# Toxicological Evaluation of Nanoparticles Using Prokaryotic Model Organisms

## 14

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**Abstract**

The extensive application of nanoparticle (NP) synthesis and expansion in recent advances in biological and material science have been of considerable scientific interest from the last century as they possess properties to measure and influence the physical substance from an atomic and molecular point of view compared with bulk materials. NPs are usually coated with metal ions, chemical surfactants, polymers, and smaller molecules. Due to these properties nanoparticles tend to get the toxic value that is largely estimated triggering both the environmental and human health risks. The number size distribution of nanoparticles of 1–100 nm is the main cause of these substances affecting the environment and health system where the passage into the ecological food chains via microorganisms has been easy, disturbing the biological balance. Hence, it is vital to evaluate the toxicity of NPs associated with microorganisms beforehand. Though the eukaryotic model was renowned, in recent developments, the use of prokaryotic models especially bacteria is considered the most convenient, rapid, and cost effective. Evaluating the toxicity of NPs using microorganisms gives an insight into the toxic impacts of NPs. Bacterial species such as *Escherichia coli*, *Pseudomonas* sp., *Bacillus* sp., and mainly magnetotactic bacteria intracellularly can synthesize the tiny crystals referred to as nanocrystals. The mechanism associated with the toxicity of NPs is mainly the oxidative stress and generation of reactive oxygen species that results in membrane disorganization, impair reproduction, and growth inhibition. This chapter in detail will give out the different approaches to evaluate the toxicity of NPs and also the use of different prokaryotic models that produce eco-friendly nanoparticles that are of greater importance in the biological system.

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## 14.1 Introduction

In recent days, the target on nanoparticles, their origin, activity, and biological toxicity by the researchers has been of much interest due to revolutionary developments in the field of nanotechnology with novel and diverge application coming into view in our daily lives. Nanotechnology is a well-known advanced technology in the field of research since the twentieth century. The word "nano" is derived from a Latin word, which means "dwarf." The productions of nanoscale level materials have become major innovatory developments in the field of nanotechnology. The nanostructures show substantial innovative and enhanced physical, chemical, and biological properties and develop owing to their size (Kumar et al. 2011). Extensive application of nanoparticle (NP) synthesis and expansion in recent advances in biological and material science have been of great scientific interest from the last century as they possess properties to measure and influence the physical substance from an atomic and molecular point of view compared with bulk materials. NPs are usually coated with metal ions, chemical surfactants, polymers, and smaller molecules. Due to these properties, nanoparticles tend to get the toxic value that is largely estimated, triggering both the environmental and human health risks. Facts of the toxicity effects of these small molecules are restricted, but according to latest research, it is swiftly budding (Ai et al. 2011).

Prokaryotes especially bacteria are the most common module of all known ecosystems. They play a significant role in biological cycles, degradation of impurities, the root of food webs or chains, and also soil health. The choice of researchers to use prokaryote models extensively is due to their simple structure and functional organization, short generation time compared with other organisms, nonpathogenic nature (harmless), an efficient genetic, and experimental model (easily manipulated) since the genetic background of these organisms is clear (i.e., the genome levels are fully sequenced and studied). Bacterial species intracellularly can synthesize the tiny crystals referred to as nanocrystals. Wiesner et al. (2009) have avowed that "microbial ecotoxicity" is predominantly an important consideration in assessing the toxicity mechanism of NPs that extrapolate to eukaryotic cells.

## 14.2 Nanoparticles

Nanoparticles (NPs) are a wide class of miniaturized particles with a range of $10^{-9}$ and 1–100 nm dimensional size (Laurent et al. 2008). Nanoparticles vary in their chemical, physical, mechanical, and electrical properties that differ significantly as of their corresponding bulk material due to their broad distribution in size (Biswas and Wu 2005; Lowry et al. 2012). For this reason, a material acknowledged to be nontoxic in bulk can subsist toxic at the nanometer scale due to its characteristic properties (Karlsson et al. 2009). Nanosized materials are allied to various scientific and advanced application technologies in the field of biosciences, namely, environment, chemical, pharmaceutical, health, and electrical engineering and also in the area of life and applied sciences (McDonald et al. 2005; Tripathi et al. 2016; Tiwari et al. 2017). Taken as a whole, based on the shape size and structure, these materials are grouped from 0D to 3D (Tiwari et al. 2012). NPs are complex molecules with three distinct layers: (a) the topmost being surface layer (functionalized with an array of small molecules, metal ions, surfactants, and polymers), (b) the shell layer, and (c) the core layer. The shell and core layer differ chemically in every characteristic. The core, the central portion, is by itself the nanoparticle (Singh et al. 2017).

## 14.3 Classification of Nanoparticles

Nanomaterials (NMs) can be classified by different looms. NMs are stratified based on their dimensions, type of material, and their origin.

### 14.3.1 Classification Based on Dimensions

Pokropivny and Skorokhod (2007) have given out a new classification for NMs based on the dimensions of the particle structure that included 0D, 1D, 2D, and 3D.

#### 14.3.1.1 Zero-Dimension Nanoparticles (0D NPs)

Zero nanomaterials or 0D refers to the measurement of all the dimensions within nanoscales (no dimensions larger than 100 nm). The general representation of 0D nanomaterials is the nanoparticles.

#### 14.3.1.2 One-Dimension Nanoparticles (1D NPs)

One-dimension system has been in use for decades. The utterance of "nano" has been allocated to refer the digit $10^{-9}$ (Hickey et al. 2013) which means one billionth of any unit that fallouts in the development of 1D NPs resembling a thin film or monolayers (range 1–100 nm in size) which is exploited in electronics; chemical and biochemical sensors (Alivisatos 2004; Kong et al. 2000); information storage system; pharmaceuticals; bioengineering; fiber and magneto-optic systems (Kong and Dai 2001; Cui et al. 2001); construction of nanowires, rods, tubes, belts, and ribbons; and nano-hierarchical models (Tolani et al. 2009; Wang 2000; Duan et al. 2001; Cui and Lieber 2001; Huang et al. 2001; Xia et al. 2003).

#### 14.3.1.3 Two-Dimension Nanoparticles (2D NPs)

Two-dimension structures are not confined to the nanoscale. They usually have two dimensions exterior to the range that of nanometric size with a particular shape. 2D materials habitually exhibit platelike shapes and successive exploitation as building blocks for the manufacture of nanodevices (Jibowu 2016). Two of the dimensions have potential application in the field of nano-containers, nano-reactors, photocatalysts, and as a template for other 2D structures. The most known 2D nanostructures are the carbon nanotubes (CNTs). Carbon nanotubes (CNTs) which are cylindrical hollow fibers consist of pure graphite surrounded by a hexagonal net of carbon atoms (Novoselov et al. 2004).

#### 14.3.1.4 Three-Dimension Nanoparticles (3D NPs)

3D nanoparticles or bulk nanomaterials are not confined to nanoscale in any dimensions. These materials are differentiated and said to have three arbitrarily dimensions beyond 100 nm. Bulk nanostructures have gained a broad interest in research. 3D nanostructures are classifed as dendrimers (highly branched, star-shaped macromolecules), fullerenes (also named as carbon 60 (C60), resemble soccer ball), and quantum dots (Bhatia 2016).

### 14.3.2 Classification Based on Material Type

Current NPs are grouped into four material-based categories: organic, inorganic, carbon, and composite-based materials.

#### 14.3.2.1 Organic-Based Nanomaterials

These types of NMs are of organic substance that is eco-friendly and nonhazardous. These are mostly preferred in drug delivery systems due to their unique property as a nanocapsule which is sensitive to thermal and electromagnetic radiation (Tiwari et al. 2008). The weak bonding (noncovalent) for self-assembly and design of molecules facilitates the transformation of organic NMs into the preferred formation, for example, dendrimers, micelles, liposomes, and NP polymers.

