Chapter 7
Nanomaterials and Human Health: An Overview

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Abstract With the advent of nanotechnology in commercial products, the risk of exposure of nanomaterials to humans and the environment is increasing at an accelerating rate. The impact of nanomaterials on humans is complex and not yet fully understood. A comprehensive understanding of the adverse effect of long-term exposure to nanomaterials on humans is warranted, and a balance between benefits and risks is required before nanomaterials are unleashed in large quantities as a part of commercial products. Most data on the consequences of nanomaterial exposure are obtained using *in vitro* and *in vivo* studies using animal models. The risk to human health is implied by these studies. In this chapter, the possible methods of exposure of humans to nanomaterials, the effect of some frequently used nanomaterials on human cells, and animal models are discussed. The primary methods of exposure to nanomaterials include oral, dermal, intravenous, and inhalation. The route of exposure can cause variation in the adverse effect on the human health. Nanomaterials elicit different negative effects/damage repair pathways depending on the type of cell, and the toxicity may vary vastly based on the type of nanomaterial. Also, the psychochemical parameters of nanomaterials such as size, shape, functionalization, and defects as well as the gender of the person can significantly alter the adverse effect on biological entities.

Keywords Human health · Toxicity · Nanomaterials · Risks · Exposure

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7.1 Introduction

Nanomaterials possess at least one dimension less than 100 nm. Nanotechnology is playing a vital role in innovation and the economy. Submicron-scale particles are ultrafine particles (UFPs) that are released into the environment by fossil fuel combustion or industrial emissions, whereas engineered nanomaterials are manufactured through controlled processes (Li et al. 2016). Both types of particles could have adverse impacts on humans, such as asthma, allergy, inflammation, DNA damage, and interference with signaling pathways. They could also adversely affect cardiovascular and respiratory systems (Li et al. 2016; Jain et al. 2018). Engineered nanomaterials have become attractive in various applications due to their unique properties imparted by the nano-scale size (Merwe and Pickrell 2018). Both commercial production and use of engineered nanomaterials are on the rise (Merwe and Pickrell 2018). It is predicted that titanium dioxide nanoparticle production could rise from 5000 tons in 2010 to 58,000 tons in 2020 (Smolkova et al. 2015). The market for graphene was estimated to be US$12 million in 2013 (Zurutuza and Marinelli 2014). In 2006, the production of synthetic amorphous silica was estimated to be one million tons per year (Fontana et al. 2017). According to the European consumer market, most engineered nanomaterial-containing products belong to the healthcare and fitness area (Mebert et al. 2017). With the rising use of engineered nanomaterials in consumer products, concerns have been raised regarding their impact on the human health and environment. Data is lacking regarding the production volume of engineered nanomaterials and their distribution in various products (Piccinno et al. 2012). Production volume, distribution, product life cycle, and product fate are an integral part of the risk assessment of engineered nanomaterials (Piccinno et al. 2012). Risk assessments to the environment and humans are very important as the use of engineered nanomaterials continues to rise since they can elicit toxicity. The risk/benefit assessment of nanomaterials before they are incorporated into consumer goods is also a very important issue and should be considered by industries (Fransman et al. 2017). Numerous studies have been carried out regarding the toxicity of engineered nanomaterials, but the fact that the toxicity of engineered nanomaterials can vary based on the size, psychochemical factors, route of administration, method of dispersion, etc., makes the investigation of impacts of engineered nanomaterials on human health complex. For example, despite numerous past studies on the cytotoxicity of engineered carbon nanomaterials, the results remain contentious (Yuan et al. 2019). Moreover, the lack of data and a comprehensive understanding of the mechanisms involved make it extremely challenging to develop regulations for engineered nanomaterial (Ganguly et al. 2018). Nanotoxicology is a new branch of the toxicology field which focuses on the understanding of toxicity of nanomaterials (Ganguly et al. 2018). The effect of nanomaterials on ecology and co-exposure of toxicant with engineered nanomaterials are also important to understand the risk posed by them (Merwe and Pickrell 2018). Developmental toxicity due to engineered nanomaterials, and the underlying mechanisms play an important role in the risk assessment of nanomaterials during

pregnancy (Dugershaw et al. 2020). Past studies have indicated that engineered nanomaterials can cause both direct and indirect developmental toxicity (Dugershaw et al. 2020).

Exposure of humans and the environment to nanoscale materials in quantities that may draw adverse biological response will continue to rise with increased use of nanomaterials in industries and consumer products (Merwe and Pickrell 2018). The method of exposure and possible impact on humans are discussed here. Most studies related to the toxicity of engineered nanomaterials have been carried out *in vitro* or *in vivo* using animal models. Therefore, most of the discussion on toxicity could be indirectly related to humans. The major focus here is on the impact of carbonaceous, silica, titanium dioxide, and silver nanomaterials on various cell lines and animal models.

