REVIEWS

Respiratory effects of manufactured nanoparticles

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Summary  Nanotechnology is the set of techniques used to engineer, characterize, and produce materials that have at least one dimension within the nanoscale. These nanomaterials, or nanoobjects, include nanoparticles and nanotubes. As dictated by the laws of quantum physics, a size within the nanoscale results in unique physicochemical properties and distinctive behaviors. Nanotechnology has a host of applications in fields ranging from cosmetology to the industry and medicine. The production and use of nanomaterials are expanding at a brisk pace. However, concerns are emerging about the potential health effects of nanoparticles in the short and long terms. These concerns are rooted in data on the harmful health effects of micrometric airborne particulate matter. Conceivably, these adverse effects might be amplified when the particles are within the nanoscale. This article is a nonexhaustive overview of current data on the penetration, deposition, translocation, and elimination of inhaled nanoparticles and on the respiratory effects of metallic nanoparticles (with special attention to titanium dioxide) and carbon nanotubes. Both in vivo and in vitro studies consistently found biological effects of nanoparticles on the respiratory system, including oxidative stress generation, proinflammatory and prothrombotic effects, pulmonary fibrosis and emphysema, and DNA damage. Improved knowledge of the potential biological effects of nanoparticles is needed to guide preventive strategies for the workplace and/or general population if needed.

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Introduction

The term “nanotechnology” holds connotations of innovation and technological progress. Nanotechnology is the set of techniques required to engineer, characterize, produce and use nanostructures, nanodevices, and nanosystems. Nanotechnology is now viewed as the highest point of miniaturization achieved by integrating technology, biology, chemistry, and physics. Nanoscience is the study of phenomena generated by nanomaterials. As dictated by the laws of quantum physics, particles within the nanoscale exhibit unique properties compared to larger particles having the same chemical composition. Thus, a characteristic inherent in the nanoscale is a large proportion of surface atoms, which may result in high surface reactivity, high resistance, and modified electrical properties. Nanoparticles have a broad range of applications, particularly in innovative sectors such as the cosmetic industry (e.g., sunscreens, lipstick, and toothpastes) [1,2], automotive industry (e.g., paints, tires, lubricants, and windshields) [3,4], and healthcare industry (e.g., drug pharmacokinetics and bioavailability, prosthetic materials, and molecular imaging) [5,6].

Nanotechnology has a major impact on the world economy. Thus, public funding for nanoresearch has increased from slightly less than 500 million US dollars in 1997 to 3.5 billion in 2004 [7]. The National Science Foundation had estimated that by 2015 nanotechnology will generate an income of about 1000 billion US dollars worldwide [8].

The rapidly growing place occupied by these new and promising technologies in our everyday life is raising many questions regarding potential effects on human health (at the workplace and in the general population) and on the environment. Concern has been voiced about the very properties responsible for the appealing characteristics of nanomaterials (e.g., high surface reactivity and ability to cross cell membranes). This concern is rooted in data on the harmful health effects of micrometric airborne pollutant particles [9–15]. Conceivably, these adverse effects might be amplified when the particles are within the nanoscale. Robust research efforts are focusing on the toxicology of nanomaterials and on their potential effects on human health and the environment. Nevertheless, many questions are still unanswered. Thus, insufficient data are available on situations associated with nanoparticle exposure (e.g., nanoparticle manufacturing, use, and application) and levels of exposure to manufactured nanoparticles. Furthermore, risk evaluations must assess not only the potential effects of native nanomaterials, but also their behavior throughout their life cycle (manufacturing method, use, aging, and biodegradability). Most of the currently available information on adverse health effects of nanoparticle comes from in vitro studies and in vivo animal studies. Given the complexity of the topic, this article is a detailed but nonexhaustive overview of current data on the penetration, deposition, translocation, and elimination of inhaled nanoparticles and on the respiratory effects of metallic nanoparticles (with special attention to titanium dioxide [TiO2]) and carbon nanoparticles.

- A size within the nanoscale is associated with an increase in the proportion of surface atoms, which enhances surface reactivity and produces new properties.
- Although extremely promising, nanotechnology is generating major concern about potential harmful effects on human health (at the workplace and in the general population) and on the environment.

