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Toxicity of nanomaterials found in human environment: A literature review

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Abstract

The US National Nanotechnology Initiative (NNI) defines nanotechnology as "the understanding and control of matter at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications." Recent scientific reports available in the literature clearly demonstrate the potential benefits of nanotechnology in consumer and industrial products. More and more nanomaterials are expected to be used in consumer products. This is expected to lead to increased human exposure to nanomaterials in their daily lives. Therefore, the effect of nanomaterials present in human environment is an area of increasing scientific interest. The information presented in this review is obtained from the current literature. It indicates that nanomaterials found in human environment may have potential for toxicological effects. However, the current literature on toxicological effects of nanomaterials is diverse. The current data are presented from studies without harmonization. These studies have used different in vitro and in vivo test models, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, these data are hard to interpret. More research on nanomaterial characterization, biological interaction, toxicity, and health effects is needed. The test methods need to be validated. Positive and negative controls for nanotoxicity need to be identified. Toxicity data harmonization needs to be done. Therefore, general information is not currently available for risk evaluation of certain nanomaterials that might be present in consumer products or that may enter into the market in future. Standardized and validated methods are necessary for toxicity assessment of nanomaterials. Therefore, in the absence of standardized validated methods any specific regulatory testing requirements for nanomaterials are currently premature. We conclude that the benefits of nanomaterials found currently in human environment are many, but their overall adverse effects on human health are limited.

Keywords

Nanotoxicity, nanomaterials, nanoparticles

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Introduction

Nanotechnology is a developing new technology. It offers many benefits in numerous fields including food,[1,2] cosmetics,[3] medicine,[4] and agriculture.[2,5] The US National Nanotechnology Initiative (NNI) defines nanotechnology as "the understanding and control of matter at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications".[6–8] In addition to varying in size they can also have different shapes and exhibit unique physical, chemical, and biological properties that are different from similar materials having larger mass and

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size. The extremely small size and high surface area of nanomaterials are associated with the potential for greater strength, stability, chemical, physical, and biological activity. Therefore, they have a wide-range of possible applications in the modern world. The projected increase in the use of nanomaterials will consequently lead to their increased presence in human environment and, therefore, potentially increasing human exposure. As a consequence, there is a great public interest in understanding the adverse health effects of nanomaterials present in human environment.

Nanomaterials found in human environment essentially fall into four classes: carbon-based nanomaterials, metal-based nanomaterials, dendrimers, and composites. The carbon-based nanomaterials such as fullerenes and nanotubes are used in films, coatings, and electronics. The metal-based nanomaterials such as nanosilver, nanogold, and metal oxides (i.e. titanium dioxide (TiO_2)) are used in food-, cosmetics-, and drug-related products. The dendrimers are nano-polymers. They are used for drug delivery. Composites such as nano-clays combine one nanoparticle with other nano-size or larger particles. They are used in packaging materials. Different classes of nanomaterials differ in shape, size, and chemical as well as biological properties.

Current understanding of adverse effects of nanomaterial exposure on human health is limited. Because of their extremely small size and, in some cases, greater stability they may remain in human environment and in body for longer period of time compared to their larger counterparts. Certain nanomaterials may be inhaled, ingested, or penetrated through the skin more readily. Therefore, interest is growing to understand their potential toxicity. For the last few years, efforts have been made to develop methods for evaluating their toxicity and health effects. These efforts have led to the birth of a new branch of toxicology called "nanotoxicology",[9,10] an emerging and rapidly developing branch of modern toxicology dealing with nanomaterial toxicity, specifically. Scientific peer-reviewed journals such as *Nanotoxicology*, *Journal of Nanotoxicology and Nanomedicine*, and *Journal of Nanoparticle Research* are dedicated exclusively to this emerging branch of toxicology.

Because of the potential exposure to broad range of nanomaterials present in human environment, their potential for toxicological effect is of increasing interest. The purpose of this review was to put together the current toxicity information available in the literature on nanomaterials that might be present in environment, while considering the diversity of toxicological end points to which humans may be exposed to in their daily lives.

Potential routes of human exposure to nanomaterials

Ingestion is an important route of human exposure to nanomaterials, both directly through food or indirectly via nanoparticle dissolution from food containers or by secondary ingestion of inhaled particles.[11] Once they enter the body they may be translocated throughout the body by blood circulation. It is possible that their distribution in the body may be a function of their size and surface characteristics such as polarity, hydrophilicity, lipophilicity, and catalytic activity.[12,13] As the particle size decreases, surface area per unit mass increases and, therefore, the nanoparticles are expected to exhibit increased chemical and biological activity in the body.[14,15] As a consequence, it is hypothesized that smaller nanoparticles might be more toxic than their larger counterparts. It is generally believed that the smaller nanoparticles are taken up by the cells faster than the larger ones. Inhalation is also an important route of human exposure to the airborne nanomaterials.[16,10] Skin absorption is another import route of human exposure to nanomaterials.[9,16]