7.2 Sources and Route of Engineered Nanomaterial Exposure to Humans

Due to their unique properties associated with their size, engineered nanomaterials have triggered an outburst of their exploitation in industrial applications. This has raised concerns about their safety and fate in the environment. The use of engineered nanomaterials is thriving in consumer as well as commercial/advanced products such as food, additives, supplements, feed, biocides, veterinary drugs, agriculture, water purification, soil cleaning, information technology, energy production, shampoo, and sunscreen (Martirosyan and Schneider 2014). Engineered nanomaterials are being considered for improving plant germination and growth, pesticides, pesticide/pathogen detection, fertilizer, etc. (Kah and Hofmann 2014; Khot et al. 2012; Parisi et al. 2015; Liu and Lal 2015). Although the majority of nanopesticides on the market exceeds the 100 nm upper size limit, as the research and nanotechnology field advances, it is possible that more and more agriculture-related products will fall into the nanoscale size range (<100 nm) (Kah 2015). This could lead to the trophic transfer of engineered nanomaterials to humans and the possibility of biomagnification (Lead et al. 2018; Judy et al. 2010). Moreover, engineered nanomaterials could end up in agricultural areas through their accumulation in sludge during wastewater treatment (Judy et al. 2010). A greener approach towards nanopesticides could be polymer-based nanoformulations (Kah and Hofmann 2014). Some studies have shown an enhanced germination rate and biomass in some plants in the presence of nanomaterials as well as their adverse impact (Khodakovskaya et al. 2009; Zheng et al. 2005; Rico et al. 2011). Hence, a greater understanding of the mechanisms involved in the use of engineered nanomaterials in agriculture causing beneficial and deleterious impacts is necessary.

Silica nanoparticles are found in processed food production and storage, and it was found that about 43% of amorphous silica is in the nanoscale range (Mebert et al. 2017). Silica is found in anticaking agents, antifoaming agents, and clarifying/

fining agents in food. Silica particles found in milk powder, instant soup, and spices may range from 50 to 200 nm in size (Mebert et al. 2017). Silica is also used as a nanofiller in food packaging, and may migrate when it comes in contact with food. Silica is extensively used in cosmetics, including hairstyling products, eyeliner, eyeshadow, lipstick, toothpaste, sunscreen, and antiperspirant commodities. Silica also paved its way in drug delivery and biomedical imaging. Silica nanoparticles can be advantageous in targeted drug delivery, imparting enhanced solubility and drug loading, whereas in the case of imaging, they can facilitate entrapment and functionalization of the imaging agents (Mebert et al. 2017). Therefore, dermal, oral, and intravenous exposure of humans to silica nanoparticles is inevitable. Workers in industries manufacturing these products are susceptible to exposure by inhalation as well.

Food-grade titanium dioxide may contain some particles in the nanosize range. Titanium dioxide nanocomposites are used as oxygen sensors in food packaging. Titanium dioxide and magnesium oxide nanoparticles are used as food preservatives and to facilitate the handling of food. The former is also used as an anticaking agent in powdered food products (Smolkova et al. 2015). Titanium dioxide nanoparticles are also used as a colorant in confectionery food items, non-dairy creamer, etc. They are used as photocatalysts in water treatment applications (Smolkova et al. 2015) and can be found in toothpaste, sunscreen, paints, and glazes (Weir et al. 2012), as well as photovoltaics, electrode material in lithium-ion batteries, and catalysts (Fröschl et al. 2012).

Silver nanoparticle coating is used in food as an antimicrobial agent as well as in cellulose pads that are often placed in packages of meat products (Smolkova et al. 2015). Silver nanoparticles are also used in bedding, water purifiers, toothpaste, nipples and nursing bottles, shampoos, fabrics, deodorants, kitchen utensils, etc. Aluminum nanoparticles are used in aluminum foil as an anti-adhesive agent (Smolkova et al. 2015).

Carbon nanotubes have applications in supercapacitors, metal composites, field emission displays, organic electrolytes, ionic liquids, and lithium batteries (Bianco et al. 2005; Zhang et al. 2013). Appropriately functionalized carbon nanotubes are also being considered for vaccine-delivery systems and protein transporters (Bianco et al. 2005). They have the potential to be used in nanoelectronic technology (Chen et al. 2016; Avouris et al. 2003). Graphene-based products that are already commercially available include tennis rackets, phone touchscreens, and battery straps (Zurutuza and Marinelli 2014). Graphene is being considered in applications such as metal alloys, filtration systems, printed electronics, flexible transparent conductors, polymer composites, multifunctional coatings, oil, etc. (Zurutuza and Marinelli 2014). Other carbon nanoparticles are found in caramelized sugar, bread, and corn flakes (Smolkova et al. 2015).