Background

Definitions

Nanomaterials, or nanoobjects, have at least one dimension within the nanometric range (1–100 nm). Nanomaterials having a single nanometric dimension are nanosheets (e.g., made of graphite). Two nanometric dimensions define nanotubes and nanowires, of which the most extensively studied are carbon nanotubes, first described in 1991 [16]. Carbon nanotubes measure a few nanometers in diameter and up to several micrometers in length. Finally, nanoparticles have all three dimensions within the nanometric range.

Nanoparticle sources

Nanoparticles are either produced naturally (e.g., by volcanic eruptions, wildfires, or marine pollution) or manufactured. Manufactured nanoparticles may be generated unintentionally (that is, as pollutants, produced for instance by manufacturing processes, diesel engines, various forms of combustion, and building materials) or intentionally (e.g., by the industry or by research laboratories).

The chemical nature of nanoparticles varies in complexity. Nanomaterials may be composed of minerals (e.g., graphite, hematite, and silica sol), metals (silicon dioxide [SiO2] and TiO2), or organic compounds (carbon compounds such as buckminsterfullerene [C60], single-wall carbon nanotubes [SWCNTs], and multiple-wall carbon nanotubes [MWCNTs]; and polymers such as polystyrene, nylon, and dextrane). Nanoparticles may also be mixtures of variable complexity depending on the method used to generate them (heating of polytetrafluoroethylene [PTFE or Teflon], welding fumes, and soot produced by the combustion of hydrocarbons or polymers). Furthermore, a nanoparticle may have a particulate core surrounded by a shell of adsorbed pollutants such as transition metals, hydrocarbons, or biological substances (e.g., endotoxins).

Determinants of harmful health effects of nanoparticles

Data about ultrafine airborne pollutants suggests that the health effects of nanomaterials may depend on a number of frequently intercorrelated factors including size, number and/or surface, shape, chemical composition, surface treatment, and potential for aggregation/agglomeration. Few data are available on the toxicity of nanoparticles to humans [17–19].
Nanoparticle size

A 1994 model of discrete inhaled particles of well-defined size, with no aggregates suggests that nanoparticle deposition in each of the three regions of the airway (nasopharynx, tracheobronchial tree, and alveolar region) may depend chiefly on nanoparticle size, which may therefore influence potential effects [20]. This point is discussed in detail in the section entitled "Inhalation, penetration, and deposition of nanoparticles in the respiratory system". Nanoparticle size is also intrinsically related to relative surface, at least for solid nanoparticles.

Nanoparticle shape

The biological effects depend on the shape of the nanoparticles. Manufactured nanoparticles come in many different shapes (e.g., spheres, fibers, tubes, rings, and disks). Experimental in vitro and in vivo studies of micrometric spherical and fibrous materials have convincingly demonstrated that fibers, whether occurring naturally (e.g., asbestos) or manufactured (e.g., fiberglass), have greater cytotoxicity and genotoxic potential and generate higher risks of lung fibrosis and lung cancer [21,22]. The critical parameters are the dose, size, and biopersistence of the fibers. Biopersistence of fibrous materials is an important characteristic, because longer material-cell contact times are associated with stronger biological effects [22]. Biopersistence is greater with fibers than with spherical particles. Toxicological studies of TiO$_2$ have shown greater toxicity with fibers than with spheres. In a study of rat alveolar macrophages exposed to similar concentrations of fibrous and particulate TiO$_2$ (1–2 μm), electron microscopy showed vacular changes and cell surface damage with the fibrous form but no significant changes with the particulate form [23]. In keeping with this finding, LDH release into the medium, used as a cytotoxicity index, increased significantly after exposure to fibrous TiO$_2$ but not to particulate TiO$_2$ [23]. These data indicate that the cytotoxic potential of TiO$_2$ depends on the shape of the material. Although they were obtained with TiO$_2$ fibers and particles larger than 100 nm, they strongly suggest an influence of shape on the biological effects of nanoparticles.

