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Immunotoxicological impact of occupational and environmental nanoparticles exposure: The influence of physical, chemical, and combined characteristics of the particles

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Abstract

While nanotechnology is growing exponentially, the knowledge of the impact of nanoparticles (NPs) on public health and the environment is limited so far. Current nanomaterial research is focused on the applications of nanotechnology, whereas there is little information on exposure assessment and risk characterization associated with NPs. Therefore, it is essential that the factors influencing NPs associated hazards be studied. This review seeks to survey and evaluate the current literature in order to better understand the impact of both airborne and engineered NPs exposure, the mechanisms at the cellular level, and the factors influencing their immunotoxicity. In fact, NPs do have immunotoxicological significance, as immune cells in the bloodstream and tissues do act to eliminate or interact with NPs.

Proper characterization of the NPs as well as understanding the processes occurring on the NPs surface when in contact with biological systems is crucial to predict or exclude toxicological effects.

Keywords

environmental exposure, health effects, immunotoxicity, nanoparticle exposure, occupational exposure

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Introduction

Two decades of nanotoxicology research have shown that the interactions between nanoparticles (NPs) and cells, animals, humans, and the environment are remarkably complex; this complexity derives from NPs' ability to bind and interact with biological matter and change their surface characteristics.

Humans are continually exposed to NPs generated by both natural processes and industrial activities. Photochemical reactions in the troposphere, crustal erosion, volcanic eruptions, and forest fires represent the main natural sources of NPs. NPs are also produced as toxic by-products[1,2] during industrial activities, i.e. combustion processes, or they are produced and designed intentionally with very specific properties related to shape, size, surface properties, and chemical composition to be used in many applications in our common life such as cosmetics, sporting goods, tires, stain-resistant clothing, sunscreens, toothpaste, food additives,

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etc.[3,4] Engineered NPs are produced in the gas phase by chemical transformation at high temperatures or as powders or slurries/suspensions/solutions from chemical solution deposition (sol-gel processes).[5–7] It should be noted, finally, that NPs are generated in indoor environment by vacuum cleaners, office printers, ironing, candles, hair dryers, or steam irons.[8–10]

There have been several studies demonstrating that NPs have biological effects on the cardiopulmonary, neurological, gastrointestinal, and skin systems; however, the effects on the immune system have not been fully identified, due to paucity of studies to date.[11–15] Studies have revealed that the same properties that make NPs so unique could also be responsible for their potential toxicity.[16,17] Compared to larger particles, NPs have a higher deposition rate in the peripheral lung, they can cross the pulmonary epithelium and reach the interstitium, and may thus be systemically distributed in the bloodstream, raising the possibility of increasing the level of inflammation. NPs have an enhanced capacity to produce ROS and, consequently, have a widespread toxicity since ROS generation by particles can exert protein, lipid, and membrane damage.[18–20]

It is hypothesized that exposure to NPs may induce immunotoxicity resulting in detrimental effects on immune function. NPs can stimulate and/or suppress the immune responses: inadvertent suppressed immunological function caused by NPs exposures may result in increased incidence and severity of infectious diseases and cancer. In contrast, inappropriate enhancement of immune function or the generation of hypersensitivity could possibly exacerbate the development of allergic and/or autoimmune diseases.[21] The NPs tropism with the immune system is largely determined by their physico-chemical properties, such as higher degree of surface charge and proton exchangeability.[22,23]

This review seeks to evaluate the current literature in order to better understand the impact of both occupational and environmental NPs exposure and the factors influencing their immunotoxicity. The focus will be put on the mechanics and biochemistry of toxicity, as well as toxicity-related risk factors and health implications.

The review of the most relevant contributions to the literature in the fields of toxicology (including *in vitro* and *in vivo* studies), industrial hygiene, and epidemiology is reported starting with the information retrieved from PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (http://scholar.google.com), and ScienceDirect (www.sciencedirect.com) using the following keywords: nanoparticle AND immunotoxicity AND source OR environment OR occupation AND health OR physico-chemical characteristic.

The search yielded more than 150 articles which were further reviewed; at the end of this selection process, 96 articles were deemed relevant to this review and were examined with a particular emphasis on two topics: chemical and physical properties, and mechanism of toxicity in relation to the influence of physical, chemical, and combined characteristics.

Physical, chemical, and combined characteristics of NPs affecting cellular uptake, translocation, and discharge

The mechanism of cellular uptake of NPs may depend on their specific physico-chemical properties such as size, shape, chemical structure, particle density, solubility, colloidal stability, agglomeration state, and surface charge.

