. Sayes, "Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: differential responses related to surface properties," *Toxicology*, vol. 230, no. 1, pp. 90–104, 2007.

[63] A. Nemmar, K. Melghit, and B. H. Ali, "The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO_2 nanorods in rats," *Experimental Biology and Medicine*, vol. 233, no. 5, pp. 610–619, 2008.

[64] K. Melghit and S. S. Al-Rabaniah, "Photodegradation of Congo red under sunlight catalysed by nanorod rutile TiO_2," *Journal of Photochemistry and Photobiology A*, vol. 184, no. 3, pp. 331–334, 2006.

[65] M. Zhou, J. Yu, and B. Cheng, "Effects of Fe-doping on the photocatalytic activity of mesoporous TiO_2 powders prepared by an ultrasonic method," *Journal of Hazardous Materials*, vol. 137, no. 3, pp. 1838–1847, 2006.

[66] E. M. Rossi, L. Pylkkänen, A. J. Koivisto et al., "Airway exposure to silica-coated TiO_2 nanoparticles induces pulmonary neutrophilia in mice," *Toxicological Sciences*, vol. 113, no. 2, pp. 422–433, 2009.

[67] A. Nemmar, K. Melghit, S. Al-Salam et al., "Acute respiratory and systemic toxicity of pulmonary exposure to rutile Fe-doped TiO_2 nanorods," *Toxicology*, vol. 279, no. 1–3, pp. 167–175, 2011.

[68] P. H. N. Saldiva, M. King, V. L. C. Delmonte et al., "Respiratory alterations due to urban air pollution: an experimental study in rats," *Environmental Research*, vol. 57, no. 1, pp. 19–33, 1992.

[69] M. Lemos, A. J. F. C. Lichtenfels, E. Amaro Jr. et al., "Quantitative pathology of nasal passages in rats exposed to urban levels of air pollution," *Environmental Research*, vol. 66, no. 1, pp. 87–95, 1994.

[70] K. Yoshizaki, J. M. Brito, A. C. Toledo et al., "Subchronic effects of nasally instilled diesel exhaust particulates on the nasal and airway epithelia in mice," *Inhalation Toxicology*, vol. 22, no. 7, pp. 610–617, 2010.

[71] T. Kampfrath, A. Maiseyeu, Z. Ying et al., "Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways," *Circulation Research*, vol. 108, no. 6, pp. 716–726, 2011.

[72] J. A. Deiuliis, T. Kampfrath, J. Zhong et al., "Pulmonary T cell activation in response to chronic particulate air pollution," *American Journal of Physiology*, vol. 302, no. 4, pp. L399–L409, 2012.

[73] S. I. Rennard, "COPD: overview of definitions, epidemiology, and factors influencing its development," *Chest*, vol. 113, pp. 235S–241S, 1998.

[74] F. D. T. Q. S. Lopes, T. S. Pinto, F. M. Arantes-Costa et al., "Exposure to ambient levels of particles emitted by traffic worsens emphysema in mice," *Environmental Research*, vol. 109, no. 5, pp. 544–551, 2009.

[75] C.-W. Lam, J. T. James, R. McCluskey, and R. L. Hunter, "Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intractracheal instillation," *Toxicological Sciences*, vol. 77, no. 1, pp. 126–134, 2004.

[76] D. B. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. A. M. Reynolds, and T. R. Webb, "Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats," *Toxicological Sciences*, vol. 77, no. 1, pp. 117–125, 2004.

[77] J. B. Mangum, E. A. Turpin, A. Antao-Menezes, M. F. Cesta, E. Bermudez, and J. C. Bonner, "Single-walled carbon nanotube (SWCNT)-induced interstitial fibrosis in the lungs of rats is associated with increased levels of PDGF mRNA and the formation of unique intercellular carbon structures that bridge alveolar macrophages In Situ," *Particle and Fibre Toxicology*, vol. 3, article 15, 2006.

[78] A. A. Shvedova, E. R. Kisin, R. Mercer et al., "Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice," *American Journal of Physiology*, vol. 289, no. 5, pp. L698–L708, 2005.

[79] A. A. Shvedova, E. Kisin, A. R. Murray et al., "Inhalation versus aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis," *American Journal of Physiology*, vol. 295, no. 4, pp. L552–L565, 2008.

[80] L. A. Mitchell, J. Gao, R. V. Wal, A. Gigliotti, S. W. Burchiel, and J. D. McDonald, "Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes," *Toxicological Sciences*, vol. 100, no. 1, pp. 203–214, 2007.

[81] D. Elgrabli, S. Abella-Gallart, F. Robidel, F. Rogerieux, J. Boczkowski, and G. Lacroix, "Induction of apoptosis and absence of inflammation in rat lung after intratracheal instillation of multiwalled carbon nanotubes," *Toxicology*, vol. 253, no. 1–3, pp. 131–136, 2008.

[82] J. Pauluhn, "Multi-walled carbon nanotubes (Baytubes): approach for derivation of occupational exposure limit," *Regulatory Toxicology and Pharmacology*, vol. 57, no. 1, pp. 78–89, 2010.

[83] A. Nemmar, M. F. Hoylaerts, P. H. M. Hoet, and B. Nemery, "Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects," *Toxicology Letters*, vol. 149, no. 1–3, pp. 243–253, 2004.

[84] G. Oberdörster, Z. Sharp, V. Atudorei et al., "Translocation of inhaled ultrafine particles to the brain," *Inhalation Toxicology*, vol. 16, no. 6-7, pp. 437–445, 2004.