Nanoparticle surface characteristics and surface reactivity

Several in vivo animal studies of ultrafine airborne pollutants showed that total particle surface area correlated with various parameters including neutrophil influx into the lungs, changes in lung epithelium permeability, and accumulation within the lymph nodes [21,24,25]. The small size and correspondingly large specific surface area of solid nanoparticles produce specific properties such as the ability to catalyze chemical reactions. The atoms and molecules at the surface play a crucial role in determining the physicochemical properties of nanomaterials [19]. The ratio of particle surface area over total number of atoms or molecules increases exponentially as particle size decreases. Given that chemical reactions occur at the particle surface, nanomaterials are expected to show greater reactivity than larger particles of identical chemical composition. For instance, in an inhaled volume of 10 μg/m$^3$ of air, the number of 5-μm particles is 0.15/mL but the number of 5-nm nanoparticles is 10$^6$ times higher (153 × 10$^6$/mL). However, the total surface areas are 12 μm$^2$/mL for 5-μm particles and 1000 times higher (12,000 μm$^2$/mL) for nanoparticles. This far greater surface area available for contact with cells and biological molecules explains the greater biological reactivity of nanoparticles compared to the same mass concentration of larger particles. Thus, nanoparticles are associated with greater free oxygen radical production, oxidative stress, and proinflammatory potential compared to larger particles [26].

Chemical composition of nanoparticles

The chemical composition of nanoparticles also influences their biological effects. Thus, one determinant of biological activity is the presence of metals in the composition of the nanoparticles or of transition metals produced as impurities during the manufacturing process. Transition metals in naturally occurring nanoparticles are involved in the production of free oxygen radicals, which are highly reactive molecules capable of modulating various biological processes and causing cell damage [19]. For instance, iron contributes to the harmful effects of particulate matter in urban air pollution [27,28]. The bioavailability of iron from particles that contact epithelial cells decreases as particle size increases [29]. Iron can induce the proinflammatory cytokine interleukin-8. Similarly, data suggest that the redox properties of iron may be involved in the cytotoxicity of carbon nanotubes for human keratinocytes [30].

Degree of nanoparticle aggregation/agglomeration

Nanoparticles tend to aggregate and/or agglomerate to varying degrees. Nanoparticles subjected to Van der Waals forces, electrostatic forces, or surface tension forces tend to agglomerate [31]. Aggregates form when nanoparticles are subjected to stronger forces and are therefore more difficult to separate. Nanoparticle agglomerates or aggregates, which may reach the microscale, exhibit complex shapes and are difficult to characterize. Agglomeration or aggregation changes the aerodynamic properties of nanoparticles and therefore probably influences airway deposition. Data obtained in mice suggest that gastrointestinal toxicity may be influenced by aggregation [32]. Thus, oral administration of zinc nanoparticles caused the death of some of the animals, in which nanoparticle aggregation was found, whereas microscale zinc particles caused no death [32].

Interestingly, the ability of nanoparticles to form aggregates via the adsorption of proteins has a major impact on clearance of the nanoparticles and on their immunological and toxicological effects. When carbon black nanoparticles (25 to 100 nm) subjected to different surface treatments were placed with dipalmitoylphosphatidylcholine (DPPC) in culture medium, agglomeration occurred within 1 hour [33]. The size distribution of the immersed nanoparticles differed significantly from that obtained with phosphate buffer used as a control. DPPC concentrations decreased in a nanoparticle surface- and size-dependent manner, indicating that surface adsorption was responsible for the agglomeration and decrease in phospholipid concentrations [33].
occurrence of similar interactions within the alveolar surfactant, which contains phospholipids crucial to normal lung mechanical properties, may contribute to the toxicity of nanoparticles for the respiratory system.

**Surface treatments of nanoparticles**

Surface treatment is a major parameter that is probably more relevant than particle type to the effects of human exposure. Surface treatments may either increase or decrease the toxicity of nanoparticles. Nanoobjects known as quantum dots, composed of cadmium selenide, are cytotoxic for primary hepatocytes under specific conditions [34]. The cytotoxicity of quantum boxes, which are three-dimensional nanoobjects, is modulated by a number of production parameters including ultraviolet radiation exposure and coating. Cytotoxicity correlates with the release of free Cd$^{2+}$ ions related to deterioration of the cadmium selenide structure. However, appropriately treated cadmium selenide quantum dots are no longer cytotoxic. These quantum dots are currently used in biomedical research to monitor in vitro cell migration and reorganization.