#### 14.3.2.2 Inorganic-Based Nanomaterials

Inorganic NMs are more often than not made of carbon. They usually include metal ions such as aluminum (Al), silver (Ag), gold (Au), zinc (Zn), copper (Cu), cobalt (Co), cadmium (Cd), iron (Fe), and lead (Pb) and metal oxides, namely, iron oxide ($Fe_2O_3$), aluminum oxide ($Al_2O_3$), titanium oxide ($TiO_2$), zinc oxide (ZnO), silicon dioxide ($SiO_2$), cerium oxide ($CeO_2$), and magnetite.

#### 14.3.2.3 Carbon-Based Nanomaterials

Commonly, these nanoparticles are made of carbon, hence known as carbon-based materials (Bhaviripudi et al. 2007). Carbon NMs are classed into graphene, CNTs, C60, carbon nanofibers, carbon black, and onions (Kumar and Kumbhat 2016).

#### 14.3.2.4 Composite-Based Nanomaterials

These nanosized materials are multiphase NPs in combination with metal-, carbon-, and organic-based NMs that can combine any NP with neighboring NP or a combination of bulk-type materials (e.g., hybrid nanofibers) or added complex structure (e.g., metalorganic frameworks).

### 14.3.3 Classification Based on Origin

Based on either natural or incidental or synthetic source, the NPs are classified into natural and synthetic nanomaterials.

#### 14.3.3.1 Natural Nanomaterials

In the natural world by either biological species or through anthropogenic activities, natural nano-objects are formed. NMs occur naturally through the Earth's spheres which constitute the total atmosphere, hydrosphere, lithosphere, and biosphere that cover up micro- and higher organisms, together with humans (Hochella et al. 2015; Sharma et al. 2015).

#### 14.3.3.2 Synthetic Nanomaterials

Synthetic nanosized materials are also called engineered nanomaterials (ENMs) since they are formed by mechanical actions (manufactured) either by physical, chemical, or biological hybrid methods by humans. These are generally toxic to the environment where researchers are highly forecasting the risk behavior.

#### 14.3.3.3 Incidental Nanomaterials

These are by-products incidentally produced from industrial processes such as engine exhaust, smoke from forest fires, welding fumes, and combustion processes.

## 14.4 Nanotoxicology

Nanotoxicology is an emerging new branch of bionanoscience that deals with the study of toxicity of structures smaller than 100 nm (nanomaterials) which affects both the environment and the human health as a result from manufacturing processes (engineered NMs), natural processes such as geological processes (volcanic ashes), atmospheric actions, and combustion practices (Haynes 2010; Maynard et al. 2011). Improvement in the fields of nanotechnology has benefits, but weighing the risk against benefits is needed and evaluating the level of toxicity is to be focused on reducing the risk assessed. Early million years ago, mankind and the living beings on Earth were claimed to be exposed to naturally produced NMs that result from the natural processes taking place every day around us (Nel et al. 2006; Buzea et al. 2007). NMs turned out from industrial and manufacturing practices by man have been probably toxic. They enter the ecosystem or the food web and tend to show direct risk for exposition. Few examples of natural and human-made NP by-products are soil erosions, ocean water evaporation, volcanic ashes, biogenic magnetite, quantum dots, catalysts, cosmetics, coating, consumer products, and building demolition, respectively. Also noticeable is that merging metals cause complex toxicity, which is not shown with single metals. Earlier in 1975, a study reported oxidative stress in asbestosis and cell structure disconfirmation due to the nanoparticle asbestos.

## 14.5 Nanomaterials and Biological System Interaction

Engineered NPs (ENPs) produced as a result of human activities furtively makes a way into the environment through water, soil, and air as a source. Application of NPs for green management intentionally instils or dumps ENP into the soil or aqua bodies. Nearly all nanoparticles are nondegradable and live longer than years in our surrounding environment (Navarro et al. 2008). This has consequentially engrossed an increasing alarm for all the stakeholders.

Commercially synthesized NPs have pioneered a way into our daily lives. One such example is the most widely used nanomaterial, ZnO, that has a leading application with industries and commercial productions such as products of personal care, ceramic goods, and paints (Brar et al. 2010; Blinova et al. 2010; Dechsakulthorn et al. 2008; Fan and Lu 2005). Another paradigm is the most common nanoparticle $TiO_2$; it is extensively used in food additives and drug delivery systems in personal care products (Ray et al. 2009; Kangwansupamonkon et al. 2009).

Living beings, especially humans, are exposed to these nano-objects through inhalation, dermis, blood circulation, ingestion, and translocation to various organs and tissues (Oberdörster et al. 2005a). The passageway of nanomaterials through cell membranes and other natal barriers causing cellular dysfunction is due to their tiny size of the so-called nanostructures (Nel et al. 2006; Xia et al. 2008). The typical example in the human body is the respiratory system which is an inimitable target for the NMs toxicity as it has a dual function of inhaling and gets complete cardiac output (Ferreira et al. 2013). The inhaled nanoparticles with the help of Brownian movement are put down in the alveolar region. As the surface area of the alveoli is high and has rigorous blood contact, the target system on the subject is more likely to be exposed to environmental influences (Maynard and Kuempel 2005; Aillon et al. 2009; Chidambaram and Krishnasamy 2012). The digestive stimulation due to ingestion of NPs in the digestive tract is because of the increase in macromolecular absorption due to the massive upload of nanoparticles (Hagens et al. 2007). The skin, the body's largest organ, is the first line of defense against external aggressors. The mechanism underlying the nanoparticle's entry into the dermis is that when in contact to the outside environment, the tiny particles are expected to mount around the hair follicles and cross the threshold keen into the body (Stern and McNeil 2008).

Studies have shown that most NPs do release reactive oxygen which in turn causes oxidative stress and inflammation by the reticuloendothelial system. The outcome on inflammatory and immunological systems may perhaps result in pro-inflammatory cytotoxic activity and oxidative stress in the lungs, liver, and brain, pre-thrombosis, and paradox effects on the circulating system (Ai et al. 2011). Nanoparticles are capable of reorganizing the protein concentration which depends on the size, twist, shape and surface charge, free energy, and functionalized groups. Due to this complex binding, adverse biological outcomes arise in the course of protein unfolding, fibrillation, thiol cross-linking, and reduced enzyme activity. Another instance is the discharge of toxic ions, while the thermodynamic traits of materials favor particles suspension in a biological surrounding (Xia et al. 2008).

Though few studies have addressed the toxicity effect of nanomaterials on animals and plant cells, the mechanism relating to the toxicological studies has not yet concluded. Silver (Ag) nanoparticles produced from consumer products in the dissolved form highly sediment in aquatic bodies exerting a toxic effect on marine organisms together with bacteria, algae, fish and daphnia (Navarro et al. 2008).

NPs have a propensity to amass in the sea and hard water and are very much powered either by specific type of organic matter or other biological particles herein freshwater. The state of distribution alters the ecotoxicity; however, several abiotic factors influence the dispersion. These factors are, namely, pH and salinity, and the existence of organic substances remains to be analytically examined as a part of ecotoxicological studies (Handy et al. 2008).

