

Toxicity of metal nanoparticles with a focus on silver

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Properties of nanoparticles are significantly different from their bulk counterparts, including their biological and toxic activity. The properties of silver nanoparticles governing their toxicological effects are summarized. These basic characteristics can be used as descriptors for modelling the toxic properties of nanoparticles.

Within the past decade, nanomaterials have been deeply integrated into our everyday life. There are numerous examples of already established and possible applications of nanoparticles (NPs). According to the recent research¹ over 1000 different manufactured NPs were developed and introduced to the market. Some of them, after being released to the environment, may cause toxic effects in terraneous and aquatic organisms. Although the nanotechnology field is growing rapidly (Figure 1), all the potential harmful effects of nanomaterials on human's health or the environment have not yet been identified.² Thus, there is a clear need for assessment of such potentially dangerous toxic effects in a short-term period.

Inorganic metal-based nanomaterials are one of the emerging areas of modern knowledge and technology and already found an ever increasing number of applications. Metal NPs (nanopowders, free-standing or supported, noble metals, as well as Ni, Fe) are used as catalysts, sensors, components of electronic materials

and devices, drug delivery agents and other medical applications, materials for production of special alloys, and as materials for remediation of ground water and soil. The main purpose of this article is to give a brief overview of the data about toxicity of metal NPs, in particular, silver NPs, as the most representative type of metal NPs. Silver is widely used as an anti-bacterial agent and an additive in customer products and pharmacy. The short list of applications of silver NPs is presented below:^{3,4}

- medical products;
- medical treatment;
- computer hardware;
- household application;
- mobile devices;
- products of personal care;
- cleaning processes;
- filtration processes;
- storage equipment;
- supplements;
- coating substances;
- baby care;
- furnishing;
- textile;
- wash and dishwashing machine;
- food packaging;
- healthcare;
- detergents
- sensors;
- solar cells, etc.

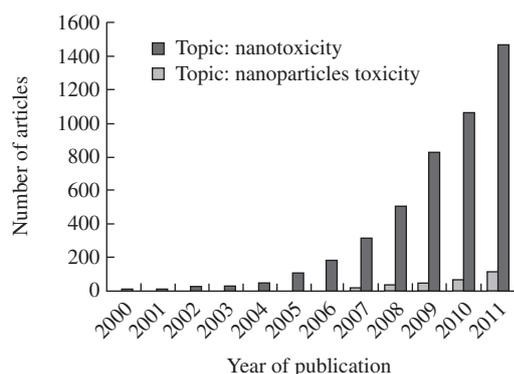


Figure 1 Number of publications per year indexed on Web of Science topically related to NPs toxicity.

Such a broad variety of applications opens a lot of possibilities to contact and penetrate into the human body, including dermal exposure, ingestion and inhalation. Besides leaking nanosilver



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particles can lead to their accumulation in wastes and they are released to the environment with wastewater. In reservoirs with stagnant water, NPs can settle down and their concentration can exceed the allowable value. As a part of sediments, they can be involved in the trophic chains as a food for bottom animals and go up through the chain. Another point of concern is the long-term consequences of such accumulation. It is known that the toxicity of silver NPs can increase with the course of time due to dissolution leading to the release of silver ions.⁵ The latter process produces additional toxic effects, because it leads to the increased concentration of silver ions in the environment. The silver ion is the most toxic form of silver.⁶

General matters

The Organization for Economic Co-operation and Development (OECD) has specified a number of physical and chemical properties of NPs that are probably most important for toxicity testing:⁷

- agglomeration/aggregation;
- water solubility;
- crystalline phase;
- dustiness;
- crystallite size;
- morphology;
- particle size distribution;
- specific surface area;
- zeta potential (surface charge);
- surface chemistry;
- photocatalytic activity;
- pour density;
- porosity;
- octanol–water partition coefficient;
- redox potential;
- radical formation potential.

Such characteristics as the particle size and the size distribution in the wet and dry state and purity of the sample are extremely important to consider in hazard studies.⁸

A single particle characteristic cannot be an overwhelming indicator of nanotoxicity. One has to select the right combination of properties providing a well-described toxic profile. Some characteristics play a significant role in the interaction of NPs with environment, others are less important or can be described through the first-priority set. Now we will consider the most important properties from toxicological point of view (from the top list) for NPs one by one with a focus on silver NPs.

