Original Article

A Review of Nanoparticles Toxicity and Their Routes of Exposures

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Abstract

The new scientific innovation of engineering nanoparticles (NPs) at the atomic scale (diameter<100nm) has led to numerous novel and useful wide applications in electronics, chemicals, environmental protection, medical imaging, disease diagnoses, drug delivery, cancer treatment, gene therapy, etc.. The manufactures and consumers of the nanoparticles-related industrial products, however, are likely to be exposed to these engineered nanomaterials which have various physical and chemical properties at levels far beyond ambient concentrations. These nanosized particles are likely to increase unnecessary infinite toxicological effects on animals and environment; although their toxicological effects associated with human exposure are still unknown. To better understand the impact of these exposures on health, and how best to formulate appropriate monitoring and control strategies, this review seeks to examine various toxicological portal routes associated with NPs exposures. In fact, these ultrafine particles are capable of entering the body through skin pores, debilitated tissues, injection, olfactory, respiratory and intestinal tracts. These uptake routes of NPs may be intentional or unintentional. Their entry may lead to various diversified adverse biological effects. Until a clearer picture emerges, the limited data available suggest that caution must be exercised when potential exposures to NPs are encountered. Some methods have been used to determine the portal routes of nanoscale materials on experimental animals. They include pharyngeal instillation, injection, inhalation, cell culture lines and gavage exposures. This review also provides a step by step systematic approach for the easy identification and addressing of occupational health hazards arising from NPs.

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1. Introduction

The term "nanotechnology" encompasses the manipulation of matter on a near-atomic scale to produce new structures, materials, and devices. It builds nanoparticles (NPs)

whose diameter is below 100 nm by manipulating matter at the atomic level [1]. According to Stern and McNeil [2], NPs can be categorized as either engineered or incidental depending on origin (Figure 1). Engineered NPs such as the quantum dots, dendrimers, carbon nanotubes and fullerene which have diameters<100 nm can be compared to sizes of living things (Figure 1). Also, NPs like diesel particles are generated incidental while living things such as viruses are natural living cells with diameters<100 nm (Figure 1).

Technology can be applied to biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use at the nanoscale levels. It, therefore, encompasses a wider range and history of procedures with useful industrial and biological processes in modifying the needs of humanity at the nanoscale level. Some studies have also shown that microorganisms can as well be used as potential developers of NPs [3, 4]. With the development of these new approaches and techniques, nanotechnological industries are acquiring new horizons enabling them to improve the quality of products and life with uncertain health safety issues (Figure 2).

The emphasis on the benefits of nanotechnology has been offset by considerable debate about the uses and safety of nanotechnologies [1, 5]. Occupational illnesses, however, are full with examples of respiratory dust particle exposures causing diseases. This has been focused largely on ultrafine particles, which represent only one aspect of nanotechnology [6]. These occupational diseases tend to be characterized by temporal or permanent physiological dysfunction with only a few visible symptoms. On the other hand, there is a possibility that they may gain access to the body and pose serious toxicological problem. These NPs may enter the body through the lungs (respiratory tract), skin (dermal contact), exposed wound tissues, intestinal tract (gut) either intentionally or unintentionally depending on the type of exposure [7-10]. NPs can enter the environment and animals system through different pathways. For instance, it could be through effluent, spillage, consumer products and disposal. The intake is usually tolerated by the organism's system, but when a certain range is exceeded, it would cause toxic effects and even deaths. Since NPs can cause risk to the environment and human health, therefore, research must be undertaken to understand and anticipate such
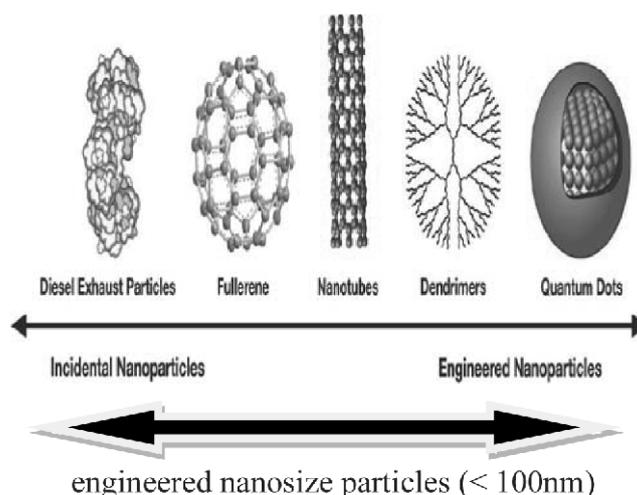

**Figure1.** Structures of some nanoparticles [2].

risks through risk assessment and risk management. However, given the limited amount of informa¬tion about the health risks of NPs, it is prudent to take measures to minimize workers' exposures and to the environment.

