# Evaluation of Toxicity of Nanoparticles Using Cell Lines

15

Sudhakar Pola and Anusha Konatala

**Abstract**

Nanoparticles range from 1 to 100 nm in size, and though the size is in nanometers, its application is in broad areas such as biomedical, industry, food, and cosmetics. With increasing utilization, the toxicity of the nanoparticle has been a great concern to evaluate their potential. To use the nanoparticle effectively, it is necessary to know the toxicity of NP and different evaluation methods and characteristics. "Dosimetry: Too complicated to consider, too important to ignore" as stated by Dr. Philip Demokritou in the seventh International Nanotoxicology Congress; Dosimetry is one of the important factors besides the surface area and high reactivity to determine the toxicity nature of the NP. Every NP may not show the same toxicity; it varies with the material it is made up of, site of its action, and exposure routes. This chapter addresses the current knowledge of evaluation of nanosized particles toxicity using in vitro derived cell lines from different literature, as a primary step for screening their toxicological effects, which contributes to the further development and advancement of nanotechnology on a safe, unbiased level. The in vitro derived cell lines however does not ensure the same cell habitat as in the tissue, as nanoparticles interact with proteins and physiological barriers, immune response in the tissue has a more complex environment. Hence, these in vitro evaluation methods give us a base for further considering the nanoparticle potential and its toxicity.

**Keywords**

Toxicological effects · Biological systems · Reactive oxygen species · Genotoxicity · *In vitro* assays

S. Pola (✉)
Department of Biotechnology, Andhra University, Visakhapatnam, Andhra Pradesh, India
e-mail: sudhakar@andhrauniversity.edu.in

A. Konatala
University of Windsor, Windsor, ON, Canada



D. B. Siddhardha et al. (eds.), *Model Organisms to Study Biological Activities and Toxicity of Nanoparticles*, https://doi.org/10.1007/978-981-15-1702-0_15

## 15.1 Introduction

A promising research interest in the delivery of biomolecules using particular delivery systems, which act as carriers for small and large molecules, especially for drugs is being carried on from the past few years. These delivery systems are not only found to be efficient for transporting drug molecules but also help in improving the pharmacokinetic and pharmacodynamic properties (Mohanraj and Chen 2006). Drug delivery is a procedure of administering a pharmaceutical compound to acquire a therapeutic effect in humans. The development of drugs is expensive, time consuming, and labor intensive (Tiwari et al. 2012). Various drug administration methods are introduced, likely with targeted, controlled, sustained delivery of pharmaceutical products (Tiwari et al. 2012). Among the different delivery systems, nanoparticles have played a promising role in accomplishing the need in every field of science. For the past 50 years, nanotechnology is into commerce, and many new directions are in progress. Within the past 15 years, nanoparticles revolutionized different delivery mechanisms. The first report of adverse effects of nanoparticles has been published within the last 10 years from in vivo and in vitro studies (Takenaka et al. 2001; Oberdörster et al. 2004; Bermudez et al. 2004; Lam et al. 2004; Geiser et al. 2005; Oberdörster et al. 2005a, b; Shvedova et al. 2005; Elder et al. 2006; Mercer et al. 2008). A knowledge gap between the technological progress and potential hazards of new developing nanotechnology creates a mystifying experience (Schulte et al. 2008). To resolve this, one needs to evaluate the toxicity of nanoparticle through different in vitro methods, and they are discussed in this chapter for a proper assessment of nanoparticle safety.

## 15.2 Nanoparticles

Nanoparticles are particulate dispersions or solid particles with a size range of 10–100 nm (Jeevanandam et al. 2018). The chemical synthesis of metallic nanoparticles dates back from fourteenth and thirteenth century BC, where the Mesopotamians and Egyptians started making glass using metals—beginning of nanoparticle metallic era (Schaming and Remita 2015). The Lycurgus cup is a fourth-century Roman glass cup made up of dichroic glass which displays various colors: green when light is passing from the front of the cup and red when passing from behind (Leonhardt 2007). These cups contain silver–gold alloy nanoparticles with a 7:3 ratio in addition to 10% of Cu (Jeevanandam et al. 2018). Clay minerals of few nanometers in thickness are best examples of natural nanomaterial usage since ancient times (Rytwo 2008). Michael Faraday reported the colloidal AuNP synthesis in 1857, which is known as the first scientific description report of nanoparticle preparation in the scientific arena (Jeevanandam et al. 2018).

An ancient history of nanoparticle and its benefits represents an active research area and a technoeconomic sector with full expansion in many domain applications. The Standard British Institution (Jeevanandam et al. 2018) has given the following definitions for scientific terms that have been used:

- Nanoscale: Measurement of approximately 1–1000 nm in size.
- Nanotechnology: Manipulation and control of matter on a nanoscale dimension by using scientific knowledge of various industrial and biomedical application.
- Nanomaterial: Material with any external or internal structures on nanoscale dimension.
- Nanoparticle: A nano-object with three external nanoscale dimensions. The terms nanorod or nanoplate are employed when the NP of the longest and shortest axes length of a nano-object is different.

The following nanoparticle types are obtained by a different method of preparation:

- Nanocapsules: A system in which drug is confined to the cavity, covered by a unique polymer membrane
- Nanospheres: A matrix system in which drug is physically and uniformly dispersed
- Nanoparticle: A system that is coated with a hydrophilic polymer-like PEG (polyethylene glycol)

Liposomes, being potential carriers with inherent problems such as low encapsulation efficiency, poor storage stability, and leakage of water-soluble drugs in the human environment led to a scope for designing nanoparticles as delivery system over liposomes. NPs are found to be the best fit in drug delivery systems with more flexible characteristics (Mohanraj and Chen 2006). Characteristics of nanoparticles in drug delivery systems include:

- Particle size and surface characteristics of nanoparticle can be easily manipulated.
- They control and sustain the release of drug at the site of localization, altering organ distribution of drug and subsequent clearance of drug to achieve high therapeutic efficacy and reduce the side effects.
- Site-specific targeting by attaching a ligand to the surface of particles or use of magnetic guidance.

Nanoparticles have gained prominence in technological advancements due to their physiochemical characteristics such as melting point, thermal conductivity, light absorption, catalytic activity, and scattering of light which assist in improving the performance of bulk counterparts (Jeevanandam et al. 2018).

Nanoparticles are prepared from a variety of materials such as protein, polysaccharides, and synthetic polymers. Nanoparticle has been prepared mostly by three common methods (Mohanraj and Chen 2006):

- Dispersion of preformed polymer
- Polymerization of monomer
- Ionic gelation or coacervation of hydrophilic polymer

Other methods such as supercritical fluid technology (Reverchon and Adami 2006) and PRINT

(particle replication in no wetting templates) are also used for nanoparticle preparation (Khan et al. 2017).