Chemical composition

Usually there is no direct correlation between the toxicity of NPs and the toxic properties of bulk materials.^{9,10} However, the composition of NPs is an important parameter.¹¹ It is expected that the toxicity of metal-containing NP should follow the trends:

Metals: Au < Pt < Fe < Ir, Rh < Pd < Co < Ni < Ag < Os

Oxides: Al₂O₃ < MgO < ZrO₂ < SiO₂ < TiO₂ < others

Chalcogenides and similar materials (III–V semiconductors): ZnS < GaN < GaP < CdS < CdSe < GaAs

Until now, no sufficiently integrated classification for all nanomaterials was developed. Some classes have been suggested, but it is not quite well established. The purity of the nanomaterials is a very important issue. Any impurity may affect the initial characteristics of the pure material.

Average particle size and size distribution

Nanoscale materials are defined as materials that have a feature scale at least in one dimension less than 100 nanometers. This

parameter seems to be at least as important as the chemical composition as far as we move towards the nano-range, and the dependences of the toxicity of diverse inorganic materials *versus* the particle size can exhibit either a volcano-shape behaviour or an exponential curve descending with the increase in the particle size.

These properties are common for all nano-objects. In most experimental works, researchers perform experiments using only a limited number of the mono-dispersed samples, or even multi-dispersed samples. In the latter case, they look at the size distribution and work with an average (median) size. Panacek *et al.*^{12,13} studied the influence of the size and different stabilizers on the antibacterial activity of nano-silver and found that this activity is dependent on the size of silver particles. This dependence was observed for the interval between 25 and 50 nm. Other authors¹⁴ assume that the smallest particles (5 nm) may be responsible for the toxic effect of NPs. Unfortunately, the impact of this factor is still not well rationalized. Though a great number of studies are based on this correlation, most of researchers compare the influence of the size on the toxicity of the NPs and bulk materials.

But what happens with the NP in a medium or in tissues? Are silver NPs present as isolated particles, agglomerates, or dissolved ions?

Agglomeration state

Considering the particle size effects on the toxicity, one should not forget about possible agglomeration and aggregation. Agglomeration is the process in which the nano-sized particles clump together to form much bigger particles of substances, causing the loss of the original small size. The main property of NPs is their size that imparts other unique properties (Figure 2).

If under certain circumstances the nano-sized state will be destroyed, the special properties will be lost also. Generally scientists observed the agglomeration state to verify the NP stability and obtained different shapes of NPs. To avoid agglomeration and coagulation in solution of NPs, one should add capping agents or stabilizers.

Shape of NPs

The role of this factor is definitely underexplored and underestimated compared to the first three factors which may be accounted for by the lack of the methods for the control of the particles shape in the range of sizes below the region of coherent scattering (<5 nm depending on the material nature) limiting the possibilities of XRD analysis, limited access to high-resolution TEM or even by the ignorance of the importance of this factor. Usually transmission electron microscopy is used to determine the form of particles and to visually define the shape. Grazing incidence X-ray diffraction and UV/visible spectrometry can be also used.

NPs, in particular nano-silver, can be nanoscale in zero dimension (atomic clusters, filaments and cluster assemblies), one

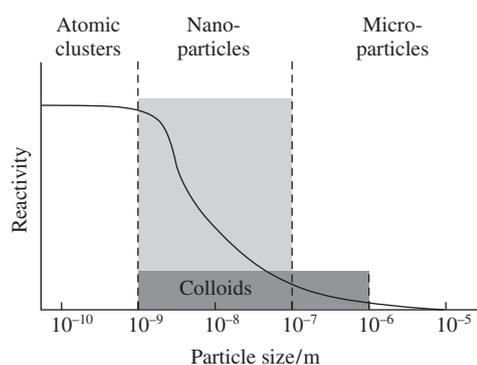


Figure 2 Reactivity of a material is strongly dependent on size.¹⁵

dimension (surface films, multilayers), two dimensions (strands, ultrafine-grained overlayers or buried layers and fibres), or three dimensions (particles, nanophase materials consisting of equiaxed nanometer sized grains). In addition, nanostructures can be produced as spheres, cubes, rods, tubes, wires, multifacets or films. In a first approximation, the majority of researchers in toxicological studies assume that silver NPs have a spherical shape. On the other hand, nanomaterials (gold, carbon, metals, metal oxides and alloys) with a variety of morphologies (shapes) are known in the art.¹⁰