The ability of nanoscale materials to en¬ter the body, however, is amongst several factors that scientists need to examine in determining whether such materials may pose any health risk. Ultrafine materi¬als have the greatest potential to enter the body if they are in the form of NPs, agglomerates of NPs, NPs aggregates and particles from nanostructured materials that become airborne or come into con¬tact with the skin [1, 11]. Once NPs are in the body, they can transverse the cells by persorption, interact with the local tissues and cause or provoke dysfunctions of the organs [8]. Based on the results from ani¬mal studies, airborne nanomaterials can be inhaled and deposited in the respira¬tory tract. From there, NPs can enter the blood stream, and translocate to other organs [12]. To better understand the impact of these exposures on

health, and how best to formulate appropriate exposure monitoring and control strategies, this review seeks to examine various toxicological portal routes associated with nanoparticles exposures. Until a clearer picture emerges, the limited data available suggest that caution must be exercised when potential exposures to NPs arise. Studies on workers exposed to aerosols of manufactured microscopic and nanoscale particles have reported lung function decrements and adverse respiratory symptoms. Already, uncertainty exists about the role of ul¬trafine particles relative to other air¬borne fine particles in causing adverse health effects [6, 13, 14]. Therefore, the portal routes of entry of engineered NPs, whose physi¬cal and chemical characteristics are like those of ultrafine particles, need to be understood.

2. Health exposure concerns of nanoparticles

As earlier stated, NPs are particles having diameter between 1 and 100 nm. They may be suspended in a gas (nanoaerosols), suspended in a liquid (colloid or nano-hydrosol), powder
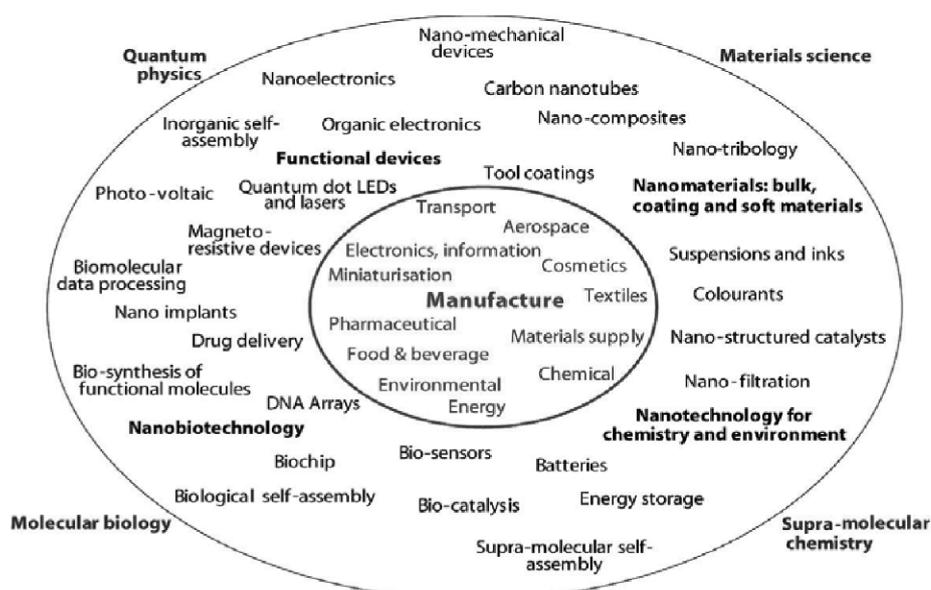
Source: ftp.cordis.lu/pub/nanotechnology/docs/nanoscience_presentation_022002_en.ppt

Figure 2. A perspective of nanotechnology applications as related to discipline. Adapted from the EU Sixth Framework Programme (www.cordis.lu/nmp/home.html).

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or embedded in matrix (nanocomposite materials). The precise definition depends on the particle shape and the diameter measurement method. The particle morphologies may vary widely at the nanoscale. For instance, carbon fullerene NPs represent identical dimensions in all directions (spherical), whereas single-wall-carbon nanotubes (SWCNTs) typically form convoluted, fiber-like (cylindrical) with a diameter below 100 nm [12].

The insufficiency of scientific knowledge obliges us to face a major uncertainty concerning the risks raised by NPs. Therefore, the processes of generating nanoscale materials in the gas phase, or using or producing nanoscale ma¬terials as powders or solutions pose the risk for releas¬ing NPs [1, 8]. Potential exposure to NPs could occur during their manufacturing, development, use-consumer product or during disposal [2]. Also, there is likely exposure to NPs if it involves disturbing de¬posited nanoscale material. There is also the possibility that the following workplace tasks increase the risk of exposure to NPs:

Working with ultrafine particles in solution without adequate protec¬tion (gloves, gowns, masks) will increase the risk of skin exposure.

❖ Working with nanoscale materials in solution during pouring or mixing op¬erations, where a high degree of agitation is involved, will lead to an increased possibility of inhalation of droplets formed.

❖ Generating NPs in the gas phase in non-enclosed systems will enhance the likelihood of aerosol expose to the workplace.

❖ Using ultrafine powders will lead to the risk of aero¬solization.

