Front. Mater. Sci. 2021, 15(4): 512–542
https://doi.org/10.1007/s11706-021-0570-8

REVIEW ARTICLE

Pathways of nanotoxicity: Modes of detection, impact, and challenges

Deepshikha GUPTA (✉), Parul YADAV, Devesh GARG, and Tejendra K. GUPTA

Amity Institute of Applied Sciences, Amity University Uttar Pradesh, Noida 201301, India

ABSTRACT: Nanotoxicology has become the subject of intense research for more than two decades. Thousands of articles have been published but the space in understanding the nanotoxicity mechanism and the assessment is still unclear. Recent researches clearly show potential benefits of nanomaterials (NMs) in diagnostics and treatment, targeted drug delivery, and tissue engineering owing to their excellent physicochemical properties. However, these NMs display hazardous health effect then to the greater part of the materials because of small size, large surface area-to-volume ratio, quantum size effects, and environmental factors. Nowadays, a large number of NMs are used in industrial products including several medical applications, consumer, and healthcare products. However, they came into the environment without any safety test. The measurement of toxicity level has become important because of increasing toxic effects on living organisms. New realistic mechanism-based strategies are still needed to determine the toxic effects of NMs. For the assessment of NMs toxicity, reliable and standardized procedures are necessary. This review article provides systematic studies on toxicity of NMs involving manufacturing, environmental factors, eco-toxic and genotoxic effects, some parameters which have been ignored of NMs versus their biological counterparts, cell heterogeneity, and their current challenges and future perspectives.

KEYWORDS: nanomaterial; nanotoxicity; cytotoxicity, genotoxicity; *in-vivo* and *in-vitro* toxicity; reactive oxygen species

Contents

Received July 23, 2021; accepted September 11, 2021

E-mail: dgupta2@amity.edu

1 Introduction

Nanoscience and technology have advanced exponentially in the last three decades for several applications like in industries, science, medicine, electronic equipments, and communication products. The unique features like mechanical, optical, thermal, and electrical properties of nanomaterials (NMs) due to their small size, quantum size effects and high surface area-to-volume ratio led to biological effects compared with their greater counterparts. Nanotechnology takes part continuously in several uses in all kinds of the human activities such as healthcare, water treatment, food, nutrition, engineering, and production and in daily life. Now, it has become very important in our regular use due to its advantageous effects on the main issues such as energy creation, in technological devices and consumer goods to generate new features. The possible benefits of NMs make them useful for diagnosis and treatment, personal care products and healthcare. With the development of NMs in large scale, the toxic effects have also become the main issue. With the advancement in nanotechnology, the problems of toxicity of NMs have come in the picture. The interaction of NMs with biological structures such as cell, tissue and micro-environment can show the dangerous effects and these dangerous effects are not visible with chemically indistinguishable and bigger counterparts in biological organisms. Therefore, the nanotoxicology is discussed here in detail which is the study of the ill effect on the environment and health of human being [1]. This review article discussed about NMs, their importance, and toxic effects on health of the human and environment and important challenges for future medicine.

Several biological models including plant and animal sources have been identified to study the toxicity of novel NMs, correlating the physio-chemical properties. Biological interaction of NMs and its mediated physiological functions are studied using conventional cell/molecular biological assays to understand the expression levels of genetic information specific to intra/extra cellular enzymes, cell viability, proliferation, and function. However, modern research still demands advanced bioassay methods to screen the acute and chronic effects of NMs at the real-time. In this regard, bio-electrochemical techniques, with the recent advancements in the microelectronics, proved to be capable of providing the non-invasive measurement of nanotoxicity effects (*in-vivo* and *in-vitro*) at both single cellular and multicellular levels [2]. Nanotechnology is the field of interdisciplinary science where we manufacture and design the NMs with extraordinary characteristics such as electrical, thermal, physical, chemical and mechanical properties for specific applications due to their low dimensions in the range of 1–100 nm and high surface area-to-volume ratio [3].

1.1 Nanomaterials and their sources

Nanoparticles (NPs) are categorized based on their dimension, shape, source, and composition. They can be 0-dimensional (0D; quantum dots (QDs)), 1-dimensional (1D; carbon nanotubes (CNTs)), 2-dimensional (2D; graphene and other 2D materials), and 3-dimensional

(3D; graphene foam). According to the content, they can be inorganic-, carbon-, organic-, and composite-based [4]. The most widely used for these purposes are zinc oxide (ZnO) NPs, titanium oxide (TiO_2) NPs, silver (Ag) NPs, silicon nanotubes (SiNTs), CNTs, and so on. The arrangement of atoms in NPs has two different forms like crystalline and amorphous. These two forms may operate as drug and chemical transporters, and they have a far greater impact on living cells than their bulk counterparts due to their distinct physicochemical features.

Over the last 15 to 20 years, the quantity of these NMs has increased from few kilograms to thousands of tons and now the use of these NMs is uncontrolled into the environment and this is still expected to grow dramatically in the near future [5]. Several natural processes produce natural NPs which include forest fires, volcanic eruptions and erosions [6]. Human has created various NMs along with natural NPs through the burning of fuel oil or coal and through the chemical manufacturing process. At present, several engineered nanoparticles (ENPs) such as ZnO, CNTs, and TiO_2 have been used in many applications such as cosmetic products, sporting goods, tires, stain-resistant clothing, teeth cleaning cream, and food additives, and these products are available in the market [3]. Depending upon the types of applications, quantity of NPs per year can be used in small amount (fluorescent QDs for bio-imaging) or millions of tons (e.g., carbon black for tire production) [1,7].

1.2 Importance of toxicity evaluation of nanomaterials

NPs are currently used in diagnosis and treatment of many human diseases, including autoimmune diseases and cancer. However, the cytotoxic effect of NPs on normal cells and living organs is a severe limiting factor that hinders their use in clinic [8]. The inhalation or consumption of NMs is the main way of the human exposure, and it could enter into the body directly via food and indirectly through dissolution of NMs from food containers. Once these NMs enter into the body, they can diffuse throughout the body by the circulation of blood. They can be distributed in the body due to their size, polarity, hydrophilic nature, and catalytic behavior. But due to the rise in the surface area, the chemical and biological activity of NMs can be increased in the body. The synthetic method regulates post-synthetic change and specific applications for *in-vivo* and *in-vitro* imaging or diagnosis and pharmacotherapy and administration. Special insights have been provided on bio-distribution, pharmacokinetics, and toxicity in a living system, which is imperative for their wider application in biology [9].

These particles create toxic effect in the body because the human cells can take small NPs with faster rate as compared to larger particles. Inhalation of airborne NMs and skin absorption are another way of human exposure to NMs.

Exposure of NMs to human is increasing because these NMs are available in the environment and the opposing effect of these NMs on the healthiness of a human is of public concern. Nanotechnology generates functional materials, systems and devices by monitoring the matters at the scale of atomic and molecular level [7]. The conventional toxicity tests generally used for chemical compounds are not suitable for NMs. At present, there is no well-known toxicity test or conventions available for the NMs safety test [10]. Several institutes are currently doing research on the standardization part of testing of NMs toxicity but till date, no standards are approved at international level for toxicity measurements. These standards can provide benefits for toxicity testing of NMs and it can provide the common testing protocols.

A careful strategy for testing of toxic NMs can enhance our understanding of toxic health effects of NMs. Therefore, more research related to toxic effects of NMs and relevant biomarkers tests are needed to identify the toxic effects of NMs on biological system [11–12]. When the NM interacts with the body or nature, some NMs such as Ag NP and Cu NP (metallic NPs) and ZnO NP and Fe_2O_3 NP (metal oxide (MO) NPs) can slash quickly, while other NPs such as TiO_2, SiO_2, CNTs and graphene are more persistent. In any case, soluble NMs impose more toxicity threat, when they are disguised by the cells, because these NMs will get solubilized and discharge poisonous metals from a system called “Trojan horse”.

There are three methods for nanotoxicity tests which are the traditional method, novel metabolomics-based method and bioluminescence-based method for the determination of the toxicity of NMs [13]. Even though, there is no specific protocol available for toxicity measurement of NMs by traditional methods, and three key parameters for screening methods of toxicity should include the physico-chemical characterization of NPs, cellular and non-cellular *in-vivo* assays test and *in-vitro* examinations [14]. It is important that the design must be mechanism-based and realistic. Currently, the toxicity effect of NMs has mostly been studied on cultured cell lines and few reviews give complete information about the effect of the concentration

of NPs on the morphology of the cells and its metabolism when coming in the exposure to the NPs. There are several biological approaches such as proteomics, metabolomics and genomics that have been widely used in toxicological field and the valuable data have been received. Amongst them, metabolomics is a fast growing discipline and this has the collection of the world metabolic information and their spectral and biochemical interpretation which uses modern spectroscopic techniques along with appropriate statistical approaches [15]. Luminescent bacteria, *Vibrio fischeri*, are recognized as the species which emit bright bioluminescence [16]. The bacterial population's metabolic activity is directly proportional to the degree of bioluminescence. This one-of-a-kind characteristic makes determining the amount of toxicity of diverse substances based on their bioluminescence inhibition simple [13,17].

2 Relationship of toxicity with physiochemical properties of nanoparticles

Toxicity assessments of NPs indicate the strong relationship among toxicity of NPs and their exceptional physiochemical properties like size and shape, agglomeration, solubility, aspect ratio and defects [18–19]. Therefore, these NPs with smaller size can enter into the cellular membrane and damage the mitochondria as well as the cells. Thus, it is crucial to have correct information of physiochemical properties of the NMs. Similar NPs may cause different cellular responses in several cells and therefore the severity of toxicity differs depending upon the type of the exposed cell [20]. Due to this conflicting data, more research must be carried out on the toxicity of NMs. However, it seems that the exposure to NPs may cause a variety of acute and chronic effects, like inflammation, high fever, fibrosis and cancer. The toxicity of NPs can be measured by the physical properties and surface features, like size, structure, external charge, production process, and core–shell topologies [19]. Most of the NMs accumulate in the liver and causing irritation and severe side effects. Cadmium selenide (CdSe) QDs also cause hepatotoxicity as they remain stable in the body tissues for up to 8 months. These characteristics of NMs depend upon the surface chemistry and particle size. In people, enormous inhalable NMs with molecular size beyond 2.5 μm tend to store usually inside the nose and throat while little NPs having molecular size less than 2.5 μm could find their ways to the upper respiratory routes [21].

2.1 Size of nanoparticles

Various experiments suggest that the size of NPs influences its toxicity. The size of NPs and their toxicity have an inverse relationship. Smaller NPs have high ratio of the surface area-to-volume which might clarify this opposite connection among size and toxicity. For several NMs, a normal size is around 30 nm and the size smaller than this enhances the surface vitality and therefore the chance of surface responses expand which can prompt thermodynamic instability of the particle and enhance toxicity [22]. Likewise, the mechanisms of the NPs toxicity with various sizes are unique. For example, 1.4 nm particles generally cause cell rot (necrosis) and 1.2 nm particles may cause dangerous cellular apoptosis. The NPs magnitude & exterior zone decide the one kind of component of NPs association with organic frameworks [23]. NPs are portrayed by an enormous explicit surface zone, which decides their high response limit and synergist action [21]. The sizes of NPs (range 1–100 nm) are equivalent with the scale of globules of protein (2–10 nm), DNA helix dimension (2 nm), and cell layer thickness (10 nm), permits them effectively to penetrate cells and organelles of cells. Huo et al. [24] have exhibited that Au NPs size less than 6 nm viably penetrates the cell core, while enormous NPs (10 or 15 nm) just infiltrate via the film of cell and are discovered distinctly in the cytoplasm. This implies that the NPs with very lesser nanometer in size are more harmful than those with 10 nm or bigger, which cannot penetrate in the core [25–26]. Contini et al. [27] studied the interactions between 5 and 60 nm citrate-stabilized Au NPs and large unilamellar vesicles act as a model membrane system. It was also reported that the smaller size (5–10 nm) Au NPs formed aggregates on the bilayer surface, larger size (25–35 nm) Au NPs adsorb on the outer surface with an observable bending of membrane while particles between 50 and 60 nm size increase the tension in membrane due to the decrease in the surface area-to-volume ratio of the liposome/Au NPs. They have followed the dependency of the toxicity of Au NPs on their sizes ranging from 0.8 to 15 nm. In a study, it was observed that NPs of the size 1.4 nm which is a comparative size for epithelial cells, fibroblasts, macrophages, and that of large DNA channels permits more interaction and cell damage within the period of 12 h leads to apoptosis. This proposes that NPs can enter the nucleus and interaction takes place with the sugar-phosphate DNA backbone having negative charge and it also block the transcription [20]. It is a similar property that

prompts expanded injurious impacts, since smaller size infers more prominent surface region and more noteworthy zone to volume proportion per given mass. Along these lines, smaller NPs went with expanded organic reactivity because of the expanded number of mass material atoms they comprise of [28].

2.2 Shape and structure of nanoparticles

NPs exhibit different levels of toxicity based on existence of different shapes like spherical, cylinders, cubes, sheets, and rods. Spherical NPs are highly inclined to endocytosis as compared to nanotubes and nanofibers. In comparison to sphere-shaped fullerenes, single-walled carbon nanotubes (SWCNTs) can more easily obstruct calcium channels [29]. In a comparative study of the impacts of hydroxyapatite (HA) NPs with different shapes like needle, plate, pole and round on cultured BEAS-2B cells, it has demonstrated that needle- and plate-shaped NPs lead to the damage of a larger number of cells than sphere- and rod-like NPs [29]. Hu et al. [30] carried out studies to check the impact of graphene-like materials on the damage of mammalian cells. Different forms of these NPs were used to measure their toxicity since it allowed them to physically harm the cell membrane [31]. However, with the rise in concentration of fetal calf serum in the culture media, the toxicity of NMs diminished due to covering of surface of the NPs with protein particles leading to the shape change.

2.3 Surface area and toxicity of nanoparticles

A bigger surface region is the reason of increased reactivity with the nearby particles which bring about the harmful impacts when utilized in fillers, beauty care products and as drug carriers. Small sized particles acquire small volume so that several particles can occupy a unit region which results in the increase in pathophysiological mechanism of toxicity like reactive oxygen species (ROS) generation, mitochondrial perturbation, and oxidative stress. The total number of particles per unit volume plays a significant role in determining the toxicity, too. A team of researchers tested acute lung inflammation with various NP surface regions and specific reactivity to better understand the link between an NP's surface region and its biological toxicity [21]. The surface area played a serious role in lungs inflammation and explains about 80% of the observed variability in acute pulmonary toxicity. The materials without intrinsic toxic behaviors are called "low-solubility and low-toxicity (LSLT) materials" [32]. These LSLT materials show low surface-specific toxicity with the EC50 dose of 175 $m^2 \cdot g^{-1}$-lung (here EC50 means the dose inducing 50% of the maximum effect). This examination infers that surface-related methods of activity are driving intense pneumonic harmfulness for the kinds of NPs researched [18,21,23].

2.4 Charge assessment of toxicity of nanoparticles

The charge on NPs surface plays a very crucial role in toxicity because surface charges strongly govern the interaction of NMs with biological molecules. Positively charged particles show high toxicity compared to the negatively charged and neutral particles because positively charged particles can enter in the cell easily because of the electrostatic force of attraction as cell membrane is negatively charged [33]. All types of cells can generally absorb the positively charged and neutral NPs at the same rate, although negatively charged NPs mainly gather in the tumor cells. Therefore, the charge modification of the NPs allows the localization of these particles, and the toxicity can be controlled. This controlled toxicity can be beneficial for the growth of proper delivery systems for chemotherapeutic drugs to the tumors [33].

2.5 Nature of coating and effect on toxicity

The shell and secondary coating is an important factor that influences NMs toxicity [34]. It is used to enhance the solubility and biocompatibility of NPs in water and other bio-based fluids, which decreases the agglomeration capacity and increases their stability [1]. The shell decreases the level of toxicity of NMs and allows selective interaction with different cells and biological molecules. The pharmacokinetics of NPs can be affected by shell, and it also changes the pattern of distribution of NPs on the body. In the report of Cho et al. [35], lectin-coated NPs with sialic acid-modified surface selectively bound with tumor cells. This finds application of NPs in precisely labeling of the cancer cells. The shell is generally used to improve the solubilization and diminishing the toxicity of QDs because their metal cores consisting of heavy metals like Cd, Te and Hg are generally hydrophobic enhancing the stability of QD core and preventing their oxidative or photolytic degradation and desalination. Therefore, this reduces the toxicity of QDs as the leakage of metal particles outside of the QD core is reduced [36]. It has been realized that the closeness of oxygen, ozone, oxygen radicals on the surface of metal NPs results into ROS production [37].

Fubini et al. [38] revealed that silica's cytotoxicity is inextricably linked to the appearance of surface radicals and ROSs. Surface coating, generally, can result in unfavorable NPs effects [38].

