



Exposure and risk assessment of engineered nanomaterials to the human body

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Abstract

21st century brings out a revolution in a field of nanotechnology. Multiple technological applications on nanoscale can be created, manipulated or utilized according to ones need. Engineered Nano materials (ENM) have a lot of benefits and implementation in different fields. Different materials can be used to create an ENM for different purposes. These materials show different physical and chemical properties (Schmid *et al.*, 2010). Although ENM has wide range of applications, but during its manufacturing and usage the chances of vulnerability for the workers and their future effects cannot be anticipated (Cordier & Stewart, 2014). Previous finding showed a group of patients were exposed to nanoparticles which were having a coating of polyacryl paint. The exposed workers showed peculiar symptoms like pleural effusions, progressive pulmonary fibrosis and can lead to death. At that time the reason for the death and symptoms was not clear. Researchers gave different opinions few argued that this is due to gas or raw materials whereas others had different reasons. But after examining biopsies and chest fluids of the patients with Transmission Electron Microscopy and Energy Dispersive X-rays (EDX) nanomaterials were found to be the reason (Song *et al.*, 2011). Nanomaterials showed deleterious effects on human lungs. It was expected that by the end 2020, 6 million workers can be affected by ENMs, thus major guidelines must be follow to avoid the toxic effects of ENMs. (Roco, 2011).

Keywords: ENMs, nanoparticles, exposure, toxicity, risk assessment

Introduction

Different exposure assessment approaches have been made in order to investigate the ways in which the humans are exposed to ENMs. In an urban environment the motor vehicles are the richest source of nanoparticles. Mainly diesel vehicles emit nanoparticles in the range of 30-60 nm. Nanoparticles of this size exhibit concentration effects as the speed of wind increases (Charron & Harrison, 2003) [3]. Moreover, fossil fuel combustion like in case of diesel engine their By Products also produces nanoparticles. This constitutes a major source of nanoparticles emission (Oberdörster *et al.*, 2005) [19]. Similarly, Nanoparticles can also enter into the environment due to several reasons. Manufacturing is the major reason, as due to technological advancement there is large production of nanomaterials every year. The left over residues, spillage, their use and disposal can directly lead to environment exposure. Various self-care products like cosmetics and sunscreen contains nanomaterials and they enter into the environment as we washed off. But still there is no huge data available for environment exposure. Studies showed that nanomaterials can travel up to 20 meters in a ground water and can remain active for more than a month (Khan *et al.*, 2017) [12]. Through the study of Institute of Occupational Medicine for the UK health and safety executive it can be presumed that industrial activities like Nanotechnology primary research and development groups, chemical and pharmaceutical industries, paint, cement and pigment manufacture and welding are the activities in which exposure can occur. Industrial by products having nanomaterials can be potentially hazardous as they stay in the workplace air; major examples are metal fumes, polymer fumes, meat smoker fumes, welding fumes, bakery oven emission etc

(Plitzko, 2009) [21].

Exposure Routes of Engineered Nanomaterials In Humans

Their properties showed marked challenges. Behavior of engineered nanoparticles can be difficult to access. The inhalation route is the prominent one followed by dermal and oral routes. In future oral route can also be a difficult challenge as food items contain nanoparticles. More than 1600 nanoenabled are there in the market. ENM which include Titanium dioxide, different metal oxides and carbon material cause pulmonary inflammation (Kinaret *et al.*, 2017) [13]. Other side effects like granuloma formation and fibrosis of the lungs can also occur. It has been observed that carbon nanotubes can cause collagen deposition in the pleural space of lungs. A part from that studies has been shown that crystalline ENM can induce genotoxic effect and can potentially act as a carcinogen. The most important exposure takes place in workplaces only. Whereas, for consumer and environment exposure there are less case reported. ENM based activities are pursued more in industries than in research labs. Throughout the year, from per kg to thousands of tons ENMs are used in industries whereas few grams to kg in the research labs.