❑ Maintenance on equipment and processes used to produce or fab¬ricate nanosize materials or the clean-up of spills or waste material will pose a potential for exposure to workers performing these tasks [1, 15].

❖ Cleaning of dust collection systems used to capture NPs can pose a potential for both skin and inhalation exposure.

❖ Machining, sanding, drilling, or oth¬er mechanical disruptions of mate¬rials containing nanoscale materials can potentially lead to aerosol of NPs.

❖ The transfer of nanomaterials in open systems is likely to increase exposure potentials even for relatively hydrophobic NPs [16]. Open systems during NPs processing may increase exposure to human beings.

3. Ultrafine particles

Ultrafine particles are not purposefully manufactured nor are they necessarily of a constant composition or size although they are less than 100 nm, so they are nano-sized. The ultrafine particles have been used to define aerosol, occupa¬tional and environmental health communities such as airborne particles smaller than 100 nm in diameter. Although no formal distinction exists between ultrafine particles and nanoparticles, the term ultrafine is frequently used in the context of nanome¬ter-diameter particles that have not been in¬tentionally produced but are the incidental products of processes involving combustion, welding, or in diesel engines [1, 13]. Likewise, the term nanoparticle is frequently used with respect to particles demonstrating size-dependent physicochem-ical properties, particularly from a materials science perspective, although no formal definition exists. As a result, the two terms are sometimes used to differentiate be¬tween engineered (nanoparticle) and inciden¬tal (ultrafine) nanoscale particles [17]. However, this does not necessarily imply that significant differences exist among the properties of these particles as related to hazard assessment, measurement, or control of exposures, and this remains an active area for research. The term ultrafine has been in existence for a long time, for example some intentionally-produced particles with primary particle sizes

in the nanosize range (TiO2) are often called ultrafine in the literature [17].

According to Borm and Kreyling [14], the effects of ultrafine particles absorbed by inhalation postulate that researchers should focus on the 5 "Ds": dose, deposition, dimension, durability and defence mechanisms. In the case of NPs, this dose-dimension relationship links toxicity to surface concentration. The dose at the pulmonary site determines the potential toxicity which is determined by the concentration and the dimensions of the particles. Deposition of NPs in the pulmonary tract is extremely dependent on particle size. The durability of a group of particles will be greater if they are insoluble and cannot be degraded or eliminated by the lung. If there is sustained exposure, there will then be a large local accumulation. However, the respiratory system has different defence mechanisms designed to eliminate undesirable particles. Toxicity often will depend on the efficiency of these mechanisms in taking charge of NPs [17].

When ultrafine dust particles are present in sufficient quantity, they can activate or destroy the macrophages or the epithelium and produce an inflammatory mechanism that is pathogenic to pulmonary function [17]. Experimental studies in rats have shown that equivalent mass doses of insoluble ultrafine particles (smaller than 100 nm) are more potent than large particles of similar composition in causing pulmo¬nary inflammation and lung tumors in those laboratory animals [1, 18]. According to Seaton and Donaldson [19], dusts found in work environments, often around one micrometer in size, can accumulate in the lungs and lead to several occupational lung diseases, such as pneumoconiosis (asbestosis, silicosis), smelters' fever, occupational asthma, berylliosis and lung cancer.

4. Engineered nanoparticles

Engineered NPs are nanoscale particles which are products of pro¬cesses such as combustion and vaporization. Engineered NPs are designed with very specific properties (including shape, size, surface properties, and chemistry), and collec¬tions of the particles in an aerosol, colloid, or powder will reflect these properties. Incidental nanoscale particles are generated in a relatively uncontrolled manner and are usually physi¬cally and chemically heterogeneous compared with engineered NPs [1]. In the last decade, engineered NPs have become an important class of new materials with several properties that make them very attractive for commercial development [20]. In fact, they have been increasingly used for manufacturing diverse industrial items such as cosmetics or clothes and for infinite applications in electronics, aerospace and computer industry. The development of engineered NPs with substantial biomedical significance has led to new opportunities and challenges in the fields of pharmacology and therapeutics, electronics, environmental and other disciplines. Nanomaterials in particular NPs are likely to be cornerstones of innovative nanomedical devices to be used for drug discovery and delivery, discovery of biomarkers and molecular diagnostics [19, 20]. Due to their small size and large surface area, engineered NPs have chemical, physical, and biological properties distinctly different from fine particles of similar chemical composition, thus making them attractive for commercial development and application. These properties may include a high rate of pulmonary deposition, the ability to travel from the lung to systemic sites, the ability to penetrate dermal barriers, and a high inflammatory potency per mass [1, 20]. Engineered NPs whose physi¬cal and chemical characteristics are like those of ultrafine particles need to be studied to determine if they pose health risks similar to those that have been as¬sociated with the ultrafine particles.