There are two different classification schemes describing the possible forms of the NPs.^{16,17} Figure 3 indicates the theoretical shapes of nanoobjects. But they are rather different from those observed in experimental measurements. Shevchenko¹⁶ suggested a classification based on a geometrics apology between shapes of the particles and figures. Most of the figures are well known mathematical objects and can be easily used to calculate some descriptors for structural modelling. Maynard¹⁷ looked at the classification from another point of view. He took as a model some kind of conceptual subjects taking into account the reactivity. NPs were divided into nine categories depending on their structure and properties: (i) spherical or compact particles (compositionally homogeneous), (ii) high aspect-ratio particles (compositionally homogeneous), (iii) complex non-spherical particles (compositionally homogeneous), (iv) compositionally heterogeneous particles with core/surface compositional variation, (v) compositionally heterogeneous particles with distributed compositional variation, (vi) homogeneous agglomerates (agglomerates of a single particle class), (vii) heterogeneous agglomerates (aggregates of diverse particle types), (viii) active particles (particle behaviour and properties depend on external stimuli), and (ix) multifunctional particles (particle behaviour and properties depend on functional responses to local environment and stimuli).

Unfortunately, both these descriptions are mainly a nice theoretical approach. When one moves to the practice, then it is not

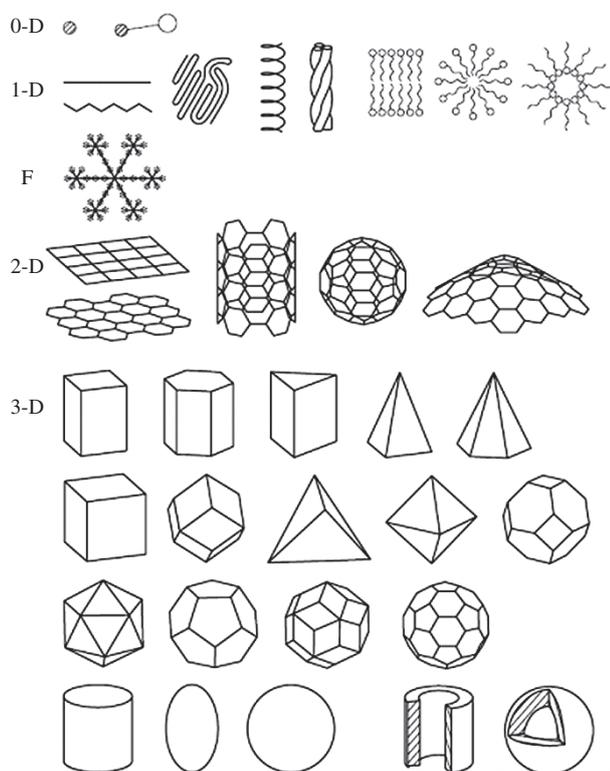


Figure 3 Examples of the forms of NPs (F stands for fractal dimension). Figure from ref. 16 ©2003 Pleiades Publishing, Inc. Reproduced with permission.

easy to figure out how these classification schemes can be applied. Very few authors mention the shape of the particles in their works and even then there is no unity between the authors. For instance, if we consider titania or SiO_2 NPs, some authors define their shape like a nanoflower and other call the same form as sea-urchin-like shape.

Depending on the synthesis conditions, the following shapes can be reached: spherical, hexagonal, triangular and truncated/filleted triangular,^{18,19} icosahedral, decahedral, elongated and octahedral,²⁰ desert-rose or nanoflower-like structure and silver-NP films,²¹ nanoprisms and nanorods,^{22,23} nanoplates, nanowires, nanocubes, flake-like shapes.²⁴ The role of the shape of NPs is less studied from the viewpoint of nanotoxicity. One of the reasons is the absence of the common system for classification and definition of the shapes of the particles. Another one is related to the difficulties with identification of the shape of the nano-substances by available physical and spectroscopic methods. Nevertheless, some data and the common sense lead to the following trends in the toxicity effect as a function of the NP morphology: spheres < hemispheres < rafts (discs) < needles (nanotubes). However, we can find some examples of practical works on this topic with nanosilver particles. Researchers from South Korea observed the interference of the shape of nano-silver on the antibacterial activity against gram-negative bacterium *Escherichia coli* and demonstrated that silver NPs induced a shape-dependent interaction on it. Truncated triangular silver nanoplates with a {111} lattice plane as the basal plane demonstrated the strongest biological response, compared with spherical and rod-shaped NPs and with Ag^+ (in the form of AgNO_3).²⁵ Other authors point out the shape-dependent electrocatalytic behaviour of silver NPs.²⁶ Scientists from the University of California, Los Angeles, who have studied the effects of different-shaped nano-sized silver on fish gill epithelial cell line (RT-W1) and on zebrafish embryos, showed that nanoplates are significantly more toxic than other particle shapes (spheres and wires).²⁷ The important role of crystal defects (stacking faults and point defects) in contributing to silver NP toxicity was confirmed in addition to the established roles of silver ion leaching from silver NPs.²⁷