Respiratory Exposure

Respiratory system can act as a primary route of exposure as inhalation is in direct contact with it. The respiratory system has several compartments like nose, larynx, airways and alveoli. They all act as a fractional filter system, through which ENMs in the occupational environment can be exposed (Savolainen *et al.*, 2010) [24]. Of course other less prominent routes are also there like Eyes, Skin and

Gastrointestinal tract. The particle size of the ENM plays a predominant role for their deposition in the lungs and distribution in the body. ENMs can easily enter to the body in an aerosol form directly to lungs through inhalation and sometimes it can enter to alveolar region of lungs also (Kreyling *et al.*, 2010) ^[14]. The Particle size of ENM will decide how deep in the lung a particle can invade. When a particle enters in the lungs, it comes in contact with the biological surrounding of the lungs and gets self surrounded by protein and albumin. As a result of this surrounding, the size of the particle increases which have a direct connection with the movement of particle in the airways. Larger particles (micrometer scale) are catch up in the nose and are easily removed by macrophages and mucociliary escalator from the airways. Small ENMs which are in the range of approximately 10 nm can easily deposit in the alveolar region of the lungs. Here, pulmonary action takes place (Monopoli *et al.*, 2012) ^[17]. The particles are engulfed into the macrophages. However, if there is damage or stress to the macrophage, it produces cytokines, chemokines as a result of differentiation and expression of other NF- κ B-dependent genes and production of lipid mediators leading to inflammation. If the inflammation is for a long period, it can be persistent to pathogenic particles as a result fibrosis can develop. Fibrosis is identified by the fibroblast proliferation, accumulation of collagen and myofibroblast transformation. Along with this the level of growth factors also increases at the site where lesions are developing. Carbon nanotubes can cause fibrosis without inflammation. ENMs exhibit genotoxic and carcinogenic properties also (This *et al.*, 2013). Genotoxicity action of the ENM can be showed in two ways- Primary and Secondary. In primary genotoxicity, nanoparticles and target cells are in direct contact with each other. Whereas, secondary genotoxicity follows the route of inflammation and then use ROS to damage DNA. In case of primary genotoxicity nanoparticles directly enters into the nucleus and interacts with the DNA and damage, it also lead to chromosome segregation (Donaldson *et al.*, 2010) ^[6]. This can also happen via indirect process as particles increases the steady state of oxidative stress as a result of oxidative damage in mitochondrial activity, there is inhibition of DNA repair. Recently it has been demonstrated that carbon nanotubes shows both genotoxic as well as carcinogenic effects (Kreyling *et al.*, 2010) ^[14] (Landsiedel *et al.*, 2009) ^[15].

Now, of those ENMs which have size approximately few nano meters can further go for systemic circulation by passing the alveolar wall. ENMs show high surface reactivity that is why where they deposit, they generate Reactive Oxygen Species (ROS) which leads to local injury and induce inflammation. Smaller ENMs via blood and lymphatics pass to the internal organs and organ system and utilize direct or indirect mechanism to cause damage. Researcher explains that the Biologically Active Dose standards are utilized in the hazard assessment and exposure. BED is an amount of Nano particles that really reaches out the target organ and their shows its effect like oxidative stress, inflammation or genotoxic via indirect-direct mechanisms (Abbas *et al.*, 2020) ^[1]. In pleuro pulmonary system, Nanoparticles induces cellular stress in epithelial cells and macrophages. Oxidative stress is the major stress inducing process, it creates imbalance between oxidants and antioxidants between cells in tissues or lung lining. As a result there is no more oxidation. Nanoparticles

produce ROS as a result of intrinsic chemical reactions. Inflammatory cells actively produces free radicals, during inflammatory responses they exhibit oxidative stress. The cell and tissue damage which lead to chronic inflammation and association of different disease is the result of ROS released from phagocytes. The redox sensitive particle plays an important role in inflammation (Warheit *et al.*, 2004) ^[30]. Sometimes it is capable of showing frustrated phagocytosis and activation of in inflammasome. The nanoparticles undergo surface modification which is measured by zeta potential shows that this is responsible or local inflammation. The positive ZP on NP is immediately neutralized on formation of a corona after deposition in lung lining fluid but inside the lysosomes, the acid and protease/lipase activity may be able to remove this, revealing the charged surface. This can the interact with the lysosomal membrane leading to rupture and release of lysosomal contents with concomitant activation of the NALP3 inflammasome, as has been shown for quartz particles, Whereas, in frustrated phagocytosis the phagosome is failed to close and there is phagolysosomal dysfunctioning as a result of it lysosome destabilized and inflammasome activates. These long fibers like carbon nanotube, titanium dioxide follows such fiber pathogenicity paradigm model (Maynard *et al.*, 2004) ^[16].