[85] A. Nemmar, P. H. M. Hoet, B. Vanquickenborne et al., "Passage of inhaled particles into the blood circulation in humans," *Circulation*, vol. 105, no. 4, pp. 411–414, 2002.

[86] A. Nemmar, H. Vanbilloen, M. F. Hoylaerts, P. H. M. Hoet, A. Verbruggen, and B. Nemery, "Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster," *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 9, pp. 1665–1668, 2001.

[87] G. Oberdörster, E. Oberdörster, and J. Oberdörster, "Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles," *Environmental Health Perspectives*, vol. 113, no. 7, pp. 823–839, 2005.

[88] J. Vermylen, A. Nemmar, B. Nemery, and M. F. Hoylaerts, "Ambient air pollution and acute myocardial infarction," *Journal of Thrombosis and Haemostasis*, vol. 3, no. 9, pp. 1955–1961, 2005.

[89] A. Seaton and K. Donaldson, "Nanoscience, nanotoxicology, and the need to think small," *The Lancet*, vol. 365, no. 9463, pp. 923–924, 2005.

[90] G. Oberdörster, Z. Sharp, V. Atudorei et al., "Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats," *Journal of Toxicology and Environmental Health Part A*, vol. 65, no. 20, pp. 1531–1543, 2002.

[91] A. Elder, R. Gelein, V. Silva et al., "Translocation of inhaled ultrafine manganese oxide particles to the central nervous system," *Environmental Health Perspectives*, vol. 114, no. 8, pp. 1172–1178, 2006.

[92] W. G. Kreyling, M. Semmler, F. Erbe et al., "Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low," *Journal of Toxicology and Environmental Health Part A*, vol. 65, no. 20, pp. 1513–1530, 2002.

[93] J. G. Wallenborn, J. K. McGee, M. C. Schladweiler, A. D. Ledbetter, and U. P. Kodavanti, "Systemic translocation of particulate matter-associated metals following a single intratracheal instillation in rats," *Toxicological Sciences*, vol. 98, no. 1, pp. 231–239, 2007.

[94] S. Takenaka, E. Karg, C. Roth et al., "Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats," *Environmental Health Perspectives*, vol. 109, no. 4, pp. 547–551, 2001.

[95] J. E. Eyles, V. W. Bramwell, E. D. Williamson, and H. O. Alpar, "Microsphere translocation and immunopotentiation in systemic tissues following intranasal administration," *Vaccine*, vol. 19, no. 32, pp. 4732–4742, 2001.

[96] T. Kato, T. Yashiro, Y. Murata et al., "Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries," *Cell and Tissue Research*, vol. 311, no. 1, pp. 47–51, 2003.

[97] N. Kapp, W. Kreyling, H. Schulz et al., "Electron energy loss spectroscopy for analysis of inhaled ultrafine particles in rat lungs," *Microscopy Research and Technique*, vol. 63, no. 5, pp. 298–305, 2004.

[98] M. Geiser, B. Rothen-Rutishauser, N. Kapp et al., "Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells," *Environmental Health Perspectives*, vol. 113, no. 11, pp. 1555–1560, 2005.

[99] N. L. Mills, N. Amin, S. D. Robinson et al., "Do inhaled carbon nanoparticles translocate directly into the circulation in humans?" *American Journal of Respiratory and Critical Care Medicine*, vol. 173, no. 4, pp. 426–431, 2006.

[100] N. L. Mills, K. Donaldson, P. W. Hadoke et al., "Adverse cardiovascular effects of air pollution," *Nature Clinical Practice Cardiovascular Medicine*, vol. 6, no. 1, pp. 36–44, 2009.

[101] A. Nemmar, S. Zia, D. Subramaniyan, I. Al-Amri, M. A. Al Kindi, and B. H. Ali, "Interaction of diesel exhaust particles with human, rat and mouse erythrocytes *in vitro*," *Cellular Physiology and Biochemistry*, vol. 29, no. 1-2, pp. 163–170, 2012.

[102] B. H. Simon, H. Y. Ando, and P. K. Gupta, "Circulation time and body distribution of 14C-labeled amino-modified polystyrene nanoparticles in mice," *Journal of Pharmaceutical Sciences*, vol. 84, no. 10, pp. 1249–1253, 1995.

[103] A. R. R. Péry, C. Brochot, P. H. M. Hoet, A. Nemmar, and F. Y. Bois, "Development of a physiologically based kinetic model for 99m-Technetium-labelled carbon nanoparticles inhaled by humans Human PBPK model for carbon nanoparticles," *Inhalation Toxicology*, vol. 21, no. 13, pp. 1099–1107, 2009.

[104] T. R. Nurkiewicz, D. W. Porter, M. Barger, V. Castranova, and M. A. Boegehold, "Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation," *Environmental Health Perspectives*, vol. 112, no. 13, pp. 1299–1306, 2004.

[105] T. R. Nurkiewicz, D. W. Porter, M. Barger et al., "Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure," *Environmental Health Perspectives*, vol. 114, no. 3, pp. 412–419, 2006.

[106] T. R. Nurkiewicz, D. W. Porter, A. F. Hubbs et al., "Nanoparticle inhalation augments particle-dependent systemic microvascular dysfunction," *Particle and Fibre Toxicology*, vol. 5, article 1, 2008.

[107] T. R. Nurkiewicz, D. W. Porter, A. F. Hubbs et al., "Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling," *Toxicological Sciences*, vol. 110, no. 1, pp. 191–203, 2009.

[108] A. Nemmar, M. F. Hoylaerts, P. H. M. Hoet et al., "Ultrafine particles affect experimental thrombosis in an *in vivo* hamster model," *American Journal of Respiratory and Critical Care Medicine*, vol. 166, no. 7, pp. 998–1004, 2002.