Therefore, it can be seen that humans can come in direct contact with engineered nanomaterials through food, cosmetics, household commodities, pharmaceuticals, water filtration, etc., leading to dermal, oral, and intravenous routes of exposure. Workers in engineered nanomaterial-related industries may be exposed directly through inhalation. Engineered nanomaterials in products such as composites,

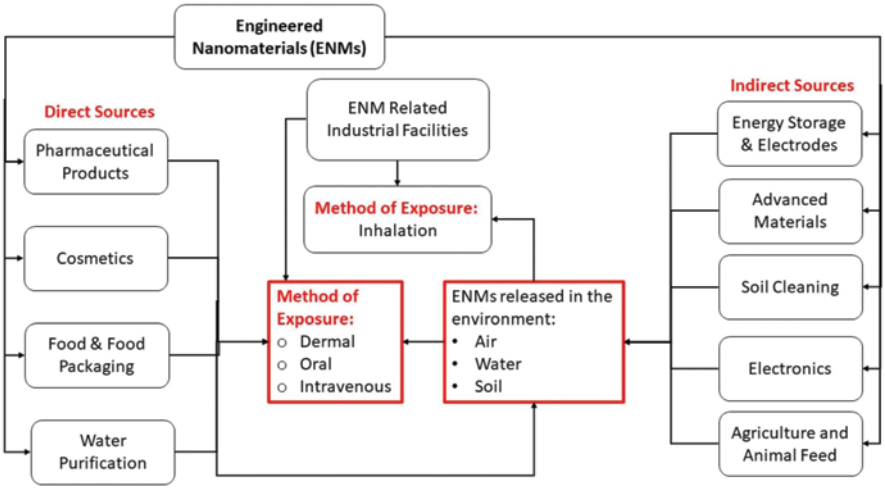

Fig. 7.1 Examples of possible sources of products containing engineered nanomaterials and methods of human exposure

batteries, electronics, paint, coatings, etc. could be released into the environment through their use and disposal, and are likely to end up in rivers, streams, air, and soil. Engineered nanomaterials released into the air could be inhaled by humans, and engineered nanomaterials released into the soil and water can enter the food chain, and eventually reach humans. Figure 7.1 shows a schematic for various sources and routes of exposure of engineered nanomaterials to humans.

7.3 Impact of Engineered Nanomaterials on Human Health

Direct data on how engineered nanomaterials influence human health is limited. There is also lack of data on the exposure of workers in industries handling nanomaterials. Most studies in this area were done on animal models. Risk factors in humans are governed by exposure level, routes of exposure and the type, size, reactivity, distribution and shape of the engineered nanomaterial (Aschberger et al. 2011). Figure 7.2 summarizes some of the different types of toxicities caused by nanomaterials and factors that may impact the type and level of toxicity. A suitable and well-established method to determine engineered nanomaterial exposure levels is very limited. Therefore, there are uncertainties and reliability issues relative to conclusions made about the health risk of engineered nanomaterials in humans (Aschberger et al. 2011). Based on the existing available database, Aschberger et al. reported the risk of four types of nanomaterials: fullerenes, carbon nanotubes, metals, and metal oxides (Aschberger et al. 2011). To assess the risk to human health, they used the indicative no-effect level (INEL), indicative no-effect

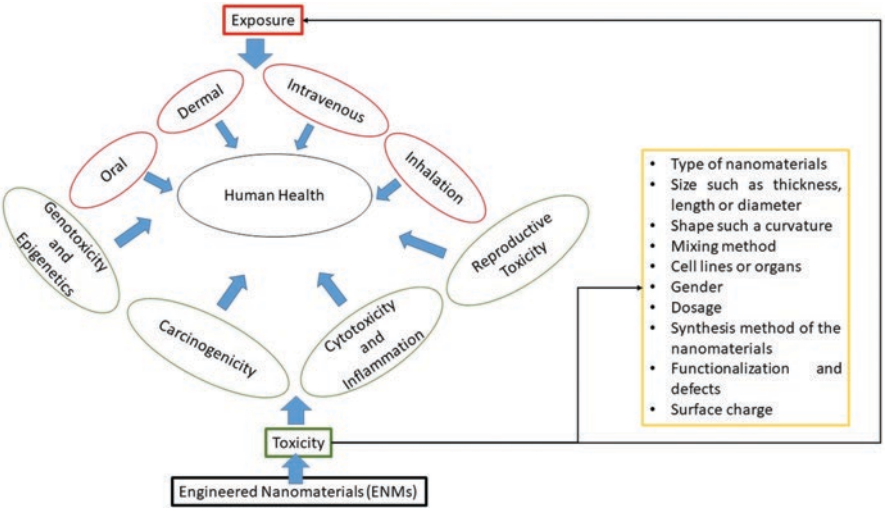

Fig. 7.2 Summary showing factors that should be taken into account when considering the toxicity of engineered nanomaterials

concentration (INEC), and predicted environmental concentration (PEC) (Aschberger et al. 2011).