ZrO_2 NPs are also used for biomedical applications due to the affordable and scalable production. Surface modifications of ZrO_2 NPs are very easy with multiphase stability. ZrO_2 NPs with good surface roughness possess promising biomedical applications, which can effectively change the cellular and subcellular functions. Chemical etching treatment is the preferred method to make the rough surface of ZrO_2 NPs. Commonly available etching acids that have been used to etch zirconia successfully include hydrochloric acid (HCl), hydrofluoric acid (HF), phosphoric acid (H_3PO_4), etc. Amongst these, HF is considered as one of the most promising etchants for zirconia. Chemical etching of zirconia with strong acids exerts substantial impacts on the surface characteristics. It can produce surfaces with nanoscale roughness, lower cytotoxicity level, and superior biocompatibility and when combined with microscale roughening treatments, it could also generate synergistic effects [39].

2.6 Solubility of nanoparticles

Solubility of NPs changes from thermodynamic prediction which has been deduced from large size particles. The big surface area of NPs is important to address the NPs dissolution. The water solubility of the NPs is upgraded by a few significant degrees after the exposure of surface edge. When the cell is exposed by the NPs, the cytotoxicity was found to be limited but detectable. The cytotoxicity response of all the materials using human mesothelioma MSTO cell (both DNA and MTT) after 3 d of treatment is in the order of $Fe_2O_3 \approx ZnO > TiO_2$, which remain consistent with 6 d of treatment.

There is lack of suitable NP characterization in several ecotoxicological studies to date. The NPs can form aggregates and the minimal primary particle size alone is insufficient to address the risks of specific NP. The amount of aggregation can affect the availability of NPs for uptake into cells. Similarly, surface area, surface charge, morphology, purity, solubility, and coating of NPs play important roles in having toxic effects of NPs in the aquatic system. The toxicity of soluble ionic metal NPs requires special attention. CdSe NPs release free cadmium, which is also responsible for the *in-vitro* cytotoxicity. Limbach et al. [34] found that the solubility of the materials strongly affect the cytotoxicity of many different oxide NPs in which highly soluble NPs such as FeO and ZnO show greater acute toxicity than that of NPs with the lowest solubility such as TiO_2 and CeO_2 [40–41]. The association between the polar NP and the moisture water is more grounded than the nonpolar particles, which should encourage the disintegration of the NPs [37]. The surface extremity additionally decreases the collaboration of hydration water with the other water atoms and improves the cooperation between the NPs which may prevent their scattering [42]. In addition, the formation of the surface extremity upsets and even reworks the hydration structure of nonpolar NP [37]. Strikingly, the polar NP with a less arranged hydration structure will, in general, have higher water solubility because the solvency is a significant parameter to evaluate the engineered NMs. As of now, there are no standard techniques for surveying the solubility of NMs. The particle size is a crucial factor in influencing the NMs solubility contrasted with their mass analogs dependent on proof that solubility will, in general, improve with the diminishing molecule size. Schmidt and Vogelsberger [43] saw that TiO_2 NMs with the undefined structure have more solubility than that of TiO_2 in the crystalline form and that unadulterated nano-anatase was more dissolvable than blended nano-anatase and nano-rutile, showing that the crystalline structure can be another significant factor in impacting the solubility of engineered nanomaterials (ENMs) [40]. Information on ENMs solvency helps with deciphering potential connections of ENMs with natural environmental factors, bioavailability and constancy, take-up rates and poisonousness [44–45].

3 Types of nanoparticles showing toxicity

Several metallic NPs have been used in several applications, but these NPs also show nanotoxicity. NPs have found their ways in the targeted drug delivery system [46]. As shown in Fig. 1, there are several factors and mechanisms which show nanotoxic effects of metallic NPs.

Zn is useful as an important part of the human nutrition. The proper amount of Zn in the human body is important for the functioning of immune system, synthesis of protein and DNA and liver function. ZnO NPs have good chemical sensing and electrical properties and are good for antimicrobial and antifungal properties. These ZnO NPs are also widely used in cosmetic products, pigments and coatings, electronic devices and as catalysts. Due to their increased exposure, ZnO NPs-based products have created problems of toxicity and safety. Numerous *in-vitro*

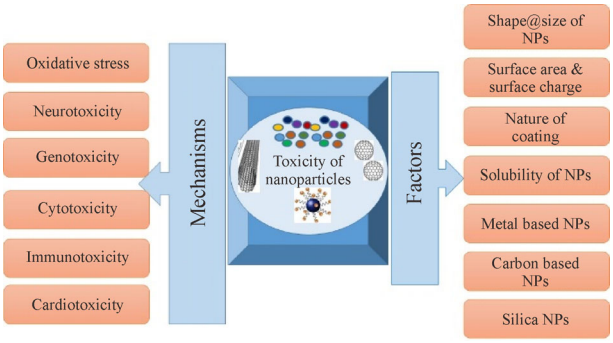

Fig. 1 Mechanisms and factors for toxicity of NPs.

researches showed the unwanted harmful consequences of ZnO NPs like the generation of autophagic cell death, oxidative stress, cellular damage, inflammatory responses and genotoxicity [47]. While skin exposure is due to consumer items like sunscreens, cosmetics to paints and workplace exposure to NPs dusts is due to processing plants producing NPs. Regardless of the broad utilization of ZnO NPs, the safe use of this NM is still uncertain. The increase in the toxic effect of ZnO NPs is owing to the dissolution of Zn ions (Zn^{2+}) in the acidic system. Such a type of toxic nature creates cytotoxicity, oxidative pressure and mitochondrial brokenness. The toxic behavior of ZnO NPs can be reduced by the complexation effect with Zn^{2+} in phosphate, phosphoric acid, and glutathione systems. *In-vivo* airways exposure due to inhalation and accumulation of the NPs show the lung inflammation and systematic toxicity. ROSs is created by mitochondrial oxidative metabolism and the cellular reaction to xenobiotics. The NPs transmit fundamentally; target organs include the liver, spleen, lung, kidney, and heart. *In-vitro* studies of BEAS-2B bronchial epithelial cells and A549 alveolar adenocarcinoma cells reveal cytotoxicity, increased oxidative stress, expanded intracellular (Ca^{2+}), diminished mitochondrial film potential, and interleukin (IL)-8 creation [48]. Transient study of skin cells brings about apoptosis, however, not in an incendiary reaction, while long haul introduction prompts expanded ROSs age, diminished mitochondrial movement, and development of rounded intercellular structures. Macrophages, dendritic cells and monocytes are affected due to the exposure to NPs, and macrophages phagocytize the NPs which are then solubilized in lysosomes. Both the comet assay and the cytokinesis-blocked micronucleus assay indicate genotoxicity *in-vitro*, whereas the Ames test does not [37,49].

CuO is a monoclinic structured semiconducting material showing a variety of physical properties like superconductivity effect, electron correlation effect and spin dynamics. It has beneficial properties such as photovoltaic and photoconductive effects due to the narrow band gap in the CuO crystal structure. CuO NPs also hold unique characteristics such as improved thermal conductivity and enhanced fluid viscosity. CuO NPs have these unique properties, making them a possible contender for an energy-saving material that could improve the efficiency of energy conversion. These NPs are also useful in different fields such as solar energy conversion, field emission, gas sensors, catalysis, batteries, and high-temperature superconductors [50]. These NPs reduce the manufacture cost and improve the catalytic efficiency. From experimental data, Cu NPs also show toxic effects on the liver and kidney. Cu ions produce toxicity exceeding above physiological tolerance level *in-vivo* studies. As a result, public and scientific researcher groups are concerned about the potential health risks and harmful effects of CuO NPs. Histopathological evaluation indicated that CuO NPs can induce serious inflammatory changes in the lungs of rat when extraordinary doses of these materials is provided, and it is chronic at low doses. Karlsson et al. [51] reported the toxic properties of CuO NPs that cause DNA damage in the human lung epithelial cell line (A549). The oxidation-sensitive fluoroprobe 2′,7′-dichlorofluorescein diacetate (DCFDA) can be used to measure ROSs by the *in-vitro* method. Researchers compared the connection between DNA damage and ROSs generation, and found that the primary effect of toxicity was attributed to the oxidative stress. When exposed to CuO NPs, cells showed reduced catalase and glutathione reductase (GR) enzyme activity and increased glutathione peroxidase activity compared to cells maintained in normal media. CuO NPs are not only generating ROS, but also obstructing the cellular antioxidant defense, according to the observed rise in oxidation ratio to total glutathione. The *in-vivo* toxicity level of nano-Cu particles was found to be more than that of micro-Cu particles [52].

Ag NPs are generally utilized in consumer products such as food supplements, coatings on medical products, water disinfectants, plastic food storage boxes, air filters, electronic instruments, textile fabrics, beauty care products, body spray because of their antimicrobial properties [53]. Ag NPs are also used as attractive materials for drug delivery and cancer therapeutics, but show *in-vitro* genotoxicity and cytotoxicity in human bronchial epithelial cells (HBEpC). Furthermore, due to the size and shape of Ag NPs, they can also be taken up by the human skin

keratinocytes and adopted by the cell. Further research is required to determine the contribution of the surface area and the size of the particles to NP dissolution by shedding of Ag particles, which contributes to the toxicity of Ag NPs. Ag NPs caused the development of relative spleen weight and impacted hepatocyte decay and release, similarly as multifocal peribiliary microhemorrhages, periodic entry vein endothelial damage, which accordingly impacts the liver [13,54].

TiO_2 is a commonly used MO having low poisonousness. The characterization as bio-inactive material (>100 nm) has made TiO_2 NPs to be broadly utilized in food items, pharmaceutical items and cosmetics like sunscreens and toothpastes. Introduction in humans may happen through the ingestion and dermal entrance, or through inhalation. The harmful effect of TiO_2 NPs appears to include the ROSs creation, oxidative stress, aggravation, genotoxicity, metabolic change and conceivably carcinogenesis. The degree and sort of cell harm emphatically rely upon concentration and physical attributes of TiO_2 NPs, including size, crystal structure and photocatalytic nature. TiO_2 NPs incite phototoxicity upon ultraviolet (UV) irradiation. They are known to instigate apoptosis by actuating apoptosis-initiating factor (AIF) in human keratinocyte cells. In addition, TiO_2 NPs have been exhibited to cause pericardial oedema and incubation of Japanese medaka rice fish (*Oryzias latipes*) undeveloped organisms when treated with TiO_2 suspensions at 0 and 14 $\mu g \cdot mL^{-1}$. The genotoxicity, apoptosis, and mitotic inhibition are brought about by both nano- and microparticles of TiO_2 in different tissues of mice [55].

The growth in Au NPs innovation holds incredible guarantee for future applications. Au NPs are used as an important promising vehicle for a wide extent of biomedical applications, while they are found to invigorate hepatic macrophages causing susceptible hepatitis and liver injury. Au NPs have arisen as the materials to develop the drug delivery systems as well as nanoscale therapeutics. It was found from animal studies that the Au NPs modified with types of thiol monolayers like tiopronin can be the reason of renal complications and morbidity. Endorsement of Au NPs for different biomedical applications by the Food and Drug Administration (FDA) has prompted expanded applications as drug carrier, malignant growth treatment and organic applications. An investigation by Tsoli et al. exhibited that Au NPs of roughly 1 nm in measurement could enter the cell and atomic films and append to DNA without cell injury and cell demise [56]. The Au NPs' modest size stimulates their consolidation into organic frameworks, which operate as excellent nanoreactor assemblies for direct plasmonic photocatalytic nitrogen fixation using visible light under ambient conditions [57]. Exceptional properties of Au NPs have driven them to cell research where some are accounted for either as poisonous or harmless. This along these lines, Au NPs contributes a simple adaptable pathway of infiltration and reactivity in natural framework than bulk gold material [58].

QDs are 0D semiconductor NMs (from ~2 to 10 nm) having extraordinary optical and electrical properties that find their use in electronic industries and biomedical imaging systems. Their fluorescence properties make them ideal fluorophores for biomedical imaging. QDs with fluorescent properties can be coupled with antibodies and receptor ligands to target cellular structures such as identifying cell membrane receptors, neoplastic cells peroxisomes and DNA. These QDs have been discovered as an important means for site-specific gene and drug deliver-systems and these QDs are the most promising candidates among several other materials for a diversity of information and visual technologies. Quantum dot light-emitting diodes (QD-LEDs) have unique features like wide color gamut, great color purity, high brightness with low turn-on voltage, and ultrathin form factor and are used in the next-generation displays [59]. QDs are used for the making advanced flat-panel LED displays and in ultrahigh density quantum information processing and data storage [60]. The QDs toxicity depends upon size, charge, external covering bioactivity, oxidative, photolytic and mechanical dependability of different components obtained from the physicochemical properties of QDs and their characteristic conditions [61]. Due to their cytotoxic and oxidative nature, the QDs dose fixation and their units of estimation (such as mg per mL, molarity, mg per kg body weight, and number of QDs per cell), connected dose across current assessments in testing are required. *In-vivo* and *in-vitro* studies have also proven the potential toxic effect of QDs to vertebrates [62]. Current advances in the use of non-metallic NPs (CNT, graphene, fullerenes, and SiO_2) for drug delivery application, tissue regeneration, biocompatibility studies and bio-imaging and bio-sensing purposes have been discussed by Erol et al. [63].

CNTs are cylinder-shaped 1D NM with extraordinary mechanical, thermal, electrical, optical properties. CNTs are formed when graphene sheets are draped into a cylindrical shape either as a solitary layer forming

SWCNTs or as multiple layers forming multi-walled carbon nanotubes (MWCNTs) [64]. CNTs are used in scratch less coating, automobile and aerospace industries, electromagnetic interference (EMI) shielding [65], optical instruments, capacitors, lithium-ion batteries (LIBs) and biomedical applications [64]. Study shows that various properties of CNTs are accounted to influence the human body. Micron-sized carbon-based particles, incorporation of CNTs, provoked aspiratory aggravation in animals, which has been studied [13,66]. The inward breath introduction of CNTs actuated threatening mesothelioma, proposing that CNTs may present risks like asbestos. It does harm to different organs of animals causing aggravation, fibrosis, and increment in pneumonic tumor rate because of aspiratory introduction to CNTs.

Fujita et al. [67] demonstrated that SWCNTs with slender bundles and short cylindrical forms caused lung inflammation that was delayed and took a long time to recover. SWCNTs with thick bundles and long cylindrical forms, on the other hand, elicited cellular responses in alveolar macrophages and sparked acute lung inflammation soon after inhalation. As a result, it has been found that the size of the bundles affects the pulmonary toxicity of SWCNTs. In the instance of MWCNTs, a 13-week breath research revealed that MWCNTs show started epithelial cell hyperplasia following perception periods when MWCNT grouping was high.

In the study by Frank et al. [68], the lung pathological profiles in mice were compared by the repeated exposure to MWCNTs and crocidolite asbestos (CA). The exposures caused neutrophilic and elevated interstitial collagen, with CA exposures causing primarily bronchoalveolar hyperplasia and exposures from CNTs causing alveolar hyperplasia of type II pneumocytes (T2Ps). When T2Ps are exposed to CNTs, proinflammatory genes, including IL-1 ss, are increased in lungs, in contrast to CA-exposed T2Ps. The cancer-causing nature of CNTs was seen on account of needle-like elongated structures of CNTs, whereas the cancer-causing nature of CNTs was constricted for shorter fiber sizes. Short CNTs initiated less aggravation, fibrosis, and *in-vivo* genotoxicity in the interminable stage [69]. CNTs may also incite formative harmfulness, for example, teratogenicity in developing embryos [70–71].

Graphene is one of the allotropic forms of carbon which is a single atomic thick layer and formed from the exfoliation of graphite. Graphene is 2D sheet in which all carbon atoms are arranged in a sp^2-hybridized hexagonal structure. It is the thinnest possible configuration of carbon molecules. The varying extraordinary properties of this material, like high mechanical stiffness, extraordinary strength, ballistic transport of electrons, thermal properties, optical transparency, and elasticity makes graphene the extraordinary material and has generated significant excitement to the research communities since the initial discovery. Graphene and its derivatives are right now being investigated for many various applications. Graphene can penetrate through the physiological boundaries into the cell structures by various exposure methods bringing *in-vivo* and *in-vitro* toxicity [31]. The various routes of administration, distinct patterns and sites of cell absorption, as well as variable tissue distribution and excretion can determine the degree of the graphene toxicity [13,72]. Airway exposure is the most method for graphene exposure in the workplace. Graphene exposure leads to lung deposits, accumulating to a very high level that can be kept in the lungs for more than 3 months following intratracheal instillation with delayed clearance. But graphene oxide (GO) and its derivatives have limited adsorption of intestinal and quickly desorb in adult mice upon oral administration. The GO exposure may induce oxidative stress at a concentration of ~10 $\mu g \cdot mL^{-1}$. GO-initiated cytotoxicity, genotoxicity and oxidative pressure have been explored in typical human lung fibroblast cells. The methyl thiazolyl tetrazolium (MTT) examine demonstrated a critical diminishing in cell reasonability and an expansion in poisonousness following a drawn out treatment time, just as an apoptotic impact of GO at a centralization of 100 $\mu g \cdot mL^{-1}$ [73].