[109] A. Nemmar, P. H. M. Hoet, D. Dinsdale, J. Vermylen, M. F. Hoylaerts, and B. Nemery, "Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis," *Circulation*, vol. 107, no. 8, pp. 1202–1208, 2003.

[110] A. Nemmar, B. Nemery, P. H. M. Hoet, J. Vermylen, and M. F. Hoylaerts, "Pulmonary inflammation and thrombogenicity caused by diesel particles in hamsters: role of histamine," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 11, pp. 1366–1372, 2003.

[111] A. Nemmar, P. H. M. Hoet, J. Vermylen, B. Nemery, and M. F. Hoylaerts, "Pharmacological stabilization of mast cells abrogates late thrombotic events induced by diesel exhaust particles in hamsters," *Circulation*, vol. 110, no. 12, pp. 1670–1677, 2004.

[112] V. M. Silva, N. Corson, A. Elder, and G. Oberdörster, "The rat ear vein model for investigating *in vivo* thrombogenicity of ultrafine particles (UFP)," *Toxicological Sciences*, vol. 85, no. 2, pp. 983–989, 2005.

[113] G. M. Mutlu, D. Green, A. Bellmeyer et al., "Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway," *Journal of Clinical Investigation*, vol. 117, no. 10, pp. 2952–2961, 2007.

[114] M. Yamashita and M. Yamashita, "Tumor necrosis factor alpha is involved in the induction of plasminogen activator inhibitor-1 by endotoxin," *Thrombosis Research*, vol. 87, no. 2, pp. 165–170, 1997.

[115] G. R. S. Budinger, J. L. McKell, D. Urich et al., "Particulate matter-induced lung inflammation increases systemic levels of PAI-1 and activates coagulation through distinct mechanisms," *PLoS ONE*, vol. 6, no. 4, Article ID e18525, 2011.

[116] A. Nemmar, D. Subramaniyan, and B. H. Ali, "Protective effect of curcumin on pulmonary and cardiovascular effects induced by repeated exposure to diesel exhaust particles in mice," *PLoS ONE*, vol. 7, Article ID e39554, 2012.

[117] Q. Sun, P. Yue, Z. Ying et al., "Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of Rho/ROCK," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 10, pp. 1760–1766, 2008.

[118] Z. Ying, P. Yue, X. Xu et al., "Air pollution and cardiac remodeling: a role for RhoA/Rho-kinase," *American Journal of Physiology*, vol. 296, no. 5, pp. H1540–H1550, 2009.

[119] A. Nemmar, S. Zia, D. Subramaniyan, M. A. Fahim, and B. H. Ali, "Exacerbation of thrombotic events by diesel exhaust particle in mouse model of hypertension," *Toxicology*, vol. 285, no. 1-2, pp. 39–45, 2011.

[120] A. Nemmar, D. Subramaniyan, S. Zia, J. Yasin, and B. H. Ali, "Airway resistance, inflammation and oxidative stress following exposure to diesel exhaust particle in angiotensin II-induced hypertension in mice," *Toxicology*, vol. 292, no. 2-3, pp. 162–168, 2012.

[121] A. Nemmar, S. Al-Salam, D. Subramaniyan et al., "Influence of experimental type 1 diabetes on the pulmonary effects of diesel exhaust particles in mice," *Toxicology Letters*, vol. 217, no. 2, pp. 170–176, 2013.

[122] A. Nemmar, D. Subramaniyan, J. Yasin, and B. H. Ali, "Impact of experimental type 1 diabetes mellitus on systemic and coagulation vulnerability in mice acutely exposed to diesel exhaust particles," *Particle and Fibre Toxicology*, vol. 10, no. 1, article 14, 2013.

[123] A. Nemmar, S. Al-Salam, S. Zia, J. Yasin, I. Al Husseni, and B. H. Ali, "Diesel exhaust particles in the lung aggravate experimental acute renal failure," *Toxicological Sciences*, vol. 113, no. 1, pp. 267–277, 2010.

[124] A. Nemmar, S. Al-Maskari, B. H. Ali, and I. S. Al-Amri, "Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats," *American Journal of Physiology*, vol. 292, no. 3, pp. L664–L670, 2007.

[125] A. Nemmar, S. Dhanasekaran, J. Yasin et al., "Evaluation of the direct systemic and cardiopulmonary effects of diesel particles in spontaneously hypertensive rats," *Toxicology*, vol. 262, no. 1, pp. 50–56, 2009.

[126] A. Nemmar, S. Al-Salam, S. Zia, S. Dhanasekaran, M. Shudadevi, and B. H. Ali, "Time-course effects of systemically administered diesel exhaust particles in rats," *Toxicology Letters*, vol. 194, no. 3, pp. 58–65, 2010.

[127] C. Moon, H.-J. Park, Y.-H. Choi, E.-M. Park, V. Castranova, and J. L. Kang, "Pulmonary inflammation after intraperitoneal administration of ultrafine titanium dioxide (TiO_2) at rest or in lungs primed with lipopolysaccharide," *Journal of Toxicology and Environmental Health Part A*, vol. 73, no. 5-6, pp. 396–409, 2010.

[128] J. Geys, A. Nemmar, E. Verbeken et al., "Acute toxicity and prothrombotic effects of Quantum dots: impact of surface charge," *Environmental Health Perspectives*, vol. 116, no. 12, pp. 1607–1613, 2008.