Need for toxicity evaluation of nanomaterial

Greater human exposure to nanomaterials present in environment and their adverse effect on health is of public concern. The unique size, shape, morphology, composition, distribution, dispersion, surface area, surface chemistry, and reactivity of nanomaterials are expected to affect their toxicity[9] making their toxicity evaluation complex.[17,18] The established toxicity tests available for screening classical chemical compounds may not be suitable for safety evaluation of nanomaterials. Currently, there are no internationally accepted toxicity tests or validated protocols available for safety evaluation of nanomaterials. National Center for Toxicological Research and National Institute of Standards and Technology are currently working on providing positive standards for toxicity testing of nanomaterial[19–22]; however, to date no internationally agreed upon nanomaterial standards are available to be used as positive controls. The establishment of standard reference materials and standard dispersion protocols would be of great benefit to nanotoxicity testing. It would provide investigators a common protocol to follow for nanotoxicity testing. Good laboratory practice may be used in lieu of standard protocols. Currently, it is apparent that no single vehicle is optimal for nanoparticles of all types and from all sources. The dispersion characteristics of any given nanomaterial can be optimized only by experimenting with different vehicles and with proper experimental observations. Therefore, a careful toxicology testing strategy for nanomaterials would increase our general understanding of the potential health effects of nanomaterials. More studies are required to identify relevant biomarkers and tests to better understand the effects that nanomaterials have on biological systems.

Characterization of nanomaterials

Proper characterization of nanomaterials is a critical component of their toxicity evaluation.[9,23–25] The quality of the

dispersion of nanoparticles is dependent on the medium used to suspend them, which may affect their biological activity. These characteristics are dependent on the way the nanomaterials are prepared and solubilized.[26] Toxicity testing of nanomaterials in aqueous medium requires a stable dispersion containing accurate particle size and distribution. Their biological effect can be impacted by the quality of their dispersion in the medium. The state of dispersion of a nanomaterial determines the extent to which particles are agglomerated into clusters. Nanoparticle agglomeration is a function of particle shape, size, surface area, and their proximity to each other.[27] The physiological and biological media commonly used in toxicity studies may affect their state of dispersion. Therefore, it is essential to measure the particle size distribution both "as-received" and "as-dosed." Protein coronas impact nanoparticle uptake into cells.[28,29] Toxicity of nanomaterials is largely dependent on their cellular uptake,[30] and, therefore, the protein corona may have a significant impact on their toxicological profile.

Currently, no internationally accepted protocol(s) for the characterization of nanomaterials has been agreed upon. Lack of proper characterization protocols, comparing toxicity data, and/or recognize the parameters that influence toxicity are challenging. Rigorous characterization of the test material for each individual safety testing study is essential.[9,23,25] Both dose- and time-dependent toxicity evaluation of well-characterized nanomaterials is needed.

There is no well-defined battery of tests for toxicity testing and characterization of nanoparticles in aqueous media. The review of existing literature indicates that studies are performed with different sources and types of nanoparticles, different cell lines, media, tissues, animal models, and/or method of administration. It is important that the nanoparticles are characterized thoroughly to make sure that they exist as nanoparticles in aqueous medium without agglomeration.[23] More research is required on the effects of size and state of agglomeration of nanomaterials on their biological activity.

Electron microscopy is often used to determine particle size, shape, and structure. Bourdon et al.[31] have used dynamic light scattering and transmission electron microscopy to determine the hydrodynamic nanoparticle size. Particle shape, namely aspect ratio, has been implicated in several toxic effects, mainly related to inhalation of certain inorganic fibers such as asbestos.[9]

Nanoparticle characterization techniques in aqueous solutions include ultra-high illumination light microscopy and disc centrifuge sedimentation. However, these techniques are limited by the measurement size range. Murdock et al.[23] characterized a wide range of nanomaterials including metals, metal oxides, and carbon-based materials in water and cell culture media, with and without serum, using dynamic light scattering and transmission electron microscopy. Their results show that many metal and metal oxide nanomaterials agglomerate in solution. Their corresponding toxicity results demonstrate that the addition of serum to cell culture medium can have a significant effect on particle toxicity possibly due to changes in agglomeration or surface chemistry, including surface charge. It was also observed that sonication has only a minimal effect on particle surface charge but does slightly reduce agglomeration. Finally, they showed that the stock solution experienced significant changes in particle agglomeration and surface charge over time.[23]