## 14.6 Evaluation of Toxicity

Evaluation of nanomaterial effect on biological organisms and ecosystem showed no general concord on techniques and protocols despite the many efforts done (Reineke 2012). Many tools have been established in evaluating the toxicity of NPs. As discussed already in the above sections, engineered nanomaterials (ENMs) have more odds for toxicity as testing these materials necessitate special attention and contemplation (Dusinska et al. 2015). EMEA and FDA in Europe and the USA regulated chemicals under the process of REACH (registration, evaluation, authorization, and restriction) for nanomedicine and pharmaceutical products (Dusinska et al. 2009, 2015; Seaton et al. 2010). Several tools have been in existence for testing toxicity, preferably using in vitro, in vivo, and in silico approaches. Characterization, bioavailability, and uptake of NPs and mechanism of toxicity should be evaluated stepwise. However, huge sets of data are required for budding and confirming different strategies in case of ENMS risk assessment; this normally is based on grouping and read-across approaches (Oomen et al. 2015). Strong and consistent data can be issued by using high-throughput methods. The use of high-throughput methods in testing ENM toxicity allows the testing of several ENMs at different concentrations, cells, and conditions exposed, reduces inter-experimental variations effects, and makes considerable savings in rate and time (Collins et al. 2017).

The measurement of environmental hazards due to NPs is taken for ecotoxicity test, an alternative tool framed for assessing intrinsic dangers of chemical substances which may be freed into nature (Crane et al. 2008). Methods for testing of NMs and their impact on the environment and living systems are assembled into four categories, namely, chemical and physical characterization, a microbiological assay using prokaryotes, in vitro and in vivo assays.

In vitro and in vivo studies generally are used to test the toxicity of chemicals and to know their primary mechanism, for example, oxidative stress, immunotoxicity, and genotoxicity. Some of the said methods are already established and approved by OECD guidelines. Nevertheless, methodologies concerning dosimetry, dispersion, short of washout, uptake, and ENMs interaction with cells and tissues are to be concerned.

### 14.6.1 Physicochemical Factors of Nanotoxicology

Physical and chemical characterization of nano-objects plays a crucial role in toxicity. The size and surface area of the nanostructures function as a key cause in the occurrence of some diseases, for example, respiratory diseases. Besides the size of the particle, features such as crystallinity, surface chemistry, oxidative stress, surface coating, porosity, purity, and the longevity of particles play a significant role in nanotoxicity (Ai et al. 2011).

#### 14.6.1.1 Size

Toxicity of a particle lies basing on the size and chemical compounds. A drop in the size of nanosized objects results in enhancing the particle surface area. Consequently, a large number of chemical substances attach to the surface which in turn increases the reactivity resulting in increased toxicity (Linkov et al. 2008). An example of this type of mechanism was seen in mucus where the absorbed nanoparticles travel through tissues before reaching the bloodstream. A different study by Hyuk et al. showed 33% of 50 nm, 26% of 100 nm, and 10% of 500 nm in mucosal and lymphatic tissues of the intestine. Nanomaterials larger than 1 and 3 μm were seen as weaker and rare, respectively, in lymphatic tissues. The conclusion drawn by the researchers on particle size is that: (a) Nanoparticles <100 nm and not ≥300 nm are absorbed by intestinal cells. (b) The absorption of smaller NPs in the lymphatic tissue is greater than intestinal cell but cannot absorb particle size of 400 nm and above. (c) Nanomaterials below 500 nm are said to enter the circulatory system. Crossing the cell membranes reaching the bloodstream via many organs is because of their small size and larger surface-to-volume ratio than bigger nano-substances. Hence, this is the sole basis for the presence of more chemical molecules on the surface; this by reason gives the more toxic effect for small NPs than larger components of the same composition (Hyuk Suh et al. 2009).

#### 14.6.1.2 Particle Surface Chemistry

Nanoparticles cover a slightly high proportion of surface atoms basing to their geometry, and this ratio as well depends on the particle size, porosity, surface coarseness, and smoothness. For example, the biocompatibility of nanoparticle is higher for porous than nonporous silica. Furthermore, the hemolytic activity of the porous silica is considerably lower than nonporous (Slowing et al. 2009). Another study showed higher toxicity levels in case of Ag nanosheets judged against nanospheres and nanowire; this is because reactions on the surface were known to have large defects (George et al. 2012).

The presence of high or no impurities shows an effect on toxicity levels for a nanomaterial. Changing electrical property may vary the toxic effect. A study demonstrated the cytotoxic reactions by NPs as a source depend merely on purity. For instance, zinc and copper oxide were the two NPs upshot (Xu et al. 2010). Ease of surface plays a part in nonspecific bindings that enhances cellular uptake of NMs and is futile in the reaction rate of NMS with cells.

#### 14.6.1.3 Chemical Composition

Chemical constituents have a greater impact on NMs as they respond to other metals. Any modifications in the nanoparticle surface will reduce the toxicity. A case in point is the reduction of toxicity of nanoparticle super-paramagnetic iron oxide on the coating with pullulan (Singh et al. 2007; Clift et al. 2008; Oberdo 2010).

#### 14.6.1.4 Dose-Dependent Toxicity

The amount or quantity at which a particle or a substance enters the biological system is defined as "dose." The dose is directly proportional to exposure or concentration of a particle in the appropriate medium (e.g., air, water, food, or soil) multiplied by the duration of contact. However, the dose whether low or high is harmful to health.

#### 14.6.1.5 Aspect Ratio

Aspect ratio is defined as the ratio of length to the diameter of a particle or a substance. The higher the aspect ratio, the higher is the toxicity (Lippmann 1990). The best exemplar is the carbon-based nanoparticles (e.g., CNTs have high aspect ratio).

### 14.6.2 Nanoparticle Uptake

Uptake of NPs through barriers, for instance, the skin, blood–brain barrier, pulmonary mucosa, and placenta, can alter significantly with a decrease in size. Hence, toxicological data have to be acquired particularly for nanosized particles (Simko and Mattsson 2010; Schleh et al. 2012; Lehr et al. 2011). Reactivity boosts concurrently with the reduction of size and subsequently increased surface area. Surface area and composition robustly determine reactivity, dispersion, interaction with biological environments as well as cellular macromolecules, and as result toxicity of ENM (Warheit et al. 2008; Kunzmann et al. 2011; Dhawan and Sharma 2010). ENMs once taken have the potential to be deposited in any area of the body (Borm and Muller-Schulte 2006; Oberdörster et al. 2005b). This is mainly due to their unique factors: size and specific functionalization. NPs materialize in various shapes and also cover diverse modifications such as restricted transformations of the interface properties and modifying the dissolution and degradation by controlled changes of surface functionalization, routine stabilization in course of macromolecules absorption, utilization of oxygen, light or reducing agents from the particle surrounding in amendment of catalytic activity, and surface area enhancement for molecule adhesion by dissolution and recrystallization of reactive material (Nel et al. 2006).

## 14.7 Effect of Nanoparticles on Prokaryotes

Reports existing from the researchers show that NPs can conjugate with the biological species in nature, making the nanomaterials gain soluble properties that may have adverse effects on prokaryotic and other aquatic organisms. The interaction of carbon NPs such as fullerenes and carbon nanotubes with a biological system is well familiar mainly with DNA, RNA, phospholipids, and proteins (Ke and Qiao 2007). Kang et al. (2007) were the first to give out the connection between the break of the bacterial cell membrane and cell death with purified single-wall carbon nanotubes (SWNTs) using antimicrobial activity. Similarly, studies on the toxicity of CNTS using *Staphylococcus aureus* and *S. warneri* illustrated antimicrobial activity, inhibition of microorganism connection, and biofilm arrangement (Narayan et al. 2005). The study of Ghafari et al. (2008) reported the inability of *T. thermophila* (protozoa) to swallow and digest their prey (bacterial species), permitting free movement of SWNTs in the food chain. From the statement, it is proved that CNTs have an adverse effect on the aquatic system that eventually leads to ecological imbalance. The consequence of nanoparticles on microbes is a lot more widespread and assorted than for the plants, invertebrates, and vertebrates (Oberdörster et al. 2007).