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5. Nano-aerosol

Aerosol is a suspension of fine solid particles or liquid droplets in a gas phase. For example: smoke, air pollutants, and perfume spray are aerosol. The word aerosol is derived from the fact that matter "floating" in air is a suspension. A nanoaerosol is, therefore, a collection of NPs suspended in a gaseous phase. The particles may be pres¬ent as discrete NPs, or as assemblies of NPs. These assemblies may have diameters larger than 100 nm. In the case of an aerosol consist¬ing of micrometer-diameter particles formed as assemblies of NPs, the definition of nanoaerosol is open to interpretation. It is generally accepted that if a nanostruc¬ture associated with the NPs is ac¬cessible through physical, chemical, or bio¬logical interactions, then the aerosol may be considered a nanoaerosol. However, if the nanomaterial within individual micrometer-scale diameter does not directly influence particle activity, the aerosol would not be described as aerosols [18].

6. Nano-agglomerate

Agglomerate is a group of coarse accumulations of material particles held to¬gether by relatively weak forces, including van der Waals forces, electrostatic forces and sur¬face tension [11, 21]. Nanomateri¬als have the greatest potential to enter the body if they are in the form of agglomerates of NPs, and particles from nanostructured materials that become airborne or come into con¬tact with the skin [1]. Agglom¬erates of NPs are usually deposited according to the diameter of the agglomerate, not that of constit¬uent NPs [11]. Deposition of agglom¬erates result in development of granulomas, while deposition of more dispersed nanotube structures result in a rapid development of interstitial fibrosis (within 7 days), which pro¬gress over a 60 day post-exposure period [1]. However, research is still ongoing to determine the physical factors that contribute to the agglomeration and de-agglomeration of NPs and the role of agglomerates in the toxicity of inhaled NPs.

7. Nano-aggregate

An aggregate is a heterogeneous particle in which various components are held to¬gether by relatively strong forces and thus not easily broken apart [17, 21]. Aggregates can bond to each other to form agglomerates. These aggregates tend to adhere to each other because of Van der Waals forces that only act over short distances, electrostatic forces present in the particles and adhesion forces related to the liquids adsorbed to their surface. Aggregation is thermal when caused by Brownian motion, and it is kinetic when caused by an external force, such as gravity, electrical forces or aerodynamic effects. Van der Waals forces are weak forces that hold aggregates together. Murr *et al.* [22] clearly showed that airborne particles were mainly aggregates of aerodynamic diameters ranging from a few nanometres to several micrometres.

7. Nano-portal routes

Due to the increased use of nanotechnology, and the potential risks associated with exposure to NPs, it is inevitable that the potential routes of entry need to be well understood. In fact, these tiny particles are able to enter the body naturally (unintentionally) or induced artificially (intentionally) through the skin, lungs or intestinal tract, and its deposition in several organs and may cause adverse biological effects [7-9, 23, 24]. Other potential routes of exposure to NPs include parental administration such as intravenous, intradermal and peritoneal injections [25]. In addition, the toxicity of NPs will also depend on whether they are persistent or cleared from different organs of entry and whether the host can raise an effective

response to sequester or dispose the particles [26]. Factors that my influence NPs entry includes size, charge, surface area and shape [27]. According to Auffan *et al.* [27] nanosize particles have an elevated surface/ volume ratio of approximately 35-40% of atoms localized at the surface of a 10 nm NPs compared with less than 20% for particles larger than 30 nm.

Nanomaterials have the greatest potential to enter the body if they are in the form of NPs, agglomerates and aggregates of NPs, and particles from nanostructured materials that become airborne or come into con¬tact with the skin. Airborne nanomaterials can be inhaled and deposited in the respira¬tory tract. Based on animal studies, NPs can enter the blood stream, and translocate to other organs [1]. There is also a seemingly countless number of NPs already present in the environment. They include carbon-based (nanotubes, nanowires, fullerenes) and metal-based (gold, silver, quantum dots, metal oxides such as titanium dioxide and zinc oxide), and those that are arguably more biological in nature (liposomes and viruses designed for gene or drug delivery). They also include consumer products such as sunscreens, cosmetics as well as those from combustion in engines, grilling, forest fires or volcanic eruptions, welding and car wax, and many more [6, 26]. These NPs represent a target for potential toxicity. As such, there is potential exposure of NPs that are introduced to the body through the act of breathing and by any other exposure routes that may result in systemic distribution, including dermal and gastrointestinal absorption and direct injection [26].

8. Nano-respiratory route

Much research has been done with NPs toxicity of the respiratory tract. These nanomaterials can be inhaled [7, 8, 12] naturally in the form of aerosol, powders or artificially by instillation into the respiratory

tract for toxicity studies. For instance, findings by Warheit *et al.* [12] and Li *et al.* [23] have found that NPs can be instilled via intratracheal, oropharyngeal and intrapha-ryngeal routes when determining the toxicity of NPs of the respiratory tract in experimental animals. The respiratory system is the part of the organs that deal with the process of respiration that is, moving from the nose through the trachea to the bronchioles. The system is responsible for taking in and sending out air from living animals. Most of the respiratory tract exists merely as a piping system for the air to travel into the lungs. The lungs are parts of the respiratory tract responsible for exchange of gases (oxygen and carbon dioxide) with the circulatory system (blood). Moving down the respiratory tract starting at the trachea, the tubes get smaller and divide more and more. Even though the cross-sectional area of each bronchus or bronchiole is smaller, the total surface area is larger because of the large number of bronchioles. This means there is less resistance at the terminal bronchioles.