- Top-down synthesis is a destructive approach in which large molecules are broken down into smaller units and converted into nanoparticles.
- Bottom-up synthesis is a constructive approach in which the nanoparticles are obtained from smaller molecules.

### 15.2.1 Types of Nanoparticles

A nanoparticle is generally classified based on their material, size, morphology, and physicochemical properties. Based on their physicochemical properties, nanoparticles are classified as (Khan et al. 2017; Jeevanandam et al. 2018):

- **Carbon-based nanoparticle:** NPs made up of carbon, as hollow tubes, ellipsoids, or spheres such as fullerenes (C60) carbon nanotubes, carbon nanofibers, carbon black, graphene, and carbon onions. Laser ablation and chemical vapor deposition (CVD) are some of the important production methods for carbon-based nanoparticles.
- **Inorganic-based nanoparticle:** NPs are made up of metal and metal oxides. These NPs may be synthesized from different metals such as Au or Ag nanoparticles, metal oxides such as $TiO_2$ ZnO, and semiconductors such as silicon and ceramics. They have a unique optoelectrical property. The size, shape, and facet-controlled synthesis of metal nanoparticle are critical in the current cutting-edge material (Dreaden et al. 2012).
- **Organic-based nanoparticle:** Nanoparticles are made up of organic matter, excluding inorganic or carbon-based material. The noncovalent interaction for self-assembly transforms organic nanoparticles into structures such as dendrimers, liposomes, micelles, and polymer nanoparticles. Lipid-based nanoparticles are 10–1000 nm of diameter in range. Surfactants and emulsifier stabilized the external core of nanoparticles.
- **Composite-based nanoparticle:** Nanoparticle with multiphase, with one phase on nanoscale dimension that may combine with other NPs or with large-type materials such as hybrid nanofibers.

Based on the origin, the nanoparticles are classified (Jeevanandam et al. 2018) into:

- **Natural nanoparticle:** They are produced in nature by biological species or anthropogenic activities. These nanoparticles are naturally present throughout the earth's sphere (hydrosphere, atmosphere, lithosphere, and biosphere).
- **Synthetic (engineered) nanoparticle:** They are produced by engine exhaust and smoke synthesized by physical and chemical, biological, or hybrid methods. Various risk assessment strategies are highly helpful in forecasting the behavior of synthetic nanoparticles in various environmental media. New schemes have focused on synthesizing other semiconductors (SCs) to avoid toxic ion-generating elements such as Se, Cd, and As and also to avoid the low availability elements (e.g., Te, Ga, and In) (Thomas et al. 2011).

### 15.2.2 Applications of Nanoparticles

Nanoparticles are effectively utilized in multiple domains. The key properties of nanoparticles designated them as a vital delivery system, in medications, where a broad scope of research on mechanism of its action is necessary. The following are some of the important applications in various fields:

- **Drugs and medication.** In field of medicine, a high interest in nanoparticles to deliver drugs in low dosage, high therapeutic effects, and negligible side effects and to improve patient compliance (Alexis et al. 2008). Superparamagnetic iron oxide nanoparticles with surface chemistry was used for in vivo applications as MRI contrast enhancement, tissue repair, cell separation, and for many more applications (Khan et al. 2017).
- **Materials and manufacturing.** In material science, nanocrystalline acts as a good substance, as their properties deviate in a size-dependent manner. Resonance energy transfer (RET) consists of noble metal nanoparticles and organic dye molecules and is important in material science and biophotonics.
- **Environment.** Nanoparticles have increased their scope in environment protection owing to their its eco-friendly characteristics. They are widely used in sensors for environment prediction, remediation of materials, contaminated with hazardous substances, and photodegradation (Khan et al. 2017). Nanoparticles are involved in degradation process in fluorescence and optical fields (Rogozea et al. 2016; Olteanu et al. 2016a, b).
- **Energy harvesting and mechanical industries.** The nonrenewable resources such as fossil fuels, a typical issue is in the synthesis and storage of energy; using nanoparticles to generate energy is widely utilized in photoelectrochemical (PEC) and electrochemical water splitting method (Avasare et al. 2015; Ning et al. 2016). In energy storage, different applications to reserve energy in nanoscale as nanogenerators are available (Greeley and Markovic 2012; Liu et al. 2015). In mechanical industries, nanoparticles are involved in tribological

properties of materials, to enhance the mechanical strength of polymer matrix and metals. Nanoparticles are also involved in the lubrication, coating, and resistance of metals (Khan et al. 2017).

### 15.2.3 Toxicity of Nanoparticles

Besides tons of nanoparticles enter the environment, and very little is known about its possible interactions with biological systems, nanotoxicology has emerged as a new discipline to investigate the potential adverse effects of nanoparticles (Bakand et al. 2012). Besides many applications in different domains, several kinds of toxicities are associated with NPs (Khan et al. 2017; Khlebtsov and Dykman 2010, 2011). Different types of nanoparticles are available in the bioapplications with early acceptance and rapid progress of nanobiotechnology. Even then, severe health effects that occurred due to prolonged exposure of humans to nanoparticles have not been established yet (Khan et al. 2017). The environment exposure of nanoparticles seems to increase in the future and thus the toxicity. The state of nanoparticle dispersion will alter the ecotoxicity and many factors of abiotic influence such as salinity, presence of organic matters, and pH (Handy et al. 2008). Health hazards of nanoparticles are always a concern due to their extended use and discharges to the natural environment in order to make it more environment friendly and more reliable (Khan et al. 2017). It is necessary to gain basic knowledge about nanotoxicology to overcome their toxicity efficiently. The toxicity of nanoparticles is majorly associated with their physiochemical properties, affecting their behavior in biological systems (Seaton 2006).

Existing and possible toxicities of nanoparticles are associated with their different characteristics such as small size distribution, large surface area, surface characteristics, insolubility, and aggregation. Modification in these characteristics would allow a convenient, efficient, and safe method to employ in major domain applications.

Nanoparticle properties are unique characteristics which are related to their synthesis (Jeevanandam et al. 2016).

Different data from the literature for the toxicological studies reveal that nanomaterial toxicity depends on various other factors, namely:

- **Dose and exposure time effect** – The number of nanoparticles that penetrate cell directly varies with its molar concentration in the medium multiplied with its exposure time (Buzea et al. 2007).
- **Particle size and shape effect** – Nanoparticles exhibit a size- and shape-dependent different levels of toxicity at aspect ratio (Jeevanandam et al. 2018).
- **Surface area effect** – Toxicity of nanoparticles increases with decreasing particle size and increasing surface area (Jeevanandam et al. 2018).