## 14.8 Prokaryotes as Model Organism

Model organisms rather known as non-human species have turn out to be essential in biological study processes by many researchers, with anticipation that the discoveries ended in the organism model might provide insight to understand the specific phenomenon of organisms and can be studied and used to gain knowledge of other organisms or other species within their own variety giving a central pose in evolutionary development. Model organisms are in vivo models with typical characteristics including generation time, easy manipulation, accessibility, genetics, possible economic advantage, and management of mechanisms (Ankeny and Leonelli 2011). Common model organisms in use are prokaryotes, plants, protists, fungi, and animals. On one hand, microbes especially bacteria constitute as a major domain of prokaryotic organisms, with an ability to stay alive in any extreme circumstances (i.e., from optimal to extremely high environmental conditions). On the other hand, the simplest bacteria have a significant competence either to mobilize or immobilize and also is capable of reducing metal ions at the nanometer scale (Sharma et al. 2018). Synthesis of NPs such as Au, Ag, Pt, Pd, CdS, $TiO_2$, $Fe_3O_4$, and so forth can be potentially synthesized by cell biomass and cell extracts of bacteria (Iravani 2014). Few microbes, namely, *magnetotactic* and *S- layer bacteria*, are capable of synthesizing inorganic materials. A choice of bacterial species, for instance, *Bacillus cereus, E. coli, B. subtilis,* and *P. aeruginosa* has been detailed in support of removing silver, cadmium, copper, and lanthanum from solution and also including a binding facility of metallic anions and cations (Mullen et al. 1989). The impact of nanotoxicity on microorganism remains in its infancy stage. Before testing the toxicity of nanomaterials on microorganisms, it is indeed crucial to understand the physiochemical properties of the so-called nano-object (Niazi and Gu 2009).

Different perspectives are in use for evaluating the nanotoxicity with prokaryotic cells. They are, namely, disk diffusion toxicity (Ruparelia et al. 2008), minimum inhibitory concentration (Qi et al. 2004), colony viable count, viability assay for cells (Rodea-Palomares et al. 2009a, b, 2010), quantification (Cuahtecontzi-Delint et al. 2013), superoxide dismutase activity or luminescence quantification (Lyon et al. 2008; Dumas et al. 2009), and microarray hybridization assay for gene expression (Yang et al. 2009). The best and better conditions for nanomaterial determination and standardization for toxicity are pH; ion presents (cation/anion); micro- and macronutrients such as amino acids, vitamins, sugars, lipids, and nucleotides; bacterial species in use; and temperature variations. Both gram-positive and gram-negative bacteria were used as models for toxicity evaluation.

### 14.8.1 Prokaryotic Models in the Evaluation of Silver Nanotoxicity

The use of Ag in the form of silver nitrate ($AgNO_3$) as an antimicrobial agent has been recognized from centuries (Klasen 2000). Since then, the $AgNO_3$ has been exploited widely in many applications, for example, in medical and industrial products and also in domestic products such as cleaning agents, clothing, and cosmetics. Due to its varied application directly or indirectly in the living system and environment, it is mandated to determine the toxic levels. The antibacterial activity of Ag NPs depends purely on the physicochemical characterization of the substance. That is to say, Ag NPs that are more soluble are more toxic and thus are likely to release more silver ions to be bonded to sulfhydryl groups coupled with protein and low molecular weight antioxidants such as glutathione. However, by contrast, Ag NPs of less soluble also show a toxic effect by way of oxidative stress (Yang et al. 2011).

Aerobic conditions increase silver nanoparticle suspension as a result of nanomaterial oxidation (Liu and Hurt 2010; Molleman and Hiemstra 2015). This phenomenon enhances the antibacterial activity of AgNPs by the release of ionic silver (Xiu et al. 2012) and the development of ROS (reactive oxygen species) (Joshi et al. 2015). Other effects that enhance antimicrobial activity are the disruption of cell membranes due to NP membrane interaction; this, in turn, activates the uptake of silver ions freely (Taglietti et al. 2012; Bondarenko et al. 2013). Antibacterial susceptibility of silver nanomaterial is species specific (Morones et al. 2005; Tamboli and Lee 2013) with gram-negative bacteria more resistant than gram-positive microbes. Echavarri and his colleagues in their study recommended the use of natural marine microbes *Cellulophaga fucicola, Pseudoalteromonas aliena,* and *Streptomyces koyangensis* as model organisms for assessing nano-silver particle (Echavarri-Bravo et al. 2017).

A study by Bowman et al. (2012) using *E. coli*, a standard prokaryotic model, supported the statement that the toxicity of Ag NM is due to the suspension of Ag ions from the surface of the particles. Bowman et al. analyzed the toxicity of Ag NM in two different ways: one is the mortality curve based on mass concentration and total surface area of particles demonstrating the dose response, and the other way is surface area-based toxicity. The conclusion drawn from the first parameter in Bowman study showed (a) toxicity to bacteria is dependent purely on particle size, with toxicity increasing as the size of the particle decreases basing on mass concentration analysis and (b) a diverge conclusion when assessed basing on total surface area, showing no or little variation in toxicity among particles of varied sizes and with same surface area. When the same species are tested for surface area-based toxicity, it sighted that the total exposed surface area of the particle is the source driving toxicity, implying that dissolution of $Ag^+$ from the surface is causing toxicity. This statement was explained and supported by Radniecki et al. (2011) with *Nitrosomonas europaea* (gram-negative bacteria) as a model organism.

Few other species apart from the above were reported as model organisms in evaluating silver nanoparticles. They are *Shewanella oneidensis* (Suresh et al. 2010), nitrifying bacteria (Choi and Hu 2008), and *P. putida* (Fabrega et al. 2009) with standard protocols such as live/dead viability assay using flow cytometry, cytotoxicity assay by spectrophotometer at 600 nm, and also accordingly by disk diffusion method.

### 14.8.2 Prokaryotic Models in the Evaluation of Inorganic Nanomaterials

Zinc oxide (ZnO) is one of the currently used compounds in the food and drug administration. It plays an important role in treating zinc deficiency (Lopes de Romana et al. 2002). Some studies concluded antibacterial activity of ZnO which characterizes ROS generation (Sawai 2003; Sawai and Yoshikawa 2004) and also is ably a strong component resisting microorganisms (Hirota et al. 2010). However, by contrast, ZnO as a nanoparticle has a toxic effect on living organisms. The antibacterial activity by microtiter plate method with E. coli, *P. aeruginosa,* and *S. aureus* is tested for ZnO nanotoxicity (Premanathan et al. 2011). ZnO toxicity assessment using *Salmonella typhimurium* as the model organism was reported by the use of the Ames test (Yoshida et al. 2009) and cytotoxicity assay (Wahab et al. 2010). Other microbial species, namely, *Streptococcus agalactiae* (Huang et al. 2008), *Vibrio fischeri* (Mortimer et al. 2008; Heinlaan et al. 2008), *Mycobacterium smegmatis, Shewanella oneidensis, Cyanothece* (Wu et al. 2010), *Thalassiosira pseudonana, Chaetoceros gracilis,* and *Klebsiella pneumonia* (Wahab et al. 2010), as model organisms were shown to be detailed in assessing the toxicity of ZnO nanomaterial via cytotoxicity assay, luminescence inhibition test, and growth inhibition assay.