The coating may influence nanoparticle penetration into cells. Albumin, the most abundant protein in the plasma and interstitial compartment, may promote endocytosis of nanomaterials. Similarly, polystyrene particles (240 nm) coated with the membrane phospholipid lecithin can cross through the alveolar-capillary barrier, in contrast to the uncoated particles [35]. In rabbits, intravenously injected colloidal gold particles coated with rabbit serum albumin underwent transcytosis mediated by receptors (albumin binding proteins) via the caveolae [36]. Thus, the presence in the alveolar epithelial lining fluid of albumin and phospholipids makes a major contribution to epithelial absorption of nanomaterials deposited in the alveolar spaces.

- Among nanoparticles, some occur naturally while others are produced by human activity, either intentionally or unintentionally.
- Factors that influence nanoparticle toxicity include size, number, surface characteristics, shape, chemical composition, surface treatment, and potential for aggregation/agglomeration.

**Inhalation, penetration, and deposition of nanoparticles in the respiratory system**

The airways constitute the main route by which nanoparticles enter the body. In general, the penetration and deposition of inhaled particles into the respiratory system can occur via five mechanisms: gravitational sedimentation, inertial impaction, interception (particle-surface contact), diffusion, and electrostatic deposition. The predominant mechanism depends on the size of the particles. Three other factors with major effects on particle deposition are airway geometry and branching pattern, breathing rate, and predominant breathing pattern through the mouth or through the nose.

Deposition of inhaled nanoparticles on the airway walls occurs chiefly via diffusional displacement by the thermal motion of inhaled and exhaled air molecules in contact with the nanoparticles. Importantly, nanoparticles can form aggregates and/or agglomerates, thus increasing in size from the nanoscale to the microscale. In contrast to microscale particles, nanoparticles exhibit increasing nasopharyngeal and tracheobronchial deposition as their size diminishes [17,37–42]. Mathematical models have been developed to predict particle deposition in the human airways. The model developed by the International Commission on Radiobiological Protection (ICRP) can be used to compute the proportions, by mass, of inhaled nanoparticles deposited in the airways of an individual breathing through the nose. Nanoparticles 1 nm in diameter are predicted to show about 90% deposition in the nasopharynx, 10% in the tracheobronchial tree, and 0% in the alveolar spaces; corresponding proportions for 5-nm particles are 30%, 30%, and 30%; and for 20-nm particles, 15%, 15%, and 50%. With 20-nm particles, the distribution of deposition according to lung surface concentration indicates that alveolar space deposition is 100 times greater than nasopharyngeal deposition and 10 times greater than tracheobronchial deposition [40,41].

**Fate of nanoparticles in the respiratory system**

**Retention of nanoparticles within the lung**

Nanoparticle lung retention depends chiefly on particle size and clearance capacity. Compared to larger-sized particles having the same chemical composition, inhaled nanoparticles show greater lung retention. In rats, identical concentrations of TiO$_2$ seems to have been intratracheally instilled led to greater lung retention with 20-nm nanoparticles compared to 250-nm particles [43]. Nanoparticle lung retention is increased in patients with obstructive airway diseases such as asthma and chronic obstructive pulmonary disease [44,45].

**Nanoparticle clearance from the airways**

Particles deposited in the airways can be cleared via two mechanisms, namely, physical processes, which vary across the three regions of the respiratory system; and chemical processes capable of eliminating more or less soluble particles, which are identical throughout the respiratory system.

Physical clearance of inhaled nanoparticles is via the mucociliary escalator that sweeps the particles up to the pharynx where they are swallowed, phagocytosis by macrophages, and translocation through the epithelium. This last mechanism will be discussed later on. The mucociliary escalator clears particles from the tracheobronchial and nasopharyngeal airways, within 24–48 hours, as shown by a study in rats exposed intratracheally for 1 hour to nanoparticles of radiolabeled iridium (15 and 80 nm) [46]. Phagocytosis of insoluble nanoparticles by macrophages occurs in the tracheobronchial tree and alveolar spaces. The efficacy of this mechanism depends largely on nanoparticle
Translocation and distribution of nanoparticles in the body

Conflicting results have been reported regarding the translocation of inhaled nanoparticles [50]. Several studies support epithelial, interstitial and neuronal translocation of insoluble or nearly insoluble nanoparticles to other compartments in the body [51–53].

