Nanomaterials: A Challenge for Toxicological Risk Assessment?

Andrea Haase, Jutta Tentschert, and Andreas Luch

Abstract Nanotechnology has emerged as one of the central technologies in the twenty-first century. This judgment becomes apparent by considering the increasing numbers of people employed in this area; the numbers of patents, of scientific publications, of products on the market; and the amounts of money invested in R&D. Prospects originating from different fields of nanoapplication seem unlimited. However, nanotechnology certainly will not be able to meet all of the ambitious expectations communicated, yet has high potential to heavily affect our daily life in the years to come. This might occur in particular in the field of consumer products, for example, by introducing nanomaterials in cosmetics, textiles, or food contact materials. Another promising area is the application of nanotechnology in medicine fueling hopes to significantly improve diagnosis and treatment of all kinds of diseases. In addition, novel technologies applying nanomaterials are expected to be instrumental in waste remediation and in the production of efficient energy storage devices and thus may help to overcome world's energy problems or to revolutionize computer and data storage technologies. In this chapter, we will focus on nanomaterials. After a brief historic and general overview, current proposals of how to define nanomaterials will be summarized. Due to general limitations, there is still no single, internationally accepted definition of the term "nanomaterial." After elaborating on the status quo and the scope of nanoanalytics and its shortcomings, the current thinking about possible hazards resulting from nanoparticulate exposures, there will be an emphasis on the requirements to be fulfilled for appropriate health risk assessment and regulation of nanomaterials. With regard to reliable risk assessments, until now there is still the remaining issue to be resolved of whether or not specific challenges and unique features exist on the nanoscale that have to be tackled and distinctively addressed, given that they

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A. Luch (ed.), *Molecular, Clinical and Environmental Toxicology*,
Experientia Supplementum 101, DOI 10.1007/978-3-7643-8340-4_8,

substantially differ from those encountered with microsized materials or regular chemicals. Based on the current knowledge, we finally provide a proposal on how risk assessment in the nanofield could be achieved and how it might look like in the near future.

Keywords Agglomeration · Aggregation · Bioavailability · Coating · Consumer products · Inhalation exposure · *In vitro* · *In situ* analytics · *In vitro* · *In vivo* · Nanoanalytics · Nano-bio interface · Nanocomposites · Nanomedicine · Nano-objects · Nanoparticles · Nanoparticle space · Nanosensors · Nanosystems · Nanotoxicology · Particle corona · Quantum dots · Read-across · Skin penetration · Structure–(re)activity relationship · Surface plasmonics · UV filters · Waste remediation

Introduction

Rarely any technology has gained such a tremendous scientific and economic interest within such a short period of time. Although it looks that the first hype on nanotechnology is already gone, the annual investment in this technology still is increasing albeit with smaller slope [1, 2]. It is often stated that nanotechnology will be the "Technology of the 21st century," which is supposed to influence our daily life and lead to an industrial revolution within a short time frame. In the workshop report "Nanotechnology Research Directions" from the Interagency Working Group on Nanoscience, Engineering and Technology (IWGN) of the National Science and Technology Council (NSTC), drafted in 1999, it is stated that "nanotechnology will be a strategic branch of science and engineering for the next century, one that will fundamentally restructure the technologies currently used for manufacturing, medicine, defense, energy production, environmental management, transportation, communication, computation, and education" [3, 4].

So, nanotechnology looks like a very young technology. Nevertheless, nanomaterials actually existed, were synthesized, and used long before the term nanotechnology was coined [5, 6]. The size of the particles usually serves as main criterion to define what a nanoparticle is supposed to be. The properties of nanomaterials are very different compared to their bulky counterparts. This may refer to physical, chemical, or electrical properties such as extraordinary strength or highly advanced optical or catalytical properties. This is what makes nanomaterials so interesting for a wide range of application fields. As the use of nanomaterials is steadily increasing and many products furnished with nanotechnology are released to the market, the concerns about the safety of this technology and about possible risks for humans and the environment gain heavy weight as well. Certainly, while the majority of the investment is still spent on basic science such as for the development of new materials, the awareness of a proper safety assessment has led to increased efforts

in the development of toxicological assays, in the advancement of exposure monitoring measures, as well as in the development of risk assessment strategies. Partially this is also due to undesirable developments in other fields, such as genetically modified organisms, where fearful public perception and unsubstantiated concerns strongly opposed the industrial use and further development of this technology. From the very beginning, in the nanotechnology field, a substantial amount of money was invested in safety aspects and in the understanding of the public perception and awareness about this technology, as well as in communication strategies [7].

Much more than in any other scientific field, the development and advancement of nanotechnology strongly depend on interdisciplinary cooperation. Expert knowledge is needed from material sciences, physics, analytics, chemistry, pharmacy, biology, medicine, toxicology, and many more highly specialized subdisciplines. Here, we want to provide a glimpse on the history of nanotechnology, to explain the issues related to definitions, and to introduce the extraordinary properties of nanomaterials and their current and possible future application fields. We will focus on "nanoanalytics" and "nanotoxicology" and how these areas could be reasonably combined. This chapter is not intended to comprehensively cover all topics related to nanotechnology. Rather, we will provide an overview on issues related to risk assessment and otherwise refer the interested reader to the excellent literature in this field (e.g., [8–12]). It should be already emphasized at this point that future efforts should be focused on *in situ* analytics and toxicological characterization. These fields are just about to emerge and might be the key for the development of structure–activity relationships applicable in health risk assessment.