Gastrointestinal Exposure

Nanomaterials which are present in consumer items like toothpaste, cosmetic, sunscreen and food lead to unknowing ingestion of large amount of population. Those materials which enter through inhalation route may enter into gastrointestinal tract after the clearance of respiratory tract. Nanomaterials in gastrointestinal tract are majorly responsible for implication of toxicity locally and nearby organs. Some studies indicate that M cells are responsible for the uptake of the nanomaterials, while other suggested villus epithelium is responsible (Geiser & Ph, 2010) ^[9]. However, till date very less data reported on nanomaterial toxicity in gastrointestinal tract. Investigations have been done in order to determine the nanomaterials ingested and its uptake from gut lumen. When nanoparticles are present in gut, they show local toxic effects. Systemic effects may induce by coming in contact with gut microorganisms. Metal nanoparticles such as silver, copper and gold showed some sort of toxicity in rat models (Jepson & Bouwmeester, 2017) ^[11]. The acidic environment of stomach triggers the toxicity of copper and silver nanoparticles by releasing the ions. Cationic nanomaterials tend to entrap in negatively charged mucus whereas repulsive carboxylated latex particles are able to diffuse the layers. Studies showed that silver nanoparticles raised important impact on gastrointestinal tract function. The dysregulation, which is a result of gastric disease induced by nanomaterials trigger inflammatory bowel disease. In this condition there is identification of pigmented cells in the peyer patches of the humans, which is formed as the macrophages accumulates mineral material like titanium dioxide (Pietrousti & Magrini, 2014) ^[20].

Skin Exposure

Skin is the largest organ of the human body. It serves as a prime barrier to absorb anything from the environment. The skin can be major route of exposure as we use cosmetics on daily basis. Exposure to undefined amount of chemical

continuously can target the nanomaterials or other toxic elements. The engineered nanomaterials sometimes elicit dermatotoxic properties; they are able to penetrate into the skin and interact with the biological environment of skin and promote a toxicological response. American Conference of Governmental Industrial Hygienists (ACGIH) labeled many of the toxic substance as 'skin notable', which have ability to total systemic exposure (Rancan *et al.*, 2012) [22]. European projects in their study showed that titanium dioxide and zinc oxide nanoparticles are unable to penetrate in the skin whereas few studies showed their penetration through skin. For a healthy skin a compound can use four properties to penetrate- intercellular (between cells), through hair follicles, transcellular (through cells) and sweat glands. Irritant detergents, chemicals and mechanical actions may result in increasing the skin adsorption. Mechanical actions like hand movement which led to exerts biomechanical forces as a result structural organization alters and skin become more permeable to nanomaterials. Moreover, study showed that when zinc isotope containing sunscreen is applied to UV damaged skin for five days; it shows high level of zinc in urine and blood of the person who is applying the sunscreen. ENMs can be potentially toxic and cause local irritation also (Setiyawan, 2013) [23]. After exposure to carbon nanofibres, carbon fibers dermatitis has been reported in humans. Nanoparticles which further enter into the epidermis in the presence of sensitization substances like nickel, cobalt and chromium may cause allergic reactions which can further induce asthma (Erdely *et al.*, 2013) [8]. Fullerene may promote IgE mediated allergic response and lead to blockage of histamine release. They can also inhibit mast cells and may lead to anaphylaxis.