Translocation into the epithelium and interstitium may allow nanoparticles to penetrate the blood and lymph and, therefore, to distribute throughout the body. Endocytosis by airway epithelial cells may occur at all three levels of the airway tree, providing nanoparticles with direct entry into the blood and lymph [17,52,53]. Other nanoparticle translocation mechanisms are generating debate, such as luminal vesicular transport via caveolae at the surface of epithelial and endothelial cells. Caveolar openings range from 40 to 100 nm, which should allow nanoparticles to cross the alveolar-capillary barrier into the systemic circulation [38]. Several studies have evaluated the potential systemic translocation of various nanoparticles administered by inhalation or intratracheal instillation [38,53,54].

Studies in animals have shown rapid translocation of several nanoparticle types from the lung to the bloodstream [17,40,43,46,53,54]. This mechanism can redistribute the nanoparticles in the organs. In a rat study, nanoparticles generated by heating PTFE were detected in the bronchial submucosa and juxta-pleural lung interstitium only 15 minutes after inhalation [55]. In other rat studies, only a small proportion of 192Ir-labeled nanoparticles administered by inhalation penetrated into the bloodstream [46]. Rats exposed in inhalation chambers to insoluble 13C nanoparticles (20–29 nm) for 18–24 hours exhibited marked radioactivity of the liver and lungs starting only 30 minutes after exposure initiation [53]. These findings indicate systemic translocation of the inhaled nanoparticles. After 24 hours’ exposure, no radioactivity was detected in the heart, olfactory bulb, brain, or kidneys. The discrepancies across studies may be ascribable to differences in administration modalities or in the nanomaterials used. In a study involving exposure of rats to inhaled TiO2 nanoparticles (20 nm) and fine particles (250 nm), nanoparticles showed greater accumulation within the lymph nodes, indicating penetration of the nanoparticles into the interstitial spaces [43]. However, in rats exposed to nanoparticles (15–20 nm) radiolabeled with iodium, fewer than 1% of the nanoparticles entered the bloodstream and reached the liver, spleen, kidneys, brain, and heart [46]. A study of hamsters given intratracheal colloidal nanoparticles of denatured serum albumin radiolabeled with 99m-technetium (99mTc) (<80 nm) showed marked pulmonary radioactivity within 5 minutes concomitantly with very small amounts of radioactivity in the blood, kidneys, spleen, and brain [54]. The size of the translocated fraction may vary with the physicochemical properties of the nanoparticles [56]. In ahamster model, physicochemical parameters such as bipolar charge at the nanoparticle surface markedly affected translocation through the airway epithelium to the bloodstream [54].

Whether nanoparticles undergo translocation to the bloodstream in humans is debated. An experimental study suggests that nanoparticles may readily cross the alveolar-capillary barrier into the pulmonary bloodstream [57]. Five healthy volunteers inhaled 5- to 10-nm carbon nanoparticles radiolabeled with 99mTc. Radioactivity was rapidly detected in the blood, indicating passage across the alveolar-capillary membrane to the pulmonary and systemic circulations, and 3 to 5% of the total dose was found in various organs (liver, heart, spleen, and brain) [57]. In contrast, two other studies involving inhalation of 99mTc-labeled carbon nanoparticles showed no evidence of translocation, the radioactivity detection limit being 1% of the inhaled dose [39,58]. Nevertheless, it is reasonable to expect that inhaled nanomaterials may reach various organs in the body.

Neuronal translocation of nanoparticles has been suggested. In rats exposed in an inhalation chamber to insoluble C13 nanoparticles for 6 hours, radioactivity counts on the first day indicated translocation of the nanoparticles to the cerebrum, cerebellum, and olfactory bulb, with persistence of the radioactivity until day 7 in the olfactory bulb [59]. The authors of this study put forward two hypotheses, namely, translocation from the lungs to the systemic circulation with passage across the blood-brain barrier, and neuronal translocation via the olfactory bulb followed by retrograde migration along the axons to the central nervous system. Studies in animals suggest that neuronal translocation to the central nervous system can occur via the sensory neurons in the airway epithelium [21,40,60]. However, it is worth noting that the olfactory mucosa in humans represents only 5% of the total nasal mucosal surface area, compared to 50% in rats.