In vitro toxicity of nanomaterials

Alternative in vitro screening assays are important for rapid, cost-effective, and high-throughput toxicological screening and characterization of nanomaterials to complement and/or supplement the more costly and time-consuming in vivo animal tests. Human cell culture systems have the potential to eliminate the need for interspecies extrapolation to increase efficiencies in testing and to reduce the use of animals.[32] Nonetheless, in vitro systems have limitations.[33] Because of limited metabolic capacity, the biotransformation of a chemical in vitro may be minimal compared to in vivo systems. However, development of high-throughput, predictive, and mechanism-based assays for evaluating the potential toxicity of nanomaterials that humans are exposed to, but for which little toxicity information is available, is important and desirable.[34] The unique physical and chemical properties of nanomaterials drive the need to develop and validate in vitro tests to assess their potential toxicity. The exposure–effect relationship results determined by in vitro models may be predictive of the toxicological and pharmacological activities in vivo[35,36] provided they are appropriately validated. The same nanomaterial obtained from different sources can produce contradictory results.[27]

In vivo toxicity of nanomaterials

The interactions between nanomaterials and in vivo systems can affect their toxicity.[37,38] Nanomaterials obtained from different sources produced different results in mouse lung exposed by inhalation.[27]

Recently, zebra fish (*Danio rerio*)[39] and *Caenorhabditis elegans*[40,41] have been used as in vivo models for nanoparticle toxicity testing. Meyer et al.[42] showed the uptake and growth inhibition of three silver nanoparticles with different sizes and polyvinylpyrrolidone or citrate coatings using *C. elegans*. Hunt et al.[40] have demonstrated growth suppression and oxidative DNA damage in *C. elegans* exposed to nanosilver.

Orally administered gold nanoparticles in mice were captured by the gastrointestinal tract and translocated by blood to other organs such as liver, spleen, kidney, heart, lungs, spleen, and brain.[43] Studies in mice suggest that most nanomaterials accumulate in the liver following oral, inhalation, and intravenous exposures.[44,45] van der Zande

et al.[46] exposed rats orally to 15–20 nm nanosilver for 28 days. They observed nanosilver present in all examined organs with the highest levels in the liver and spleen. Silver concentrations in the organs were highly correlated to the amount of Ag ion in the silver nanoparticle suspension, indicating that mainly Ag ion, and to a much lesser extent of silver nanoparticles, passed the intestines in the silver nanoparticle exposed rats.

Metabolism of nanomaterials

Our understanding of the metabolism of nanoparticles is limited. Hepatocytes play a major role in overall metabolism of nanomaterials.[47] The breakdown of nanomaterials may elicit unique molecular responses that are unpredictable, and thus, the understanding and cataloging of what, when, and how such nanostructures degradation occurs are important. The data from in vivo rodent models indicate that most nanomaterials tend to accumulate in the liver[44] when administered by the oral route where the nanoparticles have been shown to be retained, leading to tissue injury.[45] Wang et al.[48] have reviewed the metabolism of nanomaterials in vivo with emphasis on blood circulation and organ clearance profiles in the lung, liver, and kidney. Once the nanomaterials enter the bloodstream directly during their application or indirectly via inhalation, ingestion, and dermal exposure, their extremely small size allows them to be transported to different parts of the body. In vivo studies indicate that the lung, liver, and kidney are the major distribution sites and target organs for nanomaterial exposure.[48] Therefore, the clearance patterns of nanomaterials in these organs are critical for understanding of their fate in vivo.

Cytotoxicity of nanomaterials

An assessment of the cytotoxicity of nanomaterials is helpful for proper interpretation of their biological activity. The quality of nanoparticle dispersion is dependent on the medium used to suspend them, which may affect their cytotoxicity potential. Therefore, proper preparation and characterization of nanomaterials are critical for their cytotoxicity evaluation.

Current cytotoxicity evaluations of nanomaterials are largely limited to the measurement of cell viability.[49–52] Sohaebuddin et al.[50] evaluated the cytotoxicity of TiO_2 and silicon dioxide (SiO_2) nanoparticles in three cell lines, 3T3 fibroblasts, RAW 264.7 macrophages, and telomerase-immortalized bronchiolar epithelial cells. They exposed the cells to nanomaterials of different composition and sizes after characterizing their properties in PBS and serum containing culture medium. They concluded that composition and size of the nanomaterial as well as the target cell type are critical determinants of intracellular responses, degree of cytotoxicity, and potential mechanisms of toxicity. Gerloff et al.[49] investigated the cytotoxic effects of several nanoparticles TiO_2, SiO_2, zinc oxide (ZnO), and Magnesium oxide (MgO) on human Caco-2 cells. They found that all particles, except for MgO, were cytotoxic. Uboldi et al.[51] investigated the cytotoxic effects of SiO_2 nanoparticles on Balb/3T3 mouse fibroblasts. Their studies showed no cytotoxicity as measured by the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Using fluorescence microscopy, they observed internalization of nanoparticles by the cells. The nanoparticles were located exclusively in the cytoplasmic region. Shen et al.[52] have reported cytotoxicity of ZnO nanoparticles on human immune cells. Their study shows a strong correlation between the cytotoxicity induced by ZnO nanoparticles and free intracellular zinc concentration. This observation suggests that the intracellular solubility of ZnO nanoparticles into zinc ions is a requirement necessary for cytotoxicity. Sahu et al.[53] evaluated the cytotoxic potential of 20-nm nanosilver using human liver HepG2 and colon Caco2 cells in culture as in vitro models. They observed a significant concentration-dependent cytotoxicity in both HepG2 and in Caco2 cells compared to the vehicle control. Also concurrently, they observed a concentration-dependent increase in mitochondrial injury as well as loss of double-stranded DNA in both cell types. They found no nanosilver-induced cellular oxidative stress as determined by the dichlorofluorescein assay in cell type suggesting that cellular oxidative stress did not play a major role in the observed cytotoxicity of nanosilver in HepG2 and Caco2 cells. The HepG2 cells were more sensitive to nanosilver than the Caco2 cells. Their results suggested that a differential rather than a universal response of different cell types exposed to nanoparticles may play an important role in the mechanism of toxicity. Hunt et al.[40] have shown growth suppression and oxidative DNA damage in *C. elegans* exposed to nanosilver. A recent report by Uboldi et al.[54] shows that TiO_2 nanoparticles induce cytotoxic and genotoxic effects in Balb/3T3 mouse fibroblasts.