[129] L. M. Y. Akinaga, A. J. Lichtenfels, R. Carvalho-Oliveira et al., "Effects of chronic exposure to air pollution from sao paulo city on coronary of swiss mice, from birth to adulthood," *Toxicologic Pathology*, vol. 37, no. 3, pp. 306–314, 2009.

[130] L. C. Chen and J.-S. Hwang, "Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice—4. Characterization of acute and chronic effects of ambient air fine particulate matter exposures on heart-rate variability," *Inhalation Toxicology*, vol. 17, no. 4-5, pp. 209–216, 2005.

[131] Q. Sun, A. Wang, X. Jin et al., "Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model," *Journal of the American Medical Association*, vol. 294, no. 23, pp. 3003–3010, 2005.

[132] L. C. Chen and C. Nadziejko, "Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice—5. CAPs exacerbate aortic plaque development in hyperlipidemic mice," *Inhalation Toxicology*, vol. 17, no. 4-5, pp. 217–224, 2005.

[133] Q. Sun, P. Yue, R. I. Kirk et al., "Ambient air particulate matter exposure and tissue factor expression in atherosclerosis," *Inhalation Toxicology*, vol. 20, no. 2, pp. 127–137, 2008.

[134] Q. Sun, P. Yue, J. A. Deiuliis et al., "Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity," *Circulation*, vol. 119, no. 4, pp. 538–546, 2009.

[135] G. S. Kang, P. A. Gillespie, A. Gunnison, A. L. Moreira, K.-M. Tchou-Wong, and L.-C. Chen, "Long-term inhalation exposure to nickel nanoparticles exacerbated atherosclerosis in a susceptible mouse model," *Environmental Health Perspectives*, vol. 119, no. 2, pp. 176–181, 2011.

[136] J. Emmerechts, V. De Vooght, S. Haenen et al., "Thrombogenic changes in young and old mice upon subchronic exposure to air pollution in an urban roadside tunnel," *Thrombosis and Haemostasis*, vol. 108, no. 4, pp. 756–768, 2012.

[137] A. Peters, B. Veronesi, L. Calderón-Garcidueñas et al., "Translocation and potential neurological effects of fine and ultrafine particles a critical update," *Particle and Fibre Toxicology*, vol. 3, article 13, 2006.

[138] R. G. Lucchini, D. C. Dorman, A. Elder, and B. Veronesi, "Neurological impacts from inhalation of pollutants and the nose-brain connection," *NeuroToxicology*, vol. 33, pp. 838–841, 2012.

[139] A. I. Totlandsdal, J. I. Herseth, A. K. Bølling et al., "Differential effects of the particle core and organic extract of diesel exhaust particles," *Toxicology Letters*, vol. 208, no. 3, pp. 262–268, 2012.

[140] J. Topinka, A. Milcova, J. Schmuczerova, M. Mazac, M. Pechout, and M. Vojtisek-Lom, "Genotoxic potential of organic extracts from particle emissions of diesel and rapeseed oil powered engines," *Toxicology Letters*, vol. 212, no. 1, pp. 11–17, 2012.

[141] J. Topinka, P. Rossner, A. Milcova, J. Schmuczerova, V. Svecova, and R. J. Sram, "DNA adducts and oxidative DNA damage induced by organic extracts from $PM_{2.5}$ in an acellular assay," *Toxicology Letters*, vol. 202, no. 3, pp. 186–192, 2011.

[142] R. K. Saxena, M. I. Gilmour, and M. D. Hays, "Isolation and quantitative estimation of diesel exhaust and carbon black particles ingested by lung epithelial cells and alveolar macrophages *in vitro*," *BioTechniques*, vol. 44, no. 6, pp. 799–805, 2008.

[143] P. E. Schwarze, J. Øvrevik, R. B. Hetland et al., "Importance of size and composition of particles for effects on cells *in vitro*," *Inhalation Toxicology*, vol. 19, no. 1, pp. 17–22, 2007.

[144] N. Amara, R. Bachoual, M. Desmard et al., "Diesel exhaust particles induce matrix metalloprotease-1 in human lung epithelial cells via a NADP(H) oxidase/NOX4 redox-dependent mechanism," *American Journal of Physiology*, vol. 293, no. 1, pp. L170–L181, 2007.

[145] M. Ciganek, J. Neca, V. Adamec, J. Janosek, and M. Machala, "A combined chemical and bioassay analysis of traffic-emitted polycyclic aromatic hydrocarbons," *Science of the Total Environment*, vol. 334-335, pp. 141–148, 2004.

[146] R. Barouki, M. Aggerbeck, L. Aggerbeck, and X. Coumoul, "The aryl hydrocarbon receptor system," *Drug Metabolism and Drug Interactions*, vol. 27, no. 1, pp. 3–8, 2012.

[147] C. A. Pope III, R. T. Burnett, M. J. Thun et al., "Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution," *Journal of the American Medical Association*, vol. 287, no. 9, pp. 1132–1141, 2002.

[148] P. Vineis, F. Forastiere, G. Hoek, and M. Lipsett, "Outdoor air pollution and lung cancer: recent epidemiologic evidence," *International Journal of Cancer*, vol. 111, no. 5, pp. 647–652, 2004.

[149] J. Geys, L. Coenegrachts, J. Vercammen et al., "*In vitro* study of the pulmonary translocation of nanoparticles: a preliminary study," *Toxicology Letters*, vol. 160, no. 3, pp. 218–226, 2006.

[150] B. Rothen-Rutishauser, F. Blank, C. Muhlfeld, and P. Gehr, "*In vitro* models of the human epithelial airway barrier to study the

toxic potential of particulate matter," *Expert Opinion on Drug Metabolism & Toxicology*, vol. 4, pp. 1075–1089, 2008.