The small size of NPs gives them an increased opportunity for uptake into biological systems, allowing them, for example, to enter tissues, cells, and subcellular organelles, and enabling their direct binding to functional biomolecular structures. As the size decreases, the surface area per unit mass increases and NPs may experience very strong surface forces like van der Waals, electrostatic forces, and chemical bonding.[24]

An inhalation study in rats showed that 20 nm NPs of titanium dioxide (TiO_2) are characterized by longer retention time in the lungs and increased translocation to interstitial sites compared to the larger NPs (250 nm) of the same material. Small NPs that evade the alveolar macrophages penetrate the alveolar epithelium, resulting in a slower clearance rate from the lung and possibly later translocation to the circulatory and lymphatic system.[25] Alveolar macrophages on the surface of the lungs appear not to be able to recognize particles of less than 70 nm as being "foreign", thus allowing them to gain access to the pulmonary interstitium, and then capillary blood flow.[26] Moreover, fiber- or tube-shaped NPs can present a problem for macrophage-mediated clearance if the NPs exceed the width of the engulfing phagocyte. For example,

migration of macrophages containing carbon nanotube (CNT) across the pleura could cause DNA damage to mesothelial cells similar to asbestos fiber.[20]

Surface charge also plays an important role in governing cellular uptake of NPs. The plasma membrane is negatively charged, as is the intracellular environment, thus anionic NPs may be endocytosed at a lower rate than the cationic ones. Additionally, DNA is negatively charged, thus cationic NPs are more likely to interact with the genetic material.[27–30]

A number of studies have found that the shape of NPs can highly influence their rate of uptake. Spherical NPs show higher uptake than nanorods, while internalization of these cylindrical shaped materials is strongly influenced by their dimensions so that high-aspect ratio particles are internalized considerably faster than low-aspect ratio particles, i.e. more spherical particles.[31–33]

Moreover, some studies have found that particle shape may also interfere with the clearance mechanisms. Nanofibers measuring more than 20 μm in one axis are too long to be phagocytosed; they induce a rather general non-specific pulmonary inflammatory response, including release of chemokines, cytokines, reactive oxygen species (ROS), and other mediators, which can result in sustained inflammation and eventually fibrotic changes.[34,35]

NPs that reach the lung are predominantly cleared via: mucociliary escalator into the gastrointestinal tract;[36] lymphatic system;[37] and circulatory system.[38] From the lymphatic and circulatory systems, NPs may be distributed to organs, including kidneys, from where partial or total clearance may occur.

When NPs reach the systemic circulation, the particles can interact with plasma proteins, coagulation factors, platelets, and red and white cells. In particular, the binding to plasma components may have a substantial effect on the distribution and excretion of NPs.[39]

Several studies show that interstitially injected NPs pass preferentially through the lymphatic system and not the circulatory system, probably due to permeability differences. After entering the lymphatic system, they are located in the lymph nodes. NPs that are able to enter the circulatory system can also gain access to the interstitium and, from there, they can be drained through the lymphatic system to the lymph nodes as free NPs and/or inside macrophages.[40]

In vivo pharmacokinetic studies of radiolabeled solid lipid NPs revealed significant lymphatic uptake and a high rate of distribution in peri-aortic, axillar, and inguinal lymph nodes after inhalation in rats.[41] The deposited NPs that are insoluble in the lining fluid of the lungs are taken up less efficiently by the macrophages.[42] The phagocytosed NPs may be destroyed once within the lysosomes of phagocytic cells. Therefore, it is evident that for the NPs consisting of protein drug, macrophage engulfment usually means eventual digestion of the protein.[43] The NPs sequestered by the macrophages may also be transported to regional lymph nodes and may subsequently migrate to systemic circulation. [23]

The dermis has a rich supply of blood and macrophages, lymph vessels, dendritic cells, and nerve endings. Therefore, the particles that cross through the stratum corneum and into the epidermis and dermis are potentially available for recognition by the immune system. NPs with a dye penetrated deeper into hair follicles of massaged porcine skin *in vitro* and persisted longer in human skin *in vivo* than the dye in solution. Carboxylated quantum dots of 30 nm applied to the skin of mice were localized in the folds and defects in the stratum corneum and hair follicles. A small amount penetrated as deep as the dermis.[44] The NPs that penetrate to the dermis might enter the lymphatic system, and the NPs or dissolved components distribute systemically.[45,46]

Mechanism of toxicity and influence of physical, chemical, and combined properties

Toxicity of NPs has been thought to originate from NPs' size and surface area, composition, shapes, and so forth as reviewed in the following sections.

Particle size and surface area play a major role in the interaction with biological system; Liu et al. tested the inhibiting effect of different sized nano-TiO_2 on the cell survival rate for rat astrocytes *in vitro* and *in vivo*: nano-TiO_2 is toxic to rat neuroglia cells; this toxicity may be related to particle size and the mechanism may be associated with its induction inflammation.[47]

Particle chemistry is significant in determining NPs toxicity; depending on their chemistry, NPs can show different cellular uptake, subcellular

localization, and ability to catalyze the production of ROS.[48] Though particles may have the same composition, they may have different chemical or crystalline structure. The toxicity of a material depends on its type of crystalline form. NPs can change crystal structure after interaction with water or liquids.[49]

Nanomaterial purity is also an important factor as residual contaminating metals may actually be responsible for (geno) toxicological responses rather than the actual nanomaterial itself, the quantity of which depends upon the synthesis procedure employed. However, where metals are present as impurities, iron is one of the primary sources of damage as it induces oxidative stress through Fenton or Haber-Weiss reactions. Indeed, iron contaminants in CNT have been shown to result in a substantial loss of glutathione and increased lipid peroxidation in alveolar macrophages indicators of oxidative stress.[50,51]

Inflammation and immunological effects. The predominant process underlying the pathological effects of NPs is inflammation, involved in atherothrombosis, lung disease, neurodegenerative diseases, and cancer.[52,53] While the exact mechanism in which NPs induce pro-inflammatory effects is not known, it has been suggested that they create ROS, and thereby modulate intracellular calcium concentrations, activate transcription factors, and induce cytokine production. Therefore, the ability of NPs to initiate, prolong, or worsen inflammation can be seen as a key property.