History of Nanotechnology

Nanotechnology existed long before people knew about nanoparticles. Already in the fifth century B.C., colloidal gold was known in Egypt or China and, for instance, applied in medicine [13]. In roman times, nanosized silver and gold particles were used to paint glass; a well-known example is "The Lycurgus Cup." This application was also very popular in medieval times as the wonderful colored church windows at many places still allow us to recognize. For a long time the chemical nature of the applied gold and silver preparations was unknown. It was only speculation that these preparations contained gold "in such a degree of communition that it is not visible to the human eye" as Johann Kunckel wrote in 1679 [14]. In 1857, Michael Faraday was the first to synthesize tiny, nanosized gold particles intentionally by reduction of gold chloride ($AuCl_4^-$) [15]. This was the beginning of colloidal sciences. Although both terms are not synonymous and not all nanomaterials are colloids and *vice versa*, still what we call nanoscience today has its origins in the science of colloids [16]. The concept about what is possible at the nanoscale and thus the first concept of nanotechnology was introduced in 1959 by the Nobel laureate Richard Feynman in his famous lecture "There is plenty of room at the

bottom" [17]. He explained: "The principles of physics as far as I can see, do not speak against the possibility of maneuvering things atom by atom." He was also the first to use the word "nanostructures." At this time, it was still a more philosophical or theoretical problem. The technologies to manipulate and analyze materials at the nanoscale still had first to be developed. Feynman speculated about exciting new discoveries which would be possible at the nanoscale. But only with the invention of the scanning tunneling microscope (STM) in 1981 or the atomic force microscopy (AFM) in 1986, there was a possibility to understand materials far down to the nanoscale. So, truly nanotechnology in a sense of understanding that we share today was actually emerging in the second half of the twentieth century [18, 19]. The term "nanotechnology" was first used in 1974 by the Japanese university professor Norio Taniguchi [20]. In the 1970s, the idea of using nanoparticles as drug delivery systems became very popular, and substantial work in this field was done by Kreuter and coworkers [21]. Up to now, nanomedicine is still one of the main and most fascinating fields of nanoscience.

As a general consensus, the term nanoparticle or nanomaterial should only be used for intentionally produced materials. Nanoparticles can also be formed unintentionally or incidentally either by human activities (e.g., during combustion processes) or naturally. Such particles can be found in aquatic and terrestrial environments as well as in the atmosphere. At several ocean sides (e.g., around Cape Horn or at the West Coast of Ireland), significant numbers of particles in the size range of 10–100 nm were detectable, but also in other natural environments, such as the boreal forest Hyytiälä (Southern Finland), high numbers of nanoparticles of natural origin have been measured [22]. So, a significant proportion of the naturally occurring colloids are nanosized. They are heterogenous in size, shape, chemical composition, and property. They may be inorganic (e.g., mostly based on aluminum phyllosilicates or iron oxides/hydrous ferric oxides) or organic (e.g., so-called naturally occurring matter) [23, 24]. Furthermore, several biostructures belong to this size range. A typical protein is around 4–6 nm in size, and protein assemblies such as ribosomes are nanoscaled. A typical virus which is about 100 nm in size clearly belongs to the nanostructured world, while bacteria ($\geq$1 μm) usually do not.

Definitions

Nanoscience is the science that deals with materials at the nanoscale, meaning to synthesize, manipulate, or study nanoscaled materials. According to most definitions, the nanoscaled world covers the size range between approximately 1 and 100 nm [25]. Nanotechnology is defined as "the research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1–100 nm; the creation, and use of structures, devices and systems that have novel properties and functions because of their small size; and ability to be controlled or manipulated on the atomic scale" [26]. Similar definitions exist

from the International Organization for Standardization (ISO; http://www.iso.org/iso/home.htm) and other international or national bodies. Nanomaterials, on the other hand, are defined as materials that are—at least in one dimension (external or internal)—in the size range between 1 and 100 nm. However, there are also few exceptions from this size range known, such as, for instance, graphene, which is made of carbon sheets thinner than 1 nm [27]. Due to their tiny size, nanomaterials have novel, so-called nanospecific, properties that differ from those of the same material at larger sizes. Indeed, most of the definitions for nanomaterials are based solely or mainly on its size. According to this, the "nanoworld" covers the range between the world of atoms and molecules on one side and the world of bulk material at the other. However, it should be kept in mind that there is still no comprehensive definition that would be generally and internationally accepted and legally binding; current efforts are great to reach an internationally harmonized version [28].

According to the European Committee for Standardization (CEN) Technical Specification 27687 [29], nanomaterials encompass nanoobjects and nanostructured materials (Fig. 1). Nanoobjects are nanoscaled at least in one dimension; this might be the case just for one external dimension (i.e., nanoplates), for two (i.e., nanorods), or for all three dimensions (i.e., nanoparticles). Nanorods can be further separated into nanofibers (flexible nanorods), nanotubes (hollow nanofibers), or nanowires (electrically conducting or semiconducting materials). On the other hand, nanostructured materials may contain either nanocomposites or nanoparticles, or they display nanostructured surfaces (Fig. 1).

Recent discussions in the ISO Technical Committee (TC) working group TC 229/JWG 1 focusing on "Terminology and nomenclature" (http://www.iso.org/iso/iso_technical_committee?commid=381983) even suggest that the term "nanomaterial" should be replaced by "nanoparticulate material" to avoid misunderstandings. Furthermore, TC 229/JWG 1 proposed to include an additional subgroup

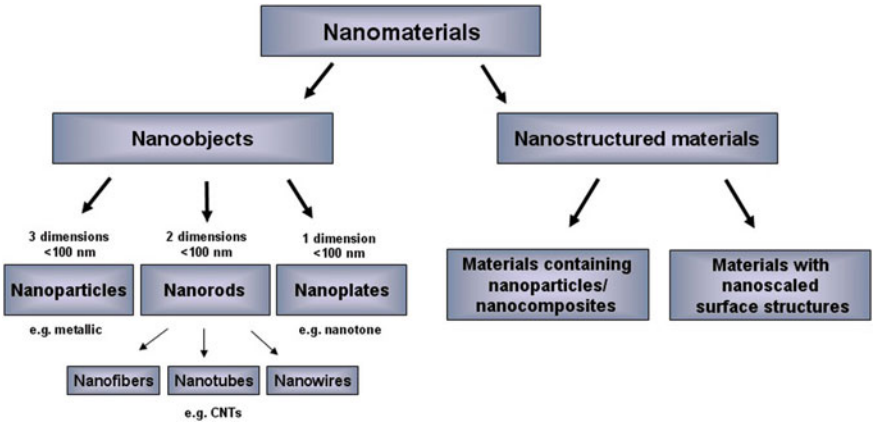

Fig. 1 Different nanomaterials according to CEN/ISO TS 27687 [29]

among nanoobjects, which could represent the novel, more complex forms of nanomaterials like core–shell structures that recently gained interest due to their multifunctionality.