The results of the cytotoxicity studies reported in the literature are diverse, but they do indicate that nanomaterials might be potential cytotoxins. It may be noted that these studies have used different models for nanomaterial testing, nanomaterials obtained from different sources, different methods for nanomaterial characterization, and different experimental conditions. Therefore, it is difficult to interpret these data.

Hepatotoxicity of nanomaterials

Liver is the primary organ involved in metabolism and detoxification of xenobiotics. Blood carrying toxicants is filtered by liver before being distributed to other parts of the body. The high rate of blood flow to the liver leads to delivery of high concentrations of the toxicant to this organ. The high levels of exposure and the high metabolic activity make liver a major target organ of toxicants. Once the nanomaterial, whether ingested, inhaled, absorbed through the skin or administered by intravenous injections, and medical devices,

reaches the circulation, it may be translocated to the liver. Some studies have suggested that nanoparticles are entrapped by the reticuloendothelial system suggesting liver and spleen as the main target organs.[43] Therefore, nanomaterials might be potential hepatotoxicants, and, therefore, hepatotoxicity testing is an important testing strategy for safety assessment of nanomaterials, where appropriate.

Hepatotoxicity studies in vitro reported in the literature are limited. However, a couple of in vitro studies have suggested that certain nanomaterials may be hepatotoxic. Hussain et al.[55] used the rat liver cell line BRL 3A as an in vitro model to assess toxicity of silver and titanium oxide nanoparticles. They used cytotoxicity, mitochondrial dysfunction, and oxidative stress as the end points of hepatotoxicity. The study concluded that silver was highly cytotoxic compared to titanium oxide. Sahu et al.[53] have observed cytotoxicity of nanosilver in human liver HepG2 and colon Caco2 cells.

Jeon et al.[56] screened the differentially expressed proteins in mouse liver caused by toxicity of titanium nanoparticles. They identified 15 proteins that showed greater than a two-fold expressional change in response to TiO_2 nanoparticles by liquid chromatography–mass spectrometry. Of these, 12 proteins were downregulated and 3 proteins were upregulated upon treatment with TiO_2 nanoparticles. The 15 differentially expressed proteins might be used for detection of inflammation, apoptosis, and antioxidative reaction in the treatment of acute hepatic damage by TiO_2 nanoparticles.

Results of limited hepatotoxicity studies reported in the literature demonstrate that nanomaterials might be potential hepatotoxins. However, it may be noted that these studies have used different models for nanomaterial testing, nanomaterials obtained from different sources, different methods for nanomaterial characterization, and different experimental conditions. Therefore, it is difficult to interpret these data.

Nephrotoxicity of nanomaterials

Kidney is one of the common target organs for nanomaterial toxicity. It has been reported that kidney is an important target organ for toxicity and the primary organ for clearance nanomaterials.[57,58] Yan et al.[58] evaluated the nephrotoxicity in rats exposed to ZnO nanoparticles. Their results showed that the ZnO nanoparticles induced mitochondrial and cell membrane damage in rat kidney leading to nephrotoxicity. Liao and Liu[59] investigated mechanism of nanomaterial-induced nephrotoxicity in rats exposed to copper nanoparticles. They observed nanocopper-induced renal proximal tubule necrosis in kidneys analyzed by renal gene expression profiles.

Studies on nephrotoxicity of nanomaterials reported in the literature are limited. The results of these studies suggest that certain nanomaterials have the potential to induce nephrotoxicity. However, it must be recognized that these studies have used different models for nanomaterial testing, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, the conclusions of these studies may not be comparable.