To date, there are many studies demonstrating that NPs can exhibit inflammatory responses. Their small size, the shape, and thus large surface area appear to be centrally involved in promoting inflammation. The surface area dose-related inflammatory response is partly related to transition metals but is also found in low-toxicity materials and the cellular mechanism is not well understood.[54]

It has been reported that the shape of some NPs can also play important roles in the toxic and immunological effects. Human lung epithelial A549 cells were exposed to Ag nanowires (length, 1.5–25 μm; diameter, 100–160 nm), spherical Ag NPs (30 nm), and Ag microparticles (<45 μm), producing distinct effects. It was found that Ag nanowires resulted in calcium influx, the strongest cytotoxicity (reduced cell viability and increased LDH release) and immunological responses (cytokine production and increased activation of NF-κB), whereas spherical Ag particles had negligible effects on cells. The possible reason is that the wires can exclusively contact with the cell surface rather than being internalized.[55]

The larger surface area enhances the catalytic activity of the material and thus has been well reported to increase its reactivity because surface atoms have a tendency to have unsatisfied high energy bonds.[56,57] These NPs are able to gain access to the cellular environment and there is a much greater chance that the enhanced surface area as compared to counterpart micron-sized particles will result in interaction with biomolecules, causing direct cellular damage and promoting oxidative stress, as demonstrated by *in vitro* experiments.[58,59]

A wide range of NPs has been found to have the ability to cause pro-inflammatory effects.[58] Studies have shown that ultrafine carbon black and TiO_2 NPs are associated with greater inflammatory potency in the lungs of rats following intratracheal instillation as compared to their fine counterparts.[60–62] Gui et al. showed increased expression of IL-1β, IL-2, IL-4, IL-6, IL-10, and IL-18, in nephritic inflammation caused by TiO_2 NPs intragastrically administered to mice.[63] Moon et al. showed that the levels of pro-inflammatory mediators, such as IL-1β, TNF-α, and macrophage inflammatory protein (MIP)-2, in BALF and mRNA expression of TNF-α and IL-1β in lung tissue were elevated post-exposure to TiO_2 NPs in mice. TiO_2 exposure resulted in significant activation of inflammatory signaling molecules, such as c-Src and p38 MAP kinase in lung tissue and alveolar macrophages, and the nuclear factor (NF)-kappa B pathway in pulmonary tissue.[64]

Regarding the impact on innate immunity, recently, several studies have demonstrated the effects of NPs on innate immunity via toll-like receptors (TLRs) signaling pathways. Several nanoparticles (e.g. TiO_2, ZnO, zirconium dioxide [ZrO_2], and silver) modulated immune responses via TLRs. TLRs may have important roles not only in different NPs uptake but also in their cellular response. Moreover, the mechanisms of interaction between NPs and TLR are still unclear. The TLRs signaling cascade results in the activation of transcription factors, nuclear factor κ light chain enhancer of activated B cells (NF-B), interferon-regulatory factors (IRFs), and mitogen-activated protein kinase; these factors affect the transcription

of genes involved in inflammatory and immune responses.[65]

As for adaptive immunity, due to their position at physiological-environmental interfaces the immune cells will interact with NPs. Specifically, NPs may facilitate allergic diseases via IgE-dependent response and/or interactions with dendritic cells, lymphocytes, eosinophils, and mast cells/basophils.[21,67] In particular, mast cells are well recognized for their role in facilitating allergic responses in many disease conditions through allergen crosslinking of the high affinity IgE receptor and subsequent release of histamine and a multitude of inflammatory mediators. Due to their location and immune regulatory role, mast cells can contribute significantly to NPs toxicity and may represent a key off-target cell type that could contribute significantly to allergic immune responses following NPs exposure.[21,66]

Inoue et al. found that dosing mice with diesel NPs exacerbates lung inflammation induced by LPS (endotoxin or lipopolysaccharide). Lung homogenates derived from the LPS + NPs mice tended to have an increased tumor necrosis factor-α level and chemotaxis activity for polymorphonuclear leukocytes.[67]

Results on pro-inflammatory effects of NPs have also been reported from clinical studies in humans. In a recent explorative analysis, the increase of particulate and gaseous air pollution was associated with multiple changes in the differential white blood cell count in patients with chronic pulmonary diseases. The researchers found an immediate decrease of polymorphonuclear leukocytes in response to an increase of particulate pollutants. Lymphocytes increased within 24 h in response to with all gaseous pollutants but showed only minor effects in regard to particulate air pollution. Monocytes showed an increase associated with ultrafine particles and nitrogen monoxide. The effect had two peaks in time, one 0–23 h before blood withdrawal and a second one with a time lag of 48–71 h.[68,69]