[151] A. D. Lehmann, F. Blank, O. Baum, P. Gehr, and B. M. Rothen-Rutishauser, "Diesel exhaust particles modulate the tight junction protein occludin in lung cells *in vitro*," *Particle and Fibre Toxicology*, vol. 6, article 26, 2009.

[152] H. S. Rosenkranz, N. Pollack, and A. R. Cunningham, "Exploring the relationship between the inhibition of gap junctional intercellular communication and other biological phenomena," *Carcinogenesis*, vol. 21, no. 5, pp. 1007–1011, 2000.

[153] L. M. Weis, A. M. Rummel, S. J. Masten, J. E. Trosko, and B. L. Upham, "Bay or baylike regions of polycyclic aromatic hydrocarbons were potent inhibitors of gap junctional intercellular communication," *Environmental Health Perspectives*, vol. 106, no. 1, pp. 17–22, 1998.

[154] L. Bláha, P. Kapplová, J. Vondrácek, B. Upham, and M. Machala, "Inhibition of gap-junctional intercellular communication by environmentally occurring polycyclic aromatic hydrocarbons," *Toxicological Sciences*, vol. 65, no. 1, pp. 43–51, 2002.

[155] J. J. Sharovskaja, A. V. Vaiman, N. A. Solomatina, and V. A. Kobliakov, "Inhibition of gap junction intercellular communications in cell culture by polycyclic aromatic hydrocarbons (PAH) in the absence of PAH metabolism," *Biochemistry*, vol. 69, no. 4, pp. 413–419, 2004.

[156] J. Song, S. Ye, W. Sheng, and Y. Jiu, "Effects of diesel exhaust particle on gap junction intercellular communication," *Wei Sheng Yan Jiu*, vol. 26, no. 3, pp. 145–147, 1997 (Chinese).

[157] G. M. Alink, M. Sjögren, R. P. Bos, G. Doekes, H. Kromhout, and P. T. Scheepers, "Effect of airborne particles from selected indoor and outdoor environments on gap-junctional intercellular communication," *Toxicology Letters*, vol. 96-97, pp. 209–213, 1998.

[158] E. Rivedal, O. Myhre, T. Sanner, and I. Eide, "Supplemental role of the Ames mutation assay and gap junction intercellular communication in studies of possible carcinogenic compounds from diesel exhaust particles," *Archives of Toxicology*, vol. 77, no. 9, pp. 533–542, 2003.

[159] W. L. Wendy Hsiao, Z.-Y. Mo, M. Fang, X.-M. Shi, and F. Wang, "Cytotoxicity of $PM_{2.5}$ and $PM_{2.5-10}$ ambient air pollutants assessed by the MTT and the Comet assays," *Mutation Research*, vol. 471, no. 1-2, pp. 45–55, 2000.

[160] H. Bayram, K. Ito, R. Issa, M. Ito, M. Sukkar, and K. F. Chung, "Regulation of human lung epithelial cell numbers by diesel exhaust particles," *European Respiratory Journal*, vol. 27, no. 4, pp. 705–713, 2006.

[161] M. Gualtieri, J. Øvrevik, S. Mollerup et al., "Airborne urban particles (Milan winter-PM2.5) cause mitotic arrest and cell death: effects on DNA, mitochondria, AhR binding and spindle organization," *Mutation Research*, vol. 713, no. 1-2, pp. 18–31, 2011.

[162] C. Furuta, A. K. Suzuki, G. Watanabe, C. Li, S. Taneda, and K. Taya, "Nitrophenols isolated from diesel exhaust particles promote the growth of MCF-7 breast adenocarcinoma cells," *Toxicology and Applied Pharmacology*, vol. 230, no. 3, pp. 320–326, 2008.

[163] S. M. Oh, B. T. Ryu, and K. H. Chung, "Identification of estrogenic and antiestrogenic activities of respirable diesel exhaust particles by bioassay-directed fractionation ," *Archives of Pharmacal Research*, vol. 31, no. 1, pp. 75–82, 2008.

[164] S. L. Tannheimer, S. L. Barton, S. P. Ethier, and S. W. Burchiel, "Carcinogenic polycyclic aromatic hydrocarbons increase intracellular Ca^{2+} and cell proliferation in primary human mammary epithelial cells," *Carcinogenesis*, vol. 18, no. 6, pp. 1177–1182, 1997.

[165] S. L. Tannheimer, S. P. Ethier, K. K. Caldwell, and S. W. Burchiel, "Benzo[α]pyrene- and TCDD-induced alterations in tyrosine phosphorylation and insulin-like growth factor signaling pathways in the MCF-10A human mammary epithelial cell line," *Carcinogenesis*, vol. 19, no. 7, pp. 1291–1297, 1998.

[166] M. Plísková, J. Vondrácek, B. Vojtesek, A. Kozubík, and M. Machala, "Deregulation of cell proliferation by polycyclic aromatic hydrocarbons in human breast carcinoma MCF-7 cells reflects both genotoxic and nongenotoxic events," *Toxicological Sciences*, vol. 83, no. 2, pp. 246–256, 2005.

[167] K. Chramostová, J. Vondrácek, L. Sindlerová, B. Vojtesek, A. Kozubík, and M. Machala, "Polycyclic aromatic hydrocarbons modulate cell proliferation in rat hepatic epithelial stem-like WB-F344 cells," *Toxicology and Applied Pharmacology*, vol. 196, no. 1, pp. 136–148, 2004.