The most common route of exposure to NPs in the workplace is by inhalation. Once inhaled, the NPs can be carried by electrostatic force of the air from the upper respiratory tract to the lower respiratory tract in the bronchioles [8, 15]. The particles are usually inhaled in the form of gases, aerosols and liquid particles, and also through systemic delivery of drugs, chemicals and other compounds to the lungs through direct cardiac output to the pulmonary arteries [26]. Inhaled nano-sized powdered mineral sunscreens or sprays pose health risk during application. According to Geiser [28] some of the products made with NPs are specifically advertised for use on the face which can act as potential routes of inhalation.

The deposition of these discrete NPs in the respiratory tract is usually determined by the particle's diameter (particle size). According to NISOH [1] agglomerates of NPs can be

deposited in the respiratory tract according to the diameter of the agglomerate. Reports, according to ICRP [29], have shown that discrete NPs are deposited in the lungs to a greater extent than larger respiratory particles. Other studies by Daigle *et al*. [30] have also shown that deposition increases due to increase in breathing rate and change from nasal to mouth breathing and among persons with existing lung diseases or conditions. Immediately the NPs are in the pulmonary sites, translocation to blood circulation through the lymphatic pathways can occur depending on the nanomaterial size. Earlier reports by Berry *et al*. [31] described the translocation of 30 nm gold NPs across the alveolar epithelium of rats by interstitial instillation. This report was further supported by Ballou *et al*. [32] when they showed the fast appearance of quantum dots (10 nm) in liver, spleen, lymph nodes and bone marrow of mouse. Also when the ultrafine particles are deposited in the alveolar region, they are usually attacked by alveolar macrophages, through the process of phagocytosis. This also led to chemotactic activities which signal the complement cascade fashion serum proteins and the inflammatory cell response to the site to remove the NPs. According to Oberdorster *et al*. [8], this may take up to 70 days in rat and 700 days in humans to be cleared. According to earlier reports of Borm and Kreyling [14], the interstitial translocation of ultrafine particles across the alveolar epithelium is more common in higher primate's species (dogs and non human) than in rodents but they assumed that the high translocation in rats can occur in human as well. Gwinn and Vallyathan [33] reported that inhaled nanosize particles may evade phagocytosis, cross cell membranes, and redistribute to other sites of the body, causing systemic health effects in experimental animals. Other animal studies, have also shown that discrete NPs may en¬ter the bloodstream from the lungs and trans¬locate

to other organs [8].

There are also reports that nanoscale viruses (30 nm) such as the polio virus found in the lungs can enter the sensory nerve endings of the olfactory organ [34]. The discrete NPs that are deposited in the nasal region may be able to enter the brain by translocation along the olfactory nerve of rats in to the brain [35]. Other reports by Oberdorster *et al*. [8] confirmed that inhaled MnO2 NPs (30 nm) can be translocated from the lungs into the olfactory organ after a 7 day post exposure in experimental rats. The olfactory system is the sensory organ used for olfaction, or the sense of smell (Figure 3), the prominent part of the face of mammals. It receives stimuli interpreted as odours from volatile and soluble substances and lies in the upper part of the nasal cavity, and that forms a mucous membrane continuous with the rest of the lining of the nasal cavity. This reveals that the nerve endings of the nasal olfactory mucosa are portal entry of nanomaterials into the host. According to earlier findings by De Lorenzo [36] silver coated colloidal gold particles of up to 50 nm can be transported through olfactory nerves as well as across the synapses of the dendrite cells. This exposure routes have not been studied in human, and research is continuing to evaluate its relevance. Taking into cognizance the nano-respiratory tract toxicity studies from animals, there is the possibility that the translocation pathways which exist in human be highly dependent on the chemical and physical properties of the NPs. It can be concluded, therefore, that the unbridled growth and use of nanotechnology in medical and human health evaluations opens society to the possibility that ultrafine particles (carbon nanotubes) could become the "asbestos" of the 21st century.

9. Nano-gastrointestinal route

The gastrointestinal tract (alimentary canal) is the system of organs in animals that takes

in food substances, and expels the remains as waste. The major functions of the intestines are ingestion, digestion, absorption and defecation. The structure of the alimentary canal of animal differs from animal to animal. Some animals have multi-chambered stomachs, while some animals' stomachs contain a single chamber. Figure 5 demonstrates a simple illustration of primates' intestinal tract.