Importantly, the size limits given are just approximative rather than absolute. Since nanospecific properties of the material do not necessarily follow exactly the size limits used for defining what "nano" is, the size-based definition alone may entail several drawbacks. In addition, the size of nanoparticles always spans a whole range and follows individual distribution curves. So, the so-called primary particle size always represents a mean value, and each type of nanoparticle displays its own polydispersity. As a consequence, even batches of nanoparticles with a mean size of $>$100 nm contain a certain fraction of particles below 100 nm.

Another issue is the likely formation of agglomerates and aggregates. Nanoparticles intrinsically tend to form agglomerates (due to rather loose interactions between individual particles; e.g., based on van der Waals forces) and aggregates (due to strong interactions between individual particles, resulting in fused or sintered particles) [29]. Some nanospecific properties such as a large surface-to-volume ratio might also retain in agglomerates and aggregates, and over time single primary particles might be released from it. Occasionally, it has been proposed to include this fact and to specify a particular nanomaterial based on the fraction (in percent) of free and individual nanoparticles it contains [30]. According to this, a nanomaterial contains particles in the size range of approximately 1–100 nm at more than x% of the total number size distribution; this means that the remaining part might be larger or aggregated. Currently, there is an ongoing and open discussion about the exact number of x [30].

While some definitions mainly focus on the size of the particles regardless of whether or not these particles exhibit so-called nanospecific properties, others concentrate on novel characteristics of the material which should be clearly different from the properties of the corresponding bulk material [31]. For instance, the latter definition has been proposed and disseminated by the British Standards Institution (BSI) in its Publicly Available Specifications (PAS) 71:2005 and 136:2007, stating that—by definition—a nanomaterial should "exhibit novel characteristics compared to the same material without nanoscale features" [32, 33]. However, neither these novel properties can be predicted or deduced from the corresponding bulk material, nor are they comparable to the physicochemical behavior of the corresponding (underlying) atoms or molecules. These nanospecific properties can pertain, but are not restricted to chemical reactivities, catalytic properties, electrical conductivity, optical or spectrophotometric characteristics, and so forth [34–37]. Some of these alterations are caused by the exponential increase of the specific surface area following decreasing sizes, the higher probability and impact of surface crystal defects, and the increasing likelihood of an incomplete coordination (bonding) of atoms at the particle surface [37]. All of these factors might contribute to the higher reactivity or catalytic activity of the respective material. In addition, due to an extremely small size, completely new features may appear. This refers to the occurrence of quantum effects such as, for instance, quantum confinement, which accounts for the special feature of so-called quantum

dots [38]. Such features do not emerge gradually with decreasing sizes but rather occur suddenly below a certain threshold. Other examples of nanospecific phenomena are wavelike transports, the predominance of interfacial phenomena, and the occurrence of surface plasmon resonance (SPR) in the case of, e.g., nanosilver or nanogold [39].

The term "nanospecific" property might be misleading as it implicates that nanomaterials as such display common properties that arise just because of their "nano" size. Rather, these properties strongly depend on the kind of material, on its actual size, but also on possible coatings and/or stabilizers applied. For instance, it has been demonstrated that the photocatalytic activity of molybdenum disulfide (MoS_2) depends on the particle size [40], and the properties of CdSe quantum dots are influenced by the coating [41]. It should be further noticed that the kind of coating also strongly affects the dissolution of soluble nanoparticles, as has been shown in the case of nanosilver [42]. Thus, nanospecific properties usually change as the size of the material changes. A given material of 80-nm size might have completely different features compared to exactly the same type of material at 10 nm or 2 nm. Currently, it is impossible to predict/deduce such properties or to describe them via math modeling. Approaches such as grouping of nanoparticles based upon similar composition and read-across, that is, the usage of toxicological data from one type of nanomaterial to predict the effects of another similar type, seem unfeasible at the moment. Nevertheless, the number of different nanomaterials is steadily increasing, and different approaches exist for their classification. Table 1 gives an overview on the most common types currently available.

The summary provided in the frame of this chapter is only a small part of what might be possible in future by means of nanotechnology. Others, more sophisticated

Table 1 Overview of the most common nanoobjects/nanoparticles currently applied (modified according to [13])

Carbon-based nanomaterials	Carbon black nanoparticles	
	Carbon nanotubes (CNTs)	Single-walled (SWCNT), multiwalled (MWCNT)
	Fullerene type (C_n)	$n = 60$, 70, or higher
Metal nanoparticles	Metal nanoparticles	For example, silver, gold nanoparticles
	Quantum dots	Classified according to core and shell composition [core: usually a metal and a p-block element (e.g., CdSe); shell: e.g., ZnS]
Metal oxides	Many different types	For example, TiO_2, ZnO, CeO_2, SiO_2, Fe_2O_3
Polymer type	Dendrimer type	Highly branched structures, based on polyamidoamines (PAMAMs)
	PLGA or PLA–PGA type, *etc.*	poly-D,L-lactide-co-glycolide, poly-lactide acid, poly-L-glutamic acid, *etc.*
	Polysaccharide type	Nanocellulose
Other types (selection)	Core–shell structures	
	Surface functionalized structures	
	Micelles, liposomes	