Recent animal and cell culture studies report that airborne NPs exert a significant effect on activating the stress axis, stimulate brain inflammatory markers, and cause neurodegeneration in oxidative stress-prone animals.[70–72] Microglia appear to play a central role in these inflammatory changes through mechanisms of innate immunity. Microglia located in proximity to fenestrated or "leaky" blood brain barriers (median eminence, area postrema, subfornical organ, and periventricular regions of the hypothalmus) can be signaled by circulating NPs particles themselves or by inflammatory cytokines/chemokines being released systemically from the chronically inflamed airways. This signaling can result in NF-κB activation, upregulation of the innate immune toll-like receptor 2 (TLR-2) and initiation of the inflammatory sequelae described in the brain and microglia exposed to concentrated air particles.[73,74]

The predisposing condition of oxidative stress appears to enhance the neurotoxicity of NPs exposure; exposure to NPs present in urban environments has been shown to induce systemic pro-oxidant and pro-inflammatory effects in apolipoprotein E knockout (ApoE–/–) mice and pro-inflammatory central nervous system (CNS) effects in BALB/c mice.[70]

Current research performed in mice investigated whether long-term exposure to ambient ultrafine airborne particulate matter affects their cognition, affective responses, hippocampal inflammatory cytokines, and neuronal morphology. Hippocampal pro-inflammatory cytokine expression was elevated and apical dendritic spine density and dendritic branching were decreased in the hippocampal CA1 and CA3 regions, respectively. These data suggest that long-term exposure to particulate air pollution levels typical of exposure in major cities can alter affective responses and impair cognition.[75]

As regards the effects of combustion-generated NPs, *in vitro* studies on keratinocyte cell lines (HaCaT) showed that organic carbon NPs formed at high temperature in combustion induced a time-dependent increase of pro-inflammatory lysophospholipid production.[76]

DNA damage. NPs have been shown to cause oxidative stress, an internal metabolic event that can induce DNA damage. This damage can invoke various cellular responses such as cell cycle arrest, apoptosis, and importantly, DNA repair. When DNA is damaged a key effector molecule that is activated is p53. This tumor suppressor gene has been described as "the guardian of the genome" as it is responsible for arresting the cell cycle and activating transcription of genes that mediate DNA repair, thus preventing the conversion of damage to mutation.[77,78] However, if the damage is extensive, then p53 triggers apoptosis in order to eliminate the individual cell for the benefit of the organism.

Eder et al. have analyzed the effect of a mixture of fine TiO_2 and ultrafine carbon black Printex 90 particles (P90) on the expression of cytochrome P450 1B1 (CYP1B1) in human monocytes, macrophages, bronchial epithelial cells, and epithelial cell lines. CYP1B1 expression is strongly downregulated by P90 in monocytes with a maximum after P90 treatment for 3 h while fine and ultrafine TiO_2 had no effect. CYP1B1 was downregulated up to 130-fold and in addition CYP1A1 mRNA was decreased 13-fold. *In vitro* generated monocyte-derived macrophages (MDM), epithelial cell lines, and primary bronchial epithelial cells also showed reduced CYP1B1 mRNA levels. The P90-induced reduction of CYP1B1 was also demonstrated at the protein level using Western blot analysis.[79]

Combustion-generated NPs exposure might be responsible for an enhancement of skin aging which involves apoptotic cell death, as suggested by *in vitro* studies on human keratinocyte HaCaT cell line.[13] These polluting NPs also induce endothelial cells (EC) death through the same apoptotic mechanism, as demonstrated in a study which provides the first evidence that exposure of EC to combustion-generated NPs could play a significant role in triggering cardiovascular events via either affecting EC functionality or promoting the inflammatory cascade.[76]

Oxidative stress. A key mechanism thought to be responsible for the genotoxic effects exerted by NPs involves oxidative stress, which refers to a redox imbalance within cells usually as a result of increased intracellular ROS and decreased antioxidants. ROS generation is considered as being the main underlying chemical process in nanotoxicology, leading to secondary processes that can ultimately cause cell damage and even cell death.[80,81] Moreover, ROS is one of the main factors involved in inflammatory processes. Intracellular oxidative stress can activate inflammation via oxidative stress-responsive signaling pathways, resulting in the expression of pro-inflammatory genes that are involved in the recruitment and activation of cytokines, chemokines, and adhesion molecules. The genes that regulate inflammation are under control of transcription factors such as NF-kB and AP-1, both of which are redox sensitive and both of which have been demonstrated to be activated in macrophages exposed to carbon black NPs.[54]

Both *in vivo* and *in vitro* studies have shown that NPs of various compositions (fullerenes, carbon nanotubes, quantum dots, and automobile exhaust) create ROS.[82–84]

It is believed that the reactivity of the surface area itself or the species (transition metals, organics) which are adsorbed to the outer surface of the particles may contribute to their reactivity and oxidative potency.[56]

The transition metals ions (such as cadmium, chromium, cobalt, copper, iron, nickel, titanium, and zinc) released from certain NPs have the potential to cause the conversion of cellular oxygen metabolic products such as H_2O_2 and superoxide anions to hydroxyl radicals (*OH), which is one of the primary DNA damaging species. In addition to composition, the high surface area associated with NPs can promote the generation of ROS. Consequently, the smaller the NPS, the higher the oxidative stress they induce.[85,86]

Moon et al. examined acute pulmonary responses in an animal study after intraperitoneal administration of TiO_2 NPs, at rest or in lungs primed with lipopolysaccharide. TiO_2 exposure increased neutrophil influx, protein levels in bronchoalveolar lavage fluid, ROS activity of BAL cells 4 h after exposure.[64]