[168] Z. Andrysík, J. Vondrácek, M. Machala et al., "The aryl hydrocarbon receptor-dependent deregulation of cell cycle control induced by polycyclic aromatic hydrocarbons in rat liver epithelial cells," *Mutation Research*, vol. 615, no. 1-2, pp. 87–97, 2007.

[169] Q. A. Khan, K. H. Vousden, and A. Dipple, "Cellular response to DNA damage from a potent carcinogen involves stabilization of p53 without induction of p21(waf1/cip1)," *Carcinogenesis*, vol. 18, no. 12, pp. 2313–2318, 1997.

[170] A. Dipple, "DNA reactions, mutagenic action and stealth properties of polycyclic aromatic hydrocarbon carcinogens," *International Journal of Oncology*, vol. 14, no. 1, pp. 103–111, 1999.

[171] Y. Nakanishi, X.-H. Pei, K. Takayama et al., "Polycyclic aromatic hydrocarbon carcinogens increase ubiquitination of p21 protein after the stabilization of p53 and the expression of p21," *American Journal of Respiratory Cell and Molecular Biology*, vol. 22, no. 6, pp. 747–754, 2000.

[172] E. Roudier, O. Mistafa, and U. Stenius, "Statins induce mammalian target of rapamycin (mTOR)-mediated inhibition of Akt signaling and sensitize p53-deficient cells to cytostatic drugs," *Molecular Cancer Therapeutics*, vol. 5, no. 11, pp. 2706–2715, 2006.

[173] M. Malmlöf, G. Pääjärvi, J. Högberg, and U. Stenius, "Mdm2 as a sensitive and mechanistically informative marker for genotoxicity induced by benzo[a]pyrene and dibenzo[a,l]pyrene," *Toxicological Sciences*, vol. 102, no. 2, pp. 232–240, 2008.

[174] J. L. Marlowe, Y. Fan, X. Chang et al., "The aryl hydrocarbon receptor binds to E2F1 and inhibits E2F1-induced apoptosis," *Molecular Biology of the Cell*, vol. 19, no. 8, pp. 3263–3271, 2008.

[175] A. Solhaug, M. Refsnes, M. Låg, P. E. Schwarze, T. Husøy, and J. A. Holme, "Polycyclic aromatic hydrocarbons induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells," *Carcinogenesis*, vol. 25, no. 5, pp. 809–819, 2004.

[176] N. E. Landvik, M. Gorria, V. M. Arlt et al., "Effects of nitrated-polycyclic aromatic hydrocarbons and diesel exhaust particle extracts on cell signalling related to apoptosis: possible implications for their mutagenic and carcinogenic effects," *Toxicology*, vol. 231, no. 2-3, pp. 159–174, 2007.

[177] J. A. Holme, B. Trygg, and E. Søderlund, "Species differences in the metabolism of 2-acetylaminofluorene by hepatocytes in primary monolayer cultur," *Cancer Research*, vol. 46, no. 4, pp. 1627–1632, 1986.

[178] U. Rannug, J. A. Holme, J. K. Hongslo, and R. Sram, "An evaluation of the genetic toxicity of paracetamol," *Mutation Research*, vol. 327, no. 1-2, pp. 179–200, 1995.

[179] X. Tekpli, J. A. Holme, O. Sergent, and D. Lagadic-Gossmann, "Importance of plasma membrane dynamics in chemical-induced carcinogenesis," *Recent Patents on Anti-Cancer Drug Discovery*, vol. 6, no. 3, pp. 347–353, 2011.

[180] D. Male, J. Brostoff, D. Roth et al., *Immunology*, Elsevier, Amsterdam, The Netherlands, 2006.

[181] E. Alfaro-Moreno, C. M. García-Cuellar, A. De Vizcaya Ruiz, L. Rojas-Bracho, and A. Osornio-Vargas, "The cellular mechanisms behind particulate matter air pollution related health effects," in *Air Pollution: Health & Environmental Impacts*, B. R. Gurjar, L. T. Molina, and C. S. P. Ojha, Eds., Taylor & Francis, 2010.

[182] H. Takizawa, T. Ohtoshi, S. Kawasaki et al., "Diesel exhaust particles activate human bronchial epithelial cells to express inflammatory mediators in the airways: a review," *Respirology*, vol. 5, no. 2, pp. 197–203, 2000.

[183] S. Hashimoto, Y. Gon, I. Takeshita et al., "Diesel exhaust particles activate p38 MAP kinase to produce interleukin 8 and RANTES by human bronchial epithelial cells and N-acetylcysteine attenuates p38 MAP kinase activation," *American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 1, pp. 280–285, 2000.

[184] M. Gualtieri, J. Øvrevik, J. A. Holme et al., "Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells," *Toxicology in Vitro*, vol. 24, no. 1, pp. 29–39, 2010.

[185] A. Baulig, M. Garlatti, V. Bonvallot et al., "Involvement of reactive oxygen species in the metabolic pathways triggered by diesel exhaust particles in human airway epithelial cells," *American Journal of Physiology*, vol. 285, no. 3, pp. L671–L679, 2003.

[186] X. J. Yin, J. Y. C. Ma, J. M. Antonini, V. Castranova, and J. K. H. Ma, "Roles of reactive oxygen species and heme oxygenase-1 in modulation of alveolar macrophage-mediated pulmonary immune responses to Listeria monocytogenes by diesel exhaust particles," *Toxicological Sciences*, vol. 82, no. 1, pp. 143–153, 2004.

[187] A. M. Knaapen, R. P. F. Schins, D. Polat, A. Becker, and P. J. A. Borm, "Mechanisms of neutrophil-induced DNA damage in respiratory tract epithelial cells," *Molecular and Cellular Biochemistry*, vol. 234-235, pp. 143–151, 2002.