Fullerenes are spherical (bucky-balls), cylindrical or ellipsoidal novel carbon-based molecules. They have a huge variety of technological and medical applications. However, their increasing production and use has revealed a concern over their potential toxicity depending on the molecular size, composition, surface properties and functionalization. Fullerenes may cause oxidative stress via ROSs, apoptosis, lysosomal layer destabilization and decrease of mitochondrial film potential, layer and DNA harm [74].

SiO_2 NPs have an assortment of uncommon properties, for example, being handily combined and having a modifiable surface, having powerful mechanical property and having a generally dormant substance creation. They have been utilized as biomaterials for a considerable length of time and frequently used in biomedical applications. Two fundamental types of SiO_2 are crystalline and amorphous. They may also be categorized into nanoporous

(<2 nm), mesoporous (2–100 nm) and microporous (>100 nm) in view of the size of the pores. The danger of human introduction to SiO_2 NPs at work environments is raising concern with respect to the poisonousness and unfavorable impacts of SiO_2 NPs. Studies have demonstrated that crystalline SiO_2 presentation prompts silicosis (a fibrotic lung illness), emphysema, and pneumonic tuberculosis in laborers. Physicochemical properties of SiO_2 NPs other than crystallinity cause distinctive toxicity impacts *in-vitro* broadly used in dietary enhancements, catheters, inserts and dental fillers [75].

All these NPs which impose the toxicity on human health and environment are shown in Fig. 2.

4 Methods of screening of toxicity

Different approaches are there for the assessment of toxicity by NPs on any organism. The toxicology of NPs is, taking everything into account, directed by their physical and engineered properties, e.g., their size, structure, surface charge, presence of shell and synergist development. The NPs can embed into endothelial and epithelial cells into the lymph and blood entering the circulatory system and lymphatic stream. It then reaches into different organs and tissues which concentrated into the kidneys, spleen, brain, heart, liver, and bone-marrow [76]. The approaches for assessment of toxicity are by *in-vivo* and *in-vitro* methods [77].

For *in-vitro* methods, the use of bacteria or cell lines is done to understand the toxicity. *In-vivo* means inside the living system like mouse or the zebra fish or some other animal models. The *ex-vivo* is the combination of *in-vivo* and *in-vitro* where organ is grown in lab to analyze the toxicity of NMs. The advantage of this method is cost effectiveness, time effectiveness with no ethical issues involved in animal models. NPs may have diverse responses on different animal models depending on the formulation, concentration, pH value, coating, exposure mode, exposure time, and targeted organ. These are created in a hydrogel matrix. *In-vitro* studies aid in the understanding of the biochemical and molecular mechanisms of nanotoxicity, as well as a better understanding of the physicochemical features of NMs contributing towards nanotoxicity. MO NPs can increase the level of oxidative stress by producing ROSs, e.g., superoxide anion ($^{\bullet}O_2^-$), hydroxide radical ($^{\bullet}OH$), and H_2O_2 in a variety of methods. These are high-energy species capable of attacking lipids, proteins, nucleic acids and other essential biomolecules. They cause damage to mitochondrial structures, impairment of the chain of electron transport, depolarization of the mitochondrial membrane, and activation of the nicotinamide adenine dinucleotide phosphate (NADPH)-like system [78].

The genotoxicity is the ability of a test agent which can induce DNA damage while cytotoxicity means if it is toxic to the cells. The genotoxicity assays can detect, quantify, and characterize the damage of DNA which is induced by a substance under investigation. The NMs release free metal ions which will induce the oxidative stress and this oxidative stress will damage the DNA. This induces the apoptosis or the inflammation. We can use the *Salmonella typhimurium* to understand mutagenic effect of NMs by the Ames assay. *Salmonella* requires histidine for its growth. In the presence of a possible mutagen like NPs, if it causes some mutation, it will cause this histidine negative to become histidine positive. If the bacteria are growing more on this plate that means the nano particle had induced some mutation in the genetic material.

The hemocompatibility is a very important factor which can decide the application of the implantable biomaterials such as orthopedic implants and artificial blood vessels. The hemocompatibility test of NMs involves hemolytic assay, anticoagulants assay, platelet adhesion and activation assay, blood coagulation time assay, and blood protein adsorption assay. For *in-vivo* assessment of NMs toxicity,

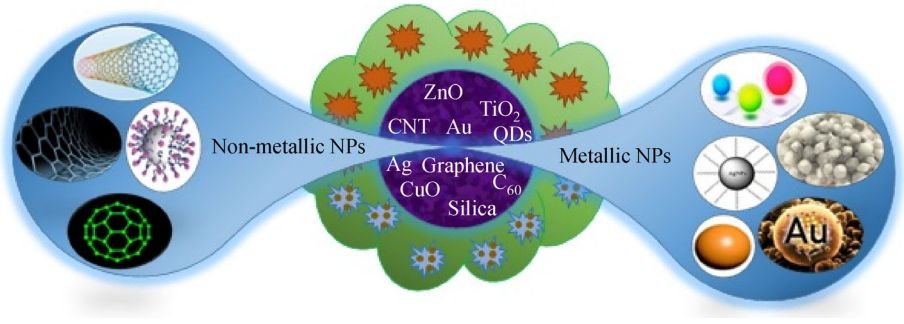

Fig. 2 The various metallic and non-metallic NMs imposing toxicity.

zebrafish model is often used as it has a clear and transparent embryo with short maturation time and functionally homologue with 70% of human disease genes. Study of toxicity of the NMs in terms of mortality rate of zebrafish or hatching as well as the heart rate and abnormal phenotypes is done.

The guidelines laid by institute/organization for the financial collaboration and growth recommend oral and dermal toxicity test to understand the toxicity of NMs. Colloidal NMs dose up to 5000 mg/kg body weight could be given to the mouse for oral toxicity test. Toxic signs for the first 3 h and after 24 h and observation over 14 d for the skin and behavioral symptoms were checked. These animals are killed after 14 d, and histopathological testing was done to check the NPs depositions in the kidney, spleen and liver.

4.1 *In-vitro* method

The various *in-vitro* methods to measure nanotoxic effects are listed in Table 1 [79–96]. Basically, cell uses two mechanisms, phagocytosis and endocytosis, for the elimination of foreign particles like viruses, bacteria or ENPs. The endocytosis transmembrane is where liquids or molecules are transported. Endocytosis is the easy approach to spread natural and modified NPs from one cell to the other, once they get entered inside the body of a human they get translocated among various parts of the body. To evaluate the *in-vitro* toxicity, the controlled dose of NPs is extremely important. Destruction to the cell by NPs can be done by any of physical or chemical methods. Chemical changes and damages can occur through the several processes such as formation of the free radicals or ROSs, release and dissolution of toxic ions, damage to surface properties by damaging the ion-exchange system of the cell membrane. Physical harm to cells can be caused by NPs by a variety of methods, including disruption of membranes and membrane activity, which can create barriers to cellular metabolism, begin protein aggregation or folding, and damage to the cells' DNA. Size and surface properties of NPs are the main factors on which damage of the cell depends. *In-vitro* testing of toxic effect should build on test models that are appropriate to protect the species [77]. Both chemical and physical interactions cause NPs to internalize and have harmful effects on live cells, and *in-vitro* studies are required to assess the toxic effects of NPs and provide the foundation for *in-vivo* investigations [97]. The cell viability and lethality studies are being used to check the toxic behavior of the NPs. *In-vitro* screening examinations are very significant for fast, low-cost and efficient nanotoxicity screening to complement or supplement the expensive and time-consuming *in-vivo* studies on animal models [97]. Assessments are further subdivided into apoptosis assay, proliferation assay, oxidative stress assay, necrosis assay, and DNA damage assay [98]. Due to the growing widespread interaction and interaction of NMs with human body, the possible hazard to human well-being and security has become the matter of worry [99].

Table 1 List of various *in-vitro* methods to measure nanotoxicity [79–96]

S/No	Organism/cell lines	Test name	Toxic effect of NPs	Ref.
1	*S. aureus*, *E. coli*	Visual turbidity assay (MIC and MBC) measuring optical density	Antibacterial efficiency of NMs	[79]
2	*Escherichia coli*	Green fluorescence protein expression assay	Antibacterial efficiency of NMs	[80]
3	*Escherichia coli*	Disc diffusion method	Antibacterial assay	[81]
4	*Eukaryotic cell*	Trypan blue cell viability assay by staining dead cells	Cell viability assay for measuring cytotoxicity of NPs	[82]
5	Eukaryotic cell lines like BHK21 (baby hamster kidney); HT29 (human colorectal-adenocarcinoma), MCF-7 (breast cancer cell line), A549 (lung cancer cell line)	MTT assay measuring absorbance using microplate reader	Cell viability assay to measure cytotoxicity (IC_{50}) of NMs	[83]
6	Eukaryotic cell (blood cells from human or animal model)	Hemocompatibility assay using light microscopy	Substrate adhesion and viability	[84]
7	*Salmonella typhimurium*, *Escherichia coli*	AMES assay	Genotoxicity, DNA oxidative damage	[85]
8	Eukaryotic cell	COMET assay, DNA laddering assay by gel electrophoresis	Genotoxicity, DNA oxidative damage	[86–87]
9	Eukaryotic cell lines BHK21; HT29	Flow cytometry uses light scattering and florescence property of cells	Necrotic, apoptotic, early and late apoptotic cells can be differentiated with fluorescence signals	[88]

(*Continued*)

S/No	Organism/cell lines	Test name	Toxic effect of NPs	Ref.
10	Eukaryotic cell lines A549, MCF-7	ROS assay using flow cytometry (IC_{50}) is measured (DCFDA, a non-fluorescent dye in presence of ROS convert into DCF that is fluorescence)	Oxidative stress of NPs can trigger the p53 mediated apoptotic pathway leading to MMP and up regulation of caspase-3 gene	[89]
11	MCF-7 and other eukaryotic cell lines	Loss of mitochondrial membrane potential (MMP) by staining with Rhodamine 123 dye	Early apoptosis is indicated by loss in red fluorescence due to Rhodamine uptake by NPs treated cells	[90–91]
12	MCF-7 and other eukaryotic cell lines	Gene expression of pro-apoptotic genes (caspase 3) and anti-apoptotic genes (bcl2) by RT-PCR analysis	Genotoxicity of NPs (expression is more in case of pro-apoptotic genes and expression is downregulated by anti-apoptotic gene expression)	[92]
13	HT29, BHK21 eukaryotic cell lines	Acridine orange/ethidium bromide (AO/EB) staining method (fluorescent DNA intercalating dye) for membrane compromised cells	Apoptosis and necrosis of cells against NPs can be checked	[93]
14	Eukaryotic cell lines A549, MCF-7	Hoechst 33342/rhodamine B staining method (rhodamine is a membrane permeable dye staining the mitochondria and cytoplasm red while Hoechst 33342 stains the double stranded DNA blue)	Live cell monitoring assay/time dependent study used to differentiate between pycnotic nuclei from normal nuclei to check the effect of NPs	[94]
15	Eukaryotic cell	Development of micro-organ from cultured nasal epithelium line embryonic heart	Gene expression and altered development	[95]
16	A549, MCF-7, BHK21 cells	SEM, TEM, AFM	Cell morphology/roughness/shape study for checking apoptotic cells	[96]

4.1.1 Proliferation assay

The evaluation of cellular metabolism can be done using proliferation assay [100]. MTT is a salt which is used for the *in-vitro* proliferation assay. This method has got many advantages because of minimum manipulation of the model cell, quick yield, and reproducible results. This test therefore measures the cell suitability in the form of reductive activity as enzymatic conversion of the tetrazolium compound takes place to a purple-colored water insoluble formazan crystal. The insoluble formazan is then solubilized using dimethyl sulphoxide (DMSO) or ethanol and the color obtained is quantified by a spectrophotometer at the wavelength between 500 and 600 nm.

4.1.2 Apoptosis assay

Apoptosis is one of the most important indications of NPs toxicity *in-vitro*. Apoptosis and DNA damage are caused primarily by the generation of excessive free radicals. Apoptosis can be measured using a variety of methods [101]. Annexin-V assay, TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay, comet assay and morphological alterations inspection are some of the methods used [102]. The endonuclease cleavage products of apoptosis are visualized using the DNA laddering technique. The apoptotic and necrotic types of cell death can be easily distinguished using agarose gel electrophoresis. Necrotic cells have genomic pieces of irregular size observed on electrophoresis and apoptotic inter-nucleosome DNA fragmentation can be indicated by the formation of ladder-like electrophoretic pattern. Propidium iodide (PI) and Annexin-V are typical markers for cell death which are used in the toxicity assessment. Changes in the morphology of nucleus and induction of apoptosis were detected when SiO_2 NPs were used for the treatment of human HepG2 hepatoma cells [103].

4.1.3 Necrosis assay

Necrosis assay is used to determine cell viability by measuring the integrity of cell membrane [104]. Neutral red (2-amino-3-methyl-7-dimethyl-amino-phenazoniumchloride), a dye which is a weakly cationic and produces deep red color at slightly acidic pH is used for this purpose [105]. This dye diffuses through the membrane in no time. It accumulates within the lysosomes and binds by with anionic sites using electrostatic hydrophobic bonds within the lysosomal matrix. Modifications of the cell surface due to the NPs interaction result in lysosomal

fragility [106], which can also end up in lowering of uptake and binding of neutral red dye that can differentiate between viable and dead cells [107].

4.1.4 Oxidative stress assay

ROSs and reactive nitrogen species (RNSs) are produced by the exposure of NPs. ROSs and RNSs can be detected by the reaction of 2,2,6,6-tetramethylpiperidine (TMP) with stable O_2^* radical which might be sensed by a high cost technique like electron paramagnetic resonance spectroscopy (EPR) [108] or fluorescent probe molecule which is thought to be good alternative as it is cost effective [109] but they also got a limitation as they react with reactive species so they are considered to be inefficient. A non-fluorescent probe, DCFDA [110], is highly reactive to radicals $HO^•$, $RO^•$, $ROO^•$ and H_2O_2 in the existence of cellular peroxidases to give highly fluorescent 2′,7′-dichlorofluorescein (DCF) [111].

4.2 Cell viability and lethality

Basically, there are two parameters (cell viability and lethality) which are being employed to measure NPs toxicity. CNTs (SWCNTs and MWCNTs) are mainly used for the evaluation of those two parameters [111]. CNTs have anti-microbial properties. A study on freshwater micro-algae (*Raphidocelis subcapitata* and *Chlorella vulgaris*) disclosed the restriction in the growth of algae using SWCNTs with median effective concentrations (EC50) of 29.99 and 30.96 $mg \cdot L^{-1}$, respectively [112]. The NPs blended with iron oxide were likewise found to be harmful in human macrophages, human hepatocarcinoma cells. NPs of iron oxide have also shown toxicity at 25–200 $\mu g \cdot mL^{-1}$ on murine macrophage cells after 2 h of exposure. The observed effects of the study show a decrease in the cell viability [113]. Many other studies have also shown drop in the cell viability. When the treatment of murine macrophages has been carried out with 0.1 $mg \cdot mL^{-1}$ Fe_2O_3 NPs for 7 d, a decrease in cell viability was observed [114].

4.3 Effect on cell lines

The effect of SWCNTs and MWCNTs were examined by different researchers on various human cell lines. Cell culture-based methods are working worldwide to measure risks to improve design and management of materials [115]. In a study, A549 cells were treated to SWCNTs at 250–500 $\mu g \cdot mL^{-1}$ for 72 h, which has resulted in damage of cell membrane [116]. The effect of MWCNTs was examined on human epidermal keratinocytes suggested toxicity facilitated proinflammatory effects by NF-κB and ROSs. MWCNTs has many other toxicological effects like oxidative stress, damages of DNA and apoptosis in mammalian cells lines which were reported by *in-vitro* studies [117]. In human glioblastoma cells, the toxicity of starch-coated Ag NPs was studied, resulting in ATP content reduction with dose, damage of DNA, and arrest of cell cycle in the G2/M phase [118].

Mammalian cells have been treated with 10–400 $\mu g \cdot mL^{-1}$ of Al NPs to test the effect of toxicity. The experimental outcomes showed that there was no major toxic effect witnessed on the cell viability within the report. In additional study cell viability showed reduction on contact with Al NPs within the range of 25–40 $\mu g \cdot mL^{-1}$ of bone marrow-derived human mesenchymal stem cells (hMSCs) [119].

Mainly, *in-vitro* analyses have exposed that the CNTs disturb the potential of membrane, integrity of membrane, cellular reproduction, and metabolic activity. *In-vitro* studies have recommended that on Ag NPs show toxicity interfering with replication of DNA, apoptosis, cytotoxicity, oxidative stress, chromosome instability and arrest in mammalian cell cycle. Fullerenes are to blame for oxidative stress and DNA damage in FE1-Muta™ mouse lung cell lines. Epithelial cells were examined to look at the consequences of C_{60}-fullerenes and SWCNTs for genotoxicity, cytotoxicity and ROS production [120]. Figure 3 depicts outcomes of *in-vivo* and *in-vitro* toxic effects of different NPs.