Manna et al. found an increased oxidative stress and inhibition of cell proliferation in response to treatment of keratinocytes with single-walled carbon nanotubes (SWCNTs) and suggest that nanotubes can activate NF-kB in a dose-dependent manner.[87]

De Marzi et al. investigated the effects of short-term and long-term ceria NPs (CeO_2-NPs) exposure to A549, $CaCo_2$, and $HepG_2$ cell lines showing that after 24-h exposure NPs can induce ROS damages. However NPs can induce genotoxic damage to the cell when present alone in the cell culture medium but can, conversely, protect the cells from oxidative stress when another genotoxic compound is also present.[86]

Reports regarding the toxicity of TiO_2 NPs under UV light irradiation have been shown to suppress tumor growth in cultured human bladder cancer cells via ROS, the data regarding their effects in the absence of UV radiation are contradictory.[88,89]

Recent studies have observed an increase in generation of ROS, activation of several kinases such as ERK and AKT, and downregulation of expression of dual-specificity phosphatases

(DUSPs) in silver NPs-exposed human neuroblastoma SH-SY5Y cells; this suggests that silver NPs could modulate the intracellular signaling pathways to lead to neuronal differentiation.[90]

Conclusion

Nanotechnology is a fast-growing field of activity that will allow for development of materials with new properties. The number of subjects exposed to NPs should increase over the next few years, in a context in which the impact of NPs on occupational health and safety is currently difficult to predict.

The main factors that determine the toxicological effects of NPs in the body are the characteristics of the exposure (e.g. penetration route, duration, and concentration) and of the exposed organism (e.g. individual susceptibility, activity at time of exposure, and the particular route the NPs follow in the body), and the intrinsic toxicity of NPs (e.g. catalytic activity, composition, electronic structure, capacity to bind or coat surface species, surface area).

The generation of ROS and oxidative injury is thought to play a significant role in many of the observed biological responses to NPs. The size, surface area, and surface of particular NPs are thought to play a role in the generation of ROS. The ROS, in addition to their damaging effects on cellular proteins, lipids, and DNA, can activate inflammation via oxidative stress-responsive signaling pathways, resulting in the expression of genes that are involved in the recruitments and activation of cells that are characteristic of inflammation. Furthermore, NPs may induce or aggravate inflammatory responses by directly influencing immune-related cells. Persistent oxidative stress and inflammation are thought to be the underlying cause of human diseases.

There are reports of toxicity following *in vitro* and *in vivo* exposure to many NPs, but there is a small amount of information about exposure assessment and the risk characterization.[91–95]

The recognized toxic effects and the physicochemical characteristics of NPs justify the immediate application of all useful measures, based on the precautionary principle, to limit exposure and protect the health of potentially exposed individuals.[96] At the same time, it would also be useful to have available analytical procedures and devices suitable for the sampling of NPs present in the ambient air. Analogously, the individuation of substances that can be used as exposure biomarkers for biological monitoring purposes should be an object of research.

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References

1. D'Anna A (2009) Combustion-formed nanoparticles. *Proceedings of the Combustion Institute* 32: 593–613.
2. Wang H (2011) Formation of nascent soot and other condensed-phase materials in flames. *Proceedings of the Combustion Institute* 33: 41–67.
3. Yokel RA and MacPhail RC (2011) Engineered nanomaterials: Exposures, hazards, and risk prevention. *Journal of Occupational Medicine and Toxicology* 6: 7.
4. Soto KF, Carrasco A, Powell TG, et al. (2005) Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. *Journal of Nanoparticle Research* 7: 145–169.
5. Baldauf R, Thoma E, Hays M, et al. (2008) Traffic and meteorological impacts on near-road air quality: Summary of methods and trends from the Raleigh near-road study. *Journal of the Air and Waste Management Association* 58: 865–878.
6. Linak WP, Miller CA and Wendt JO (2000) Comparison of particle size distribution and elemental partitioning from the combustion of pulverized coal and residual fuel oil. *Journal of the Air and Waste Management Association* 50: 1532–1544.
7. Rogers F, Arnott P, Zielinska B, et al. (2005) Real-time measurements of jet aircraft engine exhaust. *Journal of the Air and Waste Management Association* 55: 583–593.
8. Afshari A, Matson U and Ekberg LE (2005) Characterization of indoor sources of fine and ultrafine particles: A study conducted in a full-scale chamber. *Indoor Air* 15: 141–150.
9. See S W and Balasubramanian R (2006) Risk assessment of exposure to indoor aerosols associated with Chinese cooking. *Environmental Research* 102: 197–204.
10. Wallace LA, Emmerich SJ and Howard-Reed C (2004) Source strengths of ultrafine and fine particles due to cooking with a gas stove. *Environmental Science & Technology* 38: 2304–2311.