- **Crystal structure effect** – Nanoparticles exhibit a different cellular uptake, subcellular localization, and oxidative mechanism based on crystal structure effect (Jeevanandam et al. 2018).
- **Surface functionalization effect** – This effect of nanoparticles has an effect on its translocation and its oxidation processes (Oberdörster et al. 2005a, b; Sayes et al. 2004).
- **Pre-exposure effect** – Considerable cellular phagocytic activity at lower nanoparticle exposure time (Buzea et al. 2007).

Besides these, there are other characteristics and properties which affect the toxicity nature of nanoparticle. These engineered nanoparticles are established by humans and are assumed to have a different effect. Each type of engineered nanoparticles ($TiO_2$ – titanium dioxide) has severe to minimal biological effects which are of great concern on the usage of nanoparticles (Schulte et al. 2008).

The above information reveals the cause of the toxic effect of nanoparticles. It is of prior importance to know the action of the nanoparticles in biological effect in order to estimate its potentiality. The nanosize of a nanoparticle itself causes several adverse effects as they are similar to the size of natural proteins and can get access to the nucleus (in vivo case) and transfer across the placental barrier of pregnant mice (Gu et al. 2009; Chu et al. 2010). In this manner, a nanoparticle can affect the homeostasis. Some of the patterns of nanoparticles toxicity include oxidative stress, inflammation, inhibition of cell death and cell division, age, and genetic damage (Thanh and Green 2010; Verma and Stellacci 2010; Mironava et al. 2010; Lanone et al. 2009). Among these mechanisms, few are discussed with some details:

- **Reactive Oxygen Species**
- The generation of reactive oxygen species (ROS) is found to be either harmful or protective biological interaction based on its levels and further effects. They are reactive species of molecular oxygen and are key signaling molecules for homeostasis and cell signaling. They are generated extrinsically and intrinsically within the cell. Different ROS molecules contain a pool of oxidative species such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), and hydroxyl radical ($OH^{\cdot}$) (Manke et al. 2013). ROS generation happens regularly when a cell is under stress such as high temperature, pressure, and improper homeostasis leading to active oxygen-containing molecules. $O_2^{\cdot}$ is generated by molecular oxygen, which is a primary ROS by one electron reduction catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Multiple reductions of oxygen may lead to $H_2O_2$ or $OH^{\cdot}$ by dismutation and metal-catalyzed Fenton reaction, respectively (Vallyathan and Shi 1997; Thannickal and Fanburg 2000). The sources where the ROS can be generated are an inflammatory response, mitochondrial respiration, and peroxisomes, while engineered nanoparticles are known as the exogenous ROS inducers (Manke et al. 2013). Different types of nanoparticles inducing metal oxide particles induce ROS as the main mechanism of cytotoxicity (Risom et al. 2005). ROS influence further intracellular calcium concentrations and modulate

cytokine production by free radical generation (Li et al. 2010; Oberdörster et al. 2005a, b). Most of the cells can resist the ROS generation for a limit beyond the concentration with the increase of time of exposure which results in the cell damage (Soenen et al. 2011).

- The large surface area of nanoparticles and its surface molecules result in massive oxidizing capabilities. Nanoparticles generate ROS by different mechanisms (Pisanic et al. 2009):
  (a) ROS generation on exposure to an acidic environment – Nanoparticles in the acidic environment of lysosomes generates ROS by direct reactivity of the surface coating, and degradation of coating leads to the direct interaction of acidic media on metal surface or by degradation of whole nanoparticles resulting in synthesis of ions ($Fe^{2+}$, $Cd^{2+}$) inducing ROS generation (Stroh et al. 2004; Jain et al. 2018).
  (b) ROS generation on interaction with cellular organelles – Nanoparticles interacting with mitochondria result in the deregulation of electron transport chain of oxidative phosphorylation (Soto et al. 2007).
  (c) ROS generation by NADPH oxidase – Nanoparticles interact directly with NADPH oxidase, resulting in ROS generation in immune cells (Pisanic et al. 2009).
  (d) ROS generation on interaction with cell surface receptors – Nanoparticles interact with surface receptors leading to its activation triggering intracellular signaling cascades, resulting in stress response gene activation and thus ROS generation activation and change homeostasis of the cell (Pisanic et al. 2009).
- As ROS play a major role in the toxicology of nanoparticles, the evaluation of elevated ROS level is of prior importance in toxicity evaluation methods of nanoparticles.
- **Cytoskeleton and Cell Morphology Defects**
- Nanoparticles that occupy the cell lead to the alteration in its morphology or the structure of cytoskeleton (Soenen et al. 2009, 2010). Different effects in disorganization of a cell cytoskeleton are observed based on the coating of inorganic nanoparticles (Gupta and Gupta 2005). The actin and tubulin proteins of human umbilical vein endothelial cells (HUVECs) are disrupted considerably, which decreased the capacity of HUVEC for vascular network formation on nanoparticles exposure (Wu et al. 2010). More attention is focused on the effect of nanoparticles on cytoskeleton and cell morphology as it leads to inflammation, affecting the reliability of a nanoparticle. The effect of nanoparticles on cell decreases with its concentration, and therefore, no adverse effects are observed at low concentration; a variety of nanoparticles are to be tested to evaluate the maximum loading capacity without any adverse effect. It is also necessary to evaluate the secondary effects of cytoskeleton disruption and morphology by a variety of nanoparticles to use them efficiently (Soenen et al. 2011).
- **Genotoxicity**

- The size of the nanoparticles makes it more possible to enter the nucleus and interact with the nucleoproteins leading to adverse effects. Nanoparticles interfere with the cellular homeostasis resulting in a cascade of mechanisms such as:
  (a) High levels of ROS – ROS generation by nanoparticles induces point mutations leading to single- or double-strand breaks.
  (b) Perinuclear localization of nanoparticles – nanoparticle localized in perinuclear space by loaded lysosomes affecting the molecular processes of cell (transcription and translation involving with disruption in the protein synthesis and modifying gene expression).
  (c) Alteration in homeostasis – leaching of metal ion to cell cytoplasm through complexes (e.g., a divalent metal transporter) resulting in degradation of messenger RNA.
  (d) Interacting with cell surface receptors – activation of receptor and triggering signaling cascades as intracellularly, altering activation status.
  (e) Cellular stress induced by nanoparticles – ROS generation by nanoparticles induces stress indirectly affecting gene expression pattern and activation of repair genes (Soenen et al. 2011).
- Most of the nanoparticle genotoxic details are to be known. The genes involved in regulation and repair are to be evaluated for the nanoparticle toxicity for most of the biological applications. Nanoparticle can be transported to the cell interior, where it is active, and it should not induce any adverse toxic effects in the cell (Soenen et al. 2011).
- **Interaction with the Biological Molecules of Cell**
- The equivalent size of the proteins and nanoparticle seems to be an issue in interacting or misleading the cell more often, for nanoparticles as a cellular protein. When nanoparticles enter the cell, the surface charges favor the binding of available proteins, leading to protein corona (Cedervall et al. 2007). This resemblance of nanoparticle with the cellular protein affects its bioavailability by the attack of the immune system as foreign material to eliminate from the body. Besides proteins, the nanoparticles are found to interact with the lipid molecules based on the surface charge, creating a channel on cell membrane inducing a cytotoxic effect (Lin et al. 2010).