Effects of nanoparticles on the respiratory system

The results of experimental studies of nanoparticle effects should be interpreted with circumspection, for a number
Respiratory effects of manufactured nanoparticles

• Particle clearance from the tracheobronchial and nasopharyngeal airways occurs chiefly via the mucociliary escalator.
• Insoluble nanoparticles are cleared by macrophage phagocytosis in the tracheobronchial tree and alveolar spaces.
• Chemical mechanisms of nanoparticle clearance consist mainly of dissolution of soluble particles, lixiviation, and binding to proteins.
• Whether nanoparticle translocation from the respiratory system to other tissues occurs in humans remains debated.

of reasons: the number of studies is small, nanoparticles only recently became a focus of concern, the tested nanoparticles vary widely, and the nanoparticle concentrations used in vitro are usually higher than those encountered in vivo. This section emphasizes the respiratory effects of two types of manufactured nanoparticles, carbon nanotubes and metallic nanoparticles, most notably those composed of TiO₂.

In vitro experimental studies

Reactive oxygen species and oxidative stress

Carbon nanotubes

Oxidative stress is among the chief mechanisms underlying nanomaterial cytotoxicity. There is general agreement that, among carbon nanotubes, only the unpurified forms contain iron, which induces oxidative stress. This point is important when considering the toxic potential of SWCNTs. SWCNTs with high iron contents induce greater oxidative stress than do purified SWCNTs with low iron contents [61]. It was shown recently that oxidative stress in cells incubated with SWCNTs secondarily weakens the antioxidant response, diminishing the levels of glutathione and antioxidant enzymes (superoxide dismutases 1 and 2) [62]. This interesting finding completes the above-described mechanism.

Metallic nanoparticles

TiO₂ nanoparticles can produce reactive oxygen species in solution under abiotic conditions when exposed to ultraviolet radiation [19]. Studies in several models have established that various metallic and nonmetallic nanomaterials can induce intracellular oxidative stress [63–65]. A recent study highlighted the influence of particle size on the pro-oxidant and proinflammatory effects of metallic nanoparticles [66]. In vitro, human A549 cells exhibited a stronger proinflammatory response and greater oxidative stress when exposed to TiO₂ and carbon black nanoparticles than when exposed to the same mass dose of larger particles having the same chemical composition. This result suggests that the total particle surface area normalized for the total exposed cell surface area may be a better means of measuring the dose than particle mass. The dose-response relationships in this in vitro study seem consistent with the dose-response relationships found in vivo after dose standardization.

Several studies indicate that oxidative stress is among the mechanisms involved in the cytotoxic effects of nanoparticles [19]. Among them, one used a particularly elegant approach. The cytotoxic effects of various nanomaterials were compared based on correlations with intracellular oxidation caused by oxidative stress [23]. The results show clearly that cytotoxic nanoparticles consistently induce oxidative stress, whereas nanoparticles devoid of cytotoxicity produce no oxidative stress. The ability of a given nanoparticle to produce reactive oxygen species in solution, without cell contact, did not predict nanoparticle cytotoxicity in this study [23].

DNA damage

Carbon nanotubes

Two recently published studies show that DNA damage occurs when differentiated cells (V79 hamster fibroblasts, [67]) or undifferentiated cells (murine embryonic stem cells, [68]) are incubated with carbon nanotubes. These are the first studies reporting nanotube-induced DNA damage. Genetic alterations occurred in the murine stem cells, in keeping with the marked sensitivity of stem cells to agents capable of causing DNA damage. In addition, although MWCNTs were found in the nuclei of the murine stem cells [68], neither study involved a detailed evaluation to determine whether the DNA damage was related to nuclear translocation of the carbon nanotubes. Although the ability of carbon nanotubes to induce DNA damage needs to be confirmed and further evaluated, these two studies indicate a need for attention to DNA damage when evaluating the effects of carbon nanotubes.