Nano-objects titanium oxide ($TiO_2$) has also been used as an antibacterial agent despite its particle size, but this activity is enhanced when carried in nanoparticulate form. $TiO_2$ nanotoxicity using various methods such as cell viability assay, lipid peroxidation assay, cellular respiration determination test, cytotoxicity assay using spectrophotometer, and Ames test was studied using prokaryotic bacterial species specifically *E. coli* (Maness et al. 1999; Adams et al. 2006), *S. typhimurium* (Kumar et al. 2011), *S. aureus* (Mortimer et al. 2008), *B. subtilis* (Adams et al. 2006), and *Cupriavidus metallidurans* (Simon-Deckers et al. 2009).

*E. coli* and *S. aureus* as a model organisms were used in evaluating nanotoxicity for magnesium oxide using halo test and conductance assay for cytotoxicity (Sawai et al. 2000), standard plate count method, and also spectroscopic method (Jones et al. 2008). Other inorganic nanomaterials evaluated for toxicity using prokaryotic bacterial species are $SiO_2$ (*E. coli* and *B. subtilis*), $Al_2O_3$ (*E. coli, C. metallidurans,* and *S. typhimurium*), and $Co_3O_4$ (*S. typhimurium*).

In case of CuO, both prokaryotic algae (*Microcystis aeruginosa*) and prokaryotic bacteria (*S. typhimurium* and *S. aureus*) (Wang et al. 2011; Pan et al. 2010; Jones et al. 2008) were used as models for toxicological evaluation for nanotoxicity.

### 14.8.3 Prokaryotic Models in the Evaluation of Carbon-Based Nanotoxicity

Carbon 60 (C60), also known as fullerene, has been reported to slow down the antimicrobial activity (Fortner et al. 2005). However, this statement remained insufficient to prove that all nanoparticles have an antimicrobial activity or all NPs are toxic to an organism in the environment. C60 NP toxicological evaluation by *B. subtilis* using spectroscopy method at 600 nm for cytotoxicity was studied by Lyon et al. (2006). The study concluded that C60 exhibited antimicrobial activity with a minimum inhibition concentration of 0.5 ± 0.13 mg/L.

Carbon nanotubes which are the most widely used carbon nanomaterials are actively engineered nanomaterials. The NM toxicity is evaluated via dead discrimination assay by flow cytometry using PI dye with *E. coli* species as a model organism. The study gives an outcome where different shapes of CNTs exhibit growth inhibition (Kang et al. 2008).

## 14.9 Conclusion

Though nanomaterials have been beneficial with its increased application in industrial and medical health, it is shown to have harmful effects on the environment and life forms. Over a decade, extensive exploration on nanomaterials and its consequence turned out to be a major challenge. Outlining the mechanism or the exact process of the nanoparticles causing toxicity is still unclear, and estimating the overall scenario remains difficult.

This chapter, in detail, illustrated an overview of the nanoparticle origin and classification, its toxicity and effect on the biological system, and the use of the prokaryotic model for assessing the toxicity. Prokaryotic models, especially bacteria, have been a significant module due to their unique properties such as low production time, simple structure, nonpathogenic nature, and functional organization. Many studies have evaluated the toxicity of nanoparticles with microorganisms as they are the first source in the food web of all known ecosystems and also could help to extrapolate the understanding of nanomaterials on the environment and higher organisms. In order to study more about the biological toxicology of NPs, the particle characterization, uptake, and different assays are to be well learned to help assess toxicity and synthesize many additional green nanoparticles for the betterment of the society and environment.

## References

Adams LK, Lyon DY, Alvarez PJJ (2006) Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. Water Res 40(19):3527–3532. https://doi.org/10.1016/j.watres.2006.08.004

Ai J, Biazar E, Jafarpour M et al (2011) Nanotoxicology and nanoparticle safety in biomedical designs. Int J Nanomedicine 6:1117–1127

Aillon KL, Xie Y, El-Gendy N et al (2009) Effects of nanomaterial physicochemical properties on *in vivo* toxicity. Adv Drug Deliv Rev 61(6):457–466. https://doi.org/10.1016/j.addr.2009.03.010

Alivisatos P (2004) The use of nanocrystals in biological detection. Nat Biotechnol 22(1):47–52. https://doi.org/10.1038/nbt927

Ankeny RA, Leonelli S (2011) What's so special about model organisms? Stud Hist Philos Sci Part A 42(2):313–323. https://doi.org/10.1016/j.shpsa.2010.11.039

Bhatia S (2016) Nanoparticles types, classification, characterization, fabrication methods and drug delivery applications in natural polymer drug delivery systems. Springer, Cham, pp 33–93

Bhaviripudi S, Mile E, Steiner SA et al (2007) CVD synthesis of single-walled carbon nanotubes from gold nanoparticle catalysts. J Am Chem Soc 129(6):1516–1517. https://doi.org/10.1021/ja0673332

Biswas P, Wu CY (2005) Nanoparticles and the environment. J Air Waste Manage Assoc 55(6):708–746. https://doi.org/10.1080/10473289.2005.10464656

Blinova I, Ivask A, Heinlaan M et al (2010) Ecotoxicity of nanoparticles of CuO and ZnO in natural water. Environ Pollut 158(1):41–47. https://doi.org/10.1016/j.envpol.2009.08.017

Bondarenko O, Ivask A, Käkinen A et al (2013) Particle-cell contact enhances antibacterial activity of silver nanoparticles. PLoS One 8(5):e64060. https://doi.org/10.1371/journal.pone.0064060

Borm PJ, Muller-Schulte D (2006) Nanoparticles in drug delivery and environmental exposure: same size, same risks? Nanomedicine (Lond) 1(2):235–249. https://doi.org/10.2217/17435889.1.2.235

Bowman CR, Bailey FC, Elrod-Erickson M et al (2012) Effects of silver nanoparticles on zebrafish (*Danio rerio*) and *Escherichia coli* (ATCC 25922): a comparison of toxicity based on total surface area versus mass concentration of particles in a model eukaryotic and prokaryotic system. Environ Toxicol Chem 31(8):1793–1800. https://doi.org/10.1002/etc.1881

Brar SK, Verma M, Tyagi RD et al (2010) Engineered nanoparticles in wastewater and wastewater sludge–evidence and impacts. Waste Manag 530(3):504–520. https://doi.org/10.1016/j.wasman.2009.10.012

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

Chidambaram M, Krishnasamy K (2012) Nanotoxicology: toxicity of engineered nanoparticles and approaches to produce safer nanotherapeutics. Int J Pharm Sci 2(4):117–122

Choi O, Hu Z (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ Sci Technol 42(12):4583–4588. https://doi.org/10.1021/es703238h

Clift MJ, Rothen-Rutishauser B, Brown DM et al (2008) The impact of different nanoparticle surface chemistry and size on uptake and toxicity in a murine macrophage cell line. Toxicol Appl Pharmacol 232(3):418–427. https://doi.org/10.1016/j.taap.2008.06.009

Collins AR, Annangi B, Rubio L et al (2017) High throughput toxicity screening and intracellular detection of nanomaterials. Wiley Interdiscip Rev Nanomed Nanobiotechnol 9(1):e1413. https://doi.org/10.1002/wnan.1413

Crane M, Handy R, Garrod J et al (2008) Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. Ecotoxicology 17(5):421–437. https://doi.org/10.1007/s10646-008-0215-z

Cuahtecontzi-Delint R, Mendez-Rojas MA, Bandala ER et al (2013) Enhanced antibacterial activity of $CeO_2$ nanoparticles by surfactants. Int J Chem React Eng 11:1–5

Cui Y, Lieber CM (2001) Functional nanoscale electronic devices assembled using silicon nanowire building blocks. Science 291(5505):851–853. https://doi.org/10.1126/science.291.5505.851