For workers in situations of chronic inhalation exposure, it was observed that nanoscale titanium dioxide exhibited a higher indicative no-effect level (INEL) of 17 μg/m^3 followed by fullerenes. The impact of engineered nanomaterials taken in through respiration depends on their size, shape, and characteristics, as well as breathing rate, etc. Engineered nanomaterials in the size range of 10–100 nm accumulates in the alveolar region, whereas engineered nanomaterials smaller than 10 nm can accumulate in the thoracic region (Aschberger et al. 2011). For long multiwalled carbon nanotubes, the clearance mechanism from pleura may fail (Aschberger et al. 2011). Liao et al. monitored 124 engineered nanomaterial-handling workers and 77 unexposed workers for 6 months, and reported that workers with exposure to carbon nanotubes exhibited a change in antioxidant enzyme activities for glutathione peroxidase-1 and lung function (Liao et al. 2014). In the case of titanium dioxide nanomaterial, changes were observed in the antioxidant enzyme activity for copper-zinc superoxide dismutase and cardiovascular markers (Liao et al. 2014). Similar changes were observed for silver nanomaterials (Liao et al. 2014). This indicates that different types of engineered nanomaterials elicit variable adverse impacts on human health. This study showed a decreased level of serum CC16 and lung function in workers exposed to nanomaterials, which was consistent with past studies (Liao et al. 2014). The cardiovascular injury observed here was associated with the transfer of nanomaterials from respiratory epithelium to the circulatory system where they could elicit adverse changes in blood coagulation, cardiac frequency, and function. One interesting observation from this study was the lack of oxidative stress, which was contradictory to studies in the past.

According to the authors, this observation could be explained by the minimal level of worker exposure to the nanomaterials, and hence only a precursor response of the decrease in antioxidant enzyme activities was observed (Liao et al. 2014).

Studies have shown that nanomaterials could interfere with the epigenetic process, which involves modification in gene-expression levels without changes in the actual DNA itself through methylation, histone tail alteration, or microRNA mechanisms (Smolkova et al. 2015; Stoccoro et al. 2013). Epigenetic alteration has been associated with neurodegenerative diseases, cancers, cardiovascular complications, autoimmune disorders, behavioral disturbances, and psychiatric disorders (Stoccoro et al. 2013). Stoccoro et al. summarized the epigenetic impact of some nanomaterials, observing that silicon dioxide nanoparticles could lead to global DNA hypomethylation, PARP-1 hypermethylation, and PARP-1 mRNA suppression (Stoccoro et al. 2013). Quantum dots such as cadmium telluride (CdTe) could lead to global hypoacetylation and global changes in miRNAs expression, and multiwalled carbon nanotubes were observed to cause deregulation of miRNA expression (Stoccoro et al. 2013). Again, different nanomaterials exhibited different mechanisms to cause an alteration in the epigenetic process. PARP-1 initiates DNA repair by detecting defects in the chromosome, and hence its low expression is related to cancer. Some adverse effects caused by common engineered nanomaterials during *in vivo* and *in vitro* studies are discussed below.

7.3.1 Silver Nanoparticles

Due to their antimicrobial activity, silver nanoparticles have been employed in applications such as food packaging, deodorant, water purification, toothpaste, food and dietary supplements, etc., thereby leading to oral exposure. Moreover, they can be transferred through the food chain as antibiotic replacement in animal feed. The absorption of silver nanoparticles through the digestive system depends on size, surface reactivity, and hydrophobicity, and hence, agglomeration can ultimately reduce their absorption (Gaillet and Rouanet 2015). Past studies have shown that oral exposure to silver nanoparticles can lead to their transfer to various locations such as the liver, spleen, kidneys, lungs, bone marrow, brain, skin, eyes, muscles, blood, small intestine, stomach, prostate, tongue, teeth, thyroid, salivary gland, parathyroid, duodenum, heart, and pancreas. Studies on albino mice that were orally exposed to dose-dependent silver nanoparticles for 21 days exhibited weight loss, and negatively impacted microvilli and intestinal glands, leading to overall decreased absorption by the intestine (Gaillet and Rouanet 2015). Liver and kidney inflammation were observed in other studies with repeated oral administration of silver nanoparticles in the mice model (Gaillet and Rouanet 2015). An *in vivo* study with rats showed that silver nanoparticles could be transferred to offspring, and the oral administration of silver nanoparticles in doses higher than 100 mg/kg/BW/day could lead to oxidative stress in hepatic tissue during pregnancy. A dose of up to 1000 mg/kg/BW/day, revealed no toxicity related to the development of the

offspring. It was suggested that the oxidative stress caused by nanoparticles could play a dual role, as a consequence of toxicity and also as a modulator of inflammation (Gaillet and Rouanet 2015). It was also suggested that silver ions released from the silver nanoparticles were responsible for the impact observed in *in vivo* studies.