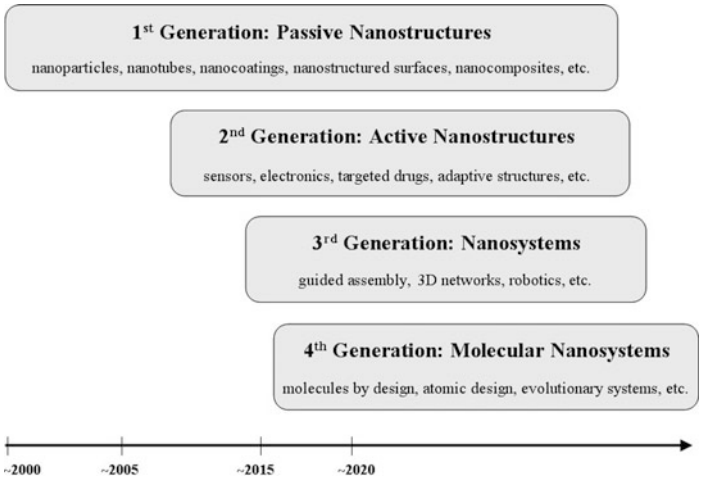

Fig. 2 Anticipated generations of nanomaterials (according to [43])

materials, are currently being the matter of R&D or are anticipated of being produced in near future. Roco predicted four overlapping generations in the advancement of nanosystems (Fig. 2) [43]. The first generation consists of passive nanostructures such as nanoparticles, nanotubes, nanocomposites, nanostructured surfaces, *etc*. Thus, the most currently used nanomaterials belong to this first generation. The second generation is made of active nanomaterials, that is, materials capable of changing their properties (e.g., shape, color, mechanical, or electrical properties) while being in use. Such materials already exist and some are also sufficiently matured for commercial application in the near future. Examples for this are sensors or drug transport systems [44].

The expected third generation will be made of nanosystems capable of self-assembly and of forming networks at the nanoscale. In the nanodevelopmental scheme proposed by Roco (Fig. 2), the fourth generation will consist of nanomaterials synthesized according to individual molecular design, combining and assembling atom by atom as already speculated by Feynman (cf. above). Regardless whether this anticipated development might become true or whether the indicated time line might be correct, the further developments of nanomaterials will continue, and many more sophisticated materials will be developed and used in all kinds of products of the future market.

Properties, Fields of Application, Benefits, Concerns

One of the most obvious characteristics of a nanoparticle is its tiny size and—in direct connection to this—the increase in its specific surface (Fig. 3). As the size of the particle drops below 10 nm, there is an exponential increase in the specific

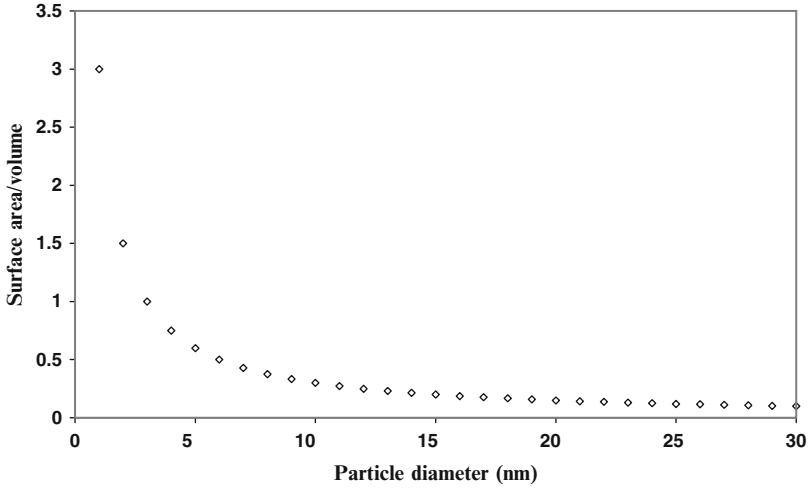

Fig. 3 Relationship between particle size (diameter) and specific surface (surface area per volume)

surface, that is, the ratio of surface to volume. As a consequence, the relative fraction of atoms located directly at the surface of the particle is significantly increasing in very small particles. While only about 0.2% of all atoms are at the surface of a 1-μm sized particle, this number increases as follows (in the case of nanogold): 3% for a 50 nm particle → 16% for a 10 nm particle → 53% for a 2.5 nm particle [37]. Therefore, surface and interfacial chemistry will be much more relevant for the understanding of nanoparticle reactivity when compared to the reactivity of the corresponding bulk material.

The chemistry of atoms at the surface clearly differs from those at the core of the particle. This is due to several reasons. In a typical crystal, there is a crystal lattice, which—in good approximation—can be assumed being infinite for bulk material. Also, each atom of a molecule has a characteristic coordination, such as, for instance, the tetrahedral coordination of saturated carbon atoms. Due to their small size and surface bending, there is no infinite lattice in nanoparticles anymore. Instead, there is a higher reactivity on the surface, a higher probability and weight of surface crystal defects, and an increased chance of undercoordination of atoms at the surface [37]. Such surface-specific chemistry is also known for bulk materials. However, due to the much higher surface/volume ratio of nanomaterials, these effects are more pronounced and decisive for particle's chemistry and physico-chemical behavior. So, all of this might contribute to the higher reactivity or to the catalytic properties of nanomaterials. A quite well-known example is the unusually high reactivity of nanogold, compared to bulk material which reveals rather inert. As a result, nanosized gold (below 10 nm in diameter) proves as excellent catalyzer, for instance, in the reduction of organic chemicals [45, 46].

Other characteristics to be taken into consideration result from the fact that nanosized particles are never "naked." Although—in theory—very small particles

might form stable suspensions without the need of further surface modification, in reality even very small particles would aggregate over time. Thus, typically, each nanoparticle requires stabilization by a certain kind of coating (either noncovalently or covalently attached to the surface) [37]. In the case of nanosilver, usually a layer of citrate or a polymer is used. Dispersions of nanoparticles principally can be stabilized via repelling surface charges (e.g., citrate coating) or via steric hindrance (e.g., polymer coating) [37]. The charging of the particle's surface, as resulting from citrate coating, induces the formation of an oppositely charged layer of ions in the dispersion medium ("Stern layer"). It seems obvious that such a charge stabilization depends on several factors, such as pH value, ionic strength of the dispersion medium, and particle concentration. The theory behind this charge stabilization has been described by Derjaguin, Landau, Verwey, and Overbeek already a while ago and became famous as "DLVO theory" (according to their first initials) [47]. Due to the wide range of influencing parameters, it usually becomes difficult to prepare highly concentrated nanoparticle stock dispersions. Also, if the stabilization of particles results from sterically demanding surface modifications, this will not lead to a universally resistant preparation either. For instance, if the particle concentration in the suspension will decrease beyond a certain threshold (below the critical micellar concentration), the polymer—if not covalently bound—might be detached and dispersed and thus all stabilizing effects will be gone [48]. To summarize, regardless by which means nanoparticles will be stabilized in suspension, an absolute stability and resistance so far remains unreachable. Instead, the fate of the suspension will depend on a range of different factors including temperature, pH, ionic strength, media composition, and others. So, over time agglomeration and aggregation of nanoparticulate suspensions is an inevitable and typical outcome.