This method is basically performed on animals like rat & mice. The assessment methods involve biodistribution, clearance, hematology, serum chemistry and histopathology [121]. Biodistribution examines the localization of NPs to tissues and organs. Radiolabels are used to detect the dead or live animals. Clearance of NPs is evaluated by sampling of excretion and metabolism upon exposure at different time intervals. Changes in the cell type and serum chemistry were examined before and after exposure. Toxicity levels of NPs in tissue can finally be concluded by histopathology of the cell, organ, and tissues after exposure. To assess the *in-vivo* poisonousness of NMs, the Organization for Economic Cooperation and Development (OECD) has suggested tests for oral harmfulness, eye irritation, corrosiveness, lethal Dose 50 (LD_{50}) and dermal toxicity [122].

For oral harmfulness test, the mice were orally

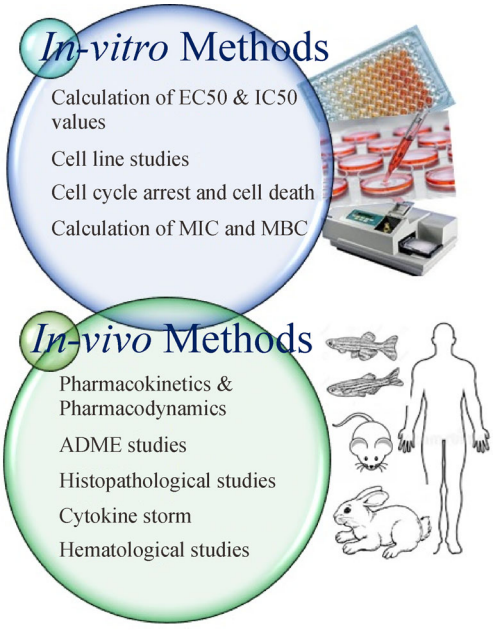

Fig. 3 Important outcomes of *in-vitro* and *in-vivo* toxicity methods.

administered 5000 mg/kg body weight (LD_{50}) of colloidal NMs. All creatures were sacrificed after 14 d and skin & liver were gathered for routine histopathological assessment [123]. After 1, 7, and 10 d of exposure, biopsies of the skin are performed for histopathological assessments and blood was taken for measuring biochemical parameters like cholesterol, triglyceride, blood glucose, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and hematological examinations.

For intense eye irritation, the creatures were treated with 1.5 and 2.5 ppm colloidal nanoparticles, individually. Briefly 0.1 mL of colloidal suspension was injected within the conjunctival sac of one eye of the creature and the other eye was filled in as a control with similar volume of refined water. The creature was observed for harmful side effects at 1, 12, 24, 48 and 72 h after treatment [124]. For intense dermal poisonousness test the creatures were arbitrarily isolated in three groups (n = 3) as follows: bunch 1 gets refined water and groups 2 and 3, get 50 and 100 ppm of colloidal solution, respectively. Every single creature analysis was performed by the OECD 434 rules. Colloidal suspensions were applied to a shaved territory of skin (4 cm × 3 cm), at that time the zone was secured with a dressing and non-bothering tape for 24 h. Following which the dressing was expelled and treated area was washed with saline with physiological pH. The creatures were kept under observation for 14 d to check skin indications (edema, erythema, ulcers, wicked scabs, staining, and scars) and poisonous signs (weight reduction, water and food utilization, conduct). At 1, 3, and 7 d after exposure, skin biopsies are performed for histopathological examinations [125].

5 *In-vivo* and *in-vitro* studies on toxicity of various nanoparticles

5.1 Ag nanoparticles

Traditionally, silver has been known to be an anti-bacterial agent. Ag NPs are employed in coating of surgical equipment, in the shape of wound dressings and prostheses. These Ag NPs enter in individual body via diverse methods and gather in different organs, crossing the blood–brain barrier (BBB) reaching the brain. By experimental findings done on rat model, traces of Ag NPs are found in spleen, liver, kidney, lungs, and brain after exposing via inhalation or injecting subcutaneously. Furthermore, compared to other NPs, Ag NPs are found to be more toxic in term of cell viability, ROS generation and lactate dehydrogenase (LDH) leakage. Mechanism of Ag NPs toxicity may involve lipid peroxidation, ROS generation casing, mitochondrial damage and apoptosis [126]. Ag NPs exist in several coatings and each coating has different degree of cytotoxicity [127]. It was evidenced that peptide-covered Ag NPs (20 nm) were progressively cytotoxic versus citrate-covered Ag NPs of an identical size. Ag NPs are commonly used in the cosmetic, industrial and medical fields due to their efficient antibacterial and unique plasmonic properties [127–128].

5.2 Al_2O_3 nanoparticles

Al_2O_3 NPs have been used in several divisions of industrial sectors. Al_2O_3 NPs show toxicity effects in several organs [129]. Al_2O_3 NPs contribute 20% to any or all nano-sized chemicals. About their poisonous impacts and dose-dependent (25–40 $\mu g \cdot mL^{-1}$) cytotoxicity of Al_2O_3 NPs (160 nm) on hMSCs were evidenced [130]. The Al_2O_3 NPs distribution can be evaluated using CRi *in-vivo* fluorescence imaging. Al_2O_3 NPs exposure resulted in alterations in cytokine levels in the spleen, thymus, and serum, as well as damage to immune organs and immune cell malfunction, resulting in aberrant immune-related cytokine expression [129]. The consequence of another examination utilizing mouse lymphoma cells line likewise propose

that Al_2O_3 NPs (< 50 nm) cause genotoxic impacts as DNA harm with no mutagenic effects [131].

5.3 Cu/CuO nanoparticles

Cu is an important and most important element which is required for normal physiological functions in humans and animals [132]. In one report, the results of Cu NPs and soluble Cu were investigated on juvenile fish *Epinephelus coioides*. Cu NPs have been found to cause extreme impairment in liver, kidney, and spleen in trial creatures. After oral administration and cooperating with digestive fluid, profoundly reactive copper ions were produced, which were then accumulated in the kidney of the uncovered animal [133]. In an *in-vitro* examination, CuO NPs with the size of 50 nm have been accounted for as being cytotoxic and genotoxic and also disturb the cell membrane integrity and inducing oxidative stress [51].

5.4 TiO_2 nanoparticles

TiO_2 NPs are manufactured universal in the large amount which is generally used in wide range of application [134]. TiO_2 also called titania is an oxide semiconductor of n-type that shows photocatalytic activity and photoconductivity [135]. Titanic oxide is artificially an inert compound; however, experiments have demonstrated that titanium NPs have some poisonous impacts in trial creatures, including DNA harm, lung inflammation and genotoxicity. TiO_2 NPs having size less than 100 nm initiate oxidative pressure and structure DNA adducts. A well understanding of TiO_2 NP toxicity in breathing organisms might promote threat assessment and safe usage practices of those NMs. Other genotoxicity TiO_2 NPs (5–200 nm) have poisonous consequences for system, kidney, liver, myocardium, spleen, and glucose and lipids homeostasis in trial animals [136].

5.5 ZnO nanoparticles

ZnO is necessary constituent of several enzymes, ointments for pain, sun screens and itch relief [137]. NPs created from ZnO have a large number of applications in wave channels, paints, gas sensors, UV locators, sunscreens, and various individual care products. Cell membrane disruption, cytotoxicity and increased oxidative stress have been reported in different mammalian cell lines as the highly recognized toxic effect of ZnO NPs. Upon exposure of human mesothelioma cells and rat fibroblast cells to ZnO NPs with high fixation (49 $mg \cdot mL^{-1}$), practically complete cell death in the cell culture was discovered [138]. MTT and comet tests can be utilized for estimating the cell suitability and DNA harm upon ZnO NPs interaction. The protection from this material for the human health is still unclear in spite of its wide use [139]. Apart from cytotoxicity, the genotoxic capability of ZnO NPs has been accounted for in both *in-vivo* and *in-vitro* examinations. To investigate the genotoxic potential of ZnO NPs, standard procedures such as comet measurement and cytokinesis-blocked micronucleus test were used. Chronic introduction to ZnO NPs (300 $mg \cdot kg^{-1}$) led to oxidative DNA harm alongside changing different proteins of the liver was estimated by utilizing the comet test strategy.

5.6 Iron oxide nanoparticles

NPs are at the forefront of rapid development in nanotechnology [140]. Iron oxide NPs are utilized in the field of biomedical sciences, tranquilizer delivery and diagnostics. Iron oxide NPs get accumulated within liver and other reticuloendothelial framework. *In-vivo* investigations have demonstrated that beyond entering the cells, iron oxide NPs stay in cell organelles, discharge into cytoplasm after breaking down, and contribute to cell iron poll. Magnetic iron oxide NPs are seen to collect within the liver, spleen, lung and brain after inward breath, demonstrating its capacity to cross the BBB [141]. Proofs show that these NPs apply their poisonous impact as cell lysis, irritation, and upsetting clotting system. Poisonous impact of iron oxide NPs in the *in-vitro* investigations showed diminished cell viability. The poisonousness of tween-covered iron oxide NPs (30 nm) on murine macrophage cells has been accounted. It was observed that iron oxide NPs at lower concentrations (25–200 $\mu g \cdot mL^{-1}$ for 2 h exposure) showed more cell harmfulness in contrast with high concentration (300–500 $\mu g \cdot mL^{-1}$ for 6 h) of exposure [142]. Cell viability was investigated by the MTT test. It is found that the iron oxide NPs show toxic effects because of the extreme production of ROSs and these extremely produced ROSs further elicit loss of DNA and lipid peroxidation [143].

5.7 Carbon-based nanomaterials

Functionalized carbon-based nanomaterials (CBNs) can be chemically activated to release their contents towards the targeted specific cells. The important benefits of these targeted drug delivery systems are: (i) small dose of strong drug is sufficient enough to use, (ii) side effects are very

less as compared to the delivery method such as chemotherapy, and (iii) they are particularly useful in overcoming the major limitation of chemotherapy, i.e., the non-specific and insufficient therapeutic concentrations deliveries that aims the tumor tissues [144]. The small size of CBNs can easily reach to the target site and it can offer the drug delivery with a separate ideal environment, which helps healthy cell avoid both reaction and degradation [145].

Many efforts have been made to determine the toxicity of CBNs *in-vivo* and *in-vitro*, and many studies have reported various toxicology profiles of CBNs. The different results of the cytotoxicity of the CBNs are related to the differences in physicochemical properties or the differences in structures of CBNs, various types of target cells, various methods of CBNs dispersion, etc. These cytotoxicity effects are due to ROS generation, DNA damage, lysosomal damage, mitochondrial dysfunction and eventual cell death via apoptosis or necrosis. The different results of the CBNs cytotoxicity may be related to their differences in physicochemical properties, types of target cells, and dispersion methods.

In biomedical applications, CBNs are becoming attractive NMs with luminescent properties [146–147]. The widespread use of CBNs in a variety of industries necessitates a thorough understanding of their harmful effects and underlying mechanisms on biological systems. The cytotoxicity of CBNs will be examined in terms of their diverse structures [17].

NMs have always attracted researchers due to their size, which are comparable to most of the biological macromolecules such as DNA, enzymes, and antibodies. Rapid advancements in nanotechnology and the discovery of CNTs in 1991 opened up new vistas in material sciences [148]. Wan et al. [149] reported that the acid-functionalized SWCNTs displayed cytotoxicity in a concentration dependent manner. In a study of Dong et al. [150], it was found that the acid-functionalized SWCNTs were engulfed by macrophages and then localized in lysosomes which leads to the damaged mitochondrial function and inhibited phagocytic activity. The present data on MWCNT-induced cellular toxicity are inconclusive. MWCNTs have different effects on different types of macrophages. MWCNTs triggered cell death in murine bone marrow-derived dendritic cells at concentrations ranging from 3 to 30 $\mu g \cdot mL^{-1}$ [151]. According to the results of an MTT assay, C_{60} fullerene did not produce cytotoxicity in alveolar macrophages. C_{60} had a modest cytotoxicity against human macrophages and did not operate as a biological inducer to cause inflammatory responses [152]. In RAW 264.7 macrophages, pristine graphene was found to cause normal cell death, including apoptosis and necrosis. Many derivatives have been formed because of the widespread use of NMs, and some of these compounds have shown cellular toxicity. According to the report from Wan et al. [149], it was found that GOs have negative effects on murine peritoneal macrophages.

Nanotoxicological research has demonstrated that the toxicity of NMs is inversely related to particle size. One study assessed the cytotoxicity of MWCNTs of various sizes (diameters < 8 nm, 20–30 nm and > 50 nm; same length, 0.5–2 μm) in 3T3 fibroblasts, bronchial epithelial cells, and RAW macrophages. In the study, MWCNTs presented the same mild degree of cytotoxicity in 3T3 fibroblasts as in bronchial epithelial cells, and MWCNTs $<$ 8 nm were more toxic than larger-diameter materials. In contrast, MWCNTs > 50 nm were more toxic than small-diameter materials in RAW264.7 cells [67].

CBNs with different geometric structures exhibit quite different toxic profiles. A cytotoxicity test protocol for SWCNTs, MWCNTs and C_{60} was performed to illustrate the influence of different geometric structures of CBNs on cytotoxicity. The cytotoxicity of SWCNTs was significantly greater than that of MWCNTs at the same concentration, and both SWCNTs and MWCNTs induced profound toxic effects at a lower concentration, whereas no toxic effect was observed in the C_{60} group [152]. Manufactured SWCNTs usually contain significant amounts of metal impurities, such as iron, which may act as a catalyst of oxidative stress. Recent studies have shown that metal impurities play a critical role in cytotoxicity and that metal-containing SWCNTs are likely more toxic than metal-free NPs [144].

6 Routes of exposure of human to nanomaterials

In present day, life has potential dangers related with introduction of human to NMs, the possible courses of passage should be surely known. These tiny particles may enter into the body by natural [unexpectedly] or artificial [intentionally] means into the skin, lungs or intestinal tract. Exposure to NMs may take place depending upon the pattern of use by any of the methods like by inhalation, oral or dermal contact. Other expected courses of exposure to

NMs incorporate intravenous, intradermal, and peritoneal infusion methods. Components that may impact NPs passage incorporate size, charge, surface territory and shape.

6.1 Respiratory route

NMs have the great potential to enter the human body when they are as NPs-agglomerates or airborne nanostructured materials come into contacts with skin. The most widely recognized course of presentation of human to NMs in the working environment is inward breath. Once breathed in, the electrostatic power of the air can transport NMs from the upper respiratory tract to the lower respiratory tract in the bronchioles [153].

6.2 Gastrointestinal route

NPs might enter the body through ingestion too. The greater part of the harmfulness contemplates relating to NPs are centered chiefly around respiratory tract (RT) exposures with barely any investigations portraying the gastrointestinal route (GI) exposures. Ingestion can happen from accidental hand to mouth movement of materials. Ingestion may likewise go along with inhalation since particles that are cleared from the respiratory tract by means of the mucociliary defense system might be gulped. Ingestion is a basic course of people's introduction to NMs, together obviously through food. Increased use of NPs may contaminate the environment and unintentional ingestion via food animals, fish, and water. When they infiltrate the body, they might be moved starting with one spot then onto the next all through the circulatory system in the body [154].

6.3 Dermal route

The "derm" is the peripheral covering of the skin (epidermis and dermis), the biggest organ of the body that protects all the internal organs. Because of its interface with the external condition, skin assumes an important job to secure (the body) against outer obstructions and site for formation of vitamin D. There are conceivable outcomes that skin hindrance or alteration [121], for example, wound, scratches, or dermatitis conditions, may influence NPs entrance of little and considerably bigger particles (0.5–7 μm). QD NPs can enter the skin if there is a scraped area, giving knowledge to potential working environment worries for medicinal services people engaged with the assembling of QDs or doing explore on expected biomedical uses of the small NPs [155–156].

6.4 Nanoparticles metabolism and uptake

After taking NPs by the cellular system, it is influenced by the physicochemical features of NPs like surface chemistry, size, shape and experimental conditions. It is notable that NPs are proficient to enter living cells, regularly through different endocytic pathways. Upon endocytosis, NPs are encased inside the early endocytic vesicles. The term "endocytosis" is broadly categorized into "pinocytosis" (cell drinking) and "phagocytosis" (cell eating). Pinocytosis includes the concealing of liquids and atoms by little vesicles, and phagocytosis serves the procedure by which monocytes, macrophages, neutrophils, and dendritic cells engulf larger particles to produce intracellular phagosomes. Pinocytosis further arranged into four distinctive fundamental classifications namely macropinocytosis, clathrin-interceded endocytosis, caveolin-intervened endocytosis, and autonomous endocytosis. These all-endocytosis components found in explicit kinds of cells have key roles in intracellular dealing and capture of NPs. Quantitative *in-vitro* assessment of the cellular uptake of NPs could be labeled by fluorescent dyes or radioisotopes. The particular selection of different endocytic processes has been established as a method to assess the cellular take-up of nanocarriers, despite the fact that it is not specific in action [157–158]. Figure 4 displays the major phagocytosis pathways for the internalization of NPs [159].