In the past, silver nanoparticles have shown a size-dependent toxicity in many cases (Miethling-Graff et al. 2014). It was demonstrated that exposure of LoVo cells to silver nanoparticles 10–100 nm in size elicited oxidative stress, thus leading to a high concentration of the reactive oxygen species (ROS). The ROS level was lower for silver nanoparticles 40–100 nm in size, compared to smaller-sized nanoparticles (Miethling-Graff et al. 2014). The mitochondrial activity of exposed cells decreased for silver nanoparticles 10 and 20 nm in size at 10 μg/ml, but for larger nanoparticles, the mitochondrial activities were observed to be similar to non-exposed cells (Miethling-Graff et al. 2014). The cell proliferation rate was observed to be size-independent and was adversely impacted by the presence of silver nanoparticles in a dose-dependent manner. It was demonstrated that the 39S ribosomal protein L50 was impacted by the 20-nm silver nanoparticles, whereas this was the 393 ribosomal protein L44 in the case of 100-nm particles (Miethling-Graff et al. 2014).

In another study, the genotoxicity and hepatotoxicity of silver nanoparticles were observed in female albino rats (El Mahdy et al. 2015). Exposure to 1 and 2 mg/kg led to hepatocellular necrosis and apoptosis. It was found that exposure to silver nanoparticles resulted in sinusoidal dilatation and leukocytosis for all *in vivo* models (El Mahdy et al. 2015). The investigators also observed chromosomal aberrations in the bone marrow metaphase cells. Both chromatid deletions and centromeric attenuations at significant levels were observed in rats exposed to silver nanoparticles at 2 and 4 mg/kg b.w. (El Mahdy et al. 2015).

Reproductive and developmental toxicity induced by silver nanoparticles were also studied. It was observed that silver nanoparticles could be passed on to the offspring, and kidneys, liver, lungs, and brain exhibited higher levels of silver nanoparticles when the parent rat orally ingested citrate-capped silver nanoparticles of approximately 7.9 nm at a concentration of 250 mg/kg/day (Ema et al. 2017). Silver nanoparticles were also observed in the maternal milk of female rats treated orally with labeled silver nanoparticles. Intravenous administration of silver nanoparticles led to their accumulation in high concentration in the maternal liver and spleen, but a nominal level was observed in the fetus (Ema et al. 2017). It was also seen that parent mice treated intraperitoneally with polyvinylpyrrolidone-coated silver nanoparticles led to silver nanoparticle accumulation in the embryo. Enhanced accumulation was observed at a lower dose than at a higher dose, thereby indicating that the higher dosage caused agglomeration, thus making it difficult to cross the placental barrier (Ema et al. 2017). In male rats, it was observed that silver nanoparticles adversely impacted Leydig cells, sperm quality, serum testosterone, and luteinizing hormone (LH) levels at 50 mg/kg a day and higher (Ema et al. 2017). Subcutaneous exposure of silver nanoparticles (average diameter 15 nm) at 1 or 5 mg/kg/day led to abnormal sperm and reduction of sperm concentration. On the other hand, intravenous exposure (average diameter 14 nm and 1 mg/kg/dose) did not lead to a significant impact on sperm concentration, fertility, and LH levels.

Intravenous administration of silver nanoparticles (average size 20 nm and 0.5 or 1 mg/kg) in female mice resulted in the reduction of follicle quantity in ovaries. At a dose level of 30 mg/kg/day, oral exposure in female rats caused apoptosis, inflammation, and degenerated follicles (Ema et al. 2017). It was also reported that fetal mortality was enhanced at a low-dose exposure to silver nanoparticles compared to a high-dose exposure, indicating that agglomeration at a higher dose prevented this adverse impact. For mice exposed subcutaneously to silver nanoparticles, the neurobehavioral development was more retarded in female offspring than in male offspring. Therefore, various factors intrinsic to nanomaterials (e.g., size and dosage) as well as factors not associated with nanomaterials (e.g., route of exposure, gender) may elicit different outcomes on health (Ema et al. 2017).