As already mentioned above, due to their extremely small size, completely new features of nanoparticles may appear. A well-known example is the occurrence of surface plasmonics in case of nanosilver or nanogold [39]. Similar as with other physicochemical properties, the SPR depends on the surface chemistry, the shape, and the size of the nanoparticles. It also will change dramatically upon aggregation and usually disappear if all nanoparticles are engaged in aggregates. Another example is quantum confinement [38]. To briefly explain this effect at the nanoscale, we have to remember what happens when atoms form a molecule. A set of bonding and antibonding molecular orbitals (MOs) form, but all electrons are placed into the bonding MOs while the antibonding MOs remain unoccupied. In a large atomic lattice (e.g., in case of metals or semiconductors), and due to their overlap, these orbitals constitute pseudo-continua called the valence band and the conduction band. For semiconductors between both, there is a band gap. As already mentioned above, in the case of nanoparticles, there is no infinite atomic lattice and—as a result—there is a loss of orbitals that could contribute to both the valence and conduction band and thus the band gap becomes larger. If an electron, by absorption of energy, is lifted from the valence to the conduction band, it can either generate electricity or recombine by releasing (emitting) light. In the case of nanoparticles, the electrons are confined within the particle, and therefore, the

probability of light emission is much higher than the occurrence of electricity [38]. Thus, the quantum efficiency (fraction of emitted light per excitation) usually is very high. This explains the properties of so-called quantum dots, which constitute a very well-suited tool for fluorescent labeling of all kinds of materials [49].

Nanomedicine

This explanation immediately leads us to the application fields. One among these with highest expectations is nanomedicine. Here, nanomaterials are used in wound dressings, for bone cements, as coatings for prostheses or surgical instruments, in catheter tubing, in imaging contrast agents for diagnostics, but also as nanomedicine *sensu stricto*, such as, for instance, enhanced chemotherapeutics [50, 51]. Through packaging of anticancer drugs into nanoparticulate vessels, higher therapeutic doses at target organs can be reached, and unwanted toxicity and side effects may be reduced simultaneously as well. For instance, the long known and widely applied anticancer drug paclitaxel has been enclosed into aluminum nanoparticle cages and traded as Abraxane^{TM} [52]. In its conventional form, paclitaxel is difficult to formulate due to its insolubility in aqueous media; therefore, additional side effects may result from conventional formulation aids. By packaging into nanoparticles, the solubility of paclitaxel is no problem anymore. Packed into small vesicles, the drug can easily penetrate into tumor tissue which usually contains leaky, fenestrated blood vessels. By contrast, healthy tissues remain unaffected since blood is supplied through vessels with intact endothelial barriers.

Another very fascinating opportunity of nanomedicine is the use of new application forms based on novel formulations (e.g., inhalable nanoaerosols) or to better reach organs such as the brain which are shielded by specific blood–tissue barriers [53, 54]. In contrast to other application fields suitable for nanotechnology, nanomedicine relates to medicine and healthcare products and, thus, is very strictly regulated and monitored.

Consumer Products

Nanomaterials are also used in many daily-life products such as cosmetics, textiles, food contact materials, and houseware goods [55]. In the cosmetics sector, “nano” is applied in two different forms: as nanoemulsion or via incorporation of nanoobjects/nanoparticles. Nanoemulsions, meaning the encapsulation of vitamins or other sensitive compounds into liposomal cages, are prepared to facilitate the uptake of these ingredients into the body. They have reached some history yet, and this type of application is generally regarded as safe [56]. Another example is the use of nanosized titanium dioxide (TiO_2) or zinc oxide (ZnO) as UV filters in sunscreens; these metal oxides are among the most commercialized nanoparticles. Table 2

Table 2 Examples of nanoparticles used in cosmetics or textiles (some are currently in the R&D phase) (according to [57, 58])

	Particle type	Purpose
Cosmetics	TiO_2 or ZnO	UV protection
	Silver	Anti-bacterial (e.g., in deodorants)
	Fullerenes (C60)	Anti-oxidant, radical scavenging creams
	Pigments	Coloring
	Silica	Absorbance of oil, long-lasting cosmetics
	Hydroxylapatite	Tooth paste (remineralizing)
	Liposomes	Supply of, e.g., vitamins
Textiles	Silver	Anti-bacterial
	ZnO or TiO_2	UV protection
	TiO_2 or MgO	Self-sterilizing (chemical, biological protection)
	SiO_2, Al_2O_3 with special coating	Water repellent
	Ceramic	Abrasion resistance
	Nanoclay	Electrical, heat, thermal resistance
	Nanocellulose	Anti-wrinkle
	Ferrum or others	Functional textiles (e.g., conductive properties)
	Carbon nanotubes (CNTs)	Stronger fibers

summarizes the nanoparticles which are mostly used or intended to be used in different consumer products [57, 58].

For the textile sector, the best-known example is the application of antimicrobial nanosilver in or on the surface of textile fibers used to produce, for instance, sportswear, underwear, T-shirts, and socks [58]. For the same reason, nanosilver is used in coatings of household products such as washing machines or other devices. One recent and new application is the use of nanomaterials in water treatment or filtering technology, mostly carbon nanotubes (CNTs; mechanical filter) or titanium dioxide (TiO_2, photocatalytic activity) [59, 60].