## 15.3 Evaluation of Toxicity

With the growing commercial interest of nanoparticle, minimal research interest is focused in evaluating the potential adverse effects of the engineered nanoparticles (Manke et al. 2013). The assessment of NP safety has been critical due to variations in:

- Types of nanoparticles (Soenen and Cuyper 2010)
- Stabilizing coating agents (Clift et al. 2009)
- Physiochemical parameters of nanoparticles (diameter, topography, surface charge) (Verma and Stellacci 2010)

- Incubation conditions such as concentration and time (Mironava et al. 2010)
- Type of assay used (Monteiro-Riviere et al. 2009)
- Type of cell used (Lanone et al. 2009)

Standardization of protocol is necessary to understand and compare the generated data from different literatures regarding the toxicity of nanoparticles. Cell viability is quite a good indication for the safety and efficacy of nanoparticle and is usually accomplished by different assays such as (Soenen et al. 2011) (1) MTT assay; (2) lactate dehydrogenase assay (LDH), trypan blue, propidium, iodine assay (to check cell membrane integrity); and (3) fluorescent annexin V (apoptosis indicator); many other assays are generally used to check the homeostasis of the cell. The results of one assay cannot be compared to another assay as each is performed on its standard parameter (Soenen and Cuyper 2009). Assays are to be performed with safety, precaution, and general controls to be included, as nanoparticles interact with components of the assay (Monteiro-Riviere et al. 2009). Animal assay, the test performed by using cell lines, is performed by routine test guidelines, and more knowledge is essential to know about the potential toxicity of vast nanoparticles and its associated complexity (Bakand et al. 2012). More business communities and research organizations continue to invest in nanoparticles, to develop an alternative test system to characterize the toxicity profile of nanoparticles (Bakand et al. 2012). Besides, in vitro models are expanding faster for evaluation in a simple, quick, and least expensive way; but the results cannot replace the in vivo studies of compound toxicity (Bakand et al. 2012). In vitro test using cultured cells generates more toxicity data than in vivo models, but high standardization is required (Blank et al. 2009). Hence, for toxicology studies, in vitro test systems with both human- and animal-based cellular needs are employed for a better evaluation. Toxicity is assessed by characterizing shape, size, and structure of nanoparticles, by high-resolution imaging techniques: transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Drobne 2007). Nanomaterial characteristics, such as high chemical bioactivity and reactivity, cellular as well as tissue and organ penetration ability, and bioavailability of nanoparticles, show both positive and negative effects on a biological environment. Thus, certain regulations are implemented by different government organizations to prevent the risk of using nanoparticles (Jeevanandam et al. 2018). There is no internationally approved protocol for manufacturing, handling, testing, and evaluating the impact of nanoparticles (Jeevanandam et al. 2018). The European Union and United States of America have regulatory and guideline legislations to control risks associated with nanomaterials. In the United States, the regulatory agencies such as Food and Drug Administration (FDA), The United States Environmental Protection Agency (USEPA), and Institute for Food and Agricultural Standards (IFAS) are associated with standard protocols to deal with the risks of nanoparticles (Jeevanandam et al. 2018). The European Medicines Agency (EMEA) and United States Food and Drug Administration (USFDA) regulate the medical usage of hazardous nanomaterials. These regulations help to control the usage of nanomaterials and nanoparticles and to determine the need for evaluation of toxicity of a nanoparticle.

## 15.4 Methods for Toxicity Evaluation of Nanoparticles Using Cell Lines

Cell culture studies are involved in awaking the knowledge of how nanoparticles react to the body. In comparison with in vivo methods, in vitro studies are less ethical, easy, fast, reliable, and less expensive to perform (Lewinski et al. 2008). Different assays are performed to check the toxicity of nanoparticles based on the characteristics such as the type of cell used in the assay and the type of toxicity/effect of the particle to be evaluated. As the nanoparticles are capable of absorbing dyes and remain in the redox state, a variety of in vitro assays are found to be efficient ways of testing nanoparticles toxicity using cell lines. Most of the test results or cell deaths are measured by the colorimetry (Lewinski et al. 2008).

Different cell lines are used for the in vitro assays. Typically, the cell cultures of human cell lines are grown in optimum conditions of 37 °C, 5% $CO_2$ atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Huo et al. 2015). A variety of human cell lines are used in in vitro assay for toxicity evaluation of nanoparticles such as human bronchial epithelial cells (HBE), human umbilical vein endothelial cells (HUVECs), human hepatocellular liver carcinoma cells (HepG2), human dermal fibroblasts (HDF), human monocyte–macrophages, human epidermal keratinocytes (HEK), and many more. Each cell type has a unique nature; and hence, all cell lines may not respond similarly to the same nanoparticle under similar optimal conditions. Eventhough the cell lines determine the in vivo environment as precise, the choice of the type of cell line for evaluating nanoparticles toxicity is critical.

Some of the in vitro assays are briefly discussed to get an overview of the types of toxicity evaluation methods:

- **Neutral Red Assay**
- Neutral red is a weak cationic dye which can diffuse the plasma membrane of the cell. It accumulates within the cell. If the integrity of the cell membrane is lost by the toxicity of nanoparticles, the uptake of dye decreases (Lewinski et al. 2008). Cytotoxicity of carbon nanotubes was assessed by neutral red assay (Flahaut et al. 2006). This assay helps in evaluating the cell membrane's permeability and its integrity in the cell lines used.
- **Trypan Blue Assay**
- A diazo dye is permeable to cells without membranes, and therefore the dead cell remains blue and live cells as colorless. The number or quantity of dead cells is evaluated by light microscopy (Lewinski et al. 2008). Gold nanoparticles and single-walled nanotubes were evaluated for the cytotoxicity by trypan blue assay (Bottini et al. 2006; Goodman et al. 2004).
- **TUNEL Assay**
- The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay detects the fragmented genomic DNA formed by endonucleases or caspases activation in apoptosis (Hengartner 2000). To count the number of cells in the tissue sample, DAPI (4′,6 diamino-2-phenylindole) was added before

mounting the coverslip and results in the staining of nuclei. Images are observed through a fluorescent microscope (Huo et al. 2015). Three different image areas of about 500 cells are counted by a microscope to know the apoptosis rate.