7.3.2 Carbon Nanotubes and Graphene

It has been observed that one of the primary routes of exposure to carbon nanotubes is through inhalation. Carbon nanotubes elicit a similar adverse impact as do asbestos, such as pulmonary inflammation, fibrosis, mesothelioma, and cancer. It has been reported that the toxicity imposed by carbon nanotubes depends on size, rigidity, impurities, method of dispersion, route, duration of exposure, and surface functionalization (Sharma et al. 2016; Orecchioni et al. 2014). Higher levels of the reactive oxygen species and low glutathione level in mice were observed for thin multiwalled carbon nanotubes. Several studies have reported that longer carbon nanotubes led to higher toxicity than shorter ones (Sharma et al. 2016). In mice, multiwalled carbon nanotubes 5–15 μm in length led to fibrosis, whereas shorter lengths in the range of 350–700 nm resulted in lower toxicity. Long multiwalled carbon nanotubes also led to genotoxicity and inflammation (Sharma et al. 2016). Van Berlo et al. investigated two different types of multiwalled carbon nanotubes, one that was longer in length and existed as rigid needle-shaped nanotubes and the other that was shorter in length and existed as entangled nanotubes (Van Berlo et al. 2014). It was demonstrated *in vitro* that rigid needle-shaped nanotubes induced cytotoxicity in RAW 246.7 cells. Exposure to both types of multiwalled carbon nanotubes led to the development of lesions consisting of nanotubes and macrophages in an animal model (mice), although rigid needle-shaped nanotubes resulted in a higher level of fibrosis. Long, thin, and rigid carbon nanotubes were able to reach bronchioles and alveoli, and were associated with impaired clearance due to the slow motility of the macrophage (Van Berlo et al. 2014). This slow motility is attributed to the intake of nanotubes in large quantities. In addition to fibrosis, alveolar inflammation and apoptosis in granuloma were also observed in both cases (Van Berlo et al. 2014).

There have been reports that the dispersion state and type of dispersant used for multiwalled carbon nanotubes impact the toxicity (Sharma et al. 2016). Higher cytotoxicity and genotoxicity were observed for multiwalled carbon nanotubes incorporating metal impurities such as iron, cobalt, etc. Functionalization of

multiwalled carbon nanotubes tends to reduce the toxicity (Sharma et al. 2016; Orecchioni et al. 2014). It was shown that carboxylate functionalized multiwalled carbon nanotubes did not stimulate an inflammatory response, whereas extensive cationic functionalization induced pulmonary fibrosis in a mouse model (Orecchioni et al. 2014). Functionalization of oxidized multiwalled carbon nanotubes with the ammonium group did not trigger the cytotoxic mechanism (Orecchioni et al. 2014). No impact on the proliferation of small airway epithelial cell (SAEC) was observed when exposed to multiwalled carbon nanotubes and nitrogen-doped multiwalled carbon nanotubes (Mihalchik et al. 2015). It was also observed that the nitrogen-doped multiwalled carbon nanotubes were less cytotoxic. Nitrogen-doped multiwalled carbon nanotubes were often shorter than the pristine ones, which could contribute to the lower cytotoxicity. Another reason for this observation could be due to the altered surface chemistry caused by nitrogen (Mihalchik et al. 2015).

Carcinogenic impacts of multiwalled carbon nanotubes were also investigated in the animal models. Multiwalled carbon nanotubes with different lengths, diameters, and curvatures were introduced to rat models by intraperitoneal injection, and these animal models were studied for 2 years (Rittinghausen et al. 2014). A high mortality rate and malignant mesothelioma were observed in all the animals exposed to multiwalled carbon nanotubes. Granulomas consisting of single fibers engulfed by macrophages and lymphocytes as well as thick connective tissue with granulomas around the liver and spleen were reported. Most of the malignant mesothelium was reported to be in the diaphragm, followed by the thoracic cavity. Sacromatoid type or biphasic (combination of sacromatoid and epithelioid types) mesothelium were more common in the multiwalled carbon naotube-treated rats. A possible pathway to mesothelioma could be associated with macrophages engulfing a large volume of nanotubes that were not able to be cleared. This could lead to their poor motility, and hence inducing chronic inflammation, oxidative stress and genotoxicity. As discussed above, longer carbon nanotubes pose greater toxicity, and nanotubes with more curvature elicit a lower toxic effect (Rittinghausen et al. 2014). It has been demonstrated that single-walled carbon nanotubes caused greater cytotoxicity and genotoxicity than multiwalled carbon nanotubes (Öner et al. 2018). In response to toxicity induced by nanotubes, epigenetic mechanisms such as hypomethylation or hypermethylation were also observed (Öner et al. 2018). Epigenetic alterations have been associated with many human diseases.