Environmental Applications

Some final examples should be given for the environmental application fields. Due to their high surface binding capacity and reactivity, nanoparticles may be useful in the removal of potentially dangerous chemicals and thus applicable in waste remediation. For instance, zero-valent iron nanoparticles turned out to be useful in the removal of arsenic from groundwater [61]. Meanwhile, iron nanoparticles are also widely applied in the decontamination of soil. Different types of dendrimers can act as chelators to bind metal ions like copper, silver, or iron [62, 63]. Other applications are nanosensors, which can sense dangerous compounds in water, air, or other surroundings. Examples are tin dioxide (SnO_2)-based gas sensors or TiO_2-based electrodes to detect chemical oxygen demand [64, 65]. The use of nanotechnology often allows to produce items with less material, which in turn may be also considered environmentally beneficial. An example is the use of nanocomposites in aircrafts or cars, which have improved mechanical properties while being much

lighter than other materials applicable in this field [66]. As a consequence, fuel can be saved and less CO_2 will be emitted. Less material is also needed, for instance, with nano-based paintings or coatings; the layers applied are thinner, and thus, material can be saved.

Concerns

Concerns about the safety of nanomaterials are high [67]. One major concern arises from the high surface binding capacity of such materials. Nanoparticles can effectively bind to other potentially toxic compounds and thus may change their bioavailability as transport vehicles within living organisms [68]. Further, nanomaterials as such may be also inherently toxic. Mainly an enhanced reactivity combined with its small size, the latter allowing the particles to reach otherwise sheltered parts of the body, is the basis of such concern [69, 70]. Currently, the data on the toxicity are by far not sufficient and resilient, as we will describe in detail below. Most of the current data are from *in vitro* or acute toxicity studies and little is known about chronic toxicity endpoints [71]. At the same time, there is also concern that novel biological responses need to be considered that, by now, we even might not be able to imagine and to anticipate. This is in principle true for any novel type of chemical compound or material. The major concern in the case of nanomaterials, however, is the enormous diversity of existing materials and the endless "nanoparticle space" which is expected to be developed and produced. A conventional testing strategy that would propose each type of material to be tested individually certainly is doomed to fail. On the other hand, currently, any kind of structure–(re)activity relationship or read-across approach seems likely unreliable and dicey. To establish such tools also in the nanotoxicology field, first of all, we need to properly and fully characterize all kinds of materials under consideration. The aim will be to learn more about the material composition and its physicochemical features that are likely to contribute or to influence the biological fate and the toxicological properties of the material once it has reached and intruded living tissues and cells. For this purpose, of course, we need to establish highly advanced and reliable nanoanalytics, which currently looks like being a problem by its own. Another current major issue is the exposure assessment of humans when confronted with nanomaterials unintentionally released from products or occurring at the workplace and in the environment. All these kinds of problems are in principle not really new. If new chemicals or substances are synthesized and introduced to the market, there should be always analytical measures at hand allowing for detection, quantification, and monitoring of these newcomers. So why should this be a problem and a challenge in the case of nanomaterials?

Nanoanalytics

The classical approach to characterize chemicals is to ask for data on identification, composition, purity, dose, or concentration. From an analytical point of view, this process is well established and comparatively straightforward. However, in order to characterize nanomaterials, this becomes far more complicated and pushes the existing analytical instrumentation right to the limits of what seems technically feasible today. To pursue the goal of getting the risk assessment of nanomaterials to a more advanced level, the following essential questions have to be asked:

- What are the properties of nanomaterials used in a specific product (e.g., nanoclay used as filler in polymers or nanosilver used as surface coating)?
- What are the properties and interactions of nanomaterial-furnished products when actually being in use (e.g., interactions of nanoparticles with biological matrices or other chemical substances through diffusion, migration, and abrasion)?
- What are the properties and interactions of nanomaterials during exposure of living organisms including humans (e.g., after oral, dermal, or inhalative uptake of nanomaterials)?

One central requirement on the metrology employed to gather necessary data is to perform *in situ* measurements under the conditions mentioned above with reliable and reproducible methods in place. For all of the three questions raised above, it has to be asked:

- Which physicochemical properties are really relevant?
- Which currently available analytical techniques are capable of measuring these characteristics?
- How is it possible to strategically combine different analytical techniques to obtain a sufficient dataset for subsequent exposure assessments?

What Does the Nanoparticle See?

The OECD Working Party on Nanotechnology (WPN) has been established in March 2007 to advise upon emerging policy issues of science, technology, and innovation related to the responsible development of nanotechnology [72]. In the following, WPN has published the following list of physicochemical properties to fully characterize individual nanomaterials:

Agglomeration/aggregation	Zeta potential (surface charge)
Water solubility/dispersibility	Surface chemistry (where appropriate)
Crystalline phase	Photocatalytic activity
Dustiness	Pour density
Crystallite size	Porosity
Redox potential	Specific surface area
Radical formation potential	Particle size distribution (dry and in media)

(*continued*)

Representative electron microscopy (TEM) picture(s)	Octanol–water partition coefficient (where relevant)

In addition, OECD also provides a list of fate properties:

Dispersion stability in water	Adsorption, desorption
Biotic degradability	Adsorption to soil or sediment
Identification of degradation products	Bioaccumulation potential
Abiotic degradability and fate	Other relevant environmental fate information (if available)
Further testing of degradation product(s) as required	

Although there are several comparable lists [73–75], the discussion which physicochemical properties are essential to fully characterize and describe nanoparticles is not finished yet. On the other hand, research undoubtedly demonstrated that in biological environments, for instance, in the human body or in food matrix, once being introduced nanoparticles are immediately covered by matrix molecules surrounding the particle, and the so-called corona is formed [76]. For some nanoparticles, several constituents of this corona have been identified yet; different proteins and lipids are among them. The configuration and properties of an individual nanoparticle (i.e., chemical composition, shape, size, and coating) decisively influence which biomolecules will be bound at its surface [77, 78]. Although some biomolecules will be bound quite strongly, forming the so-called "hard corona", others are only loosely bound, and—as a consequence—the biological identity of the particle rather underlies certain dynamics due to its constantly changing "soft corona" at the surface. Through nanoparticle's corona, the surface area, surface reactivity, and surface charge are characterized, thus determining its biological impact and fate [79].