Brain Exposure

Engineered nanomaterials can also reach to the brain primarily via inhalation, followed by dermal exposure and third route is gastrointestinal tract. The ENMs to brain go via inter and intracellular transport and then blood circulation to other organs. Sometimes it may reach to the brain via axonal transport via olfactory nerve. Nanotitania shows maximum effects on nervous system. They can alter brain electrolyte, increased acetyl choline, glutamate and nitric oxide and nitric oxide but decrease dopamine, serotonin and their metabolites ultimately increase oxidative stress and reduce antioxidant defense mechanism, increase apoptosis and impaired memory (Iavicoli *et al.*, 2012) [10]. In case of nanoalumina, excessive aluminum exposure in those patients who have problem in kidneys may cause fatal encephalopathy, anemia, adverse effect on skeleton system and brain development of neonates (Willhite *et al.*, 2014) [31].

Apart from that, nanoparticles are also known to induce toxicity during fetus development. The embryotoxicity develops as a result of structural defects, retards in development, organ toxicity, birth or growth problems and reduced body weight. It might results in abortion or delayed physical development. These signs sometimes appear after birth. Such persons are more prone to allergic reactions.

ENM's Risk Assessments

Risk assessments have been done in order to estimate the risk generated by the exposure to ENMs. The commercialization of nano particles based products was started in 2000s. Day by day the number of nanoparticles

containing products is increasing in the market. Production, use and disposal of such NPs must be done under specific guidelines. Employees, workers and whosoever working in direct contact must need proper information on the hazard of nanomaterials and its associated health risks. Quantitative Risk Assessment of ENMs have been made and it involves five steps- firstly, evaluate the data which is available. Secondly, select a specific adverse response. Thirdly, determine its critical dose. Fourthly, calculate the human equivalent dose and lastly, determine the long term exposure dose- if someone works for lifetime (Bleeker *et al.*, 2015) [2]. The Point of Departure (POD) can easily be detected by QRA, which can help in identifying the concentration of dose which shows adverse effect. When quantitative data is not available other methods are utilized like grouping based on physical and chemical properties, read-across method and many more. The trajectory of risk assessment can also be done by using those methods which have been applied in the past. There is very less knowledge of risk, hazard and exposure to ENMs (Commission, 2006). The initial approach to risk assessment was based on precautionary assessment to fulfill the need for any kind of guidance to host risk management decision. Methods have been developed to prioritize or group ENMs based on the available subchronic or chronic dose-response data for benchmark materials and the utilization of shorter-term *in vivo* data for many ENMs. The history how risk assessment for ENM is in use for over 20 years since then various approaches have been used. Scientific investigations showed the evidence for all the approaches utilized by measuring difference in dose – response relationship of respirable particles by size that means nano scale and micro scale. Dosimetry models have been utilized to estimate the deposit dose of inhaled nano particles in humans and animals. In order to study long term dosage, large data is needed for the risk assessment (Schulte *et al.*, 2018) [26].

Future Directions

The major research needed in the future is to decrease the uncertainty while assessment of risk associated with nano particles. Knowledge of different pathways and internal, external exposures everything is supported by physical and chemical characterization of materials. For an external exposure, particle's concentration and its basic properties are in different state (liquid, gas) enters must be known. The impact of different reactions like agglomeration and deagglomeration or behavior of nanoparticles needs careful attention (Monteiro-Riviere *et al.*, 2014) [18]. The dose of nanoparticles which can be enough to cross the physiological barriers must be known along with its penetration to target organ or cellular and sub cellular parts of the body. New technologies will be required in the near future to demonstrate the hazard associated with nanoparticles. The *in vitro* and *in vivo* dose comparison must be done in order to develop new screening assays. Realistic exposure to internal and external dosage must be given in order to identify realistic information. There are three research options require to enable risk assessments (Elder *et al.*, 2009) [7]. First of all determine the characteristics of ENMs like its physical and chemical properties and its biological mode of action. This step is followed by applying standardized schemes for *in vivo* testing for qualitative and quantitative analysis. Finally, finding the means to utilize the known physical and

chemical properties and *in vitro* data in order to determine the risk associated with the specific particles. PODs which are used based on earlier responses; it provides the data which is overly protected from risk or to develop any adverse effects. Many approaches have been utilized to emerge from these efforts in the future.

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