11. Di Gioacchino M, Petrarca C, Lazzarin F, et al. (2011) Immunotoxicity of nanoparticles. *International Journal of Immunopathology and Pharmacology*. 24: 65–71.
12. Lomer MCE, Thompson RPH and Powell JJ (2002) Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease *Proceedings of the Nutrition Society* 61: 123–130.
13. Pedata P, Boccellino M, La Porta R, et al. (2012) Interaction between combustion-generated organic nanoparticles and biological systems: In vitro study of cell toxicity and apoptosis in human keratinocytes. *Nanotoxicology* 6: 338–352.
14. Sharma HS and Sharma A (2013) New perspectives of nanoneuroprotection, nanoneuropharmacology and nanoneurotoxicity: Modulatory role of amino acid neurotransmitters, stress, trauma, and co-morbidity factors in nanomedicine. *Amino Acids* 45: 1055–1071.
15. Wang X, Reece SP and Brown JM (2013) Immunotoxicological impact of engineered nanomaterial exposure: Mechanisms of immune cell modulation. *Toxicology Mechanisms and Methods* 23: 168–177.
16. Arora S, Rajwade JM and Paknikar KM (2012) Nanotoxicology and in vitro studies: The need of the hour. *Toxicology and Applied Pharmacology* 258: 151–165.
17. Brown DM, Wilson MR, MacNee W, et al. (2001) Size-dependent proinflammatory effects of ultrafine polystyrene particles: A role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology and Applied Pharmacology* 175: 191–199.
18. Thompson EA, Sayers BC, Glista-Baker EE, et al. (2014) Innate immune responses to nanoparticle exposure in the lung. *Journal of Environmental Immunology and Toxicology* 1: 150–156.
19. Dick CA, Brown DM, Donaldson K, et al. (2003) The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. *Inhalation Toxicology* 15: 39–52.
20. Li N, Xia T and Nel AE (2008) The role of oxidative stress in ambient particulate matter induced lung diseases and its implications in the toxicity of engineered nanoparticles. *Free Radical Biology and Medicine*. 44: 1689–1699.
21. Shannahan JH and Brown JM (2014) Engineered nanomaterial exposure and the risk of allergic disease. *Current Opinion in Allergy and Clinical Immunology* 14: 95–99.
22. Inoue KI and Takano H (2011) Aggravating impact of nanoparticles on immune-mediated pulmonary inflammation. Special Issue: Nanoparticles and inflammation. *The Scientific World Journal* 11: 382–390.
23. Yang W, Peters JI and Williams III RO (2008) Inhaled nanoparticles: A current review. *International Journal of Pharmaceutics* 356: 239–247.
24. Johnston H, Brown D, Kermanizadeh A, et al. (2012) Investigating the relationship between nanomaterial hazard and physicochemical properties: Informing the exploitation of nanomaterials within therapeutic and diagnostic applications. *J Control Release* 164: 307–313.
25. Geiser M, Rothen-Rutishauser B, Kapp N, et al. (2005) Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environmental Health Perspectives* 113: 1555–1560.
26. Moghimi SM and Hunter AC (2001) Capture of stealth nanoparticles by the body's defences. *Critical Reviews in Therapeutic Drug Carrier System* 18: 527–550.
27. Jiang J, Oberdorster G and Biswas P (2009) Characterization of size, surface charge and agglomeration state of nanoparticle dispersions for toxicological studies. *Journal of Nanoparticle Research* 11: 77–89.
28. Lockman PR, Koziara JM, Mumper RJ, et al. (2004) Nanoparticle surface charges alter blood–brain barrier integrity and permeability. *Journal of Drug Targeting* 12: 635–641.
29. Nan A, Bai X, Son SJ, et al. (2008) Cellular uptake and cytotoxicity of silica nanotubes. *Nano Letters* 8: 2150–2154.
30. Sabbioni E, Fortaner S, Farina M, et al. (2014) Interaction with culture medium components, cellular uptake and intracellular distribution of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts. *Nanotoxicology* 8: 88–99.
31. Chithrani BD and Chan WC (2007) Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Letters* 7: 1542–1550.
32. Gratton SE, Ropp PA, Pohlhaus PD, et al. (2008) The effect of particle design on cellular internalization pathways. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11613–11618.
33. Muller J, Huaux F, Fonseca A, et al. (2008) Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: Toxicological aspects. *Chemical Research in Toxicology* 21: 1698–705.
34. Borm PJ and Kreyling W (2004) Toxicological hazards of inhaled nanoparticles potential implications for drug delivery. *Journal of Nanoscience and Nanotechnology* 4: 521–531.
35. Hoet PH, Bruske-Hohlfeld I and Salata OV (2004) Nanoparticles-known and unknown health risks. *Journal of Nanobiotechnology* 2: 12.
36. Semmler M, Seitz J, Mayer P, et al. (2004) Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhalation Toxicology* 16: 453–459.