Crystal structure

Now we can return to the above-mentioned {111} lattice plane in a truncated triangular silver nanoplates.²⁴ In this work, the authors used oriented particulate monolayer X-ray diffraction (OPML-XRD) to obtain structural information on the nanoplates.²⁵ The data for other nano-forms were not presented. The effort to describe the crystal structure of silver NP was made by Hofmeister and Tan.²⁸ They divided the following particle forms: single crystal, single-twinned, and multiple twinned particles based on the truncated octahedron or cuboctahedron shape. Although there is a certain preference for some of these forms that vary for different types of synthesis and processing, there is no principal difference in them. Even in the organized structure, some parallel twin lamellae or cyclic twinned segments can be found.

Surface area

This property is a function of two characteristics of NPs: their shape and size. The shape validates the rule for the equation and the size gives us the variable for counting. The smaller size obviously gives the larger value of the surface area. In some cases, this correlation can explain the higher reactivity of nano-substances compared with their bulk counterparts. Even some authors consider the surface area as important as the size and presume to take it as a dose marker. Duffin *et al.*²⁹ identified a relationship between the surface area, dose, and reactivity of metal NPs *in vivo* and *in vitro*. They demonstrated the possibility of estimating the potential toxicity of NPs based on the inflammatory

response of as instilled surface area dose. The researchers from Korea Advanced Institute of Science and Technology, posted these two determinants at the same rank.³⁰

Surface chemistry

Surface chemistry is a term combining surface coating, defects on the surface, and properties of grafted groups – nature, concentration and flexibility of fragments grafted to the NP (polymers, stabilizers, ligands, anchoring, capping or chelating groups). Obviously, such grafted moieties may enhance the toxicity of NP or suppress this toxicity depending on the intrinsic toxic properties of these moieties and/or their synergy with the metal NPs. Recent exploration with two manufactured surface-modified and non-modified samples showed that silver NPs have a tendency to settle out in high ionic strength media independently of the surface modification.³¹ Enhancement of the antibacterial activity of silver NPs (25 nm) caused by their stabilizing agent was revealed.¹³ Three most widely used additives were studied: sodium dodecyl sulfate (SDS), Tween 80 (polyoxyethylene sorbitan monooleate), PVP 360 – polyvinylpyrrolidone (MW = 360 000).¹³ SDS showed the greatest enhancement of antibacterial characteristics.

Surface charge

The surface charge is the electric charge existing at the NP–medium interface. Several papers were dedicated to the studies in this area. Some of them appear interesting from the viewpoint of the toxic properties, but most of researchers work with practical applications such as Raman scattering, facilitated transport in separation membranes.^{32,33} A possible cytotoxic effect of charged silver and gold NPs to Human Lung Carcinoma, A549 cells was discussed.³⁴ Positively charged NPs were found to be more toxic compared to negatively charged NPs both for silver and gold.³⁴ The same effect was found with bacillus species.³⁵ The authors presume the possibility both of a chemical and physical mechanism of toxicity of NPs.

The surface charge reflects another significant characteristic of NPs – the so-called Zeta-potential (ζ -potential) on the surface. In turn, Zeta-potential is one of the marks of the particles stability. Typically, NPs with Zeta-potentials greater than 20 mV or less than –20 mV have sufficient electrostatic repulsion to remain stable in solution.³⁶

Structure-dependent electronic configuration

Since the area of nanotoxicity is in the infant state, we could find only a few unpublished research data devoted to the electronic properties of nano-silver. The electronic and geometric properties of silver and gold NPs were studied with the goal to find possible explanation of catalytic application.³⁷ The other electronic and microwave characteristics of silver NP composites were learned as a factor of the presence of non-linear resistive and reflective behaviour.³⁸