Inhalation toxicity of nanomaterials

Nanoparticles in air can travel great distances by Brownian diffusion. Therefore, inhalation is an important route of human exposure to the airborne nanomaterials. Nanoparticles are deposited in the respiratory tract predominantly by diffusion.[60,61] Inhalation of nanoparticles results in depositing the nanoparticles within the alveolar regions of the rat lung.[60–62] Once deposited, nanoparticles may cross biological membranes and access tissue that would not normally be exposed to larger particles. Inhaled TiO_2 nanoparticles translocate into lung interstitial.[61,63] Inhalation of TiO_2 nanoparticles resulted in pulmonary overload in rats and mice with inflammation.[64]

The in vitro inhalation toxicity studies on nanomaterials reported in the literature are limited. The human lung cancer cell line A549 has been used as an in vitro cell culture model for study of nanomaterial toxicity. Foldbjerg et al.[65] used this system to investigate the cytotoxic and genotoxic effects of silver nanoparticles. They measured the cellular uptake of the nanoparticles by atomic absorption spectroscopy and flow cytometry. They measured the dose-dependent cytotoxicity of silver nanoparticles by the MTT and annexin V/propidium iodide assays. The cytotoxicity of silver nanoparticles was greatly decreased by pretreatment with the antioxidant, *N*-acetyl-cysteine. They reported a strong correlation between the levels of reactive oxygen species (ROS) and mitochondrial damage along with early apoptosis. They measured the ROS-induced DNA damage by increased DNA adducts by ^{32}P-postlabeling after nanoparticle exposure. The level of DNA adducts was strongly correlated with the cellular ROS levels and was inhibited by antioxidant pretreatment. Their results suggested that silver nanoparticles act as mediator of ROS-induced cytotoxicity and genotoxicity. Recently, Jugan et al.[66] evaluated the cytotoxic and genotoxic effects of titanium oxide nanoparticles on a cell line A549 alveolar epithelial cells representative of human lung.

Studies on inhalation toxicity of nanomaterials found in the literature are limited. The results of these studies suggest that certain nanomaterials may have the potential to induce nephrotoxicity. However, it may be noted that these studies have used different models for nanomaterial testing, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, the data from these studies are difficult to interpret.

Dermal toxicity of nanomaterials

Skin, the largest organ of the body, serves as a primary route of environmental and/or occupational human exposure for chemicals. Nanomaterials have the capability to

increase solubility, transparency, and color of cosmetic products. Therefore, they are used by the cosmetic industry. The nanomaterials with potential uses in cosmetics include nanosilver, nanogold, nanoemulsions, nanocapsules, nanocrystals, dendrimers, fullerenes, liposomes, hydrogels, and solid lipid nanoparticles.[67]

Nanomaterials are used in pharmaceuticals. They are used as medical interventions for prevention, diagnosis, and treatment for skin diseases. Their ability to improve the solubility of poorly water-soluble drugs, modify pharmacokinetics, increase drug half-life, improve bioavailability, diminish drug metabolism, controlled and targeted delivery of drugs, and the simultaneous delivery of drug combination therapy makes them ideal candidates for nanomedicine.[68] They are used for drug delivery in diseased skin and to the openings of hair follicles. Most drug delivery particles are based on lipid carriers, that is, solid lipid nanoparticles and nanoemulsions. Nanosilver is used for its antimicrobial activity and skin cancer prevention.[69]

The ability for nanomaterials to penetrate skin is an ongoing investigation. Some studies[9,70] have reported the penetration of nanoparticles through the skin, but more independent studies[71–77] did not observe nanoparticle penetration through the intact human, pig, or mouse skin. Mortensen et al.[78] evaluated the in vivo skin penetration of quantum dot nanoparticles in the murine model by irradiation of ultraviolet radiation (UVR). They reported that these nanoparticles penetrated the skin poorly. The condition of the skin, flexed or nonflexed, did not appear to alter penetration and tape-stripped skin depicted quantum dots only on the surface of the viable epidermis. They concluded that quantum dots can penetrate the dermal layer by abrasion of skin.[79] Recently, Domeradzka-Gajda et al.[80] examined percutaneous absorption of two different sizes (15 and 45 nm) of silver nanoparticles in combination with cosmetic ingredients (aluminum chloride), methyl paraben, or di-*n*-butyl phthalate using pig skin as an in vitro model. After 24 h exposure to the nanosilver, they measured silver in receptor fluid by the inductively coupled plasma mass spectrometry. They observed low, but detectable silver absorption and no statistically significant differences in the penetration between the different types of silver nanoparticles. Also they observed no significant differences for silver penetration when the silver nanoparticles were used in combinations with aluminum chloride, methyl paraben, or di-*n*-butyl phthalate. They observed that the smaller 15 nm nanosilver in combination with methyl paraben showed the highest amount of silver penetrating the pig skin. They concluded that silver nanoparticles minimally penetrated pig skin in vitro. Aluminum chloride, methyl paraben, and butyl phthalate did not modify the penetration.

Published studies on dermal toxicity of nanomaterials are limited. The results of these studies indicate that certain nanomaterials may penetrate skin. However, it is recognized that these studies have used different models for nanomaterial testing, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, the data from these studies are difficult to interpret.