- **Hemolytic Assay**
- It is a colorimetric assay of detecting red colored cyanomethemoglobin in the solution. The nanoparticles are incubated in blood, and hemoglobin released by damaged cells is oxidized by methemoglobin in the presence of bicarbonate by ferricyanide. The cyanide converts methemoglobin to cyanomethemoglobin (Neun and Dobrovolskaia 2010). The cells are then centrifuged, and the undamaged erythrocytes producing cyanomethemoglobin is measured by spectrophotometry at 540 nm. The result of this assay evaluates the hemolytic properties of nanoparticles (Neun and Dobrovolskaia 2010).
- **3D Spheroid Culture-Based NP Toxicity Testing System**
- Human hepatocarcinoma (HepG2) cells are used in preparing 3D live tissue spheroid models, as the liver is the main organ for the nanoparticle uptake (Gao et al. 2004). The inverted colloidal crystal topology is used as a 3D cell growth substrate, prepared from transparent and cell repulsive polyacrylamide hydrogel (Lee et al. 2006). The spheroid formation of HepG2 cells enhances optimal prediction through the matrix. The toxic effects of cadmium telluride (CdTe) and gold (Au) NPs were tested using different approaches to evaluate the membrane integrity, metabolic activity, and comparison with 2D cell toxicity (Lee et al. 2009). The morphological changes are observed in scanning electron microscopy, whereas the live–dead assay assesses cell viability.
- **Live–dead Viability Assay**
- The assay determines if the cell is alive or dead with different absorbing capabilities of the live and dead cells; it includes two chemicals – calcein acetoxymethyl (calcein AM) and ethidium homodimer. The former is electrically neutral; an esterified molecule can enter cells by the diffusion process (Lewinski et al. 2008). Once the calcein AM enters the cell, it converts to calcein by esterases to a green fluorescent molecule. By contrast, the dead cells get stained by ethidium homodimer, a membrane impermeable molecule, and turn to fluorescent red if it binds to the nucleic acid. The fluorescence is emitted by calcein and ethidium homodimer at a wavelength of 515 nm and 635 nm, respectively (Lewinski et al. 2008).
- **MTT Assay**
- MTT assay is a colorimetric method to determine the mechanism of cell death. MTT 3-(4,5- dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide is a pale-yellow solution that produces dark blue or purple formazan by the live cells. This color formation is due to mitochondrial dehydrogenase enzyme present in living cells (Malich et al. 1997).
- The toxicity of silver nanoparticles is tested using MTT assay on human pulmonary cell lines: THP-1 and A549. MTT assay is found to be a more sensitive test and widely used assay for evaluating cell toxicity (Lanone et al. 2009). The potential cytotoxicity of silver nanoparticles was assessed by MTT assay, using human epidermal keratinocytes (HEKs), and found the AgNPs exposure, indicating a dose-dependent decrease in toxicity (Samberg et al. 2010). The cytotoxicity

of hematite nanoparticles is detected by MTT using MCF-7, A549, and Hep3B. After incubation with nanoparticles overnight at conditions of 37 °C and 5% $CO_2$, the supernatant is removed and the MTT solution is added. The formation of formazan is quantified by spectrophotometry at 545 nm (Rajendran et al. 2017).

- **Cell Cycle Analysis**
- Pheochromocytoma cells (PC 12) were plated on a well plate in Dulbecco's minimal essential medium. The cells are then placed into the centrifuge tube on treating with glycerol monooleate nanoparticles. The cells are centrifuged at 1500 rpm for 5 min, and the pellet obtained was washed with PBS 1×, discarding the supernatant. The pellet obtained was washed frequently with saline, and samples were analyzed in cytofluorimeter to study the cell cycle. A standard optical filter at 585/542 nm was used to determine the number of cells in each phase of the cell cycle (Valente et al. 2018).
- **Analysis of Apoptotic Markers**
- PC 12 cell lines were treated with glycerol monooleate nanoparticles to evaluate proapoptotic cell stress response, by molecular mechanisms such as transcription and translation activation. Key apoptotic markers such as BCL-2 and Bax are evaluated by the real-time quantitative polymerase chain reaction (RqPCR) (Valente et al. 2018).
- **Micronucleus Assay**
- The presence of the micronucleus is detected to check the genotoxicity of the nanoparticles. The human hepatoma cell line (HepG2) was treated with nanosilver solution. Relaxin B solution is added to the sample, after 24 h of exposure, the cells are fixed with the solution (glacial acetic acid/methanol in 1:3 ratio), and then stained with Giemsa. The Type I and Type II micronucleus predicts the chromosome breakage and loss, respectively. Nuclear buds can predict gene amplification, and its change is observed on microscopy (Wang et al. 2019).
- **ROS Assay**
- The dichlorodihydrofluorescein diacetate (DCFH-DA) is an oxidative fluorogenic dye that measures the peroxyl, hydroxyl, and other ROS within the cell. A549 cells were used to evaluate the cytotoxic ROS generation by graphene oxide nanoparticles through ROS assay. The microplate reader monitors the fluorescence. The graphene oxide nanoparticles found to cause ROS generation even at low concentration (Chang et al. 2011).
- **SRB Assay**
- The sulforhodamine (SRB) detects the cytotoxicity of curcumin solid dispersions. SRB assay was performed on MCF-7 (breast cancer cell line) and NCIH 460 (non-small cell lung cancer cell lines). These cells are treated for 48 h, and toxicity was evaluated (Abreu et al. 2011).
- **TBARS Assay**
- Thiobarbituric acid reactive substance (TBARS) assay is used to predict the formation of malondialdehyde (MDH) and other reactive substance that is generated by lipid peroxidation. Porcine brain cells were utilized to evaluate curcumin

nanoparticle cytotoxicity and observed to reduce or discourage the TBARS level in the cell (Sá et al. 2019).

- **Lactate Dehydrogenase Assay**
- The enzyme lactate dehydrogenase oxidizes lactate to pyruvate and promotes the conversion of a tetrazolium salt into formazan with an absorbance at 490 nm. The amount of LDH released from cells is proportional to damaged cells (Lewinski et al. 2008). Human embryonic kidney cells (HEK)-293 on exposure to copper oxide nanoparticles cause the peroxidation of lipids (Reddy and Lonkala 2019).
- **Mitochondrial Membrane Potential (MMP) Assay**
- The silver nanoparticles are evaluated by MMP in BRL 3A cells by staining with rhodamine 123. On treatment, the qualitative effect on mitochondrial membrane potential was found to be affected by the silver nanoparticles, and the intensity of the fluorescent brightness is reduced (Hussain et al. 2005).
- **GSH Assay**
- Glutathione is a major antioxidant which is oxidized to glutathione disulfide (GSSG) in the presence of ROS (Lewinski et al. 2008). Reduced glutathione (GSH) maintains the oxidation–reduction homeostasis, and its alteration in GSH level indicates damage to the cell. In BRL 3A rat cells, the GSH level was decreased on treatment with silver nanoparticles, which is found to be significant (Hussain et al. 2005).
- **Clonogenic Assay**
- Clonogenic assay is a method to cell reproductive death. MCF-7 cells were trypsinized and seeded, followed by incubation in the presence of B26 organic nanoparticles. After incubation, for few days, the cells are fixed with methanol and crystal violet solutions. The cells are counted by an inverted microscope (Dhanwal et al. 2019).