It was observed that graphite oxide nanosheets led to apoptosis, DNA fragmentation, and elevated levels of reactive oxygen species in spermatogonial stem cells at concentrations of 100 and 400 μg/ml (Hashemi et al. 2016). The method of oxidation to synthesize graphene oxide played an important role in the nanomaterial's toxicity response to lung epithelial cells (Chng and Pumera 2013). Graphene oxide with an increased oxygen content elicited lower cytotoxicity, and vice-versa. Since higher oxidation was achieved with permanganate compared to chlorate, the former could lead to reduced toxicity (Chng and Pumera 2013). At a graphene oxide concentration of 125 μg/ml obtained through various oxidation processes, the adverse impact on the viability of lung epithelial cells was observed, although there were conflicting results regarding the toxicity of graphene oxide (Chng and Pumera

2013). Carbon nanotubes and graphene oxide have sometimes exhibited enhanced cell proliferation. It is possible that carbon nanotubes can interfere with the mitotic spindle interaction, which may contribute to enhanced proliferation (Rittinghausen et al. 2014).

7.3.3 Silica Nanoparticles

Silica nanoparticles have shown comparatively lower toxicity than other nanomaterials although their toxicity is dependent upon size, dosage, chemical stability of the crystal structure, surface charge, and functionalization. It was observed that silica nanoparticles approximately 22.5 nm and 56.9 nm in diameter led to lower FE1 cell viability after 24 h of exposure compared to nanoparticles with average diameters of 237.5 nm and 2045.4 nm (Decan et al. 2016). Dose-dependent cytotoxicity to FE1 cells up to 250 μg/ml and synthesis of reactive oxygen species at a dosage of 12.5 μg/ml and 50 μg/ml were reported for silica nanoparticles (Decan et al. 2016). Silica nanoparticles are mostly cleared by lysosomal exocytosis, and their accumulation in the lysosome is size dependent (Decan et al. 2016). Pyrogenic silica nanoparticles are more cytotoxic than precipitated ones (Fontana et al. 2017). Past studies have shown that dermal exposure to silica nanoparticles did not induce skin damage and toxicity within internal organs (Trouiller et al. 2009; Fruijtier-Pölloth 2012). In animal models, silica nanoparticles when orally administered were able to cross the gastrointestinal tract and find a path to the circulatory system. Surface functionalization such as carboxyl- and amine-functionalized silica nanoparticles as well as smaller-sized particles exhibited enhanced transport through the gastrointestinal tract (Mebert et al. 2017).

In an animal model, food-grade silica nanoparticles at a single dose of 500-mg/kg were excreted with feces, although an increased concentration of silica particles was observed in the liver, spleen, and kidneys (Mebert et al. 2017). It could be suggested that silica nanoparticles were safer, based on studies that used a higher dose of silica nanoparticles than the allowed exposure levels for humans. The intratracheal administration of silica particles was mostly cleared from the lungs, lowering its possibility to induce an adverse effect on this organ. Silica nanoparticles were reported to cause epigenetic alterations such as hypermethylation of apoptosis-related genes in human bronchial epithelial cells and hypomethylation of keratinocyte cell lines when exposed to 15 nm silica particles (Mebert et al. 2017). On the other hand, weak chromosomal aberration or effects were observed *in vitro* and *in vivo* due to the exposure to silica nanoparticles, indicating limited mutagenicity and genotoxicity (Fruijtier-Pölloth 2012). A mutagenic response to silica nanoparticles 7.172 nm and 7.652 nm in size was reported for mouse lymphoma cell lines at 100 and 150 μg/ml (Demir and Castranova 2016). The genotoxicity and mutagenicity of silica nanoparticles are dependent on the type of cells, particle size, and other psychochemical parameters requiring more in-depth exploration of the effects for clarity and consistency. One *in vivo* study showed that the oral ingestion of silica

nanoparticles did not lead to a tumor in rats and mice, indicating that most likely, silica nanoparticles were not associated with carcinogenicity (Fruijtier-Pölloth 2012). Moreover, food-grade amorphous silica did not induce reproductive and developmental toxicity in rabbits and mice at 1600 mg/kg bw/day (Fruijtier-Pölloth 2012).

7.3.4 Titanium Dioxide Nanoparticles

Oral exposure of titanium dioxide nanoparticles to maternal mice caused enhanced DNA deletion in the fetus, indicating that it can be passed on to the offspring (Trouiller et al. 2009). It also led to single- and double-strand DNA breaks in mice and chromosomal damage, which was assessed by detecting micronuclei in erythrocytes (Trouiller et al. 2009). Enhanced micronuclei frequency at concentration levels of 500 mg/kg indicates that they are clastogenic in mice. Titanium dioxide nanoparticles also caused oxidative DNA damage in the liver of mice (Trouiller et al. 2009). DNA damage caused by exposure to titanium dioxide particles was also observed *in vitro* for A549 cells, in contrast to another study carried out on the same cell line (Karlsson et al. 2009; Hanot-Roy et al. 2016). Micron-sized particles exhibited higher levels of DNA damage than did particles in the nanoscale range (Karlsson et al. 2009). Titanium dioxide nanoparticles exhibited negligible cytotoxicity to A549 cells when exposed for 18 h at 40 μg/cm^2, and a similar observation was noted in another study (Karlsson et al. 2009; Hanot-Roy et al. 2016). The oral exposure of female mice to 25 nm and 80 nm of titanium dioxide nanoparticles at 5 g/kg resulted in a significantly higher inflammation in the liver compared to that in the male mice, and in this study, myocardial and kidney damage due to the nanoparticles was also reported (Wang et al. 2007).