[188] R. Becher, A. Bucht, J. Øvrevik et al., "Involvement of NADPH oxidase and iNOS in rodent pulmonary cytokine responses to urban air and mineral particles," *Inhalation Toxicology*, vol. 19, no. 8, pp. 645–655, 2007.

[189] A. Kocbach, J. I. Herseth, M. Låg, M. Refsnes, and P. E. Schwarze, "Particles from wood smoke and traffic induce differential pro-inflammatory response patterns in co-cultures," *Toxicology and Applied Pharmacology*, vol. 232, no. 2, pp. 317–326, 2008.

[190] R. B. Hetland, F. R. Cassee, M. Låg, M. Refsnes, E. Dybing, and P. E. Schwarze, "Cytokine release from alveolar macrophages exposed to ambient particulate matter: heterogeneity in relation to size, city an season," *Particle and Fibre Toxicology*, vol. 2, article 4, 2005.

[191] E. Alfaro-Moreno, V. Torres, J. Miranda et al., "Induction of IL-6 and inhibition of IL-8 secretion in the human airway cell line Calu-3 by urban particulate matter collected with a modified method of PM sampling," *Environmental Research*, vol. 109, no. 5, pp. 528–535, 2009.

[192] K. L. Oslund, L. A. Miller, J. L. Usachenko, N. K. Tyler, R. Wu, and D. M. Hyde, "Oxidant-injured airway epithelial cells upregulate thioredoxin but do not produce interleukin-8," *American Journal of Respiratory Cell and Molecular Biology*, vol. 30, no. 5, pp. 597–604, 2004.

[193] J. Øvrevik, M. Refsnes, P. Schwarze, and M. Låg, "The ability of oxidative stress to mimic quartz-induced chemokine responses is lung cell line-dependent," *Toxicology Letters*, vol. 181, no. 2, pp. 75–80, 2008.

[194] J. Øvrevik, M. Refsnes, A. I. Totlandsdal, J. A. Holme, P. E. Schwarze, and M. Låg, "TACE/TGF-α/EGFR regulates CXCL8 in bronchial epithelial cells exposed to particulate matter components," *European Respiratory Journal*, vol. 38, no. 5, pp. 1189–1199, 2011.

[195] E. Alfaro-Moreno, R. López-Marure, A. Montiel-Dávalos et al., "E-Selectin expression in human endothelial cells exposed to PM10: the role of endotoxin and insoluble fraction," *Environmental Research*, vol. 103, no. 2, pp. 221–228, 2007.

[196] A. Montiel-Dávalos, E. Alfaro-Moreno, and R. López-Marure, "$PM_{2.5}$ and PM_{10} induce the expression of adhesion molecules and the adhesion of monocytic cells to human umbilical vein endothelial cells," *Inhalation Toxicology*, vol. 19, no. 1, supplement, pp. 91–98, 2007.

[197] A. Montiel-Dávalos, M. D. J. Ibarra-Sánchez, J. L. Ventura-Gallegos, E. Alfaro-Moreno, and R. López-Marure, "Oxidative stress and apoptosis are induced in human endothelial cells exposed to urban particulate matter," *Toxicology in Vitro*, vol. 24, no. 1, pp. 135–141, 2010.

[198] A. Montiel-Dávalos, J. L. Ventura-Gallegos, E. Alfaro-Moreno et al., "TiO_2 nanoparticles induce dysfunction and activation of human endothelial cells," *Chemical Research in Toxicology*, vol. 25, no. 4, pp. 920–930, 2012.

[199] A. Rosenberger, H. Bickeböller, V. McCormack et al., "Asthma and lung cancer risk: a systematic investigation by the international lung cancer consortium," *Carcinogenesis*, vol. 33, no. 3, pp. 587–597, 2012.

[200] I. M. Adcock, G. Caramori, and P. J. Barnes, "Chronic Obstructive pulmonary disease and lung cancer: new molecular insights," *Respiration*, vol. 81, no. 4, pp. 265–284, 2011.

[201] H. Bartsch and J. Nair, "Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair," *Langenbeck's Archives of Surgery*, vol. 391, no. 5, pp. 499–510, 2006.

[202] X. Zhou, X. W. Wang, L. Xu et al., "COOH-terminal domain of p53 modulates p53-mediated transcriptional transactivation, cell growth, and apoptosis," *Cancer Research*, vol. 59, no. 4, pp. 843–848, 1999.

[203] E. Alfaro-Moreno, T. S. Nawrot, B. M. Vanaudenaerde et al., "Co-cultures of multiple cell types mimic pulmonary cell communication in response to urban PM10," *European Respiratory Journal*, vol. 32, no. 5, pp. 1184–1194, 2008.

[204] M. D. Ramos-Godínez, B. E. González-Gómez, A. Montiel-Dávalos, R. López-Marure, and E. Alfaro-Moreno, "TiO_2 nanoparticles induce endothelial cell activation in a pneumocyte-endothelial co-culture model," *Toxicology in Vitro*, vol. 27, no. 2, pp. 774–781, 2012.

[205] S. M. Dubinett, J. M. Lee, S. Sharma, and J. J. Mule, "Chemokines: Can effector cells be redirected to the site of the tumor?" *Cancer Journal*, vol. 16, no. 4, pp. 325–335, 2010.

[206] M. D. Meek, "Ah receptor and estrogen receptor-dependent modulation of gene expression by extracts of diesel exhaust

particles," *Environmental Research*, vol. 79, no. 2, pp. 114–121, 1998.