Metallic nanoparticles

Metallic and nonmetallic nanoparticles with limited solubility in water have also been shown to induce DNA damage. A literature review on the genotoxicity of poorly hydrosoluble particles, such as TiO₂, shed light on the mechanisms involved [69]. The available data indicate only that the genotoxic effects of poorly hydrosoluble particles are related to DNA oxidation by reactive oxygen species and nitrogen. There is an urgent need for further evaluation of this effect. Whether a causal link exists between lung inflammation and genotoxic effects remains unknown. In addition, little information is available on the impact of inflammation on DNA alterations associated with mutagenic and carcinogenic effects (cell cycle arrest, DNA repair, cell proliferation, and apoptosis).

In vivo experimental studies

Carbon nanotubes

Lam et al. [70] were among the first groups to investigate the pulmonary effects of intratracheal instillation of carbon nanotube suspensions. Mice received intratracheal instillations of raw SWCNTs, SWCNTs treated to remove metallic residues, carbon black nanoparticles, and quartz nanoparticles. Histological examination of the lungs 90 days later showed the presence of these nanomaterials in the alveoli [70]. SWCNTs (with or without metallic residues) induced an inflammatory response with granulomas surrounding the nanotubes, indicating toxicity [71]. Similar granulomas were found in rats intratracheally instilled with SWCNTs, although the inflammation resolved within 3 months [72].

Subsequent studies established that inflammation occurred chiefly with poorly dispersed carbon nanotubes.
that aggregated into microscale particles. A dispersed preparation of SWCNTs given by pharyngeal aspiration to mice caused alveolar and interstitial fibrosis, whereas less well-dispersed SWCNTs produced a granulomatous reaction [72]. These findings were confirmed by two very recent studies in mice exposed to aerosolized MWCNTs (6 hours per day for 24 days). These are the first two studies of the effects of inhaled carbon nanotubes [73,74]. The pulmonary lesions ranged from absent to moderate (alveolar fibrosis without granuloma formation). Nevertheless, one of these studies [73], in which no lung inflammation or tissue damage was found, showed evidence of systemic immunosuppression after 14 days after inhalation for 6 hours/24 hours of MWCNTs in doses of 0.3 to 5 mg/m². There was a decrease in the T-cell-dependent antibody response and increased expression of interleukin-10 messenger RNA in spleen homogenates [73].

In ApoE−/− transgenic mice given a single intratracheal instillation of SWCNTs, examination of the aorta after 7, 28, and 60 days showed mitochondrial DNA damage and oxidative stress [75]. When the SWCNTs were instilled intratracheally once a week for 8 weeks, acceleration of atheroma plaque formation in the aorta was noted [75]. The fibrotic response of the lung to carbon nanotubes may involve oxidative stress, as it is exaggerated in mice deficient in vitamin E, which has antioxidant properties [76]. A recent study by the same group found evidence of another systemic effect: SWCNTs given by pharyngeal aspiration led to a substantial increase in the severity of experimentally induced Listeria monocytogenes pneumonia. Decreases in nitric oxide production and bacterial phagocytosis by alveolar macrophages were noted [77].

MWCNTs induced malignant mesothelioma in p53−/− transgenic mice [78]. MWCNTs or crocidolite (asbestos) fibers administered as a single intraperitoneal injection induced mesothelioma in 15.8% (3/19) and 31.6% (6/19) p53−/− animals, respectively. In contrast, no cases of mesothelioma were seen among p53−/− mice exposed to fullerene nanoparticles or unexposed p53−/− mice [78].

Another experimental study found a fiber-like effect of MWCNTs injected intraperitoneally to mice [79]. Each animal received a single injection of 100 µg/mL of short or long amosite (asbestos) fibers or of short or long MWCNTs. Compared to the short fibers, long amosite fibers and long MWCNTs, respectively, induced a stronger inflammatory response and larger numbers of inflammatory granulomas [79].