Ingestion is another route whereby NPs may enter the body. Most of the toxicity studies pertaining to NPs are focused mainly on respiratory tract (RT) exposures with few studies describing the gastrointestinal tract (GI) exposures. Ingestion can occur from unintentional hand to mouth transfer of materials. This can occur with traditional materials, and it is scientifically reasonable to assume that it could also happen during handling of materials that contain NPs. Ingestion may also accompany inhalation exposure because particles that are cleared from the respiratory tract via the mucociliary escalator may be swallowed [8, 9, 23]. Nanomaterials can also be ingested into the gastrointestinal tract viz water, food, cosmetics, drugs, drug delivery devices, the swallowing of inhaled particles or intentional hand to mouth transfer of particles [2, 7, 8]. Some studies have investigated the potential intestinal absorption and the translocation of NPs and generally found uptake within the GT. Studies of Jani [37] showed the absorption of titanium particles 150-500 nm larger than typically used in sunscreen into the liver and spleen through gut. More detail about the fate of ingested particles can be viewed from radioactive metal studies, which found NPs passage from the gastrointestinal system to other organs [14].

NPs administered orally can be absorbed, through the membranous epithelial cells (M-cells) of the Peyer's patches in the gut-associated lymphoid tissue (GALT) and also through the numerous gut enterocytes [9].

Earlier reports by Jani *et al*. [37] indicated that oral administration of NPs can be absorbed across the GI tract via the lymph nodes to the liver and spleen. Reports of Yoshifumi [38] showed that NPs substances are easily taken up by the recticuloendothelial cells during drug transfer. The uptake of these particles of different sizes can lead to different toxicological effects. Studies on polystyrene latex NPs in the range of 3 μm to 50 nm revealed that maximal absorption occurred with particles ranging 50-100 nm in diameter [39]. However, further studies by Hussain *et al*. [39] found that even latex particles above 1 mm can be trapped in the Peyer's patches. The uptake of ultrafine particles by the GI tract can stimulate phygaocytosis at the GI mucosa and cause antigen-antibody mediated reactions and inflammatory responses and from there systematically to other organs of the body [39].

Studies by Chen *et al*. [9] have shown that copper NPs can induce toxicological effects and heavy injuries occurred on the kidney, liver and spleen of the experimental mice. In these studies by Chen *et al*. [9] after gavaging mice with copper NPs, they discovered that the GI tract toxicity belongs to class 3 (moderately toxic) of Hodge and Sterner Scale. Other symptoms associated with the toxicity were alimentary disorder such as loss of appetite, diarrhea and vomiting. Others included hypopnea, tremor and arching of the back. Other reports by Mayank and Monsoor [10] have also showed that, gelatin NPs can traverse through the gastrointestinal tract fairly quickly with more than 85% of the administered dose per gram localizing in the large intestine within the first h. After 5 days post-administration, transgene expression can occur in the small and large intestine of rats. However, there are few more reports about the toxicology of NPs by gastrointestinal tract.

10. Nano-dermal route

The derm is the outer covering of the skin

(epidermis and dermis). It is the largest organ of the body and guards the underlying internal organs. Due to its interface with the environment, skin plays a very important role in protecting (the body) against external interferences. Its other functions are insulation temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates.

There are possibilities that skin barrier alterations - such as wounds, scrapes, or dermatitis conditions - could affect nanoparticle penetration and the skin as a potential route of exposure and should not be overlooked. Debilitated skin represents a good channel for entry of finer and even larger particles (0.5-7 μm) as reported by Blundell et al. [40]. These studies found a large accumulation of soil particles in lymph nodes of bear footed human associated with elephantiasis. Researchers at North Carolina State University have also shown that quantum dot NPs can penetrate the skin if there is an abrasion, providing insight into potential workplace concerns for healthcare workers or individuals involved in the manufacturing of quantum dots or doing research on potential biomedical applications of the tiny NPs [41]. Earlier reports by Kim et al. [42] showed that mice injected intradermally with quantum dots can localize in the lymph nodes and can systematically spread to other organs as previously described. The U.K. Royal Society and Royal Academy of Engineers have reported that NPs of titanium dioxide used in sunscreens do not penetrate beyond the epidermis [1]. However, the report also makes a number of recommendations addressing the need for further and more transparent information in the area of nanoparticle dermal penetration. Tinkle et al. [43] have shown that particles smaller than 1 μm in diameter may penetrate into mechanically flexed skin samples. Recent studies by Zhang et al. [44] reported the penetration of quantum dot (QD621) NPs

(i.e., NPs containing cadmium and selenium core with cadmium sulphite) when topically applied to weaning porcine skin (of Yorkshire pigs). The same group also used the same QD621 and found that the quantum dot could penetrate neonatal human epidermal kerationcytes leading to inflammatory responses. The QD621 were depicted in the intercellular lipid bilayers of the stratum corneum by transmission electron microscopy [44] by elevating cytokines (interleukin-6 and interleukin 8). Monteiro-Riviere [41] reported that quantum dot penetration was a function of intercellular lipid structure or hair follicle density which could modify these penetration processes. Previous studies by Ryman-Rasmussen et al. [45] showed that quantum dot could penetrate through the epidermal layers synthesized with the same core/shell and with similar surface coatings having similar hydrodynamic diameters but different penetration rates. Apart from quantum dot, Zhang et al. [44] also showed the biological interactions of functionalized single-wall carbon nanotubes in human epidermal keratinocytes stimulating cytokines.