The evolving and changing composition of nanoparticle's corona interacts with the biological material at the molecular level, thereby determining the impact and potential toxicity of the individual particle on its surrounding environment [80]. A closer look reveals that the interface between the nanoparticle and its surrounding medium is shaped by the physicochemical configuration of the material, the solid–liquid interface covering the particle, and the contact zone of the interface interacting with the biological substrate [70, 81]. Although research is mostly concentrated on the interactions of nanoparticles with proteins, a wide range of other biomolecules, like carbohydrates, amino acids, and lipids, can contribute to the composition of the nano-bio interface and thus influence the functionality and signaling of those cells affected (Fig. 4). Based on this notion, the precise characterization of nanoparticles in their particular environment, for example, blood, interstitial fluid, or food, may be one of the key elements crucial for any classification of nanoparticle-mediated risks and the prediction of possible health impacts [82, 83]. However, for industrial relevant nanomaterials, hardly anything is known about the composition of their corona [84, 85]. Furthermore, there is a great lack of systematic data that would be actually necessary to understand how the corona will change in its composition and occurrence dependent on nanoparticle properties. In principle, this situation does not only apply to nanoparticle's protein corona.

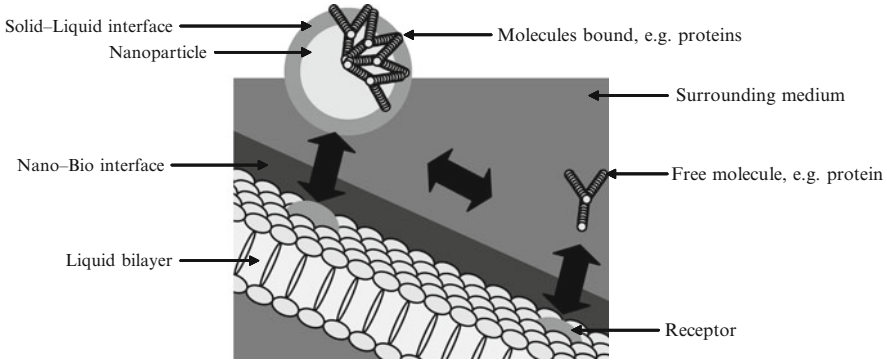

Fig. 4 Interactions at the nano-bio interface: molecules present in the extracellular matrix get adsorbed at nanoparticle's surface and then can bind to the cell membrane, for example, to receptors, just like completely free molecules dissolved in the medium (modified according to a graphic of the Center for Functional Nanostructures (CFN) at KIT, Germany; website: http://www.cfn.kit.edu/)

Rather, it is now well accepted that many physicochemical properties will change over the life cycle of nanomaterials [86, 87]. In conclusion, besides proper characterization of the material right after synthesis, we also need reliable and well-advanced *in situ* analytics of nanomaterials in all kinds of matrices. Research in this direction is only just about to emerge [88].

Measuring Nanoparticles

In analytics, it is common to distinguish between qualitative (e.g., determination of properties) and quantitative (e.g., determination of amounts) assessments. However, for nanomaterials, things reveal more complicated. For instance, determination of the size of a nanoparticle, which obviously looks like a qualitative measure, turns out to be a quantitative measure as well. As the sizes of nanoparticles in a particular preparation follow an individual distribution function, actually, the particle numbers in each size fraction have to be quantified [89].

As it is true in analytics in general, each measurement technique comes with its own limitations, but even more so, in the area of nanomaterials current methods usually offer no more than an estimation. For instance, in dynamic light scattering (DLS) measurements—a technique used to determine the size distribution of particles—readouts are size-weighted as the larger particles contribute much stronger to the scattered signals. In addition, what is actually measured is not the "size" but the hydrodynamic radius, which might result in significant differences for certain nanoparticles [90]. Nevertheless, generally there has been a rapid development for all kinds of analytical techniques in the nanotechnology field. Currently, more than 50 different techniques are used for physicochemical characterization of

nanomaterials. This range of methods encompasses all major areas of analytical chemistry [91, 92], and it would reach far beyond the scope of this introductory chapter to describe them all in more detail. Microscopy, spectroscopy, spectrometry, and separation techniques account for the main part of the analytical portfolio that enables to identify, to separate, and to visualize nanoparticles (Table 3). However, these techniques are thus far only validated for the characterization of nanomaterials under controlled conditions such as, for example, dispersed in water or other defined media or simply in its solid states [93]. Rarely, these methods have yet been tested for nanoparticles embedded or dispersed in complex environments such as human blood and tissues, food, or sewage.

In situ analytics of nanomaterials are meant to also consider and characterize the presence of the aforementioned particle corona and any changes in the aggregation behavior of the particles while being dispersed in biological media. Naturally the constraints of measuring nanoparticles in their particular environmental matrix raise the bar with respect to technological requirements and again lead to the question: Which specific parameters are actually of real importance for characterization in the given context and how to achieve this goal with existing technologies? Ideally from a risk assessment point of few, having the analytical challenges in

Table 3 Overview of currently applied analytical methods in the field of nanomaterials

Category	Technique		Sensitivity	Experience/ distribution based on applications	Costs	Parameters analyzed
Imaging techniques	TEM		h	h	h	Structure
	SEM		h	h	h	Structure
	AFM		m	h	h	Structure
Separation techniques	Chromatography (e.g., HPLC)		m	h	l	Size/structure
	AF4		m	l	l	Size
	CE		l	m	l	Size/charge
Characterization techniques	NMR		l	h	h	Composition/structure
	AU		m	m	l	Size/shape/structure
	MS	ICP	h	h	m / h	Mass/composition
		IM	m	m	m	Mass/composition
		ESI	h	h	m	Mass/composition
		MALDI	h	h	h	Mass/composition
		SIMS	h	l	h	Mass/composition
		DESI	h	l	h	Mass/composition
	DLS		m	m	m	Size/distribution
	SAXS		m	l	h	Size/shape/structure

l low, *m* middle, *h* high, *TEM* transmission electron microscopy, *SEM* scanning electron microscopy, *AFM* atomic force microscopy, *HPLC* high-performance(high-pressure) liquid chromatography, *AF4* asymmetric flow field-flow fractionation, *CE* capillary electrophoresis, *NMR* nuclear magnetic resonance (spectroscopy), *AU* analytical ultracentrifugation, *MS* mass spectrometry, *ICP* inductively coupled plasma, *IM* ion mobility, *ESI* electrospray ionization, *MALDI* matrix-assisted laser desorption/ionization, *SIMS* secondary ion mass spectrometry, *DESI* desorption electrospray ionization, *DLS* dynamic light scattering, *SAXS* small-angle X-ray scattering

mind, these techniques should be well established (validated), robust, and highly selective and sensitive enough for each given analyte.