Cui Y, Wei Q, Park H et al (2001) Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. Science 293(5533):1289–1292. https://doi.org/10.1126/science.1062711

Dechsakulthorn F, Hayes A, Bakand S et al (2008) *In vitro* cytotoxicity assessment of selected nanoparticles using human skin fibroblasts. AATEX 14:397–400

Dhawan A, Sharma V (2010) Toxicity assessment of nanomaterials: methods and challenges. Anal Bioanal Chem 398(2):589–605. https://doi.org/10.1007/s00216-010-3996-x

Duan X, Huang Y, Cui Y et al (2001) Indium phosphide nanowires as building blocks for nanoscale electronic and optoelectronic devices. Nature 409(6816):66–69. https://doi.org/10.1038/35051047

Dumas EM, Ozenne V, Mielke RE et al (2009) Toxicity of CdTe quantum dots in bacterial strains. IEEE Trans Nanobioscience 8(1):58–64. https://doi.org/10.1109/TNB.2009.2017313

Dusinska M, Dusinska M, Fjellsbø LM et al (2009) Testing strategies for the safety of nanoparticles used in medical applications. Nanomedicine (Lond) 4(6):605–607. https://doi.org/10.2217/nnm.09.47

Dusinska M, Boland S, Saunders M et al (2015) Towards an alternative testing strategy for nanomaterials used in nanomedicine: lessons from NanoTEST. Nanotoxicol 9(S1):118–132. https://doi.org/10.3109/17435390.2014.991431

Echavarri-Bravo V, Paterson L, Aspray TJ et al (2017) Natural marine bacteria as model organisms for the hazard-assessment of consumer products containing silver nanoparticles. Marine Environ Res 130:293–302. https://doi.org/10.1016/j.marenvres.2017.08.006

Fabrega J, Fawcett SR, Renshaw JC et al (2009) Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. Environ Sci Technol 43(19):7285–7290. https://doi.org/10.1021/es803259g

Fan Z, Lu JG (2005) Zinc oxide nanostructures: synthesis and properties. J Nanosci Nanotechnol 5(10):1561–1573. https://doi.org/10.1166/jnn.2005.182

Ferreira AJ, Cemlyn-Jones J, Cordeiro CR (2013) Nanoparticles, nanotechnology and pulmonary nanotoxicology. Rev Port Pneumol 19(1):28–37. https://doi.org/10.1016/j.rppnen.2013.01.004

Fortner JD, Lyon DY, Sayes CM et al (2005) C60 in water: nanocrystal formation and microbial response. Environ Sci Technol 39(11):4307–4316

George S, Lin S, Ji Z et al (2012) Surface defects on plate-shaped silver nanoparticles contribute to its hazard potential in a fish gill cell line and zebrafish embryos. ACS Nano 6(5):3745–3759. https://doi.org/10.1021/nn204671v

Ghafari P, St-Denis CH, Power ME et al (2008) Impact of carbon nanotubes on the ingestion and digestion of bacteria by ciliated protozoa. Nat Nanotechnol 3(6):347–351. https://doi.org/10.1038/nnano.2008.109

Hagens WI, Oomen AG, de Jong WH et al (2007) What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul Toxicol Pharmacol 49(3):217–229. https://doi.org/10.1016/j.yrtph.2007.07.006

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

Haynes CL (2010) The emerging field of nanotoxicology. Anal Bioanal Chem 398:587–588. https://doi.org/10.1007/s00216-010-3972-5

Heinlaan M, Ivask A, Blinova I et al (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71(7):1308–1316

Hickey RJ, Meng X, Zhang P et al (2013) Low-dimensional nanoparticle clustering in polymer micelles and their transverse relaxivity rates. ACS Nano 7(7):5824–5833. https://doi.org/10.1016/j.toxlet.2007.08.009

Hirota K, Sugimoto M, Kato M et al (2010) Preparation of zinc oxide ceramics with a sustainable antibacterial activity under dark conditions. Ceram Int 36(2):497–506. https://doi.org/10.1021/nn400824b

Hochella MF, Spencer MG, Jones KL (2015) Nanotechnology: nature's gift or scientists' brainchild? Environ Sci Nano 2(2):114–119. https://doi.org/10.1039/C4EN00145A

Huang Y, Duan X, Cui Y et al (2001) Logic gates and computation from assembled nanowire building blocks. Science 294(5545):1313–1317. https://doi.org/10.1126/science.1066192

Huang Z, Zheng X, Yan D et al (2008) Toxicological effect of ZnO nanoparticles based on bacteria. Langmuir 24(8):4140–4144. https://doi.org/10.1021/la7035949

Hyuk Suh W, Suslick SK, Stucky Galen D et al (2009) Nanotechnology, nanotoxicology, and neuroscience. Prog Neurobiol 87(3):133–170

Iravani S (2014) Bacteria in nanoparticle synthesis: current status and future prospects. Int Sch Res Notices 2014:1–18. https://doi.org/10.1155/2014/359316

Jibowu T (2016) The formation of doxorubicin loaded targeted nanoparticles using nanoprecipitation, double emulsion and single emulsion for cancer treatment. J Nanomed Nanotechnol 7(379):1–7. https://doi.org/10.4172/2157-7439.1000379

Jones N, Ray B, Ranjit KT et al (2008) Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett 279(1):71–76. https://doi.org/10.1111/j.1574-6968.2007.01012.x

Joshi N, Ngwenya BT, Butler IB et al (2015) Use of bioreporters and deletion mutants reveals ionic silver and ROS to be equally important in silver nanotoxicity. J Hazard Mater 287:51–58. https://doi.org/10.1016/j.jhazmat.2014.12.066

Kang S, Pinault M, Pfefferle LD et al (2007) Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir 23(17):8670–8673. https://doi.org/10.1021/la701067r

Kang S, Mauter MS, Elimelech M (2008) Physicochemical determinants of multiwalled carbon nanotube bacterial cytotoxicity. Environ Sci Technol 42(19):7528–7534. https://doi.org/10.1021/es8010173

Kangwansupamonkon W, Lauruengtana V, Surassmo S et al (2009) Antibacterial effect of apatite-coated titanium dioxide for textiles applications. Nanomedicine 5(2):240–249. https://doi.org/10.1016/j.nano.2008.09.004

Karlsson HL, Gustafsson J, Cronholm P et al (2009) Size-dependent toxicity of metal oxide particles—a comparison between nano-and micrometer size. Toxicol Lett 188(2):112–118. https://doi.org/10.1016/j.toxlet.2009.03.014

Ke PC, Qiao R (2007) Carbon nanomaterials in biological systems. J Phys Condens Matter 9(37):373101. https://doi.org/10.1088/0953-8984/19/37/373101

Klasen HJ (2000) A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns 26(2):131–138. https://doi.org/10.1016/S0305-4179(99)00116-3

Kong J, Dai H (2001) Full and modulated chemical gating of individual carbon nanotubes by organic amine compounds. J Phys Chem B 105(15):2890–2893. https://doi.org/10.1021/jp0101312

Kong J, Franklin NR, Zhou C et al (2000) Nanotube molecular wires as chemical sensors. Science 287(5453):622–625. https://doi.org/10.1126/science.287.5453.622

Kumar N, Kumbhat S (2016) Essentials in nanoscience and nanotechnology. Wiley, Hoboken, NJ, pp 189–236

Kumar A, Pandey AK, Singh SS et al (2011) Cellular uptake and mutagenic potential of metal oxide nanoparticles in bacterial cells. Chemosphere 83(8):1124–1132. https://doi.org/10.1016/j.chemosphere.2011.01.025