Other NPs such as TiO_2 and ZnO have also been reported as key particles that are capable of penetrating the skin when applied topically to human skin in vitro [15]. Studies by Tan et al. [46] showed the absorption of titanium through human skin with micro fine TiO_2, while studies with microfine zinc and TiO_2 particles applied to porcine skin did not show penetration. This is because pig porcine skin has collagenate (Mediskin), which can provide limited adhesion of NPs. According to the studies by Rodney and Barbara [47] porcine skin has showed limited wound adhesion and limited control of bacterial infection.

Other studies have reported that NPs with varying physicochemical properties were able to penetrate the intact skin of pigs [45]. These NPs were quantum dots of different sizes, shapes, and surface coatings. They were reported to penetrate the stratum corenum

barrier by passive diffusion and localize within the epidermal and dermal layers within 8 to 24 h. Another experiment carried out by Baroli [48] with excised human ski healthy female abdominal skin samples, exposed to NPs for a maximum of 24 h showed that penetration of NPs to the skin occurred through the stratum corneum lipidic matrix and hair follicle orifices, allowing NPs to reach the deepest layers of the stratum corneum, the stratum granulosum, and hair follicles. He also showed that in some exceptional cases, the NPs were found in viable epidermis [48].

Studies conducted *in vitro* us¬ing primary or cultured human skin cells have shown that both SWCNT and multi-walled carbon nanotubes (MWCNT) can enter cells and cause release of pro-inflammatory cyto¬kines, oxidative stress, and decreased viability [41]. Research on the dermal exposure of NPs is still ongoing and it is still unknown if skin penetration of NPs would result in adverse effects as these studies have not been reported in animal models.

Most of the penetration and distribution of nanomaterials in skin and toxicity are minimal and limited to the uppermost stratum corneum layers and areas near hair follicles. This usually led to irritation of the inflammation area in experimental animals. This is because the stratum corneum is the primary barrier for skin and that any type of perturbations to the skin such as an open wound, cut, or alteration to this skin barrier could expose NPs to viable skin cells [15, 44]. Therefore, more toxicological assessment such as abrasion should be conducted to determine if penetration to this barrier would allow an enhancement of absorption of nanomaterials. This raises the question whether nanomaterials could penetrate the dermis, be eventually absorbed systemically, and be responsible for an acute/chronic and local/systemic potential health risk. We already know that that the skin is nanoporous at the nanoscale, having orifices of hair follicles and glands open on skin surface therefore, providing alternative entrance routes.

11. Nano-ocular route

The eyes are used to detect light and sending of signals along the optic nerve to the visual areas of the brain. The human eye can be divided into the anterior and posterior anatomical segments. Only few reports are available in which the eye is used as a source of entry of NPs into the experimental animals. Drug delivery is achieved through topical application [49]. However, topical application of drugs for treatment of posterior eye disorders is not very effective due to the rapid precorneal elimination due to solution drainage, long diffusional path length, induced lacrimation, and corneal epithelial impermeability to molecules larger than 5 kDa [50]. However, NPs have generated considerable interest for drug delivery into the eye [49]. According to Herrero-Vanrell and Refojo [51], intravitreally administrations of NPs have shown to sustain drug delivery to the eye. The subconjunctival administrations of Fluorescent NPs (FluospheresTM, 20 nm) to male Sprague-Dawley rats containing sodium fluorescein, NPs were detected within 15 min. Recent reports by Farjo *et al*. [52] explained how DNA NPs can be implored to transfer genes into the mouse retina. Jani *et al*. [53] reported that albumin NPs encapsulating pCMV.Flt23K when injected into the corneas of uninjured mice the NPs were detected in the corneal keratocyte cytoplasm. The albumin NPs can be used to express intraceptors for extended periods that are effective in suppressing injury-induced corneal neovascularization. The highly efficient transfer of the reporter gene into photoreceptor cells could lead to effective treatments for conditions such as retinitis pigmentosa. Therefore, by modifying the properties of NPs, they could be made to target specific organs.