We discussed here not all the properties from the list, but only the best studied ones and presented in the open literature. Properties of NPs are very complex and interrelated. They should be considered in a combination. Without proper material characterization, toxicological studies will provide limited useful information, and will not be comparable to other studies conducted with similar types of nanomaterials.³⁹

In vivo and *in vitro* toxicity of NPs

In the next sections, we will pay attention to available toxicity data for silver NPs, observe the main research trends, tendency and future efforts to understand Ag NP nanotoxicity. One admitted reliable technique for observing toxicity of NPs does not exist yet. Classical methods for evaluating particle toxic behaviour are not satisfactory for this goal.⁴⁰ In one of OECD's reports⁴⁰ recom-

mendation was given for evaluation and validation of nanotoxicity methodology and techniques.

Toxicity of the silver NPs is one of the well studied areas, due to Ag NPs widespread inclusion in a number of diverse products (including clothes and wound dressings), which largely arises from the antibacterial behaviour of Ag NPs. Toxicological properties of silver NPs can be different from their bulk counterpart. Due to their small size, NPs can penetrate to the cell membranes and pass through immune barriers. A number of the publications proved that while NPs do obey classical mechanisms of clearance from the organs, there are transport pathways that are typical of particles within the nanosize range.^{41–47}

There are abundant toxicological investigations that are related with the inhibitory action of silver nanoparticles to microbes, bacteria and other microorganisms which is the first-priority application for Ag NPs. We want to look at a different side and research of the toxic behaviour of silver NPs with respect to bottom hydrobionts as a first target for leaking NPs.

Most toxicologists initiate their research of toxic properties of silver with monovalent silver ions (Ag^+) and elemental silver (Ag^0). A toxic profile of monovalent silver ion and elemental silver is used as a background for toxicological investigation of silver NPs. Usually the ionic form is more toxic. There is suggestion that the toxicity of NPs can be explained by their ability to release ions. Bilberg and Hovgaard⁴⁸ inspected this idea on Zebrafish (*Danio rerio*). They observed the 48-hour median lethal concentration (LC50) values for nanosilver and silver ion at the same concentration of ions in solution. The values were 84 and 25 $\mu\text{g dm}^{-3}$, respectively. The toxic response of nanosilver cannot be totally explained by liberating Ag^+ ions.^{49,50} Possible ionic and unique mechanism for NPs was discussed in detail.⁴ Fabrega *et al.*⁵¹ reviewed the behaviour and effects of nanosilver in the aquatic environment. They draw attention to bioavailability, overviewed factors influencing the bioaccumulation and discussed a probable mechanism of the biodistribution. The authors outlined a comparative table for different organisms from open sources.⁵¹ Unfortunately, the conditions of the experiments are very different to allow one to make any conclusion.

Abundance investigation was made for *Daphnia magna* due to their compatibility necessary requirements⁵² and simplicity of the experiment. In one of the later works,⁵⁰ the authors observed acute toxicity of nanosilver to *D. magna* using the 48-hour immobilization test. Two types of colloidal silver NPs and a suspension of silver NPs were used as the sources of NPs. Toxicity significantly depends on the particle type. Comparative characteristics of the received results with published data are available.⁵⁰

Assessment of the acute (96 h) and chronic (21 day) toxicity of nano- and micro-sized Ag and CeO_2 particles for *D. magna* neonates was performed.⁵³ The authors evaluated the impact of both material and size of NPs on mortality and molting. Nanosized particles revealed a higher effect on neonates of *D. magna* compared with micro-silver; and silver samples proves a stronger reply than CeO_2 . Other researchers compared the toxicity for *D. magna* and algae *Pseudokirchneriella* measuring LC50 for *Daphnia* (10 $\mu\text{g dm}^{-3}$ for nanosilver and 1.3 $\mu\text{g dm}^{-3}$ for ionic silver) and IC50 for algae (500 $\mu\text{g dm}^{-3}$ for nanosilver and 26 $\mu\text{g dm}^{-3}$ for ionic silver).⁵⁴

Information about toxicity of NPs is already present in journals, books and articles but it is too early to make sound conclusions. There is no one common point of view at the toxicity of silver NPs. Some outcome can be achieved by variation of biological responses of different nanomaterials in a variety of *in vitro* and *in vivo* model systems using validated approaches.⁵⁵ The clear toxicological differences may be observed for different materials in *in vitro* cell systems. However, the same responses do not always appear when administering the same material *in vivo*.^{56,57}

Toxic effects of the NPs are not dictated by the particle mass alone, researchers have tried to better characterize the physico-chemical characteristics of particles being chosen for experimental studies. It can help to determine how these parameters impact the toxic effects observed. It is valuable for researchers to consider not only the physical and chemical characteristics of NPs in toxicology studies, but also different exposure metrics (e.g., particle mass, number, surface area, concentration). Such information will ultimately be used to compare hazards of different NP types.