Immunotoxicity of nanomaterials

Information on immunotoxicity of nanomaterials is limited. Compatibility of nanomaterials with the immune system is largely determined by their surface chemistry.[81] A review of the current status of immunotoxicity testing of nanomaterials for their safety assessment indicates that nanoparticles can both stimulate and/or suppress the immune responses.[64] Nanomaterials can modulate cytokine production.[82,83] They induce pro-inflammatory effects in the lung in experimental animals with increased expression on Interleukin (IL)-1β, Macrophage Inflammatory Proteins (MIP)-1α, Monocyte Chemoattractant Protein (MCP)-1, MIP-2, keratinocyte chemoattractant, Chemokine (C-C motif) ligand 17 (TARC), Granulocyte-macrophage colony-stimulating factor (GM-CSF), and activation of the stress-activated mitogen-activated protein kinases (MAPKs) p38 and Jun N-terminal kinase (JNKs).[82,83] The available data suggest that through the elicitation of an oxidative stress mechanism, nanoparticles may contribute to pro-inflammatory disease processes in the lung, particularly allergy.[82,83]

Pfaller et al.[18] evaluated the cytotoxicity, immunotoxicity, and genotoxicity of gold and iron oxide nanoparticles on human cells using a panel of cell-based tests in different laboratories. Their studies showed that these nanoparticles induced very little or no cytotoxicity, immunotoxicity, and genotoxicity. However, they carefully discuss several technical issues for working with nanoparticles that can help understand this finding. More studies are needed for the development and validation of methods for studying the immunotoxicity of nanomaterials.

The results of limited studies on immunotoxicity of nanomaterials suggest that certain nanomaterials may have the potential to cause immunotoxicity. However, it has to be noted that these studies have used different models for nanomaterial testing, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, the data from these studies are difficult to interpret.

Genotoxicity of nanomaterials

The current literature search revealed limited information on the genotoxicity of nanomaterials with the majority of the literature reports limited to cytotoxicity. A number of methods found in the literature have been used for genotoxicity testing of nanomaterials. They include chromosomal aberrations, DNA strand breaks, oxidative DNA damage, DNA adducts, mutations, and micronucleus formation.[84,85] Several studies have reported that TiO_2 nanoparticles are genotoxic.[45,86–88] However, several other independent studies[49,89,90] have reported that TiO_2 is not genotoxic.

Zuzana et al.[91] have reported that iron oxide nanoparticles induce genotoxicity in vitro when determined by the comet assay. Sahu et al.[92,93] evaluated the potential genotoxicity of 20-nm nanosilver in human liver HepG2 and colon Caco2 cells in culture by the cytochalasin B-blocked micronucleus assay[92] as well as by the flow cytometric in vitro micronucleus assay.[93] They observed a concentration- and time-dependent increase in the frequency of binucleated cells with micronuclei induced by the nanosilver compared with the control. Sahu et al.[94,95] used the same genotoxicity assays, the cytochalasin B-blocked micronucleus assay[94] and the flow cytometric in vitro micronucleus assay,[95]], the same in vitro models (HepG2 and Caco2 cells), and the same experimental conditions to compare the potential genotoxicity of two different sizes (20 and 50 nm) of nanosilver of the same shape, composition, surface charge, and obtained from the same commercial source. They observed that the smaller (20 nm) nanoparticle was genotoxic to both the cell types by inducing micronuclei, but the larger (50 nm) nanoparticle produced a much weaker response. Their results demonstrated that nanoparticle size and cell types were likely critical determinants of nanosilver genotoxicity.

Sahu et al.[94,95] evaluated the contribution of ionic silver to the genotoxic potential of nanosilver. They did not find the micronucleus frequencies in HepG2 and Caco2 cells exposed to the ionic silver statistically significant from control values except at the highest concentrations for both cell types. The authors concluded that the ionic silver did not contribute to the micronucleus-forming ability of nanosilver in either cell types.[94,95]

The literature on genotoxicity of nanomaterials is clearly controversial and inconsistent. The controversy and inconsistency may be due to a lack of proper characterization of the nanoparticles including their source and configuration. Warheit and Donner[25] have discussed the rationale for genotoxicity testing of nanomaterials and requirement for nanomaterial risk assessment. They observed that standardized methods are necessary to evaluate genotoxicity of nanomaterials. They concluded that in the absence of standardized methods any specific regulatory testing requirements for nanomaterials are premature.

Cardiotoxicity of nanomaterials

Literature search for studies on cardiotoxic potential of nanomaterials resulted in very limited information.