[207] K. A. Pacheco, "Epigenetics mediate environment: gene effects on occupational sensitization," *Current Opinion in Allergy and Clinical Immunology*, vol. 12, no. 2, pp. 111–118, 2012.

[208] P. Vineis and K. Husgafvel-Pursiainen, "Air pollution and cancer: biomarker studies in human populations," *Carcinogenesis*, vol. 26, no. 11, pp. 1846–1855, 2005.

[209] P. Møller, J. K. Folkmann, L. Forchhammer et al., "Air pollution, oxidative damage to DNA, and carcinogenesis," *Cancer Letters*, vol. 266, no. 1, pp. 84–97, 2008.

[210] J. Zhang, A. J. Ghio, M. Gao, K. Wei, G. D. Rosen, and D. Upadhyay, "Ambient particulate matter induces alveolar epithelial cell cycle arrest: role of G1 cyclins," *FEBS Letters*, vol. 581, no. 27, pp. 5315–5320, 2007.

[211] P. H. Danielsen, S. Loft, A. Kocbach, P. E. Schwarze, and P. Møller, "Oxidative damage to DNA and repair induced by Norwegian wood smoke particles in human A549 and THP-1 cell lines," *Mutation Research*, vol. 674, no. 1-2, pp. 116–122, 2009.

[212] M. M. Hasegawa, Y. Nishi, H. Tsuda, N. Inui, and K. Morimoto, "Effects of diesel exhaust particles on chromosome aberration, sister chromatid exchange and morphological transformation in cultured mammalian cells," *Cancer Letters*, vol. 42, no. 1-2, pp. 61–66, 1988.

[213] S. M. Oh, H. R. Kim, Y. J. Park, S. Y. Lee, and K. H. Chung, "Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells)," *Mutation Research*, vol. 723, no. 2, pp. 142–151, 2011.

[214] O. Sevastyanova, B. Binkova, J. Topinka et al., "*In vitro* genotoxicity of PAH mixtures and organic extract from urban air particles—part II: human cell lines," *Mutation Research*, vol. 620, no. 1-2, pp. 123–134, 2007.

[215] J. Hukkanen, O. Pelkonen, J. Hakkola, and H. Raunio, "Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung," *Critical Reviews in Toxicology*, vol. 32, no. 5, pp. 391–411, 2002.

[216] S. Mollerup, G. Berge, R. Bæra et al., "Sex differences in risk of lung cancer: expression of genes in the PAH bioactivation pathway in relation to smoking and bulky DNA adducts," *International Journal of Cancer*, vol. 119, no. 4, pp. 741–744, 2006.

[217] K. Yang, Y. Huang, G. Zhao, Y. Lei, and K. Wang, "Expression of PAH-DNA adducts in lung tissues of Xuanwei female lung cancer patients," *Chinese Journal of Lung Cancer*, vol. 13, no. 5, pp. 517–521, 2010.

[218] E. Gyorffy, L. Anna, Z. Gyori et al., "DNA adducts in tumour, normal peripheral lung and bronchus, and peripheral blood lymphocytes from smoking and non-smoking patients: correlations between tissues and detection by 32P-postlabelling and immunoassay," *Carcinogenesis*, vol. 25, no. 7, pp. 1201–1209, 2004.

[219] J. A. Holme, M. Gorria, V. M. Arlt et al., "Different mechanisms involved in apoptosis following exposure to benzo[a]pyrene in F258 and Hepa1c1c7 cells," *Chemico-Biological Interactions*, vol. 167, no. 1, pp. 41–55, 2007.

[220] A. Gábelová, Z. Valovicová, G. Bacová et al., "Sensitivity of different endpoints for *in vitro* measurement of genotoxicity of extractable organic matter associated with ambient airborne particles (PM_{10})," *Mutation Research*, vol. 620, no. 1-2, pp. 103–113, 2007.

[221] M.-X. Ensell, W.-Z. Whong, Z.-C. Heng, J. Nath, and T. Ong, "*In vitro* and *in vivo* transformation in rat tracheal epithelial cells exposed to diesel emission particles and related compounds," *Mutation Research*, vol. 412, no. 3, pp. 283–291, 1998.

[222] I. Abbas, G. Garçon, F. Saint-Georges et al., "Polycyclic aromatic hydrocarbons within airborne particulate matter (PM2.5) produced DNA bulky stable adducts in a human lung cell coculture model," *Journal of Applied Toxicology*, vol. 33, no. 2, pp. 109–119, 2011.

[223] A. R. Collins and L. R. Ferguson, "DNA repair as a biomarker," *Mutation Research*, vol. 736, no. 1-2, pp. 2–4, 2012.

[224] B. Binkova, I. Chvatalova, Z. Lnenickova et al., "PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms," *Mutation Research*, vol. 620, no. 1-2, pp. 49–61, 2007.

[225] E. Longhin, E. Pezzolato, P. Mantecca et al., "Season linked responses to fine and quasi-ultrafine Milan PM in cultured cells," *Toxicology in Vitro*, vol. 27, pp. 551–559, 2013.

[226] M. Mehta, L.-C. Chen, T. Gordon, W. Rom, and M.-S. Tang, "Particulate matter inhibits DNA repair and enhances mutagenesis," *Mutation Research*, vol. 657, no. 2, pp. 116–121, 2008.

[227] J. Pourazar, A. Blomberg, F. J. Kelly et al., "Diesel exhaust increases EGFR and phosphorylated C-terminal Tyr 1173 in the bronchial epithelium," *Particle and Fibre Toxicology*, vol. 5, article 8, 2008.