Kunzmann A, Andersson B, Thurnherr T et al (2011) Toxicology of engineered nanomaterials: focus on biocompatibility, biodistribution and biodegradation. Biochim Biophys Acta 1810(3):361–373. https://doi.org/10.1016/j.bbagen.2010.04.007

Laurent S, Forge D, Port M et al (2008) Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem Rev 108(6):2064–2110. https://doi.org/10.1021/cr068445e

Lehr CM, Daum N, Schneider M et al (2011) Biological barriersda need for novel tools in nanotoxicology and nanomedicine. Preface Eur J Pharm Biopharm 77:337. https://doi.org/10.1016/j.ejpb.2011.02.006

Linkov I, Satterstrom FK, Corey LM (2008) Nanotoxicology and nanomedicine: making hard decisions. Nanomedicine 4(2):167–171. https://doi.org/10.1016/j.nano.2008.01.001

Lippmann M (1990) Effects of fiber characteristics on lung deposition, retention, and disease. Environ Health Perspect 88:311–317. https://doi.org/10.1289/ehp.9088311

Liu J, Hurt RH (2010) Ion release kinetics and particle persistence in aqueous nano-silver colloids. Environ Sci Technol 44(6):2169–2175. https://doi.org/10.1021/es9035557

Lopes de Romana D, Brown KH, Guinard JX (2002) Sensory trial to assess the acceptability of zinc fortificants added to iron-fortified wheat products. J Food Sci 67(1):461–465. https://doi.org/10.1111/j.1365-2621.2002.tb11429.x

Lowry GV, Kelvin B, Simon CA et al (2012) Transformations of nanomaterials in the environment. Environ Sci Technol 46(13):6893–6899. https://doi.org/10.1021/es300839e

Lyon DY, Adams LK, Falkner JC et al (2006) Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size. Environ Sci Technol 40(14):4360–4366. https://doi.org/10.1021/es0603655

Lyon DY, Brunet L, Hinkal GW et al (2008) Antibacterial activity of fullerene water suspensions (nC60) is not due to ROS-mediated damage. Nano Lett 8(5):1539–1543. https://doi.org/10.1021/nl0726398

Maness PC, Smolinski S, Blake DM et al (1999) Bactericidal activity of photocatalytic TiO(2) reaction: toward an understanding of its killing mechanism. Appl Environ Microbiol 65(9):4094–4098

Maynard AD, Kuempel ED (2005) Airborne nanostructured particles and occupational health. J Nanopart Res 7(6):587–614. https://doi.org/10.1007/s11051-005-6770-9

Maynard AD, Warheit DB, Philbert MA (2011) The new toxicology of sophisticated materials: nanotoxicology and beyond. Toxicol Sci 120(S1):S109–S129. https://doi.org/10.1093/toxsci/kfq372

McDonald SA, Konstantatos G, Zhang S et al (2005) Solution-processed PbS quantum dot infrared photodetectors and photovoltaics. Nat Mater 4(2):138–142. https://doi.org/10.1038/nmat1299

Molleman B, Hiemstra T (2015) Surface structure of silver nanoparticles as a model for understanding the oxidative dissolution of silver ions. Langmuir 31(49):13361–13372. https://doi.org/10.1021/acs.langmuir.5b03686

Morones JR, Elechiguerra JL, Camacho A et al (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16(10):2346. https://doi.org/10.1088/0957-4484/16/10/059

Mortimer M, Kasemets K, Heinlaan M et al (2008) High throughput kinetic Vibrio fischeri bioluminescence inhibition assay for study of toxic effects of nanoparticles. Toxicol In Vitro 22(5):1412–1417. https://doi.org/10.1016/j.tiv.2008.02.011

Mullen MD, Wolf DC, Ferris FG et al (1989) Bacterial sorption of heavy metals. Appl Environ Microbiol 55(12):3143–3149

Narayan RJ, Berry CJ, Brigmon RL (2005) Structural and biological properties of carbon nanotube composite films. Mater Sci Eng B 123(2):123–129. https://doi.org/10.1016/j.mseb.2005.07.007

Navarro E, Baun A, Behra R et al (2008) Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicol 17(5):372–386. https://doi.org/10.1007/s10646-008-0214-0

Nel A, Xia T, Mädler L, Li N (2006) Toxic potential of materials at nanolevel. Science 311(5761):622–627. https://doi.org/10.1126/science.1114397

Niazi JH, Gu MB (2009) Toxicity of metallic nanoparticles in microorganisms-a review. In: Kim YJ, Platt U, Gu MB, Iwahashi H (eds) Atmospheric and biological environmental monitoring. Springer, Dordrecht, pp 193–206. https://doi.org/10.1007/978-1-4020-9674-7_12

Novoselov KS, Geim AK, Morozov SV et al (2004) Electric field effect in atomically thin carbon films. Science 306(5696):666–669. https://doi.org/10.1126/science.1102896

Oberdo G (2010) Safety assessment for nanotechnology and nanomedicine: concepts of anotoxicology. J Intern Med 267(1):89–105. https://doi.org/10.1111/j.1365-2796.2009.02187.x

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 Fibre Toxicol 2(1):8. https://doi.org/10.1186/1743-8977-2-8

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

Oberdörster G, Oberdörster E, Oberdörster J (2007) Concepts of nanoparticle dose metric and response metric. Environ Health Perspect 115(6):A290–A294. https://doi.org/10.1289/ehp.115-1892118

Oomen A, Bleeker E, Bos P et al (2015) Grouping and read-across approaches for risk assessment of nanomaterials. Int J Environ Res Public Health 12(10):13415–13434. https://doi.org/10.3390/ijerph121013415

Pan X, Redding JE, Wiley PA et al (2010) Mutagenicity evaluation of metal oxide nanoparticles by the bacterial reverse mutation assay. Chemosphere 79(1):113–116. https://doi.org/10.1016/j.chemosphere.2009.12.056

Pokropivny VV, Skorokhod VV (2007) Classification of nanostructures by dimensionality and concept of surface forms engineering in nanomaterial science. Mater Sci Eng C 27(5–8):990–993

Premanathan M, Karthikeyan K, al JK (2011) Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomedicine 7(2):184–192. https://doi.org/10.1016/j.nano.2010.10.001

Qi L, Xu Z, Jiang X et al (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res 339:2693–2700. https://doi.org/10.1016/j.carres.2004.09.007

Radniecki TS, Stankus DP, Neigh A et al (2011) Influence of liberated silver from silver nanoparticles on nitrification inhibition of Nitrosomonas europaea. Chemosphere 85(1):43–49. https://doi.org/10.1016/j.chemosphere.2011.06.039

Ray PC, Yu H, Fu PP (2009) Toxicity and environmental risks of nanomaterials: challenges and future needs. J Environ Sci Health Part C 27(1):1–35. https://doi.org/10.1080/10590500802708267

Reineke J (2012) Nanotoxicity: methods and protocols. Humana Press, Totowa, NJ

Rodea-Palomares I, Fernández-Piñas F, González-García C et al (2009a) Use of lux-marked cyanobacterial bioreporters for assessment of individual and combined toxicities of metals in aqueous samples. In: Handbook on cyanobacteria: biochemistry, biotechnology and applications. Nova Science Publishers, New York, pp 283–304

Rodea-Palomares I, Gonzalez-Garcia C, Leganes F et al (2009b) Effect of pH, EDTA, and anions on heavy metal toxicity toward a bioluminescent cyanobacterial bioreporter. Arch Environ Contam Toxicol 57(3):477–487. https://doi.org/10.1007/s00244-008-9280-9