Du et al.[96] investigated cardiovascular toxicity of silica nanoparticles in rats by intratracheal instillation. They evaluated hematologic parameters, inflammatory reactions, oxidative stress, endothelial dysfunction, and myocardial enzymes in serum. They observed silica nanoparticles passing through the alveolar–capillary barrier into systemic circulation. Their results showed that the cardiovascular toxicity of silica nanoparticles was dependent on particle size and dosage. Oxidative stress played an important role in inflammatory reaction and endothelial dysfunction. Duan et al.[97] used both endothelial cells in vitro and zebra fish model in vivo to evaluate cardiovascular effects of silica nanoparticles. They used cytotoxicity, oxidative stress, and apoptosis as toxicological biomarkers. They reported oxidative stress and apoptosis as major factors for endothelial cells dysfunction. They concluded that silica nanoparticle exposure is a potential risk factor for the cardiovascular system failure.

Yang et al.[13] have reported a chronic cardiac toxicity in mice exposed to different sizes of gold nanoparticles by the tail vein. They evaluated an accumulation of nanoparticles in the mouse heart and their effects on cardiac function, structure, fibrosis, and inflammation. The nanoparticles did not affect systolic function. However, the left ventricular end-diastolic inner dimension, left ventricular mass, and heart weight/body weight were significantly increased in mice receiving the smallest (10 nm) nanoparticles following only 2-weeks exposure. They concluded that gold nanoparticles caused cardiac hypertrophy.

The limited studies on cardiotoxic potential of nanomaterials suggest that certain nanomaterials have the potential to induce cardiotoxicity. It may be noted that these studies have used different models for nanomaterial testing, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, the data from these studies are difficult to interpret.

Interactive toxicity of nanomaterials

Literature search demonstrates that nanomaterials interact with cellular organelles and this interaction affects their toxicity. Vallhov et al.[98] investigated the effect of spherical gold nanoparticles on the maturation of human dendritic cells in the presence of Lipopolysaccharides (LPS). They observed that the nanoparticles can be the carriers of LPS leading to toxicity. Their studies indicated a potential adverse effect of LPS-contaminated nanomaterials for medical use. Dobrovolskaia et al.[99] have shown that nanoparticles interfere with detection of the endotoxin LPS. With increased application of nanomaterials in medicine and medical devices, the potential contamination of these nanoparticles with LPS is of concern.

Zheng et al.[100] evaluated the interactive toxicity of 25 and 50 nm TiO_2 nanoparticles with bisphenol A (BPA) in human embryo L-02 hepatocytes. These nanoparticles entered cells following 24 h exposure with or without BPA. The results of this study showed that nano-TiO_2 alone did not induce significant DNA or chromosome damage, but the mixture of nano-TiO_2 and BPA increased toxicity via increasing oxidative stress, DNA double strand breaks and micronuclei formation.

Interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. Within this field, an area that has been largely unexplored is the

interaction of metal nanoparticles with viruses. Elechiguerra et al.[101] have demonstrated that silver nanoparticles undergo a size-dependent interaction with HIV-1. They showed that silver nanoparticles exclusively in the range of 1–10 nm attached to the virus. Because of this interaction the silver nanoparticles inhibit the virus from binding to host cells.

Potential mechanisms of nanomaterial toxicity

The mechanism of toxicity of nanomaterials is currently unknown. There is an ongoing debate on the contribution of metal ions to the toxicity of metallic nanoparticles. Results of some studies suggest that the metal ions may be responsible for the toxicity of their corresponding metallic nanoparticles.[24,102–105] However, Sahu et al.[53,92,93] have reported that ionic silver does not contribute to the cytotoxicity and genotoxicity of 20 nm nanosilver in HepG2 and Caco2 cell culture models. Similar results indicating that ionic silver does not contribute to the toxicity of nanosilver have been reported by other independent investigators.[24,105,106]

The mechanism of nanomaterial interaction with cellular organelles is not well understood. Oxidative stress is a major mechanism of toxicity for a wide variety of chemicals. Cellular oxidative stress induces mitochondrial membrane damage, an early indicator of cellular stress. Mitochondrial membrane damage leads to mitochondrial dysfunction, a critical step in cell injury and cell death. Oxidative stress appears to play a major role in the toxicity of nanomaterials. Nanoparticles of various size and chemical composition are able to preferentially localize in mitochondria leading to oxidative stress and cellular damage.[55,107]

Wang et al.[57] investigated the mechanism of cytotoxicity of SiO_2 nanoparticles in cultured human embryonic kidney (HEK293) cells. They observed a dose-dependent decrease in cell viability, increase in intracellular ROS level, and reduction in GSH content. Their studies concluded that the cytotoxicity of SiO_2 nanoparticles in cultured HEK293 cells was associated with oxidative stress. These studies suggested involvement of oxidative stress in nanoparticle toxicity. However, this may not be true for all nanoparticles. Recent studies of Sahu et al.[53] do not show a direct involvement of cellular oxidative stress in HepG2 and Caco2 cells exposed to 20 nm nanosilver. Similar results have been reported by other independent studies demonstrating the absence of oxidative stress in nanoparticle toxicity.[108]