Interlinks between the experimental and theoretical strategy

The classical way of assessing toxicity, e.g. by performing *in vivo* experiments, is very expensive and time consuming. Performing such tests for all possible nanoparticle types, sizes and concentrations is practically infeasible. A cheap and efficient alternative to such tests is using predictive computational models, for example Quantitative Structure–Activity Relationship (QSAR) models. Using QSARs for NPs is a new and still developing area of research.

The proper balance between the experimental work and modelling of the toxicity and health effects of nanomaterials becomes the centre of the recent and future studies related to the toxicity of nanomaterials. The practical component of the research work includes:

- A rational and systematic approach to the synthesis of diverse inorganic nanomaterials, including metal NPs such as Au, Ni, Co, Fe, noble metals and some bimetallic compositions, metal oxide NPs (TiO₂, SiO₂, CeO₂, SnO₂, Al₂O₃, ZrO₂, mixed compositions), metal chalcogenide NPs (CdS, ZnS, CdSe, ZnSe) or similar materials (GaN, GaP, GaAs), the most frequently studied NPs so far were those included in the OECD activities (Ag, TiO₂, CeO₂, Al₂O₃).
- Characterization of the nanomaterials by physicochemical methods in relation to their toxic effects with the goal to reveal the correlation between the particle size and morphology of NPs and their toxicity.
- Studies of the fate and transport of nanomaterials in the environmental ecosystems.
- Toxicity tests *in vitro* or *in vivo* using diverse cell lines and microorganisms and algae (daphnia, algae, fishes, molluscs, worms as representative organisms).
- Toxicity tests *in vitro* or *in vivo* to reveal human health effects, including inhalation and ingestion effects.

The modelling studies typically include:

- Accumulation and digestion of the available literature data on the relationship between the toxicity of inorganic nanomaterials of diverse classes listed above and their chemical composition, size of the NPs, shape (morphology) of NPs and the availability and nature of the grafted groups.
- QSAR studies related to modelling the behaviour of NPs in living organisms.
- Development of user-friendly databases on the toxic effects of NPs.
- Evaluation of predictability of the toxicity of novel nanomaterials on the basis of the developed data bases and revealed experimental dependences.

Modelling of the toxic effects of NPs of different composition can help to shorten the time of the experimental studies, reduce the overall costs and save lives of organisms used in *in vivo* tests. Literature data related to this area are scarce. The ability to screen nanomaterials with high-throughput instrumentation can be very helpful given the large number of possible nanomaterial/cell culture compositions and enable robust structure–activity relationships under controlled conditions. Shaw *et al.*⁵⁸ evaluated the toxicity of

50 NPs in a high-throughput fashion, testing multiple cell types and multiple assays that reflect different aspects of cellular physiology, and finally collected almost 24 000 data points.

By applying hierarchical clustering to the dataset, the authors identified nanomaterials with similar patterns of biological activity across a broad sampling of cellular contexts and were able to infer whether the activity profiles were dominated by either core composition or surface modifications. The authors also concluded that given the heterogeneity within each cluster, the biological activity of NPs arises from the combined effects of many aspects of their composition and is therefore difficult to predict *a priori*. However, no further studies were conducted to verify how the particles behaved *in vitro* and the materials were only characterized before entering the assays. The analysis indicated that similarity or differences in biological activity is not significantly enhanced by evaluating NPs in multiple different cellular assays and cell types. These findings disagree with Monteiro-Riviere *et al.*, who showed that due to the nanomaterials interaction with some assay markers, more than one assay might be required for determining NP toxicity.⁵⁹ Further aspects of the modelling studies by using QSAR approach are described.⁶⁰ The paper provides advice on how to develop QSARs in future for nanomaterials based on the current experiences with QSAR in ecotoxicology of regular bulk chemicals. One of the recommendations is that separate QSARs need to be developed for individual classes of nanomaterials, and some possibilities for structural descriptors are given. For instance, toxicity of 17 various metal oxide NPs was described⁶¹ as a function of the enthalpy of formation of a gaseous cation at the same oxide stage.