6.5 Factors affecting the efficacy of nanoparticles uptake

The capability of endocytosis depends on the size, shape of NPs, yet in addition the charge, and nature of covering. Optimization of the physicochemical boundaries and surface modification is required for cell specific targeting, in a particular model to each kind of NPs, to improve the capability of cellular take-up and the impacts of different potential blends of the NPs attributes must be assessed to anticipate the nano-harmfulness. Small-sized NPs having larger surface area allow more contact with biological membranes and are easily taken up by phagocytic system. Modified elongated NPs have higher efficiency in cell adhere in comparison to spherical NPs. Positively charged NPs exhibit better cellular internalization due to better interaction with negatively charged cell membrane, surface

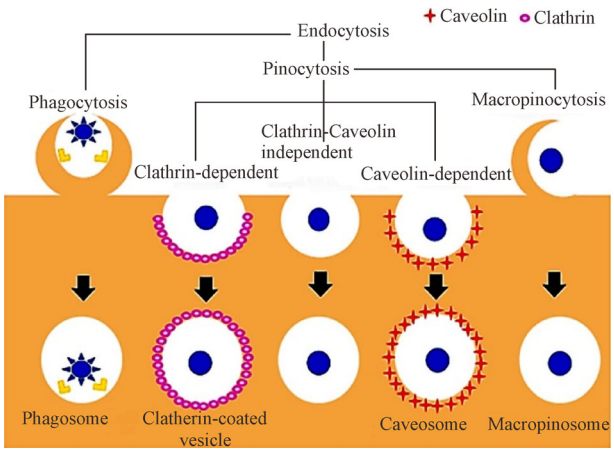

Fig. 4 The main pathways of NPs endocytosis. Reproduced with permission from Ref. [159].

modification of NPs with cell-specific ligands also lead to increased cellular uptake. Attachments of cell penetrating peptides (CPPs) to NPs have been reported as efficient drug delivery strategy for crossing biological barriers [160].

6.6 Nanomaterials-induced immunomodulation and oxidative stress

The factors which affect the immune response have complexity which includes particle size, composition, binding with plasma protein, surface chemistry, and routes of exposure. Interaction of NPs can take place with both the innate and the adaptive immune cells which can disturb their functions and can also affect the immune system. Inflammation can be evidenced by cytokines production as an important immune response induced by NPs [161]. NPs due to strong oxidative abilities may lead to events of inflammation. Redox signaling can be targeted by phospholipid metabolites because several growth parameters and cytokines generate ROSs near to the plasma membrane. Oxidative damage induced by NPs leads to immune imbalance resulting in free radical induced inflammatory response. GO can decrease cell viability and increase necrosis by TLR4 signaling pathway which is innate immune system receptor that can induce chronic inflammation and ROSs production. It has been observed that poly(acrylic acid) (PAA)-conjugated Au NPs can activate the NF-κB signaling pathway in THP-1 cells. TNF-α and IL-8 were among the inflammatory cytokines generated by the cells. When such transcription factors are activated, they cause pro-inflammatory genes to be transcribed, which lead to the creation of cytokines, chemokines, and adhesion molecules. Clear relationship between the production of intrinsic free radical due to NPs and their ability to produce inflammation in the lungs could not be laid in spite of several studies, despite the fact that the structure of NPs and their cytotoxic effects have relevant support [162].

NPs of various substance structures, for example, CNTs, fullerenes and MOs are very notable to instigate oxidative stress. Transition metals mainly including Fe, Cu, Cr, and V are associated with ROS production through instruments, for example, Haber–Weiss-type and Fenton-type responses. Fenton responses for the most part includes a change metal particle that responds with H_2O_2 to yield $^{\bullet}OH$ and the oxidized metal particle. Fe and Cu metal NPs were accounted for to prompt oxidative pressure by means of the Fenton-type response. NPs including Cr, Co and V can catalyze both Haber–Weiss-type and Fenton-type responses. A significant cancer prevention agent protein, glutathione reductase converts metal NPs into intermediates that initiate the ROSs reactions [163–164].

7 Nanomaterials inhalation toxicity

Ahamed et al. [165] reported that CuO NPs induce oxidative stress, cytotoxicity and genotoxicity in human lung cells lines. Leaching of copper ions, autophagy and ROSs generation may be the mechanisms of Cu NPs toxicity in lung cells. NPs in air can travel huge distances by the Brownian dispersion. Hence, inward breath could be a significant course of human introduction to the NMs that are air-borne. NPs are accumulated within the respiratory tract and alveoli prevalently by dissemination. Once introduced, NPs may cross natural layers & retain in tissue that might not typically be presented to these substances. The lung disease cell line A549 in humans has utilized the culture replica of *in-vitro* cell for investigation of harmfulness of NMs. Foldbjerg et al. [166] utilized this framework to explore the cytotoxic & genotoxic impacts of NPs of silver. The MTT and annexin V/propidium iodide assays were used to establish dose-dependent cellular toxicity of silver ions and polyvinyl pyrrolidone (PVP)-coated Ag NPs in humans, and evidence of Ag NPs uptake was evaluated using indirect atomic absorption spectroscopy (IAAS) and flow cytometry (FC) techniques. ROSs damages DNA, which was detected by an increase in bulky DNA adducts after exposure to Ag NPs using ^{32}P post labeling. The level of bulky DNA adducts was substantially linked with cellular ROS and it could also be decreased by antioxidant pretreatment, implying that Ag

NPs are the intermediary of genotoxicity induced by ROSs.

Lately, Jugan et al. [167] assessed the cytotoxic impacts & genotoxic impacts of TiO_2 NPs on the cell line A549 alveolar epithelial cells illustrative of lung in human. Research on inhalation of harmfulness NMs proposes that NMs of certain type can possibly prompt nephrotoxicity. TiO_2 ENPs when internalized in the human alveolar cells was found to induce cyto- and genotoxicity [168]. Currently, translocation of inhaled NPs which are passed through the membranes such as the air–blood barrier into secondary target organs (STOs) is debated [169–170].

7.1 Routes of exposure

The major pathway of concern is also known as inhalation. We have covered three primary locations where humans might be exposed to inhalation pollution: the workplace, the environment, and the consumer product.

7.1.1 Workplace

Exposure to air borne ENMs is of greatest significance within the area of related wellbeing. The potential occasions incorporate manufacture, transport, and handling of ENMs, during the development of nano-empowered items. Kuhlbusch et al. [171] provided a review about ENMs pertinent for business related exercises, which incorporate Ag, fullerenes, carbon black, CNTs, MWCNTs, SiO_2 and MOs like TiO_2, CeO_2 and Al_2O_3. The main routes of exposure are workplaces of ENPs and their aggregates and derivatives are used. These include processing pilot plants, drilling and sawing of NMs, industrial production facilities as well as research related work area settings. Handling and refining of the raw material involve production, processing steps, bagging and shipping, work processes with the nanomaterial product that can be organized using the production pathway. An assortment of consumer items is additionally liable to deliver respirable airborne ENMs in nearness to the client. Presentation to airborne ENMs may happen using showers and powders, generally applied in beautifiers, cleaning or care items. Propellant sprays produce fundamentally littler particles in compare with pump splashes. This demonstrates, from one viewpoint, that consumer items may deliver nano-sized airborne particles despite the fact that the liquid formulation does not contain any ENMs. Although more examinations on aerosol formulation by customer items are as of now being conducted, the market presentation of recent items and still uncertain information entail further research [172].

7.1.2 Environment

NPs may invade all the compartments of environment such as air, water, and land. It is important to evaluate and quantify the NMs exposure of the biotic and abiotic components of ecosystem starting from the sources of emission, utilization, recycling, and disposal. The sources of ENMs are hard to acknowledge or perhaps to judge. ENMs might be discharged into the ambient air by procedures adopted in occupational or lifestyle customs. The introduction of ENMs may happen essentially at stages involving molecule recovery, spray drying or milling [173]. ENMs may additionally be coincidentally discharged into ambient air during the handling and transport of particles. Dumping waste into landfills, incineration generating ash in air and resistance to recycling processes for example, TiO_2 NPs remain stable to acid based recycling processes increase the load of NPs exposure to environment [174]. Fume gas catalysts can also act as source of airborne ENMs which are regularly delivered with nano-sized MOs, e.g., CeO_2.

7.2 Immunotoxicity of nanomaterials

Data on NMs immunotoxicity are constrained and the testing of immunotoxicity of NMs has shown that NPs can together animate or potentially stifle the invulnerable reactions. NMs can regulate the cytokine creation [175]. They instigate expert fiery impacts within the test creatures with expanded articulation on IL-1, macrophage inflammatory protein (MIP)-1, monocyte chemoattractant protein (MCP)-1, MIP-2, keratinocyte chemoattractant (KC), chemokine (C-C theme) thymus and activation-regulated chemokine (TARC) ligand, granulocyte-macrophage colony-stimulating factor (GM-CSF), and enactment of the pressure-initiated mitogen-actuated protein kinases (MAPKs) p38 & c-Jun N-terminal kinases (JNKs). More investigations are required for the turn of events & approval of techniques for examining the NMs immunotoxicity. The *in-vivo* and *in-vitro* experiments on immunotoxicity of NMs recommend that some NMs can possibly cause immunotoxicity. Direct injury to immune cells by NMs prompts apoptosis and necrosis, while associations of NMs using the immunologic response itself can change immune specific signaling pathways, bringing about changes in immune cell function estimated by articulation

of surface markers, cytokine production, cell separation and immune activation. Along these lines, dependable testing requires pertinent *in-vivo* and *in-vitro* models that may recognize typical and neurotic reactions. The expanded conglomeration that may normally happen on contact with the organic environment may cause successful clearing or sequestering by immune cell which is able to normally perceive especially bigger particles (>0.5 mm). The responses of self-proteins with NMs and their tenacity within the living being can cause autoimmune reactions. Actuation of inflammasomes can happen through some components. Neutrophils or poly-morphonuclear granulocytes assume a key job in NM-actuated irritation [176]. Enactment of mast cells can prompt creation of histamines and different substances causing airway irritation. One factor suspected to add to the ongoing emotional increment in rate of sensitivities, lung illnesses and asthma is ecological contamination and inward breath of ultrafine particles [41]. Most *in-vitro* immunotoxicity detection models detect immunosuppression which can be caused by a number of events. An assessment of the functionality of immune cells could be measured by mediators like histamine, cytokines or activation of the complement cascade leading to hypersensitivity reactions.

7.3 Genotoxicity of nanomaterials

Genotoxicity may lead to variation in germ cells leading to health implications in future generations. NMs can cause physical and chemical damage to cells and may induce genotoxicity by indirect methods like induction of ROSs, DNA injury, chromosomal aberrations, damage to structural protein and lipids of cell membrane or induction of inflammatory responses. They also incorporate clastogenicity (chromosome breakage and rearrangements) and aneuploidy. Genotoxicity can be tested by both *in-vivo* and *in-vitro* measures. The DNA damage caused by NMs exposure can be measured by the comet assay which can detect alkali labile sites, DNA cleavage and lesion specific endonucleases. The cell cultures may be exposed to test substance and incubated with metaphase arresting agents and finally studied microscopically. Chromosomal damage is checked in interphase cell micronuclei or dividing mitotic cells and cytochalasin B blocks the cell division that lead to binucleated cell accumulation. In a study carried by Sahu et al. [138], cytochalasin B blocked assay and the stream cytometric *in-vitro* micronucleus measure, the equivalent *in-vitro* replicas (HepG2 & cells of Caco2) & the equivalent trial circumstances to analyze the possible genotoxicity of two distinct sized nano-Ag (20 and 50 nm) of the similar structure, synthesis, charge on surface, and acquired from a similar origin. It is also observed that the smaller sized NPs (20 nm) can be genotoxic to both the types of cells by actuating micronuclei. Their outcomes exhibited that the size of NPs and the type of cells were probably basic determinants of genotoxicity of nanosilver. Dusinska et al. [175] examined the method of reasoning for genotoxicity NMs testing & necessity for the NM hazard evaluation. They saw that normalized techniques are important to assess NMs genotoxicity, and reasoned that without normalized techniques, the particular administrative testing prerequisites for NMs are untimely.

7.4 Cytotoxicity

For legitimate understanding of their biological action of NPs, a valuation of the cytotoxicity of NMs is important. The *in-vitro* labeling of therapeutic cells with NPs has become a common practice but concerns about the possible effects of the NPs on the healthy cells are increasing. Hence, appropriate readiness and portrayal of NMs are basic for the cytotoxicity assessment. Existing cytotoxicity assessments of NMs are to a good extent restricted to the estimation of cell viability. The possible mechanism of cytotoxicity by formation of ROSs is depicted in Fig. 5.

Sohaebuddin et al. [177] studied the cytotoxicity of SiO_2 and TiO_2 NPs in 3 cell lines, 264.7 macrophages, 3T3 fibroblasts and telomerase-immortalized bronchiolar epithelial cells. After detailing their characteristics in phosphate buffered saline (PBS) and serum-containing materials, the cells were exposed to the NPs of various compositions and sizes. They reasoned that the NMs production, size, and the target cell type are key factors of intracellular reactions, cytotoxicity, and mechanism of poisonousness. Researches on the cytotoxicity of some NMs such as TiO_2, SiO_2, ZnO and periclase (MgO) revealed that they affect human Caco-2 cells, and it was brought into notice that all the tests of NPs show cytotoxicity expect MgO. Exploration of the cytotoxic impacts of SiO_2 NPs on Balb/3T3 mouse fibroblasts [75]. The investigations of this study indicated negligible cytotoxicity as estimated by the MTT examine [18]. Cytotoxicity of ZnO NPs shows toxic effect on the immune system of human. ZnO NPs showed strong link with free intracellular zinc content. Sahu et al. [178] assessed the Ag NPs cytotoxic capability with 20 nm size utilizing human liver HepG2 and colon Caco2 cells in

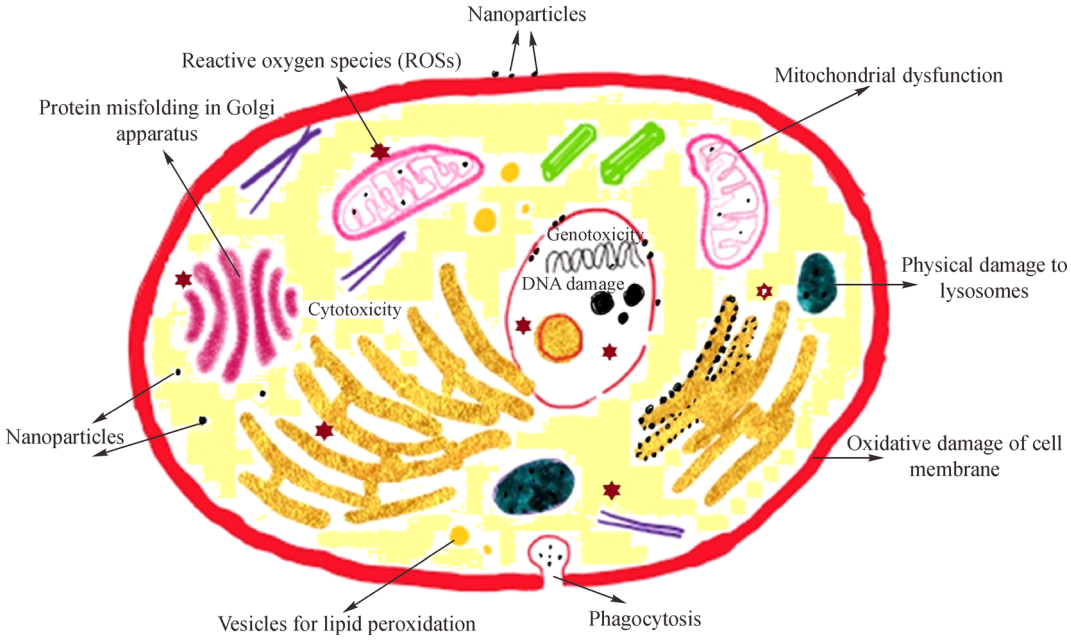

Fig. 5 Schematic illustration depicting NM-induced cytotoxicity.

culture as *in-itro* models, and found the cytotoxicity effect of Ag NPs in both the HepG2 and the Caco2 cells. In both of cell types, the increase in mitochondrial damage with concentration-dependent and the loss of double-stranded DNA was found. They detected no nano-Ag-induced cell oxidative stress in cell types where the dichlorofluorescein test indicated cell oxidative stress. Nano-Ag sensitivity was higher in HepG2 cells than in Caco2. Their findings suggested that a variable reactivity of various cell types exposed to NPs, as opposed to a generic reaction, could have a significant role in poisonousness. Hunt et al. [179] have indicated development concealment and oxidative DNA harm in *C. elegans* presented to nano-Ag. An ongoing report by Uboldi et al. [180] shows that toxicity is decreased with the particle size while it is negatively correlated with the viscosity of the media. The cytotoxicity contemplated in the article has a variety of outcomes, but they all illustrate that NMs can be predicted to be cytotoxic. These studies tried the variety of NMs testing models, NMs synthesized from a variety of methods and sources, and test circumstances.

The cytotoxicity test is a key sign of *in-vitro* biological system evaluation, and numerous experimental procedures to determine and evaluate cytotoxicity are constantly being created as current cell biology advances. NMs cause cell-specific reactions, resulting in varying toxicity and cell fate depending on the kind of cell exposed. Surface chemistry is crucial in determining how NPs interact with bio-system.