The above discussed in vitro assays are typically performed to detect either cytotoxicity or genotoxicity. A new approach to evaluate or predict the toxicity of nanoparticles easily is by using computer nanotoxicology – QSAR (quantitative structure-activity relationship) – which is a quick, mostly accurate, and no resource-intensive test to detect toxicity of nanoparticles.

### 15.4.1 QSAR

A statistical model correlates a set of the structural parameter of a compound to its activity. The parameters are mostly based on electric and steric properties of a compound. Physiological measurements of biological assay data determine the biological activity of a compound. The QSAR workflow is as follows (Burello and Worth 2011):

QSAR is most widely used in drug discovery and is still having a limited application in the evaluation of nanoparticles. More research and analysis on the toxicity of nanoparticles will be done easily by the computer nanotoxicology in the near future,

along with a collaborated work among computational scientists and nanomaterials descriptions with toxicologists to develop new computational assays for evaluating nanoparticles toxicity.

## 15.5 Conclusion and Future Perspective

This chapter reviews the toxicity of nanoparticle and its evaluation methods using cell lines. The typical interactions between the nanoparticles and the biological systems are gaining more interest to evaluate the potentiality of nanoparticles, and still it seems to be challenging to get a conclusion of underlying mechanism of toxicity. This review outlines the importance to evaluate the toxicity of nanoparticles and how easy and reliable to use those different evaluation methods. Most of the assays were performed on engineered or human-made nanoparticles as the natural nanoparticles are found to be much more safe and efficient to use biologically. It is known that nanomaterials are not hazardous particles and many are nontoxic and have some healthy beneficial effects. However, risk assessment or evaluation helps one to determine the further actions needed to assess the effect of nanoparticles on human health and environment. The use of cancer cell types is to be minimized to evaluate the toxicity nature of nanoparticle, which is less susceptible to nanoparticle-induced cytotoxicity. The toxicity evaluation methods are found to be efficient, quick, and reliable to assess the toxicity nature of the nanoparticles.

## References

Abreu RM, Ferreira IC, Calhelha RC et al (2011) Anti-hepatocellular carcinoma activity using human HepG2 cells and hepatotoxicity of 6-substituted methyl 3-aminothieno[3,2-b]pyridine-2-carboxylate derivatives: in vitro evaluation, cell cycle analysis and QSAR studies. Eur J Med Chem 46(12):5800–5806. https://doi.org/10.1016/j.ejmech.2011.09.029

Alexis F, Pridgen E, Molnar LK et al (2008) Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 5(4):505–515. https://doi.org/10.1021/mp800051m

Avasare V, Zhang Z, Avasare D et al (2015) Room-temperature synthesis of $TiO_2$ nanospheres and their solar driven photoelectrochemical hydrogen production. Int J Energy Res 39(12):1714–1719. https://doi.org/10.1002/er.3372

Bakand S, Hayes A, Dechsakulthorn F (2012) Nanoparticles: a review of particle toxicology following inhalation exposure. Inhalat Toxicol 24(2):125–135. https://doi.org/10.3109/08958378.2010.642021

Bermudez E, Mangum JB, Wong BA et al (2004) Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 77:347–357

Blank F, Gehr P, Rothen-Rutishauser B (2009) In vitro human lung cell culture models to study the toxic potential of nanoparticles. In: Nanotoxicity, pp 379–395. https://doi.org/10.1002/9780470747803.ch19

Bottini M, Bruckner S, Nika K et al (2006) Multi-walled carbon nanotubes induce T lymphocyte apoptosis. Toxicol Lett 160(2):121–126. https://doi.org/10.1016/j.toxlet.2005.06.020

Burello E, Worth A (2011) Predicting toxicity of nanoparticles. Nat Nanotechnol 6:138–139

Buzea C, Pacheco II, Robbie K (2007) Nanomaterials and nanoparticles: sources and toxicity. Biointerphases 2(4):MR17–MR71. https://doi.org/10.1116/1.2815690

Cedervall T, Lynch I, Lindman S et al (2007) Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci 104(7):2050–2055. https://doi.org/10.1073/pnas.0608582104

Chang Y, Yang S, Liu J et al (2011) In vitro toxicity evaluation of graphene oxide on A549 cells. Toxicol Lett 200(3):201–210. https://doi.org/10.1016/j.toxlet.2010.11.016

Chu M, Wu Q, Yang H et al (2010) Transfer of quantum dots from pregnant mice to pups across the placental barrier. Small 6(5):670–678. https://doi.org/10.1002/smll.200902049

Clift MJ, Boyles MS, Brown DM et al (2009) An investigation into the potential for different surface-coated quantum dots to cause oxidative stress and affect macrophage cell signaling in vitro. Nanotoxicol 4(2):139–149. https://doi.org/10.3109/17435390903276925

Dhanwal V, Katoch A, Singh A et al (2019) Self-assembled organic nanoparticles of benzimidazole analogue exhibit enhanced uptake in 3D tumor spheroids and oxidative stress induced cytotoxicity in breast cancer. Mater Sci Eng C 97:467–478. https://doi.org/10.1016/j.msec.2018.12.039

Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El-Sayed MA (2012) The golden age: gold nanoparticles for biomedicine. Chem Soc Rev 41:2740–2779. https://doi.org/10.1002/chin.201227268

Drobne D (2007) Nanotoxicology for safe and sustainable nanotechnology. Arch Ind Hyg Toxicol 58(4):471–478. https://doi.org/10.2478/v10004-007-0040-4

Elder A, Gelein R, Silva V et al (2006) Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114:1172–1178

Flahaut E, Durrieu M, Remy-Zolghadri M et al (2006) Investigation of the cytotoxicity of CCVD carbon nanotubes towards human umbilical vein endothelial cells. Carbon 44(6):1093–1099. https://doi.org/10.1016/j.carbon.2005.11.007

Gao X, Cui Y, Levenson RM et al (2004) In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 22(8):969–976. https://doi.org/10.1038/nbt994