Discussion

The current state-of-the-art illustrates that no single particle characteristic is a hallmark indicator of toxicity. Notwithstanding this, some particle characteristics play some role in directing biological fate and toxicity. First of all, the size of NPs, it is a very deniable parameter. Mechanism of action of this parameter is still not clear. Stabilizers play a substantial role in toxicity of stabilized particles as nanosilver. For TiO₂, the crystalline form has the greatest impact on pulmonary responses, whereas particle surface area, coating, shape, and, to some extent, particle size, appear to only have lesser influences on toxicity. Past experience with particles or fibers like asbestos and silica would suggest that characteristics such as surface reactivity, morphology, and biopersistence that affects the generation of reactive oxygen species, persistent inflammation, impaired macrophage clearance, and fibrotic lesions would likely be important parameters for driving the hazards of NPs. Manipulation of NPs, such as quantum dots, gold, and iridium, show that surface chemistry and size are critical determinants for influencing absorption, transport within and outside the respiratory system, retention and clearance mechanisms and rates, as well as the extent of eliciting inflammation and oxidative stress. In the case of metal NPs, the choice exists among the free-standing (naked) NPs with some toxicity information available for nano-zero-valent iron, noble metals, silver, and gold and supported or grafted NPs, which, as a rule, are much less toxic as compared to the free-standing counterparts. In spite of a quite significant number of recent publications related to the toxicity of inorganic nanomaterials containing metals (metals, metal oxides, metal chalcogenides), the knowledge of the relationships between the toxic effect and the chemical composition of regular chemicals are much better understood than the relationships between the toxic properties of this novel class of materials and their geometrical characteristics, including the size and shape of NPs.

Whereas some but scarce and non-systematic data are available on the NP size effect on the toxicity, the information about the role of the morphology or shape or crystal structure of NPs is

missing. Also, there is no general concept allowing for the prediction of the toxic behaviour of the metal NPs and their effects on the human health and environment. Results of reported toxicity testing are strongly impacted by a lack of knowledge on the fate of the materials during toxicity testing due to for instance agglomeration and aggregation of NPs and due to complexation with natural organic matter. These processes will affect the bio-availability of the NPs.

Still only random (non-systematic) toxicity tests are described in the literature, which were performed in most cases *in vitro*, whereas the number of *in vivo* tests is very limited, which is explained by the cost difference and by the early stage of development of nanomaterials. Also, the major focus in both *in vitro* and *in vivo* studies was placed on carbon nanomaterials, while the publications on metal, metal oxide, or metal chalcogenide NPs (either free-standing or supported) are quite limited.

A number of examples of the toxic behaviour of diverse nanomaterials are described in the recent book.⁶² The methods of monitoring the diverse nanoparticles (Ag, Au) and oxides (TiO₂) in different organs of rats are outlined in review.⁶³

Conclusion

It became obvious nowadays that the number of NPs manufactured is exponentially increasing. So does the diversity of NPs produced as well as the areas of their application. The so-called first-generation NPs like the ones mentioned above, are put on the market in large quantities and industrial scientists are now developing increasing applications of 3rd and 4th generations of NPs (potentially including self-assembling systems). The development of NP-tailored risk assessment methodologies of even the 1st generation of NPs is lagging way behind. This is why international organisations and institutions like OECD, EPA, and EU are increasing their efforts in mobilizing expertise (and data) to allow for development of validated risk estimates of large numbers of NPs of widely varying composition. Given the increasing numbers of NPs produced and the increased complexity in terms of NP characteristics affecting their inherent risks, it is undoable to experimentally generate the ecotoxicity profiles of 'all' NPs marketed. On the other hand, as current research efforts are increasing (as, for instance, witnessed by the exponentially growing number of publications in peer-reviewed scientific journals), more and more data are becoming available that may be used to deduce generalities across NP characteristics that determine their inherent toxicity characteristics to aquatic and terrestrial ecosystems.

The use of both experimental and theoretical data relating the toxicity effects of NPs of metals to their intrinsic properties (electronic state, coordination, chemical composition, shape and morphology of NPs and their size characteristics) should be the strategic alley of the future research in the field of nanomaterials design and application.

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