7.5 Cardiotoxicity

A review by Bostan et al. [181] represents cardiotoxic effects of NPs of iron oxide, SiO_2, Ag, ZnO and TiO_2, as well as SWCNTs and MWCNTs, when subjected to various animal models from mice, rats, and zebrafish. Increased levels of various inflammatory marker genes like CRP, TNF-α, IL-6, IL-1β, ET-1, D-dimer, LDH, CK-MB, and caspase-3 have been reported indicative of oxidative stress, increased risk of acute myocardial infarction.

Cardiovascular poisonousness of SiO_2 NPs in rodents by intra-tracheal instillation was explored by Du et al. [182]. Hematologic boundaries, inflammatory responses, oxidative pressure, endothelial brokenness, and myocardial enzymes were all measured in the serum. SiO_2 NPs passed via the alveolar–capillary barrier and reached to complete circulation. The researchers found that the toxicity of SiO_2 NPs for cardiovascular disease was highly dependent on the size of the molecule and its dose. To test the cardiovascular effects of SiO_2 NPs, *in-vitro* endothelial cells and *in-vivo* zebra fish model was used. As toxicological biomarkers, they used oxidative pressure, cytotoxicity, and apoptosis. They identified oxidative pressure and apoptosis as key-factors in the failure of endothelial cell. Yang et al. [183] reported a chronic cardiac toxicity in mice presented to various sizes of Au NPs by the tail vein. They looked studied the effects of NPs accumulation in the mouse heart on circulatory capability,

structure, fibrosis, and inflammation. In 14 d of exposure, the increase in left ventricular mass and body weight takes place in animals with NPs of 10 nm size. They inferred that Au NPs caused heart hypertrophy.

8 Nanoecotoxicity and effects on plants and animals

The release of NPs in the aquatic environment has an impact on aquatic plants and animals, while the release of NPs in the terrestrial environment has an impact on the expansion and survival of land plants and animals. Nano ecotoxicology can be considered as sub-discipline of ecotoxicology and mainly aims to spot and expect effects drawn by NMs on ecosystems. The NMs can have biological and developmental impacts on earthbound and oceanic biological systems, for example, circulation of NMs can lead to bioaccumulation and bio-magnification in biotic procedures that influence the existence of living systems. The focus was set on selected synthetic NPs such as nano-TiO_2, nano-ZnO, nano-CuO, nano-Ag, SWCNTs, MWCNTs and C_{60}-fullerenes, and organism groups representing different levels of the food chain (bacteria, algae, crustaceans, ciliates, fish, yeasts, and nematodes). Nano-TiO_2 (31%), C_{60} (18%), nano-ZnO (17%), nano-Ag (13%), SWCNTs, and nano-CuO all had a higher hazardous effect overall (both 9%) [184–185]. Built NPs may consequence for living being ontogeny and multi-generational life histories [186].

8.1 Effects of nanoparticles in plants

The schematic representation of uptake, translocation and phytotoxicity of Ag NPs is depicted in Fig. 6 [187]. Nanofertilizers are utilized to enhance plant uptake and nourishment. Bulk and NP forms of Cu and Ag were accounted for profound phytotoxicity, influencing development and transpiration when probed plants.

SiO_2 NPs have been accounted for to cause plant protection from salt worry by enhancing the antioxidant system of squash [188]. ENPs as well as aerosol (TiO_2 NPs) and colloidal silver (Ag NP) cause phytotoxicity in tomatoes (*Lycopersicon esculentum*) [189]. Nano-$TiSiO_4$ has been seen as basically phytotoxic on the development of dicotyledonous plant species including lettuce, *Lactuca sativa* and tomato *Lycopersicon lycopersicum* [190]. ZnO NPs have been accounted for to cause phytotoxic and genotoxic influences on *Allium cepa* or onion distinguished by boundaries uncovering influenced mitotic (MI), micronuclei (MN), chromosomal aberration indices and lipid peroxidation [191]. In an experimental study, it was found that the iron oxide NPs (γ-Fe_2O_3 NPs) showed physiological variations on watermelon seedlings [192].

8.2 Effects of nanoparticles in animals

Earthworms are essential for the integration and fragmentation of organic detritus, organic matter mineralization, and mineral nutrient recycling [193]. NPs may be accidently released into the dirt or conveyed with the

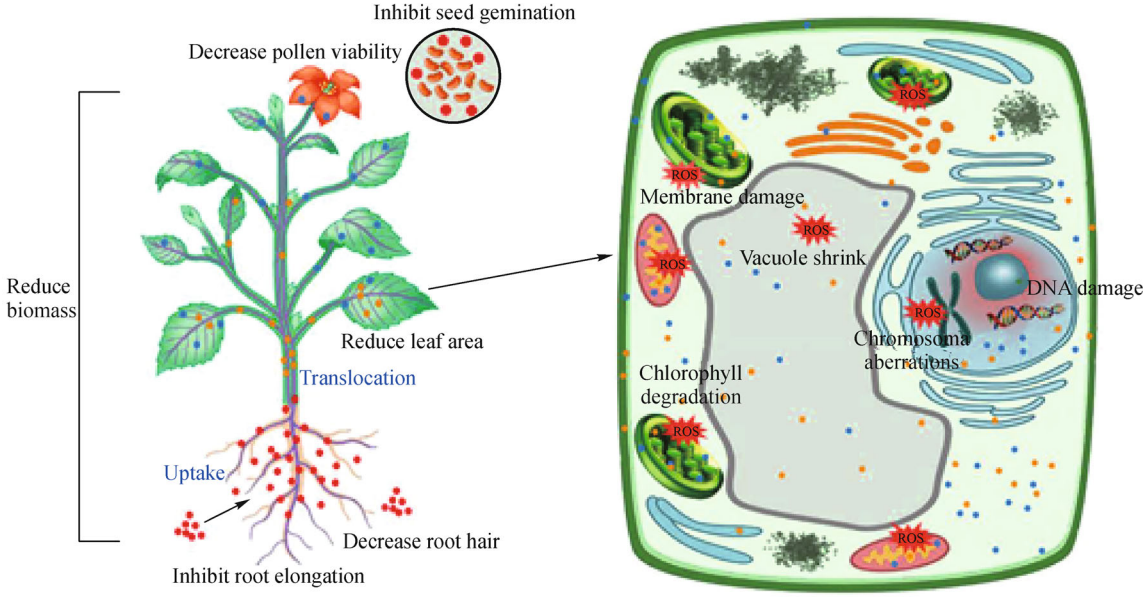

Fig. 6 Toxicity of Ag NPs in plant. Reproduced with permission from Ref. [187].

breeze. The impacts of NPs are more vulnerable within the dirt than in water. When exposed to NPs, apoptotic cells were detected in the cuticle and intestinal epithelium. As a result, it will be deduced that NPs controlled the extended barrier made up of mucus and antibacterial atoms; they also influenced the assimilation of nutrients and hence the security provided by the chloragogen tissue in earthworms [194].

The impacts of ZnO NPs were explored on *in-vitro* societies of hepatocyte strains originating from human and fish. In a study, Handy et al. [195] compared the adsorption of TiO_2 NPs and C_{60} fullerenes onto the gill microenvironment and mucus layer of fishes, endocytosis, rather than the absorption on membrane transporters or diffusion via cell membranes, was the mechanism by which NPs were taken up by epithelial cells. The gills, intestines, liver, and occasionally the brain were among the organs targeted. Hepatic excretion of NPs into the bile seems to be more probable mode of excretion renal or branchial excretion.

The Ag NPs (120 nm in diameter) infiltrated into the hepatocytes, causing an oxidative pressure due to the presence of ROS, INF expression, and endoplasmic reticulum (ER) interruption, according to a report done on *in-vivo* zebrafish and *in-vitro* culture of tumoral human hepatocytes Huh7. Half of the LC50 concentration (71.1 $g \cdot L^{-1}$) was exposed to adult zebrafish for 14 d to uncover the toxicity mechanisms of Ag NPs. In the gills and liver tissues, cytological alterations and intrahepatic localization of Ag NPs were found, and the results determined a possible indicator of oxidative stress. In addition to oxidative stress, genotoxic effects were seen in peripheral blood cells, including nuclear abnormalities, the existence of micronuclei, and a lack of cell contact with an irregular shape in liver parenchyma cells [196]. *In-vitro* tests on zebrafish revealed that Ag NPs induced neurotoxic effects that differed from Ag^+ ions. On the first stage of development, the effects of Ag NPs of various sizes (12 and 28 nm), Ag NPs covered with PVP (45, 63, 65, and 324 nm), and Ag^+ ions were also remarkable. Ag^+ slowed sac progress, causing some abnormalities in the process; Ag NPs had little effect [197]. Figure 7 depicts the aquatic food chain and other direct routes of exposure to NPs leading to toxic effects on human.

9 Concluding remarks and future challenges

In the modern world, the researchers have demonstrated several benefits of nanotechnology in several fields such as catalysis, biotechnology, environmental science, electronics, solar energy, medical and pharmaceutical industries but the toxic effect of NPs on human health and environment cannot be ruled out. Several chemical and physical methods have been used to synthesize NPs but the determination of their contaminated effects in individual health and environment is similarly important. In the present article, we have demonstrated the immense use of

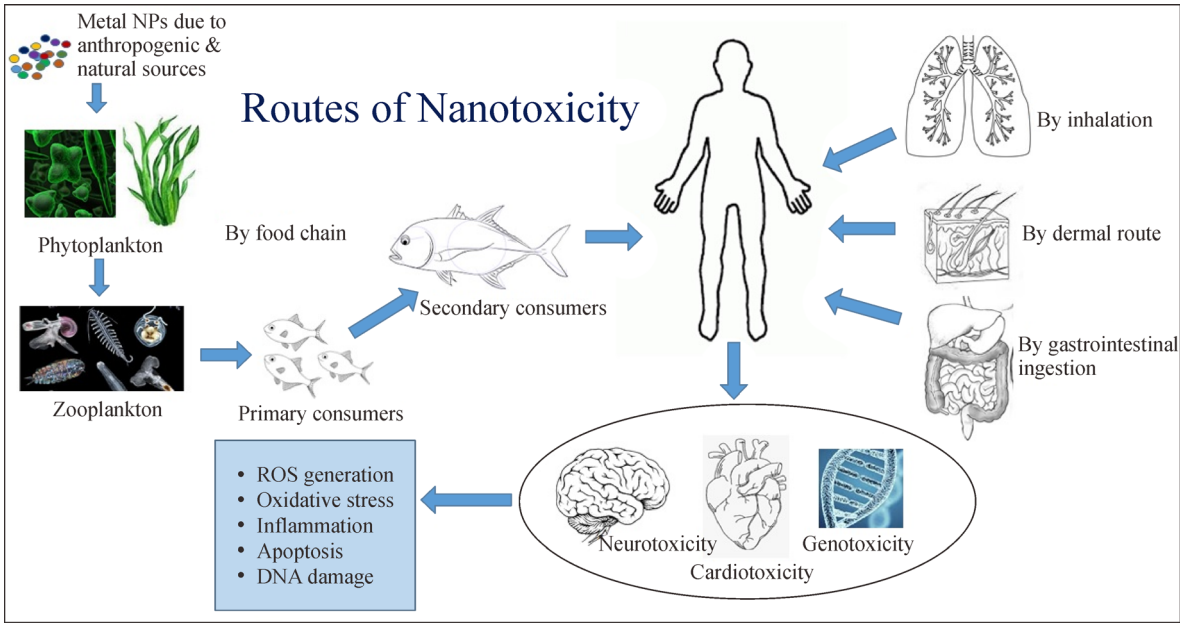

Fig. 7 Flowchart representing the routes of nanotoxicity involving the food chain and other pathways with their impact.

NPs in biomedical applications leading to direct exposure to them within the permissible limit. But, beyond the permissible concentration limit, they may cause change in gene expression and protein/lipid oxidation. Effect of NPs size, shape, type, concentration, aspect ratio, solubility and several other parameters enables the NPs for their potential route of human exposure. Several methods for screening the toxicity of NPs such as *in-vivo* and *in-vitro* effects of cell lines, routes of exposure to human, and mechanism of toxicity of NPs have been discussed in detail and compared with other studies. Progressively many nations have launched national nanotechnology programs, but unquestionably the gain and loss due to nanotechnology may rely on the power to deal with problems with the planet. Many scientists are working hard to find solutions to several pressing environmental concerns like bioaccumulation, environmental impact, toxicity and long-term issues. This article shows the advancement in metallic and non-metallic NPs and their toxic consequences on the human health. Till date, insufficient information is available for the hazard evaluation of NMs. Furthermore, there is no internationally recognized standard methodology available for assessing NMs' hazardous effects. Further research is still needed to understand the proper mechanism of nanotoxicity, standardization of the protocols of toxicity and long-term effect of NPs on persons' health and environment impact.

Authors' contribution Deepshikha Gupta: Conceptualization, outline preparation and figures, and review; Tejendra K. Gupta: Editing, review, and presentation; Parul Yadav and Devesh Garg: Data collection, resources and writing.

Disclosure of potential conflict of interest The authors declare no conflict of interest.

Acknowledgements The authors are grateful to the Department of Chemistry, Amity Institute of Applied Sciences, Amity University Uttar Pradesh, India, for allowing them to complete their study and for offering the chance to do so through a non-teaching credit course (NTCC). The authors are also grateful to Amity University for providing the essential library resources for data collection.

References

[1] Lowry G V, Gregory K B, Apte S C, et al. Transformations of nanomaterials in the environment. Environmental Science & Technology, 2012, 46(13): 6893–6899

[2] Shinde R B, Veerapandian M, Kaushik A, et al. State-of-art bioassay systems and electrochemical approaches for nanotoxicity assessment. Frontiers in Bioengineering and Biotechnology, 2020, 8: 325

[3] Jeevanandam J, Barhoum A, Chan Y S, et al. Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. Beilstein Journal of Nanotechnology, 2018, 9: 1050–1074

[4] Malhotra B D, Ali M A. Chapter 1 - Nanomaterials in biosensors: Fundamentals and applications. In: Malhotra B D, Ali M A, eds. Nanomaterials for Biosensors. William Andrew Publishing, 2018, 1–74

[5] Musee N. Nanowastes and the environment: Potential new waste management paradigm. Environment International, 2011, 37(1): 112–128

[6] Griffin S, Masood M I, Nasim M J, et al. Natural nanoparticles: A particular matter inspired by nature. Antioxidants, 2017, 7(1): 3

[7] Zuo J, Jiang T, Zhao X, et al. Preparation and application of fluorescent carbon dots. Journal of Nanomaterials, 2015, 2015: 787862

[8] Ajdary M, Moosavi M A, Rahmati M, et al. Health concerns of various nanoparticles: A review of their *in vitro* and *in vivo* toxicity. Nanomaterials, 2018, 8(9): 634

[9] Kush P, Kumar P, Singh R, et al. Aspects of high-performance and bio-acceptable magnetic nanoparticles for biomedical application. Asian Journal of Pharmaceutical Sciences, 2021 (in press)

[10] Gupta R, Xie H. Nanoparticles in daily life: Applications, toxicity and regulations. Journal of Environmental Pathology, Toxicology and Oncology, 2018, 37(3): 209–230

[11] Patra J K, Das G, Fraceto L F, et al. Nano based drug delivery systems: Recent developments and future prospects. Journal of Nanobiotechnology, 2018, 16(1): 71

[12] Buzea C, Pacheco I I, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases, 2007, 2(4): MR17–MR71

[13] Wani M Y, Hashim M A, Nabi F, et al. Nanotoxicity: Dimensional and morphological concerns. Advances in Physical Chemistry, 2011, 450912 (15 pages)

[14] Zhang Y, Ram M K, Stefanakos E K, et al. Synthesis, characterization, and applications of ZnO nanowires. Journal of Nanomaterials, 2012, 2012: 624520

[15] Erofeev A, Gorelkin P, Garanina A, et al. Novel method for rapid toxicity screening of magnetic nanoparticles. Scientific Reports, 2018, 8(1): 7462

[16] Gellert G. Sensitivity and significance of luminescent bacteria in chronic toxicity testing based on growth and bioluminescence. Ecotoxicology and Environmental Safety, 2000, 45(1): 87–91

[17] Yuan X, Zhang X, Sun L, et al. Cellular toxicity and immunological effects of carbon-based nanomaterials. Particle and Fibre Toxicology, 2019, 16: 18

[18] Shin S W, Song I H, Um S H. Role of physicochemical properties in nanoparticle toxicity. Nanomaterials, 2015, 5(3): 1351–1365

[19] Iqbal M A, Md S, Sahni J K, et al. Nanostructured lipid carriers system: Recent advances in drug delivery. Journal of Drug Targeting, 2012, 20(10): 813–830

[20] Rose J, Auffan M, Proux O, et al. Physicochemical properties of nanoparticles in relation with toxicity. In: Bhushan B, ed. Encyclopedia of Nanotechnology. Dordrecht: Springer Netherlands, 2012, 2085

[21] Sukhanova A, Bozrova S, Sokolov P, et al. Dependence of nanoparticle toxicity on their physical and chemical properties. Nanoscale Research Letters, 2018, 13(1): 44