37. Liu J, Wong HL, Moselhy J, et al. (2006) Targeting colloidal particulates to thoracic lymph nodes. *Lung Cancer* 51: 377–386.
38. Oberdörster G, Oberdörster E and Oberdörster J (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles *Environmental Health Perspective* 113: 823–839.
39. Lovric J, Bazzi HS, Cuie Y, et al. (2005) Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *Journal of Molecular Medicine* 83: 377–385.
40. Shwe TTW, Yamamoto S, Kakeyama M, et al. (2005) Effect of intratracheally instillation of ultrafine carbon black on proinflammatory cytokine and chemokine release and mRNA expression in lung and lymph-nodes of mice. *Toxicology and Applied Pharmacology* 209: 51–61.
41. Videira MA, Botelho MF, Santos AC, et al. (2002) Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. *Journal of Drug Targeting* 10: 607–613.
42. Chono S, Tanino T, Seki T, et al. (2006) Influence of particle size on drug delivery to rat alveolar macrophages following pulmonary administration of ciprofloxacin incorporated into liposomes. *Journal of Drug Targeting* 14: 557–566.
43. Lombry C, Edwards DA, Preat V, et al. (2004) Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. *American Journal of Physiology: Lung Cellular and Molecular Physiology* 286: 1002–1008.
44. Filipe P, Silva JN, Silva R, et al. (2009) Stratum corneum is an effective barrier to TiO_2 and ZnO nanoparticle percutaneous absorption. *Skin Pharmacology and Physiology* 22: 266–275.
45. Mortensen LJ, Oberdörster G, Pentland AP, et al. (2008) In vivo skin penetration of quantum dot nanoparticles in the murine model: The effect of UVR. *Nano Letters* 8: 2779–2787.
46. Gopee NV, Roberts DW, Webb P, et al. (2007) Migration of intradermally injected quantum dots to sentinel organs in mice. *Toxicological Sciences* 98: 249–257.
47. Liu Y, Xu Z and Li X (2013) Cytotoxicity of titanium dioxide nanoparticles in rat neuroglia cells. *Brain Injury* 7: 934–939.
48. Xia XR, Monteiro-Riviere NA and Riviere JE (2010) Skin penetration and kinetics of pristine fullerenes (C60) topically exposed in industrial organic solvents. *Toxicology and Applied Pharmacology* 242: 29–37.
49. Buzea C, Pacheco B and Robbie K (2007) Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases* 2: 17–71.
50. Ghio AJ, Stonehuerner J, Dailey LA, et al. (1999) Metals associated with both the water-soluble and insoluble fractions of an ambient air pollution particle catalyze an oxidative stress. *Inhalation Toxicology* 11: 37–49.
51. Singh N, Manshian B, Jenkins GJS, et al. (2009) NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials. *Biomaterials* 30: 3891–3914.
52. Donaldson K and Tran CL (2002) Inflammation caused by particles and fibers. *Inhalation Toxicology* 14: 5–27.
53. Tansey MG, McCoy MK and Frank-Cannon TC (2007) Neuroinflammatory mechanisms in Parkinson's disease: Potential environmental triggers, pathways, and targets for early therapeutic intervention. *Experimental Neurology* 208: 1–25.
54. Brown DM, Donaldson K, Borm PJ, et al. (2004) Calcium and ROS-mediated activation of transcription factors and TNF-α cytokine gene expression in macrophages exposed to ultrafine particles. *American Journal of Physiology: Lung Cellular and Molecular Physiology* 286: 344–353.
55. Stoehr LC, Gonzalez E, Stampfl A, et al. (2011) Shape matters: Effects of silver nanospheres and wires on human alveolar epithelial cells. *Particle and Fibre Toxicology* 8: 36.
56. Borm PJA, Robbins D, Haubold S, et al. (2006) The potential risks of nanomaterials: A review carried out for ECETOC. *Particle and Fibre Toxicology* 3: 11–45.
57. Roduner E (2006) Size matters: Why nanomaterials are different. *Chemical Society Reviews* 35: 583–592.
58. Donaldson K and Stone V (2003) Current hypotheses on the mechanisms of toxicity of ultrafine particles. *Annali dell'Istituto Superiore di Sanità* 39: 405–410.
59. Risom L, Moller P and Loft S (2005) Oxidative stress-induced DNA damage by particulate air pollution *Mutation Research* 592: 119–137.
60. Gojova A, Guo B, Kota RS, et al. (2007) Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: Effect of particle composition. *Environmental Health Perspective* 115: 403–409.
61. Peters K, Unger RE, Kirkpatrick CJ, et al. (2004) Effects of nano-scaled particles on endothelial cell function in vitro: Studies on viability, proliferation and inflammation. *Journal of Materials Science – Materials in Medicine* 15: 321–325.
62. Peters A, Veronesi B, Calderon-Garciduenas L, et al. (2006) Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Particle and Fibre Toxicology* 3: 13.
63. Gui S, Zhang Z, Zheng L, et al. (2011) Molecular mechanism of kidney injury of mice caused by exposure to titanium dioxide nanoparticles. *Journal of Hazardous Materials* 195: 365–370.
64. Moon C, Park HJ, Choi YH, et al. (2010) 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 A* 73: 396–409.
65. Luo YL, Chang LW and Lin P (2015) Metal-based nanoparticles and the immune system: activation, inflammation, and potential applications. *BioMed Research International* 2015: 143720
66. Smith J, Brown JM and Zamboni WC (2014) Walker from immunotoxicity to nanotherapy: The effects of nanomaterials on the immune system. *Toxicological Sciences* 138: 249–255.