Inflammation which is mediated by production of inflammatory mediators such as cytokines appears to play an important role in the toxicity of nanomaterials. Schanen et al.[109] have reported that exposure of dendritic cells to TiO_2 nanoparticles elevated levels of pro-inflammatory cytokines. Ainslie et al.[110] observed the production of inflammatory cytokines and ROS induced by titanium, silicon oxide, and polycaprolactone nanoparticles in human monocytes. Their results showed that these nanoparticles induced significantly less inflammatory response compared to the positive control, lipopolysaccharide. They found that titanium nanoparticle was more inflammatory than the silicon oxide and polycaprolactone nanoparticles. Trickler et al.[111] showed the pro-inflammatory effects of silver nanoparticles in primary rat brain microvessel endothelial cells in vitro. They observed a size- and time-dependent cytotoxicity and pro-inflammatory response induced by these nanoparticles. Their study demonstrated that the smaller 25 nm silver particles induced significantly greater toxic effects at lower concentrations and shorter exposure times compared to the larger 80 nm particles.

The limited reported studies suggest that both oxidative stress and inflammation may play important roles in the toxicity of nanomaterials. However, the exact molecular mechanisms of nanotoxicity remain to be fully understood.

Current challenges

Continued increase of human exposure to nanomaterials is expected because of increasing presence of nanomaterials in human environment and consumer products. Because of their extremely small size, nanoparticles are expected to have different biological responses compared to their larger mass counterparts. They may be metabolized or altered in vivo. Since their unique properties are dependent on size, shape, and composition, the metabolized nanomaterials may have a different effect on the biological systems compared to the parent material. This uncertainty and incomplete understanding of their interactions with biological systems requires better characterization and integration, both at the level of hazard assessment and exposure assessment, to allow for sufficiently robust assessment for regulatory policy decisions to be made in order for risk managers to properly respond.

Use and exposure route of a material is linked to its potential toxicity. Exposure information very much depends on particle behavior,[112] while hazard assessment depends on proper toxicity evaluation.[25] Our literature search indicates that insufficient reliable data are currently available for a general risk assessment of nanomaterials.[25,112–115] Furthermore, there are no internationally accepted standard methods for toxicity testing or for chemical characterization of nanomaterials. No universally accepted positive controls currently exist for nanotoxicity testing. Data on human exposure to nanomaterials remain limited. More studies are required on nanoparticle characterization, their routes of exposure, metabolism, and mechanisms of action before standardized quantitative risk assessment protocols will become available. Current risk evaluation of nanomaterials will continue to rely for the near future heavily on extrapolation of rodent dose–response data.[115]

Toxicity of a nanomaterial is the outcome of its intrinsic physicochemical properties such as size, shape, surface

properties, and chemical composition. These nanomaterial characteristics influence their cellular uptake. In general, the smaller nanoparticles are taken up by the cells faster than the larger ones. However, there might be an optimal size for efficient nanomaterial uptake into cells. The same nanomaterial of a particular size and shape may exhibit different toxicological profiles depending on its cellular environmental conditions. For example, a nanomaterial of the same size and shape may aggregate into different sizes and shapes that may decide the outcome of their biological activity and toxicity. Depending on these characteristics, the same nanomaterial may exhibit different toxic events.

As with many of their larger counterparts, the potential risk of nanomaterials present in the human environment is a challenge. Besides the data gaps identified above, there is scientific uncertainty regarding aspects of the risk assessment, including (a) particle characteristics that may affect toxicity; (b) fate and transport through the environment; (c) routes of exposure and the metrics by which exposure is measured; (d) mechanisms of translocation to different parts of the body; and (e) mechanisms of toxicity and disease.

Conclusions

Use of nanotechnology in human environment and in consumer products is growing. It is expected that human exposure to nanomaterials will continue to increase. Therefore, extensive human exposure to nanomaterials is of public concern. Thus, there is a need for better tools to evaluate the safety of nanomaterials. Current understanding of the effects of human exposure to nanomaterials is very limited. The information collected from current literature indicates that nanomaterials found in human environment may have potential for toxicological effects. However, the current literature on toxicological effects of nanomaterials is diverse. The current data are presented from studies without harmonization. These studies have used different in vitro and in vivo test models, different sources of test nanomaterials, different methods for nanomaterial characterization, and different experimental conditions. Therefore, these data are hard to interpret. More research on nanomaterial characterization, biological interaction, toxicity, and health effects is needed. The test methods need to be validated. Positive and negative controls for nanotoxicity need to be identified. Toxicity data harmonization needs to be done. Therefore, sufficient information is not readily available for risk evaluation of nanomaterials that are present in consumer products or that may enter into the market in future. Standardized and validated methods are necessary for toxicity assessment of nanomaterials. Therefore, in the absence of standardized validated methods any specific regulatory testing requirements for nanomaterials are currently premature. We conclude that the benefits of nanomaterials found currently in human environment are many, but their effects on human health are limited.

Author's note

Dr. Sahu is employed by the US Food and Drug Administration. Dr. Hayes is a consultant with adjunct academic appoints at Michigan State University and University of South Florida.

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