[22] Gliga A R, Skoglund S, Wallinder I O, et al. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: The role of cellular uptake, agglomeration and Ag release. Particle and Fibre Toxicology, 2014, 11(1): 11

[23] Albanese A, Tang P S, Chan W C W. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual Review of Biomedical Engineering, 2012, 14(1): 1–16

[24] Huo S, Jin S, Ma X, et al. Ultrasmall gold nanoparticles as carriers for nucleus-based gene therapy due to size-dependent nuclear entry. ACS Nano, 2014, 8(6): 5852–5862

[25] Viswanath B, Kim S. Influence of nanotoxicity on human health and environment: The alternative strategies. In: de Voogt P, ed. Reviews of Environmental Contamination and Toxicology. Cham, Switzerland: Springer International Publishing, 2017, 61–104

[26] Agus H H, Hornsby M, Chen M, et al. Outstanding reviewers for toxicology research in 2017. Toxicology Research, 2018, 7(3): 320

[27] Contini C, Hindley J W, Macdonald T J, et al. Size dependency of gold nanoparticles interacting with model membranes. Communications Chemistry, 2020, 3(1): 130

[28] Kim T H, Kim M, Park H S, et al. Size-dependent cellular toxicity of silver nanoparticles. Journal of Biomedical Materials Research Part A, 2012, 100A(4): 1033–1043

[29] Li Z, Hulderman T, Salmen R, et al. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environmental Health Perspectives, 2007, 115(3): 377–382

[30] Hu W, Peng C, Lv M, et al. Protein corona-mediated mitigation of cytotoxicity of graphene oxide. ACS Nano, 2011, 5(5): 3693–3700

[31] Ou L, Song B, Liang H, et al. Toxicity of graphene-family nanoparticles: A general review of the origins and mechanisms. Particle and Fibre Toxicology, 2016, 13(1): 57

[32] Schmid O, Stoeger T. Surface area is the biologically most effective dose metric for acute nanoparticle toxicity in the lung. Journal of Aerosol Science, 2016, 99: 133–143

[33] Karakoti A S, Hench L L, Seal S. The potential toxicity of nanomaterials — The role of surfaces. JOM, 2006, 58(7): 77–82

[34] Limbach L K, Li Y, Grass R N, et al. Oxide nanoparticle uptake in human lung fibroblasts: Effects of particle size, agglomeration, and diffusion at low concentrations. Environmental Science & Technology, 2005, 39(23): 9370–9376

[35] Cho J, Kushiro K, Teramura Y, et al. Lectin-tagged fluorescent polymeric nanoparticles for targeting of sialic acid on living cells. Biomacromolecules, 2014, 15(6): 2012–2018

[36] El Badawy A M, Silva R G, Morris B, et al. Surface charge-dependent toxicity of silver nanoparticles. Environmental Science & Technology, 2011, 45(1): 283–287

[37] Bahadar H, Maqbool F, Niaz K, et al. Toxicity of nanoparticles and an overview of current experimental models. Iranian Biomedical Journal, 2016, 20(1): 1–11

[38] Fubini B, Hubbard A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radical Biology and Medicine, 2003, 34(12): 1507–1516

[39] Rathee G, Bartwal G, Rathee J, et al. Emerging multi-model zirconia nanosystems for high-performance biomedical applications. Advanced NanoBiomed Research, 2021, 2100039

[40] Zhu J, Ou X, Su J, et al. The impacts of surface polarity on the solubility of nanoparticle. The Journal of Chemical Physics, 2016, 145(4): 044504

[41] Dusinska M, Boland S, Saunders M, et al. Towards an alternative testing strategy for nanomaterials used in nanomedicine: Lessons from NanoTEST. Nanotoxicology, 2015, 9(Sup1): 118–132

[42] Nel A, Xia T, Mädler L, et al. Toxic potential of materials at the nanolevel. Science, 2006, 311(5761): 622–627

[43] Schmidt J, Vogelsberger W. Aqueous long-term solubility of titania nanoparticles and titanium(IV) hydrolysis in a sodium chloride system studied by adsorptive stripping voltammetry. Journal of Solution Chemistry, 2009, 38(10): 1267–1282

[44] Donaldson K, Stone V. Current hypotheses on the mechanisms of toxicity of ultrafine particles. Annali dell'Istituto Superiore di Sanita, 2003, 39(3): 405–410

[45] Avramescu M L, Rasmussen P E, Chénier M, et al. Influence of pH, particle size and crystal form on dissolution behaviour of engineered nanomaterials. Environmental Science and Pollution Research International, 2017, 24(2): 1553–1564

[46] Thakuria A, Kataria B, Gupta D. Nanoparticle-based methodologies for targeted drug delivery — An insight. Journal of Nanoparticle Research, 2021, 23(4): 87

[47] Annangi B, Rubio L, Alaraby M, et al. Acute and long-term *in vitro* effects of zinc oxide nanoparticles. Archives of Toxicology, 2016, 90(9): 2201–2213

[48] Wang M, Zhang Y, Xu M, et al. Roles of TRPA1 and TRPV1 in

cigarette smoke-induced airway epithelial cell injury model. Free Radical Biology and Medicine, 2019, 134: 229–238

[49] Huang Y, Ding L, Li C, et al. Safety issue of changed nanotoxicity of zinc oxide nanoparticles in the multicomponent system. Particle & Particle Systems Characterization, 2019, 36(10): 1900214

[50] Assadian E, Zarei M H, Gilani A G, et al. Toxicity of copper oxide (CuO) nanoparticles on human blood lymphocytes. Biological Trace Element Research, 2018, 184(2): 350–357

[51] Karlsson H L, Cronholm P, Gustafsson J, et al. Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. Chemical Research in Toxicology, 2008, 21(9): 1726–1732

[52] Chen Z, Meng H, Xing G, et al. Acute toxicological effects of copper nanoparticles *in vivo*. Toxicology Letters, 2006, 163(2): 109–120

[53] Tulve N S, Stefaniak A B, Vance M E, et al. Characterization of silver nanoparticles in selected consumer products and its relevance for predicting children's potential exposures. International Journal of Hygiene and Environmental Health, 2015, 218(3): 345–357

[54] Lok C N, Ho C M, Chen R, et al. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. Journal of Proteome Research, 2006, 5(4): 916–924

[55] Grande F, Tucci P. Titanium dioxide nanoparticles: A risk for human health? Mini-Reviews in Medicinal Chemistry, 2016, 16(9): 762–769

[56] Tsoli M, Kuhn H, Brandau W, et al. Cellular uptake and toxicity of Au_{55} clusters. Small, 2005, 1(8–9): 841–844

[57] Chen L W, Hao Y C, Guo Y, et al. Metal-organic framework membranes encapsulating gold nanoparticles for direct plasmonic photocatalytic nitrogen fixation. Journal of the American Chemical Society, 2021, 143(15): 5727–5736

[58] Simpson C A, Salleng K J, Cliffel D E, et al. *In vivo* toxicity, biodistribution, and clearance of glutathione-coated gold nanoparticles. Nanomedicine: Nanotechnology Biology and Medicine, 2013, 9(2): 257–263

[59] Choi M K, Yang J, Hyeon T, et al. Flexible quantum dot light-emitting diodes for next-generation displays. NPJ Flexible Electronics, 2018, 2(1): 10

[60] Shirasaki Y, Supran G J, Bawendi M G, et al. Emergence of colloidal quantum-dot light-emitting technologies. Nature Photonics, 2013, 7(1): 13–23

[61] Ryman-Rasmussen J P, Riviere J E, Monteiro-Riviere N A. Penetration of intact skin by quantum dots with diverse physicochemical properties. Toxicological Sciences, 2006, 91(1): 159–165

[62] Hoshino A, Fujioka K, Oku T, et al. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. Nano Letters, 2004, 4(11): 2163–2169

[63] Erol O, Uyan I, Hatip M, et al. Recent advances in bioactive 1D and 2D carbon nanomaterials for biomedical applications. Nanomedicine: Nanotechnology Biology and Medicine, 2018, 14(7): 2433–2454

[64] Gupta T K, Budarapu P R, Chappidi S R, et al. Advances in carbon based nanomaterials for bio-medical applications. Current Medicinal Chemistry, 2019, 26(38): 6851–6877

[65] Gupta T K, Singh B P, Teotia S, et al. Designing of multiwalled carbon nanotubes reinforced polyurethane composites as electromagnetic interference shielding materials. Journal of Polymer Research, 2013, 20(6): 169

[66] Morimoto Y, Horie M, Kobayashi N, et al. Inhalation toxicity assessment of carbon-based nanoparticles. Accounts of Chemical Research, 2013, 46(3): 770–781

[67] Fujita K, Fukuda M, Endoh S, et al. Size effects of single-walled carbon nanotubes on *in vivo* and *in vitro* pulmonary toxicity. Inhalation Toxicology, 2015, 27(4): 207–223

[68] Frank E A, Carreira V S, Birch M E, et al. Carbon nanotube and asbestos exposures induce overlapping but distinct profiles of lung pathology in non-Swiss albino CF-1 mice. Toxicologic Pathology, 2016, 44(2): 211–225

[69] Francis A P D T, Devasena T. Toxicity of carbon nanotubes: A review. Toxicology and Industrial Health, 2018, 34(3): 200–210

[70] Lin B, Zhang H, Lin Z, et al. Studies of single-walled carbon nanotubes-induced hepatotoxicity by NMR-based metabonomics of rat blood plasma and liver extracts. Nanoscale Research Letters, 2013, 8: 236

[71] Zheng W, McKinney W, Kashon M, et al. The influence of inhaled multi-walled carbon nanotubes on the autonomic nervous system. Particle and Fibre Toxicology, 2016, 13(1): 8

[72] Akhavan O, Ghaderi E, Emamy H, et al. Genotoxicity of graphene nanoribbons in human mesenchymal stem cells. Carbon, 2013, 54: 419–431

[73] Wang A, Pu K, Dong B, et al. Role of surface charge and oxidative stress in cytotoxicity and genotoxicity of graphene oxide towards human lung fibroblast cells. Journal of Applied Toxicology, 2013, 33(10): 1156–1164

[74] Aschberger K, Johnston H J, Stone V, et al. Review of fullerene toxicity and exposure — Appraisal of a human health risk assessment, based on open literature. Regulatory Toxicology and Pharmacology, 2010, 58(3): 455–473

[75] Murugadoss S, Lison D, Godderis L, et al. Toxicology of silica nanoparticles: An update. Archives of Toxicology, 2017, 91(9): 2967–3010

[76] Ahamed M, Karns M, Goodson M, et al. DNA damage response to different surface chemistry of silver nanoparticles in

mammalian cells. Toxicology and Applied Pharmacology, 2008, 233(3): 404–410

[77] Parasuraman S. Toxicological screening. Journal of Pharmacology & Pharmacotherapeutics, 2011, 2(2): 74–79

[78] Marano F, Rodrigues-Lima F, Dupret J M, et al. Cellular mechanisms of nanoparticle toxicity. In: Bhushan B, ed. Encyclopedia of Nanotechnology. Dordrecht: Springer Netherlands, 2016, 498–505

[79] Teh C H, Nazni W A, Nurulhusna A H, et al. Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly via resazurin-based turbidometric assay. BMC Microbiology, 2017, 17(1): 36

[80] Soboleski M R, Oaks J, Halford W P. Green fluorescent protein is a quantitative reporter of gene expression in individual eukaryotic cells. The FASEB Journal, 2005, 19: 440–442

[81] Balouiri M, Sadiki M, Ibnsouda S K. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 2016, 6(2): 71–79

[82] Strober W. Trypan blue exclusion test of cell viability. Current Protocols in Immunology, 2015, 111: A3.B.1–A3.B.3

[83] Vinken M, Rogiers V, eds. Protocols in *In Vitro* Hepatocyte Research. Humana Press, 2015

[84] Zhao M, Li D, Yuan L, et al. Differences in cytocompatibility and hemocompatibility between carbon nanotubes and nitrogen-doped carbon nanotubes. Carbon, 2011, 49(9): 3125–3133

[85] Kim H R, Park Y J, Shin D Y, et al. Appropriate *in vitro* methods for genotoxicity testing of silver nanoparticles. Environmental Health and Toxicology, 2013, 28: e2013003

[86] Gurunathan S, Han J W, Eppakayala V, et al. Cytotoxicity of biologically synthesized silver nanoparticles in MDA-MB-231 human breast cancer cells. BioMed Research International, 2013, 2013: 535796

[87] Kumar V, Sharma N, Maitra S S. *In vitro* and *in vivo* toxicity assessment of nanoparticles. International Nano Letters, 2017, 7(4): 243–256

[88] Saadat Y R, Saeidi N, Vahed S Z, et al. An update to DNA ladder assay for apoptosis detection. BioImpacts, 2015, 5(1): 25–28

[89] Chang H Y, Huang H C, Huang T C, et al. Flow cytometric detection of reactive oxygen species. Bio-Protocol, 2013, 3(8): e431

[90] Bin-Jumah M N, Al-Abdan M, Al-Basher G, et al. Molecular mechanism of cytotoxicity, genotoxicity, and anticancer potential of green gold nanoparticles on human liver normal and cancerous cells. Dose-Response, 2020, 18(2): 1559325820912154

[91] Alsaedi I I J, Taqi Z J, Hussien A M A, et al. Graphene nanoparticles induces apoptosis in MCF-7 cells through mitochondrial damage and NF-KB pathway. Materials Research Express, 2019, 6(9): 095413

[92] Asare N, Duale N, Slagsvold H H, et al. Genotoxicity and gene expression modulation of silver and titanium dioxide nanoparticles in mice. Nanotoxicology, 2016, 10(3): 312–321

[93] Kasibhatla S, Amarante-Mendes G P, Finucane D, et al. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. Cold Spring Harbor Protocols, 2006, 2006(3): pdb. prot4493

[94] Bucevičius J, Lukinavičius G, Gerasimaitė R. The use of hoechst dyes for DNA staining and beyond. Chemosensors, 2018, 6(2): 18

[95] Wick P, Chortarea S, Guenat O T, et al. *In vitro–ex vivo* model systems for nanosafety assessment. European Journal of Nanomedicine, 2015, 7(3): 169–179

[96] Mahmood S, Mandal U K, Chatterjee B, et al. Advanced characterizations of nanoparticles for drug delivery: Investigating their properties through the techniques used in their evaluations. Nanotechnology Reviews, 2017, 6(4): 355–372

[97] Tardiff R G. *In vitro* methods of toxicity evaluation. Annual Review of Pharmacology and Toxicology, 1978, 18(1): 357–369

[98] Hillegass J M, Shukla A, Lathrop S A, et al. Assessing nanotoxicity in cells *in vitro*. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2010, 2(3): 219–231

[99] Astashkina A, Grainger D W. Critical analysis of 3-D organoid *in vitro* cell culture models for high-throughput drug candidate toxicity assessments. Advanced Drug Delivery Reviews, 2014, 69–70: 1–18

[100] Ni M, Xiong M, Zhang X, et al. Poly(lactic-co-glycolic acid) nanoparticles conjugated with CD133 aptamers for targeted salinomycin delivery to $CD133^+$ osteosarcoma cancer stem cells. International Journal of Nanomedicine, 2015, 10: 2537–2554

[101] Li G Y, Osborne N N. Oxidative-induced apoptosis to an immortalized ganglion cell line is caspase independent but involves the activation of poly(ADP-ribose) polymerase and apoptosis-inducing factor. Brain Research, 2008, 1188: 35–43

[102] Ryter S W, Kim H P, Hoetzel A, et al. Mechanisms of cell death in oxidative stress. Antioxidants & Redox Signaling, 2007, 9(1): 49–89

[103] Lu X, Qian J, Zhou H, et al. *In vitro* cytotoxicity and induction of apoptosis by silica nanoparticles in human HepG2 hepatoma cells. International Journal of Nanomedicine, 2011, 6: 1889–1901

[104] Browne S M, Al-Rubeai M. Defining viability in mammalian cell cultures. Biotechnology Letters, 2011, 33(9): 1745–1749

[105] Kumar V, Sharma N, Maitra S S. *In vitro* and *in vivo* toxicity assessment of nanoparticles. International Nano Letters, 2017, 7(4): 243–256

[106] Borenfreund E, Shopsis C. Toxicity monitored with a correlated set of cell-culture assays. Xenobiotica, 1985, 15(8–9): 705–711

[107] Magder S. Reactive oxygen species: Toxic molecules or spark of

life? Critical Care, 2006, 10(1): 208

[108] Gomes A, Fernandes E, Lima J L F C. Fluorescence probes used for detection of reactive oxygen species. Journal of Biochemical and Biophysical Methods, 2005, 65(2–3): 45–80

[109] Wagner A J, Bleckmann C A, Murdock R C, et al. Cellular interaction of different forms of aluminum nanoparticles in rat alveolar macrophages. The Journal of Physical Chemistry B, 2007, 111(25): 7353–7359

[110] Fantel A G. Reactive oxygen species in developmental toxicity: Review and hypothesis. Teratology, 1996, 53(3): 196–217