**Metallic nanoparticles**

In general, TiO₂ nanoparticles administered into the lungs produce an inflammatory response with increased total bronchoalveolar lavage fluid cell counts and a predominance of macrophages and neutrophils [49,80–83]. This inflammatory response was often described as transient, occurring 24 hours after inhalation or intratracheal instillation of the nanoparticles and usually resolving within 2 weeks [49,81]. As mentioned above, several physicochemical parameters seem involved in the inflammatory response, including dose, size, and crystalline structure. In mice, aerosols of TiO₂ nanoparticles having a primary size of 2 to 5 nm induced an exaggerated inflammatory response only when given in the highest dose of 8.88 mg/m³; lower doses (0.77 or 7.22 mg/m³) had little effect [81]. The inflammatory response was detected after 2 weeks but not after 3 weeks. Studies suggest that nanoparticle size may have a major influence on biological effects. When administered intranasally to mice, TiO₂ generates an inflammatory response only within the nanoscale (29 and 14 nm), no inflammation being seen with fine particles (250 nm) [82]. In this study, the nanoparticles but not the fine particles of TiO₂ acted as adjuvants to allergic sensitization [82]. Compared to the same mass of fine particles (250 nm) of TiO₂ anatase, 20-nm particles instilled intratracheally in rats and mice induced a considerably stronger inflammatory response (with increased neutrophil counts in bronchoalveolar lavage fluid 24 hours after the administration) [20]. However, when the dose was expressed as the surface area of retained particles, the dose-effect curve of the inflammatory response was not significantly different between the two particle sizes. Thus, surface area may be a more relevant metric than mass or particle count when investigating biological effects [20]. Finally, a major impact of crystalline structure has been reported. In rats given intratracheal fine or ultrafine rutile TiO₂ particles or ultrafine anatase/rutile (80/20) particles, the rutile crystalline structure was associated with only transient inflammation regardless of particle size, whereas the lung inflammation induced by the anatase/rutile mixture was still present 3 months after the exposure [80].

A prothrombotic effect was reported recently in rats intratracheally instilled with TiO₂ rutile nanoparticles in doses of 1 and 5 mg/kg [84]. Findings after 24 hours included increased alveolar macrophage and neutrophil counts in bronchoalveolar lavage fluid, pulmonary and cardiac edema, and systemic inflammation with increased monocyte and granulocyte counts and platelet aggregation.

Finally, an emphysema-like condition was described in ICR mice only 2 weeks after a single intratracheal instillation of TiO₂ nanoparticles (19–21 nm) [83]. The histopathological examination of the lungs showed disrupted alveolar septa, type II pneumocyte hyperplasia, epithelial cell apoptosis, and inflammation with increased macrophage counts. This is the first evidence to date of an emphysema-like effect. Interestingly, the induction of emphysema-like lesions contrasts with the predominantly fibrotic lesions seen with microscale TiO₂ particles.

**Conclusions**

Available in vivo and in vitro studies consistently showed biological effects of nanomaterials (including carbon nanotubes and TiO₂) on the respiratory system. These effects included oxidative stress generation, proinflammatory effects, prothrombotic effects, lung fibrosis, emphysema-like lung disease, and DNA damage. However, the amount of published scientific information remains scant. The expanding use of nanoparticles, most notably in the industry and in medicine, together with the industrial-scale production of nanoparticles, indicates an urgent need for additional information on the potential and established health effects of nanoparticles. The underlying pathogenic mechanisms must be elucidated. Improved knowledge of the potential biological effects of nanoparticles is required to develop
appropriate preventive methods for workers and for the general population if needed.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

KEY POINTS

- Whether nanoparticles generate meaningful toxic effects remains unclear.
- Few data are available on nanoparticle toxicity in humans. Factors that affect nanoparticle toxicity include size, shape, total surface area, chemical composition, degree of aggregation/agglomeration, and surface coating.
- The smaller size of nanoparticles compared to microscale particles is responsible for differences in deposition patterns within the respiratory system.
- Nanoparticle retention within the lungs depends chiefly on particle size, physical clearance mechanisms (mucociliary escalator, macrophage phagocytosis, and epithelial translocation), and chemical clearance mechanisms (dissolution of soluble particles, lixiviation, and protein binding).
- Whether nanoparticles can translocate from the respiratory system to other tissues in humans is still a matter of debate.
- The biological effects of nanoparticles on the respiratory system may include oxidative stress, proinflammatory effects, prothrombotic effects, and DNA damage. Lung fibrosis and emphysema may develop.

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