12. Nano-auditory route

The ear is the organ that detects sounds and plays a major role in the sense of balance and body position. It is a part of the auditory system. Vertebrates have a pair of ears, placed symmetrically on opposite sides of the head. The arrangement of the ears and the ability to localize sound sources (waves) can facilitate the entry of NPs into the inner ear and to the other parts of the body via blood. However, very few researches have been made public that the auditory pathway is a channel for NPs transport into the ear. This is due the complex nature of the anatomy of the ear which contains hollow channels filled with liquid, and contains a sensory epithelium that is studded with hair cells. The microscopic hairs of these cells are structural protein filaments that project out into the fluid and reduce NPs chances of penetrating the ear. Some preliminary reports [54] by Mamedova et al. of Hough Ear Institute showed that superparamagnetic NPs can be used as drug delivery into the inner ear of guinea pigs and into the prilymphatic fluid. Another pilot report by Xianxi et al. [55] of San Diego CA also showed that polylactic/glycolic acid (PLGA) polymer coated with iron oxide NPs, applied to the round window membrane of chinchillas, induced by magnetic field can enter the inner ear and will be found in multiple locations within the cochlea tissue. According to a recent work by Barnes et al. [56] to compare two different physiological studies that involve magnetic acceleration of superparamagnetic nanoparticles (SPION) through two round window membrane (RWM) models, through electron microscopy studies, they were able to confirm that SPION were pulled through the RWM of anesthetized guinea pigs.

13. Nano-intravenous routes

In biological assessment, intravenous administration of NPs is a very important route used in determining toxicological assessment in experimental animals. In the study of De Jong et al. [5] to determine particle size-dependent organ distribution of gold NPs, they intravenously injected gold NPs with diameters of 10, 50, 100 and 250 nm to rats, and observed oxidative stress in the rat liver cells. The 10 nm gold NPs showed the most widespread presence in various organ systems including brain, heart, kidneys, lungs, testis, and thymus [25]. Also in order to test the toxicity and biomedical imaging of layered nanohybrids consisting of magnesium/aluminium core, Flesken et al. [35] injected the NPs subcutaneously, intraperitoneally and intravenously to mice. Their histological findings showed inflammatory lesions in the lungs and dermis after intravenous and subcutaneous administration, respectively. Early experimental studies by Rocio et al. [57] who administered NPs intravenously as single doses of 20 and 100 mg/kg for 14 days, also showed the liver as a passive target tissue for NPs if given intravenously, due to the phagocytosis by Kupffer cells. Indeed, intravenous administration of NPs is followed by inflammatory responses, characterized by an increased synthesis and secretion of cytokines. Experimental animals absorb NPs from the site of injection into the lymphatic system [58] as shown in Figure 9. The subcutaneous route involves a complex sequence of nanoparticle movement, mostly involving lymph and blood. The relevance of intravenous administration of NPs into experimental animals studies to humans have been questioned not only in drug delivery but also in vaccination, a modality which requires systematic absorption of the encapsulated active drug to achieve a biological response [57] as shown in Figure 8.

14. Nano-mucus route

The mucus membrane pathway is the lining of most endodermal cells that cover the

epithelium and are involved in absorption and secretion. They line various body cavities that are exposed to the external environment and internal organs. It is continuous with the skin, nostrils, lips, ears, the genital and the anus. NPs deposited on various mucus tissues pathway, encounter mucus or epithelial lining fluid. They may then interact with macrophages, which may result in their clearance, or they may enter the interstitium where they may make contact with fibroblasts and endothelial cells or cells of the immune system. The mucus membrane is the first barrier that confronts NPs that are deposited in the conducting epithelium. Other reports by Moghimi *et al.* [59] have shown that NPs can be translocated through the mucosal lining and epithelial barrier of the intestine and do associate with the GALT (gastrointestinal associated lymphatic tissue) and the circulatory system. According to Umamaheshwari *et al.* [60] NPs deposited on the mucus membranes might lead to various kinds of interaction forces between mucoadhesive nanomaterials and the mucus surface. These forces include electrostatic attraction, hydrogen bonding, Van der Waals forces, mechanical interpenetration, and entanglement. Many methods have been used to evaluate these interactions *in vitro* and *in vivo*. A fluorescence probe method was used to measure *in vivo* mucoadhesive capacity of developed formulations [60]. Depending upon the specific mucus membrane application, NPs exposure may translocate and impart a cytological toxic effect depending on the factors earlier reported.

15. Conclusion

The safety issues derived from NPs routes of entry and their potential bio-distribution are governed by surface area, shape, agglomeration, aggregation solubility and size with protein (opsonisation) interactions within the host. The size fractions in the nanoscale range have greater lung deposition and rapid systemic translocation having various inflammatory, oxidative and cytotoxic effects on experimental animals than larger particles [2, 59]. With these discussed possible potential routes of NPs, nanotechnology research should proceed with caution. The combination of hazard and production should go hand in hand so as to reduce potential acquisition of NPs through the practice of good manufacturing practice (GMP), good laboratory practice (GLP) and International Standards Organisation (ISO). Suitable quality control procedures should be part of the process so as to ensure NPs product safety and quality and hence part of the company quality assurance scheme. Also the manufacturing industries of nanotechnology should work hand in hand with the health and hazard risk assessment so as to establish a lower health risk of any type emanating from the production and used of NPs. Though there is limited toxicological data available at the present, with the current review, there is hope to increase the awareness and safety issues of nanotechnology.

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