[111] Hussain S M, Javorina A K, Schrand A M, et al. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. Toxicological Sciences, 2006, 92(2): 456–463

[112] Sohn E K , Chung Y S, Johari S A, et al. Acute toxicity comparison of single-walled carbon nanotubes in various freshwater organisms. Biomed Research International, 2015, 2015: 323090

[113] Delcroix G J R, Jacquart M, Lemaire L, et al. Mesenchymal and neural stem cells labeled with HEDP-coated SPIO nanoparticles: *In vitro* characterization and migration potential in rat brain. Brain Research, 2009, 1255: 18–31

[114] Bahadar H, Maqbool F, Niaz K, et al. Toxicity of nanoparticles and an overview of current experimental models. Iranian Biomedical Journal, 2016, 20(1–3): 1–11

[115] Davoren M, Herzog E, Casey A, et al. *In vitro* toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. Toxicology *in Vitro*, 2007, 21(3): 438–448

[116] Choi S J, Oh J M, Choy J H. Toxicological effects of inorganic nanoparticles on human lung cancer A549 cells. Journal of Inorganic Biochemistry, 2009, 103(3): 463–471

[117] Herzog E, Byrne H J, Casey A, et al. SWCNT suppress inflammatory mediator responses in human lung epithelium *in vitro.* Toxicology and Applied Pharmacology, 2009, 234(3): 378–390

[118] AshaRani P V, Mun G L K, Hande M P, et al. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano, 2009, 3(2): 279–290

[119] Reddy A R N, Reddy Y N, Krishna D R, et al. Multi wall carbon nanotubes induce oxidative stress and cytotoxicity in human embryonic kidney (HEK293) cells. Toxicology, 2010, 272(1–3): 11–16

[120] Walker V G, Li Z, Hulderman T, et al. Potential *in vitro* effects of carbon nanotubes on human aortic endothelial cells. Toxicology and Applied Pharmacology, 2009, 236(3): 319–328

[121] Shvedova A A, Castranova V, Kisin E R, et al. Exposure to carbon nanotube material: Assessment of nanotube cytotoxicity using human keratinocyte cells. Journal of Toxicology and Environmental Health Part A, 2003, 66(20): 1909–1926

[122] Cherukuri P, Bachilo S M, Litovsky S H, et al. Near-infrared fluorescence microscopy of single-walled carbon nanotubes in phagocytic cells. Journal of the American Chemical Society, 2004, 126(48): 15638–15639

[123] Pulskamp K, Diabaté S, Krug H F. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicology Letters, 2007, 168(1): 58–74

[124] Hu F, Neoh K G, Cen L, et al. Cellular response to magnetic nanoparticles “PEGylated” via surface-initiated atom transfer radical polymerization. Biomacromolecules, 2006, 7(3): 809–816

[125] Liu Y, Chen W, Joly A G, et al. Comparison of water-soluble CdTe nanoparticles synthesized in air and in nitrogen. The Journal of Physical Chemistry B, 2006, 110(34): 16992–17000

[126] Bueno J. Chapter 6 - Nanotoxicity: The impact of increasing drug bioavailability. In: Shegokar R, ed. Nanopharmaceuticals. Elsevier, 2020, 121–133

[127] Vazquez-Muñoz R, Borrego B, Juárez-Moreno K, et al. Toxicity of silver nanoparticles in biological systems: Does the complexity of biological systems matter? Toxicology Letters, 2017, 276: 11–20

[128] Mao B H, Chen Z Y, Wang Y J, et al. Silver nanoparticles have lethal and sublethal adverse effects on development and longevity by inducing ROS-mediated stress responses. Scientific Reports, 2018, 8: 2445

[129] Li H, Huang T, Wang Y, et al. Toxicity of alumina nanoparticles in the immune system of mice. Nanomedicine, 2020, 15(9): 927–946

[130] Sadiq I M, Pakrashi S, Chandrasekaran N, et al. Studies on toxicity of aluminum oxide (Al_2O_3) nanoparticles to microalgae species: *Scenedesmus* sp. and *Chlorella* sp. Journal of Nanoparticle Research, 2011, 13(8): 3287–3299

[131] Morsy G M, Abou El-Ala K S, Ali A A. Studies on fate and toxicity of nanoalumina in male albino rats: Lethality, bioaccumulation and genotoxicity. Toxicology and Industrial Health, 2016, 32(2): 344–359

[132] Lee I C, Ko J W, Park S H, et al. Comparative toxicity and biodistribution of copper nanoparticles and cupric ions in rats. International Journal of Nanomedicine, 2016, 11: 2883–2900

[133] Wang T, Long X, Cheng Y, et al. The potential toxicity of copper nanoparticles and copper sulphate on juvenile *Epinephelus coioides*. Aquatic Toxicology, 2014, 152: 96–104

[134] Shi H, Magaye R, Castranova V, et al. Titanium dioxide nanoparticles: A review of current toxicological data. Particle and Fibre Toxicology, 2013, 10(1): 15

[135] Uekawa N, Endo N, Ishii K, et al. Characterization of titanium oxide nanoparticles obtained by hydrolysis reaction of ethylene

glycol solution of alkoxide. Journal of Nanotechnology, 2012, 102361 (8 pages)

[136] Lv K, Yu J, Cui L, et al. Preparation of thermally stable anatase TiO_2 photocatalyst from $TiOF_2$ precursor and its photocatalytic activity. Journal of Alloys and Compounds, 2011, 509(13): 4557–4562

[137] Siddiqi K S, Rahman A U, Tajuddin, et al. Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Research Letters, 2018, 13: 141

[138] Sahu D, Kannan G M, Vijayaraghavan R, et al. Nanosized zinc oxide induces toxicity in human lung cells. ISRN Toxicology, 2013, 2013: 316075

[139] Mishra P K, Mishra H, Ekielski A, et al. Zinc oxide nanoparticles: A promising nanomaterial for biomedical applications. Drug Discovery Today, 2017, 22(12): 1825–1834

[140] Ali A, Zafar H, Zia M, et al. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnology, Science and Applications, 2016, 9: 49–67

[141] Cotin G, Piant S, Mertz D, et al. Chapter 2 - Iron oxide nanoparticles for biomedical applications: Synthesis, functionalization, and application. In: Mahmoudi M, Laurent S, eds. Iron Oxide Nanoparticles for Biomedical Applications. Elsevier, 2018, 43–88

[142] Tiwari V, Mishra N, Gadani K, et al. Mechanism of anti-bacterial activity of zinc oxide nanoparticle against carbapenem-resistant *Acinetobacter baumannii.* Frontiers in Microbiology, 2018, 9: 1218

[143] Feng Q, Liu Y, Huang J, et al. Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. Scientific Reports, 2018, 8: 2082

[144] Bladh K, Falk L K L, Rohmund F. On the iron-catalysed growth of single-walled carbon nanotubes and encapsulated metal particles in the gas phase. Applied Physics A: Materials Science & Processing, 2000, 70(3): 317–322

[145] Gupta T K, Budarapu P R, Chappidi S R, et al. Advances in carbon based nanomaterials for bio-medical applications. Current Medicinal Chemistry, 2019, 26(38): 6851–6877

[146] Kokorina A A, Ermakov A V, Abramova A M, et al. Carbon nanoparticles and materials on their basis. Colloids and Interfaces, 2020, 4(4): 42

[147] Maiti D, Tong X, Mou X, et al. Carbon-based nanomaterials for biomedical applications: A recent study. Frontiers in Pharmacology, 2019, 9: 1401

[148] Mohanta D, Patnaik S, Sood S, et al. Carbon nanotubes: Evaluation of toxicity at biointerfaces. Journal of Pharmaceutical Analysis, 2019, 9(5): 293–300

[149] Wan B, Wang Z X, Lv Q Y, et al. Single-walled carbon nanotubes and graphene oxides induce autophagosome accumulation and lysosome impairment in primarily cultured murine peritoneal macrophages. Toxicology Letters, 2013, 221(2): 118–127

[150] Dong P X, Wan B, Wang Z X, et al. Exposure of single-walled carbon nanotubes impairs the functions of primarily cultured murine peritoneal macrophages. Nanotoxicology, 2013, 7(5): 1028–1042

[151] Palomäki J, Karisola P, Pylkkänen L, et al. Engineered nanomaterials cause cytotoxicity and activation on mouse antigen presenting cells. Toxicology, 2010, 267(1–3): 125–131

[152] Jia G, Wang H, Yan L, et al. Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. Environmental Science & Technology, 2005, 39(5): 1378–1383

[153] Oberdörster C, Maynard A, Donaldson K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Particle and Fibre Toxicology, 2005, 2(1): 8

[154] Bergin I L, Witzmann F A. Nanoparticle toxicity by the gastrointestinal route: Evidence and knowledge gaps. International Journal of Biomedical Nanoscience and Nanotechnology, 2013, 3(1–2): 163–210

[155] Brouwer D H, Gijsbers J H J, Lurvink M W M. Personal exposure to ultrafine particles in the workplace: Exploring sampling techniques and strategies. The Annals of Occupational Hygiene, 2004, 48(5): 439–453

[156] Bennat C, Müller-Goymann C C. Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. International Journal of Cosmetic Science, 2000, 22(4): 271–283

[157] De Jong W H, Borm P J A. Drug delivery and nanoparticles: Applications and hazards. International Journal of Nanomedicine, 2008, 3(2): 133–149

[158] Grabrucker A M, Garner C C, Boeckers T M, et al. Development of novel Zn^{2+} loaded nanoparticles designed for cell-type targeted drug release in CNS neurons: *In vitro* evidences. PLoS One, 2011, 6(3): e17851

[159] Salatin S, Khosroushahi A Y. Overviews on the cellular uptake mechanism of polysaccharide colloidal nanoparticles. Journal of Cellular and Molecular Medicine, 2017, 21(9): 1668–1686

[160] Li H, Tsui T Y, Ma W. Intracellular delivery of molecular cargo using cell-penetrating peptides and the combination strategies. International Journal of Molecular Sciences, 2015, 16(8): 19518–19536

[161] Zhang J M, An J. Cytokines, inflammation, and pain. International Anesthesiology Clinics, 2007, 45(2): 27–37

[162] Thannickal V J, Fanburg B L. Reactive oxygen species in cell signaling. American Journal of Physiology: Lung Cellular and

Molecular Physiology, 2000, 279(6): L1005–L1028

[163] Manke A, Wang L Y, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Research International, 2013, 2013: 942916

[164] Das T K, Wati M R, Fatima-Shad K. Oxidative stress gated by Fenton and Haber Weiss reactions and its association with Alzheimer's disease. Archives of Neuroscience, 2015, 2(2): e20078

[165] Ahamed M, Akhtar M J, Alhadlaq H A, et al. Assessment of the lung toxicity of copper oxide nanoparticles: Current status. Nanomedicine, 2015, 10(15): 2365–2377

[166] Foldbjerg R, Dang D A, Autrup H. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Archives of Toxicology, 2011, 85(7): 743–750

[167] Jugan M L B S, Barillet S, Simon-Deckers A, et al. Cytotoxic and genotoxic impact of TiO_2 nanoparticles on A549 cells. Journal of Biomedical Nanotechnology, 2011, 7(1): 22–23

[168] Kansara K, Patel P, Shah D, et al. TiO_2 nanoparticles induce cytotoxicity and genotoxicity in human alveolar cells. Molecular Cytogenetics, 2014, 7(1): P77

[169] Kreyling W G, Semmler-Behnke M, Seitz J, et al. Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. Inhalation Toxicology, 2009, 21(Sup1): 55–60

[170] Poh T Y, Ali N A B M, Mac Aogáin M, et al. Inhaled nanomaterials and the respiratory microbiome: Clinical, immunological and toxicological perspectives. Particle and Fibre Toxicology, 2018, 15: 46

[171] Kuhlbusch T A J, Asbach C, Fissan H, et al. Nanoparticle exposure at nanotechnology workplaces: A review. Particle and Fibre Toxicology, 2011, 8(1): 22

[172] Kessler R. Engineered nanoparticles in consumer products: Understanding a new ingredient. Environmental Health Perspectives, 2011, 119(3): a120–a125

[173] Bundschuh M, Filser J, Lüderwald S, et al. Nanoparticles in the environment: Where do we come from, where do we go to? Environmental Sciences Europe, 2018, 30(1): 6

[174] Martínez G, Merinero M, Pérez-Aranda M, et al. Environmental impact of nanoparticles' application as an emerging technology: A review. Materials, 2021, 14(1): 166

[175] Dusinska M, Tulinska J, El Yamani N, et al. Immunotoxicity, genotoxicity and epigenetic toxicity of nanomaterials: New strategies for toxicity testing? Food and Chemical Toxicology, 2017, 109(Pt 1): 797–811

[176] Bhattacharya K, Andón F T, El-Sayed R, et al. Mechanisms of carbon nanotube-induced toxicity: Focus on pulmonary inflammation. Advanced Drug Delivery Reviews, 2013, 65(15): 2087–2097

[177] Sohaebuddin S K, Thevenot P T, Baker D, et al. Nanomaterial cytotoxicity is composition, size, and cell type dependent. Particle and Fibre Toxicology, 2010, 7(1): 22

[178] Sahu S C, Zheng J, Graham L, et al. Comparative cytotoxicity of nanosilver in human liver HepG2 and colon Caco2 cells in culture. Journal of Applied Toxicology, 2014, 34(11): 1155–1166

[179] Hunt P R, Marquis B J, Tyner K M, et al. Nanosilver suppresses growth and induces oxidative damage to DNA in *Caenorhabditis elegans*. Journal of Applied Toxicology, 2013, 33(10): 1131–1142

[180] Uboldi C, Urbán P, Gilliland D, et al. Role of the crystalline form of titanium dioxide nanoparticles: Rutile, and not anatase, induces toxic effects in Balb/3T3 mouse fibroblasts. Toxicology *in Vitro*, 2016, 31: 137–145

[181] Bostan H B, Rezaee R, Valokala M G, et al. Cardiotoxicity of nano-particles. Life Sciences, 2016, 165: 91–99

[182] Du Z, Zhao D, Jing L, et al. Cardiovascular toxicity of different sizes amorphous silica nanoparticles in rats after intratracheal instillation. Cardiovascular Toxicology, 2013, 13(3): 194–207

[183] Yang C, Tian A, Li Z. Reversible cardiac hypertrophy induced by PEG-coated gold nanoparticles in mice. Scientific Reports, 2016, 6(1): 20203

[184] Rickerby D G, Morrison M. Nanotechnology and the environment: A European perspective. Science and Technology of Advanced Materials, 2007, 8(1–2): 19–24

[185] Kahru A, Dubourguier H C. From ecotoxicology to nanoecotoxicology. Toxicology, 2010, 269(2–3): 105–119

[186] Lv M, Huang W, Chen Z, et al. Metabolomics techniques for nanotoxicity investigations. Bioanalysis, 2015, 7(12): 1527–1544

[187] Yan A, Chen Z. Impacts of silver nanoparticles on plants: A focus on the phytotoxicity and underlying mechanism. International Journal of Molecular Sciences, 2019, 20(5): 1003

[188] Siddiqui M H, Al-Whaibi M H, Faisal M, et al. Nano-silicon dioxide mitigates the adverse effects of salt stress on *Cucurbita pepo* L. Environmental Toxicology and Chemistry, 2014, 33(11): 2429–2437

[189] Song U, Jun H, Waldman B, et al. Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicology and Environmental Safety, 2013, 93: 60–67

[190] Bouguerra S, Gavina A, Ksibi M, et al. Ecotoxicity of titanium silicon oxide ($TiSiO_4$) nanomaterial for terrestrial plants and soil invertebrate species. Ecotoxicology and Environmental Safety, 2016, 129: 291–301

[191] Kumari M, Khan S S, Pakrashi S, et al. Cytogenetic and

genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*. Journal of Hazardous Materials, 2011, 190(1–3): 613–621

[192] Wang Y, Hu J, Dai Z, et al. *In vitro* assessment of physiological changes of watermelon (*Citrullus lanatus*) upon iron oxide nanoparticles exposure. Plant Physiology and Biochemistry, 2016, 108: 353–360

[193] Exbrayat J M, Moudilou E N, Lapied E. Harmful effects of nanoparticles on animals. Journal of Nanotechnology, 2015, 2015: 861092

[194] Petersen E J, Huang Q, Weber W J. Bioaccumulation of radio-labeled carbon nanotubes by *Eisenia foetida*. Environmental Science & Technology, 2008, 42(8): 3090–3095

[195] Handy R D, Henry T B, Scown T M, et al. Manufactured nanoparticles: Their uptake and effects on fish — A mechanistic analysis. Ecotoxicology, 2008, 17(5): 396–409

[196] Krishnaraj C, Harper S L, Yun S I. *In vivo* toxicological assessment of biologically synthesized silver nanoparticles in adult zebrafish (*Danio rerio*). Journal of Hazardous Materials, 2016, 301: 480–491

[197] Myrzakhanova M, Gambardella C, Falugi C, et al. Effects of nanosilver exposure on cholinesterase activities, CD41, and CDF/LIF-like expression in zebrafish (*Danio rerio*) larvae. BioMed Research International, 2013, 2013: 205183