Geiser M, Rothen-Rutishauser B, Kapp N et al (2005) Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect 113(11):1555–1560. https://doi.org/10.1289/ehp.8006

Goodman CM, Mccusker CD, Yilmaz T et al (2004) Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjug Chem 15(4):897–900. https://doi.org/10.1021/bc049951i

Greeley J, Markovic NM (2012) The road from animal electricity to green energy: combining experiment and theory in electrocatalysis. Energy Environ Sci 5(11):9246. https://doi.org/10.1039/c2ee21754f

Gu Y, Cheng J, Lin C et al (2009) Nuclear penetration of surface functionalized gold nanoparticles. Toxicol Appl Pharmacol 237(2):196–204. https://doi.org/10.1016/j.taap.2009.03.009

Gupta AK, Gupta M (2005) Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles. Biomater 26(13):1565–1573. https://doi.org/10.1016/j.biomaterials.2004.05.022

Handy RD, Kammer FV, Lead JR et al (2008) The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicol 17(4):287–314. https://doi.org/10.1007/s10646-008-0199-8

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407(6805):770–776. https://doi.org/10.1038/35037710

Huo L, Chen R, Zhao L et al (2015) Silver nanoparticles activate endoplasmic reticulum stress signaling pathway in cell and mouse models: the role in toxicity evaluation. Biomater 61:307–315. https://doi.org/10.1016/j.biomaterials.2015.05.029

Hussain S, Hess K, Gearhart J et al (2005) *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro 19(7):975–983. https://doi.org/10.1016/j.tiv.2005.06.034

Jain TK, Reddy MK, Morales MA et al (2018) Nanomagnetic-mediated drug delivery for the treatment of dental disease. Mol Pharm 5(2):316–327. https://www.sciencedirect.com/science/article/pii/S1549963418300248

Jeevanandam J, Chan YS, Danquah MK (2016) Nano-formulations of drugs: recent developments, impact and challenges. Biochimie 128-129:99–112. https://doi.org/10.1016/j.biochi.2016.07.008

Jeevanandam J, Barhoum A, Chan YS et al (2018) Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol 9:1050–1074. https://doi.org/10.3762/bjnano.9.98

Khan I, Saeed K, Khan I (2017) Nanoparticles: properties, applications and toxicities. Arab J Chem 12:908. https://doi.org/10.1016/j.arabjc.2017.05.011

Khlebtsov NG, Dykman LA (2010) Optical properties and biomedical applications of plasmonic nanoparticles. J Quant Spectrosc Radiat Transf 111:1–35. https://doi.org/10.1016/j.jqsrt.2009.07.012

Khlebtsov N, Dykman L (2011) Biodistribution and toxicity of engineered gold nanoparticles: a review of *in vitro* and *in vivo* studies. Chem Soc Rev 40:1647–1671. https://doi.org/10.1039/C0CS00018C

Lam CW, James JT, McCluskey R et al (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci 77:126–134

Lanone S, Rogerieux F, Geys J et al (2009) Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Part Fibre Toxicol 6(1):14. https://doi.org/10.1186/1743-8977-6-14

Lee J, Shanbhag S, Kotov NA (2006) Inverted colloidal crystals as three-dimensional microenvironments for cellular co-cultures. J Mater Chem 16(35):3558. https://doi.org/10.1039/b605797g

Lee J, Lilly GD, Doty RC et al (2009) *In vitro* toxicity testing of nanoparticles in 3D cell culture. Small 5(10):1213–1221. https://doi.org/10.1002/smll.200801788

Leonhardt U (2007) Invisibility cup. Nat Photonics 1:207–208. https://doi.org/10.1038/nphoton.2007.38

Lewinski N, Colvin V, Drezek R (2008) Cytotoxicity of nanoparticles. Small 4(1):26–49. https://doi.org/10.1002/smll.200700595

Li JJ, Muralikrishnan S, Ng CT et al (2010) Nanoparticle-induced pulmonary toxicity. Exp Biol Med 235(9):1025–1033

Lin J, Zhang H, Chen Z et al (2010) Penetration of lipid membranes by gold nanoparticles: insights into cellular uptake, cytotoxicity, and their relationship. ACS Nano 4(9):5421–5429. https://doi.org/10.1021/nn1010792

Liu J, Liu Y, Liu N et al (2015) Metal-free efficient photocatalyst for stable visible water splitting via a two-electron pathway. Science 80(347):970–974. https://doi.org/10.1126/science.aaa3145

Malich G, Markovic B, Winder C (1997) The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. Toxicology 124(3):179–192. https://doi.org/10.1016/s0300-483x(97)00151-0

Manke A, Wang L, Rojanasakul Y (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int 2013:1–15. https://doi.org/10.1155/2013/942916

Mercer RR, Scabilloni JF, Wang L et al (2008) Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model. Am J Physiol Lung Cell Mol Physiol 294:L87–L97. https://doi.org/10.1152/ajplung.00186.2007

Mironava T, Hadjiargyrou M, Simon M et al (2010) Gold nanoparticles cellular toxicity and recovery: effect of size, concentration and exposure time. Nanotoxicol 4(1):120–137. https://doi.org/10.3109/17435390903471463

Mohanraj VJ, Chen Y (2006) Nanoparticles – a review. Trop J Pharmaceut Res 5(1):561–573. http://www.tjpr.freehosting.net

Monteiro-Riviere N, Inman A, Zhang L (2009) Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. Toxicol Appl Pharmacol 234(2):222–235. https://doi.org/10.1016/j.taap.2008.09.030

Neun BW, Dobrovolskaia MA (2010) Method for analysis of nanoparticle hemolytic properties in vitro. Method Mol Biol 697:215–224. https://doi.org/10.1007/978-1-60327-198-1_23

Ning F, Shao M, Xu S et al (2016) $TiO_2$/graphene/NiFe-layered double hydroxide nanorod array photoanodes for efficient photoelectrochemical water splitting. Energy Environ Sci 9(8):2633–2643. https://doi.org/10.1039/c6ee01092j

Oberdörster G, Sharp Z, Atudorei V et al (2004) Translocation of inhaled ultrafine particles to the brain. Inhalat Toxicol 16(6–7):437–445. https://doi.org/10.1080/08958370490439597

Oberdörster G, Maynard A, Donaldson K et al (2005a) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fiber Toxicol 2(8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1260029/):8

Oberdörster G, Oberdörster E, Oberdörster J (2005b) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–839. https://doi.org/10.1289/ehp.7339

Olteanu NL, Lazăr CA, Petcu AR et al (2016a) "One-pot" synthesis of fluorescent Au@SiO2 and SiO2@Au nanoparticles. Arab J Chem 9:854–864. https://doi.org/10.1016/j.arabjc.2015.12.014