Rodea-Palomares I, Petre AL, Boltes K et al (2010) Application of the combination index (CI)–isobologram equation to study the toxicological interactions of lipid regulators in two aquatic bioluminescent organisms. Water Res 44(2):427–438. https://doi.org/10.1016/j.watres.2009.07.026

Ruparelia JP, Chatteriee AK, Duttagupta SP et al (2008) Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater 4(3):707–716. https://doi.org/10.1016/j.actbio.2007.11.006

Sawai J (2003) Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J Microbiol Methods 54(2):177–182. https://doi.org/10.1016/S0167-7012(03)00037-X

Sawai J, Yoshikawa T (2004) Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. J Appl Microbiol 96(4):803–809. https://doi.org/10.1111/j.1365-2672.2004.02234.x

Sawai J, Kojima H, Igarashi H et al (2000) Antibacterial characteristics of magnesium oxide powder. World J Microbiol Biotechnol 16(2):187–194. https://doi.org/10.1023/A:1008916209784

Schleh C, Semmler-Behnke M, Lipka J et al (2012) Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration. Nanotoxicol 6(1):36–46. https://doi.org/10.3109/17435390.2011.552811

Seaton A, Tran L, Aitken R et al (2010) Nanoparticles, human health hazard and regulation. J R Soc Interface 7(S1):S119–S129. https://doi.org/10.1098/rsif.2009.0252.focus

Sharma VK, Filip J, Zboril R et al (2015) Natural inorganic nanoparticles–formation, fate, and toxicity in the environment. Chem Soc Rev 44(23):8410–8423. https://doi.org/10.1039/C5CS00236B

Sharma G, Pandey S, Ghatak S et al (2018) Potential of spectroscopic techniques in the characterization of "green nanomaterials". Nanomater Plants Algae Microorganisms 1:59–77. https://doi.org/10.1016/B978-0-12-811487-2.00003-7

Simko M, Mattsson MO (2010) Risks from accidental exposures to engineered nanoparticles and neurological health effects: a critical review. Part Fibre Toxicol 7(1):42. https://doi.org/10.1186/1743-8977-7-42

Simon-Deckers A, Loo S, Mayne-L'Hermite M et al (2009) Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. Environ Sci Technol 43(21):8423–8429. https://doi.org/10.1021/es9016975

Singh S, Shi T, Duffin R et al (2007) Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine $TiO_2$: role of the specific surface area and of surface methylation of the particles. Toxicol Appl Pharmacol 222(2):141–151. https://doi.org/10.1016/j.taap.2007.05.001

Singh S, Vishwakarma K, Singh S et al (2017) Understanding the plant and nanoparticle interface at transcriptomic and proteomic level: a concentric overview. Plant Gene 11(B):265–272. https://doi.org/10.1016/j.plgene.2017.03.006

Slowing II, Wu CW, Vivero-Escoto JL et al (2009) Mesoporous silica nanoparticles for reducing hemolytic activity towards mammalian red blood cells. Small 5(1):57–62. https://doi.org/10.1002/smll.200800926

Stern ST, McNeil SE (2008) Nanotechnology safety concerns revisited. Toxicol Sci 101(1):4–21. https://doi.org/10.1093/toxsci/kfm169

Suresh AK, Pelletier DA, Wang W et al (2010) Silver nanocrystallites: biofabrication using *Shewanella oneidensis*, and an evaluation of their comparative toxicity on gram-negative and grampositive bacteria. Environ Sci Technol 44(13):5210–5215. https://doi.org/10.1021/es903684r

Taglietti A, Diaz Fernandez YA, Amato E et al (2012) Antibacterial activity of glutathione-coated silver nanoparticles against gram positive and gram negative bacteria. Langmuir 28(21):8140–8148. https://doi.org/10.1021/la3003838

Tamboli DP, Lee DS (2013) Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram negative bacteria. J Hazard Mater 260:878–884. https://doi.org/10.1016/j.jhazmat.2013.06.003

Tiwari DK, Behari J, Sen P (2008) Application of nanoparticles in waste water treatment. World Appl Sci J 3(3):417–433

Tiwari JN, Tiwari RN, Kim KS (2012) Zero-dimensional, one-dimensional, two-dimensional and three-dimensional nanostructured materials for advanced electrochemical energy devices. Prog Mater Sci 57(4):724–803. https://doi.org/10.1016/j.pmatsci.2011.08.003

Tiwari M, Sharma NC, Fleischmann P et al (2017) Nanotitania exposure causes alterations in physiological, nutritional and stress responses in tomato (*Solanum lycopersicum*). Front Plant Sci 8:633. https://doi.org/10.3389/fpls.2017.00633

Tolani SB, Craig M, DeLong RK et al (2009) Towards biosensors based on conducting polymer nanowires. Anal Bioanal Chem 393(4):1225–1231. https://doi.org/10.1007/s00216-008-2556-0

Tripathi DK, Singh S, Singh VP et al (2016) Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultiver and hybrid differing in arsenate tolerance. Fron Environ Sci 4:46. https://doi.org/10.3389/fenvs.2016.00046

Wahab R, Mishra A, Yun SI et al (2010) Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. Appl Microbiol Biotechnol 87(5):1917–1925. https://doi.org/10.1007/s00253-010-2692-2

Wang ZL (2000) Characterizing the structure and properties of individual wire-like nanoentities. Adv Mater 12(17):1295–1298. https://doi.org/10.1002/1521-4095(200009)

Wang Z, Li J, Zhao J, Xing B (2011) Toxicity and internalization of CuO nanoparticles to prokaryotic alga *Microcystis aeruginosa* as affected by dissolved organic matter. Environ Sci Technol 45(14):6032–6040. https://doi.org/10.1021/es2010573

Warheit DB, Sayes CM, Reed KL et al (2008) Health effects related to nanoparticle exposures: environmental, health and safety considerations for assessing hazards and risks. Pharmacol Ther 120(1):35–42. https://doi.org/10.1016/j.pharmthera.2008.07.001

Wiesner MR, Lowry GV, Jones KL et al (2009) Decreasing uncertainties in assessing environmental exposure, risk. and ecological implications of nanomaterials Environ Sci Technol 43:6458–6462. https://doi.org/10.1021/es803621k

Wu B, Wang Y, Lee YH et al (2010) Comparative eco-toxicities of nano-ZnO particles under aquatic and aerosol exposure modes. Environ Sci Technol 44(4):1484–1489. https://doi.org/10.1021/es9030497

Xia Y, Yang P, al SY (2003) One-dimensional nanostructures: synthesis, characterization, and applications. Adv Mater 15(5):353–389. https://doi.org/10.1002/adma.200390087

Xia T, Kovochich M, Liong M et al (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2(10):2121–2134. https://doi.org/10.1021/nn800511k

Xiu ZM, Zhang QB, Puppala HL et al (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett 12(8):4271–4275. https://doi.org/10.1021/nl301934w

Xu M, Fujita D, Kajiwara S et al (2010) Contribution of physicochemical characteristics of nano-oxides to cytotoxicity. Biomaterials 31(31):8022–8031. https://doi.org/10.1016/j.biomaterials.2010.06.022

Yang S, Pappas KM, Hauser LJ et al (2009) Improved genome annotation for Zymomonas mobilis. Nat Biotechnol 27(10):893–894. https://doi.org/10.1038/nbt1009-893

Yang X, Gondikas AP, Marinakos SM et al (2011) Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in *Caenorhabditis elegans*. Environ Sci Technol 46(2):1119–1127. https://doi.org/10.1021/es202417t

Yoshida R, Kitamura D, Maenosono S (2009) Mutagenicity of water-soluble ZnO nanoparticles in Ames test. J Toxicol Sci 34(1):119–122. https://doi.org/10.2131/jts.34.119