67. Inoue KI, Takano H, Yanagisawa R, et al. (2007) Effects of inhaled nanoparticles on acute lung injury induced by lipopolysaccharide in mice. *Toxicology* 238: 99–110.
68. Brüske I, Hampel R, Socher M, et al. (2010) Impact of ambient air pollution on the differential white blood cell count in patients with chronic pulmonary disease. *Inhalation Toxicology* 22: 245–252.
69. Harishchandra RK, Saleem M and Galla HJ (2010) Nanoparticle interaction with model lung surfactant monolayers. *Journal of the Royal Society, Interface* 7: 15–26.
70. Campbell A, Araujo JA, Li H, Sioutas C, et al. (2009) Particulate matter induced enhancement of inflammatory markers in the brains of apolipoprotein E knockout mice *Journal of Nanoscience and Nanotechnology* 9: 5099–5104.
71. MohanKumar SMJ, Campbell A, Block M, et al. (2008) Particulate matter, oxidative stress and neurotoxicity. *NeuroToxicology* 29: 479–488.
72. Pedata P, Garzillo EM and Sannolo N (2010) Ultrafine particles and effects on the body: Review of the literature. *Giornale Italiano di Medicina del Lavoro ed Ergonomia* 32: 23–31.
73. Pocock JM and Kettenmann H (2007) Neurotransmitter receptors on microglia. *Trends in Neurosciences* 30: 527–535.
74. Nguyen MD, D'Aigle T, Gowing G, et al. (2004) Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. *Journal of Neurosciences* 24: 1340–1349.
75. Fonken LK, Xu X, Weil ZM, et al. (2011) Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology. *Molecular Psychiatry* 16: 987–995.
76. Pedata P, Bergamasco N, D'Anna A, et al. (2013) Apoptotic and proinflammatory effect of combustion-generated organic nanoparticles in endothelial cells. *Toxicology Letters* 219: 307–314.
77. Foley S, Crowley C, Smaihi M, et al. (2002) Cellular localization of a water-soluble fullerene derivative. *Biochemical and Biophysical Research Communication* 294: 116–119.
78. Lane DP (1992) Cancer. p53, guardian of the genome. *Nature* 358: 15–16.
79. Eder C, Frankenberger M, Stanzel F, et al. (2009) Ultrafine carbon particles down-regulate CYP1B1 expression in human monocytes. *Particle and Fibre Toxicology* 16: 6–27.
80. Xia T, Kovochich M, Brant J, et al. (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Letter* 6: 1794–1807.
81. Gou N, Onnis-Hayden A and Gu AZ (2010) Mechanistic toxicity assessment of nanomaterials by whole-cell-array stress genes expression analysis. *Environmental Science and Technology* 44: 5964–5970.
82. Bonvallot V, Baeza-Squiban A, Baulig A, et al. (2010) Organic compounds from diesel exhaust particles elicit a proinflammatory response in human airway epithelial cells and induce cytochrome p450 1A1 expression. *American Journal of Respiratory Cell and Molecular Biology* 25: 515–521.
83. Karlsson HL, Cronholm P, Gustafsson J, et al. (2008) Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. *Chemical Research in Toxicology* 21: 1726–1732.
84. Park EJ, Yi J, Chung KH, et al. (2008) Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. *Toxicology Letters* 180: 222–229.
85. Knaapen AM, Borm PJ, Albrecht C, et al. (2004) Inhaled particles and lung cancer. Part A: Mechanisms. *International Journal of Cancer* 109: 799–809.
86. De Marzi L, Monaco A, De Lapuente J, et al. (2013) Cytotoxicity and genotoxicity of ceria nanoparticles on different cell lines in vitro. *International Journal of Molecular Sciences* 14: 3065–3077.
87. Manna SK, Sarkar S, Barr J, et al. (2005) Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-kB in human keratinocytes. *Nano Letters* 5: 1676–1684.
88. Kubota Y, Shuin T, Kawasaki C, et al. (1994) Photokilling of T-24 human bladder cancer cells with titanium dioxide. *British Journal of Cancer* 70: 1107–11011.
89. Rehn B, Seiler F, Rehn S, et al. (2003) Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: Untreated and surface treated. *Toxicology and Applied. Pharmacology* 189: 84–95.
90. Dayem AA, Kim B, Gurunathan S, et al. (2014) Biologically synthesized silver nanoparticles induce neuronal differentiation of SH-SY5Y cells via modulation of reactive oxygen species, phosphatases, and

kinase signaling pathways. *Biotechnol Journal* 9: 934–943.
91. Campbell A, Oldham M, Becaria A, et al. (2005) Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology* 26: 133–140.
92. Franchini M and Mannucci PM (2011) Thrombogenicity and cardiovascular effects of ambient air pollution. *Blood* 118: 2405–2412.
93. Li R, Mittelstein D, Kam W, Pakbin P, et al. (2013) Atmospheric ultrafine particles promote vascular calcification via the NF-kB signaling pathway. *American Journal of Physiology - Cell Physiology* 304: 362–369.
94. Rim KT, Song S and Kim HY (2013) Oxidative DNA damage from nanoparticle exposure and its application to workers' health: A literature review. *Safety and Health at Work* 4: 177–186.
95. Samberg ME, Oldenburg SJ and Monteiro-Riviere NA (2009) Evaluation of silver nanoparticle toxicity in skin in vivo and keratinocytes in vitro. *Environmental Health Perspective* 118: 407–413.
96. Kuhlbusch TA, Asbach C, Fissan H, et al. (2011) Nanoparticle exposure at nanotechnology workplaces: A review. *Particle Fibre Toxicology* 8: 22.