Olteanu NL, Rogozea EA, Popescu SA et al (2016b) "One-pot" synthesis of Au–ZnO–SiO2 nanostructures for sunlight photodegradation. J Mol Catal A Chem 414:148–159. https://doi.org/10.1016/j.molcata.2016.01.007

Pisanic TR, Jin S, Shubayev VI et al (2009) Nanotoxicity: from in vivo and in vitro models to health risks. Wiley, London, pp 397–425

Rajendran K, Sen S et al (2017) Evaluation of cytotoxicity of hematite nanoparticles in bacteria and human cell lines. Colloid Surf B Biointerface 157:101–109. https://doi.org/10.1016/j.colsurfb.2017.05.052

Reddy AR, Lonkala S (2019) In vitro evaluation of copper oxide nanoparticle-induced cytotoxicity and oxidative stress using human embryonic kidney cells. Toxicol Ind Health 35(2):159–164. https://doi.org/10.1177/0748233718819371

Reverchon E, Adami R (2006) Nanomaterials and supercritical fluids. J Supercritical Fluid 37(1):1–22. https://doi.org/10.1016/j.supflu.2005.08.003

Risom L, Moller P, Loft S (2005) Oxidative stress-induced DNA damage by particulate air pollution. Mutat Res 592(1–2):119–137. http://www.sciencedirect.com/science/article/pii/S0027510705002460

Rogozea EA, Olteanu NL, Petcu AR et al (2016) Extension of optical properties of ZnO/SiO2 materials induced by incorporation of Au or NiO nanoparticles. Optical Mater 56:45–48. https://doi.org/10.1016/j.optmat.2015.12.020

Rytwo G (2008) Clay minerals as an ancient nanotechnology: historical uses of clay-organic interactions, and future possible perspectives. La Revista Macla 9:15–17

Sá IS, Peron AP, Leimann FV et al (2019) In vitro and in vivo evaluation of enzymatic and antioxidant activity, cytotoxicity and genotoxicity of curcumin-loaded solid dispersions. Food Chem Toxicol 125:29–37. https://doi.org/10.1016/j.fct.2018.12.037

Samberg ME, Oldenburg SJ, Monteiro-Riviere NA (2010) Evaluation of silver nanoparticle toxicity in skin in vivo and keratinocytes in vitro. Environ Health Perspect 118(3):407–413. https://doi.org/10.1289/ehp.0901398

Sayes CM, Fortner JD, Guo W et al (2004) The differential cytotoxicity of water-soluble fullerenes. Nano Lett 4(10):1881–1887. https://doi.org/10.1021/nl0489586

Schaming D, Remita H (2015) Nanotechnology: from the ancient time to nowadays. Found Chem 17:187–205. https://doi.org/10.1007/s10698-015-9235-y

Schulte P, Geraci C, Zumwaide R et al (2008) Sharpening the focus on occupational safety and health in nanotechnology. Scand J works Environ Health 34(6):471–478

Seaton A (2006) Nanotechnology and the occupational physician: introduction. Occupat Med 56(5):294–294. https://doi.org/10.1093/occmed/kql049

Shvedova AA, Kisin ER, Mercer R et al (2005) Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol 289(5):L698–L708. https://doi.org/10.1152/ajplung.00084.2005

Soenen SJ, Cuyper MD (2009) Assessing cytotoxicity of (iron oxide-based) nanoparticles: an overview of different methods exemplified with cationic magnetoliposomes. Contrast Media Mol Imaging 4(5):207–219. https://doi.org/10.1002/cmmi.282

Soenen SJ, Cuyper MD (2010) Assessing iron oxide nanoparticle toxicity *in vitro*: current status and future prospects. Nanomedicine (Lond) 5(8):1261–1275. https://doi.org/10.2217/nnm.10.106

Soenen SJ, Illyes E, Vercauteren D et al (2009) The role of nanoparticle concentration-dependent induction of cellular stress in the internalization of non-toxic cationic magnetoliposomes. Biomaterials 30(36):6803–6813

Soenen SJ, Nuytten N, Meyer SF et al (2010) High intracellular Iron oxide nanoparticle concentrations affect cellular cytoskeleton and focal adhesion kinase-mediated signaling. Small 6(7):832–842. https://doi.org/10.1002/smll.200902084

Soenen SJ, Rivera-Gil P, Montenegro J et al (2011) Cellular toxicity of inorganic nanoparticles: common aspects and guidelines for improved nanotoxicity evaluation. Nano Today 6(5):446–465

Soto K, Garza KM, Murr LE (2007) Cytotoxic effects of aggregated nanomaterials. Acta Biomater 3(3):351–358. https://doi.org/10.1016/j.actbio.2006.11.004

Stroh A, Zimmer C, Gutzeit C et al (2004) Iron oxide particles for molecular magnetic resonance imaging cause transient oxidative stress in rat macrophages. Free Rad Biol Med 36(8):976–984. https://www.ncbi.nlm.nih.gov/pubmed/15059638

Takenaka S, Karg D, Roth C et al (2001) Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 109(suppl.4):547–551

Thanh NT, Green LA (2010) Functionalisation of nanoparticles for biomedical applications. Nano Today 5(3):213–230. https://doi.org/10.1016/j.nantod.2010.05.003

Thannickal VJ, Fanburg BL (2000) Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 279(6):L1005–L1028. https://www.ncbi.nlm.nih.gov/pubmed/11076791

Thomas PJ, Stanfield JL, Vanitha PV (2011) Nanoparticles. Ann Rep Prog Chem 107:505–518. https://doi.org/10.1039/c1ic90030g

Tiwari G, Tiwari R, Bannerjee S et al (2012) Drug delivery systems: an updated review. Int J Pharm Investig 2(1):2. https://doi.org/10.4103/2230-973x.96920

Valente F, Bysell H, Simoni E et al (2018) Evaluation of toxicity of glycerol monooleate nanoparticles on PC12 cell line. Int J Pharm 539(1–2):23–30. https://doi.org/10.1016/j.ijpharm.2018.01.035

Vallyathan V, Shi X (1997) The role of oxygen free radicals in occupational and environmental lung diseases. Environ Health Perspect 105:165–177. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1470247/

Verma A, Stellacci F (2010) Effect of surface properties on nanoparticles cell interactions. Small 6(1):12–21. https://doi.org/10.1002/smll.20090115

Wang X, Li T, Su X et al (2019) Genotoxic effects of silver nanoparticles with/without coating in human liver HepG2 cells and in mice. J Appl Toxicol 39(6):908–918. https://doi.org/10.1002/jat.3779

Wu X, Tan Y, Mao H, Zhang M (2010) Toxic effects of iron oxide nanoparticles on human umbilical vein endothelial cells. Int J Nanomedicine 5:385–399. https://doi.org/10.2147/ijn.s10458