The International Agency for Research on Cancer (IARC) suggests that experimental evidence supports the carcinogenicity of titanium dioxide particles in animal models, although it is inconclusive in the case of humans (Hanot-Roy et al. 2016). Relevant cell lines associated with lungs such as human pulmonary microvascular endothelial cells (HPMEC-ST1.6R), alveolar macrophage (THP-1), and alveolar epithelial cells (A549) have been investigated *in vitro* (Hanot-Roy et al. 2016). In all these cell lines, a significant increase in the reactive oxygen species generation was observed, but in the case of THP-1, the production was delayed. No significant cytotoxicity was observed for A549 and THP-1 cells, whereas HPMEC-ST1.6R cells exhibited cytotoxicity starting at 50 μg/ml (Hanot-Roy et al. 2016). The A549 cells also did not exhibit significant apoptosis, but the HPMEC-ST1.6R cells showed dose-dependent apoptosis (Hanot-Roy et al. 2016). After 24 h exposure to the nanoparticles at 200 μg/ml and 800 μg/ml levels, the A549 cells did not exhibit cell signaling in response to DNA damage, but for HPMEC-ST1.6R cells, phosphorylation of H2AX was observed (Hanot-Roy et al. 2016). In the case of THP-1 cells, along with H2AX, phosphorylation of both ATR and ATM proteins was noted. This study emphasizes that the response related to cytotoxicity and cell signaling

pathways for DNA damage may vary significantly based on the cell lines (Hanot-Roy et al. 2016). The repair kinetics for DNA damage in Caco-2 cells after exposure to titanium dioxide nanoparticles has also been studied (Zijno et al. 2015). Enhanced levels of OGG1 expression suggested that Caco-2 cells were successful in repairing the oxidative DNA damage when exposed to titanium dioxide nanoparticles for 6 h at 2.5 μg/cm^2 (Zijno et al. 2015). Humans have a higher chance of exposure to titanium dioxide nanoparticles since they are frequently used in food and cosmetics. Another study also observed DNA damage with titanium dioxide nanoparticles (21 nm and 50 nm) at 1000 μg/ml on human embryonic kidney cells (HEK293) and mouse embryonic fibroblast cells (NIH/3 T3), but no oxidative DNA damage was noted (Demir et al. 2015). The authors also reported similar results for both sizes of nanoparticles, thus indicating a size-independent effect (Demir et al. 2015).

7.4 Conclusion

It is difficult to understand the deleterious impact and risk posed by nanoparticles on humans since there is a lack of data regarding the volume of nanomaterials that are being produced or used in consumer goods. There is also a paucity of information on the quantification of nanomaterials released into the environment, making it further difficult to assess the risk of nanomaterials. The deleterious impact of nanomaterials on human health is mostly extrapolated from *in vitro* and *in vivo* studies using animal models. There are also contradictory reports in the literature regarding the toxicity of nanomaterials, most probably caused by the numerous factors that can impact the toxicity study, beginning with the type of cell line, type of nanomaterial, cell medium, dosage and size, method of mixing, functionalization, synthesis process of the nanomaterials, surface charge, shape, exposure method, gender, etc. This makes it very difficult to assess the risk of nanoparticles or to determine their effect in humans. Experimental designs using animal models and *in vitro* test settings in order to conduct a comprehensive study on the toxicity of a specific nanomaterial is complex and must take into account the interference of various external factors that may impact the results. With the increasing use of nanomaterials in peoples' lives and the higher frequency of their release into the environment, it may become essential to develop a comprehensive understanding of the impact of various nanomaterials on human health. Long-term exposure to nanomaterials will also become an important topic in the near future.

Already there is regulation imposed by the European Union on cosmetic manufacturers who are required to notify authorities if nanomaterials are being used in their processes. It is very important to consider a balance between the risks and advantages posed by engineered nanomaterials. As a result, a more robust method for detecting hazards and quantifying engineered nanomaterials and the life cycle analysis of engineered nanomaterial-contained consumer goods will be necessary in the near future, as different types and large volumes of nanomaterials transition towards commercialization.

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