Nanoparticles: Any Health Risks to be Expected?

To reliably identify the hazards and to assess the potential risks of nanoparticles for humans, we have to consider the following two issues already mentioned above: First, nanoparticles might significantly change the bioavailability of other potentially toxic compounds. In the environment, contaminants are often adhered to solid matrices, one of which could also be represented by the surface of nanoparticles. The efficient binding of chemicals is well documented for many nanomaterials, and this property can be used in a beneficial way to clean, for example, water or soil. CNTs can bind many organic compounds [94] or metals like copper [95] and cobalt [68], zero-valent iron oxide nanoparticles have been shown to adsorb a variety of compounds [96], and a similar behavior is known or expected for other types of nanomaterials as well. As a consequence, potentially toxic compounds might become more bioavailable through facilitated uptake into organisms and subsequent distribution throughout the body, thereby even better penetrating blood–tissue barriers. However, only few studies on this issue (typically in the context of air pollution) were conducted so far. For instance, it could be demonstrated that $FeCl_3$ strongly increases lung inflammation caused by 14-nm carbon black particles *in vivo* [97]. In an *in vitro* study, it was shown that $ZnCl_2$ potentiates TNF-α release in macrophages upon exposure to nanoparticulate air samples [98]. Similarly, putative allergens might attach to the surface of nanoparticles, thereby enhancing allergic reactions. This had been demonstrated for general airborne pollutant particles (diesel exhaust) first and recently also been shown for engineered nanoparticles [99, 100]. Resulting from such observations, nanoparticles are currently also tested as adjuvants in vaccine development [101].

Some of the observed adverse effects might actually result from the scavenging of essential nutrients or endogenous messengers such as hormones, meaning that some nanoparticles could be able to extract physiological compounds from the blood and thus causing a critical shortage in the whole body or in single organs/tissues [102]. Biomolecules that get incorporated into nanoparticle's corona might also undergo changes in their conformation and activity, which in turn can trigger adverse reactions in the whole organism such as, for instance, aberrant plasmatic coagulation or platelet aggregation [103, 104]. However, research in this direction is still extremely limited.

Secondly, the toxic potential of the material itself inevitably is to be evaluated faithfully and comprehensively. However, also in this field, there are usually still extremely limited—if any—data available, and sometimes, the results of different studies may be even contradictory and inconsistent and thus without any value for the assessment of risks [105]. For risk assessment purposes, low-dose, chronic *in vivo* studies would be of highest value as the outcome of such studies can be

used to predict possible overall adverse effects in humans. However, up to now, only for a limited number of nanomaterials (e.g., titanium dioxide, silver, carbon black, CNTs), *in vivo* data exist that typically were obtained through acute or subchronic dosing. Conversely, a large number of *in vitro* studies on nanoparticle toxicity have been published, yet most of them are only of limited value in risk assessment. Since usually extremely high doses are applied as bolus in the *in vitro* systems used, the extrapolating of such data to predict the corresponding outcome in humans is prone to fail. Moreover, the results obtained *in vitro* frequently even reveal inconsistent as recently been summarized for the endpoint genotoxicity [106]. Usually it is hard to figure out whether the inconsistencies observed in nanotoxicological studies are due to improper characterization of the material itself (e.g., unequivocal identity of the particles applied?) or due to an inadequate or flawed design of the study conducted (e.g., proper cell model and suitable endpoint selected?). Overall, the design of studies aimed at assessing nanomaterial toxicity requires much attention to many details as explained nicely in a number of articles [69, 70, 105, 107]. By all means, prior to testing, the nanomaterial needs to undergo copious characterization by several complementary techniques, and the doses finally applied in the experiment should reflect realistic dimensions expected to occur under real-life exposure conditions [70, 105].

In light of the large number of nanomaterials already known and expected to be produced in near future, a tiered testing approach has been proposed comprising a range of different *in vivo* regimes, but also cell-free and cell-based *in vitro* methods [107]. Nevertheless, until today, there is still no final consensus on which validated toxicological assays are appropriate and meaningful enough in the case of nanoparticles, how they optionally have to be adopted to this material, or whether new assays have to be developed and validated instead [69, 107, 108]. Of course, there is great international effort to harmonize national proposals and programs, for example, in the OECD Working Party on Manufactured Nanomaterials (WPMN) [108]. As already mentioned above, only little or even nothing is known for chronic, repeated, and low-dose exposures. Moreover, existing *in vivo* data only cover a small number of organisms, which is especially problematic for ecotoxicological assessments [109]. Mostly these studies employ a small range of established invertebrate models (e.g., *Daphnia magna*) and occasionally certain fish models (e.g., *Danio rerio*). However, virtually no studies exist on plants or other terrestrial organisms [109]. Another limitation is that only a few studies employ more than one organism such that comparison would be feasible, as it was done, for example, by Oberdörster and coworkers [110].

An increasing production and widespread use of nanoparticles enhances the probability of considerable human exposure. Humans can be exposed at workplaces, as consumers or patients or via the environment. Therefore, serious public concern has been raised in recent times about the safety of these materials for humans and the environment [67, 111]. As outlined above, the toxicity of nanoparticles in humans and their environmental fate in good parts result from intrinsic properties of the material and will be influenced by certain physicochemical properties such as size, shape, solubility, or biopersistence (Table 4). To some extent,

Table 4 Selection of physicochemical properties of nanoparticles and kinds of biological responses that are likely to be affected

Composition	Inherent toxicity of the nanomaterial (e.g., nickel, cadmium)
Size	Uptake, translocation, elimination
Shape	Uptake, clearance (e.g., fiber toxicity)
Surface modification	Uptake, interaction with biomolecules
Charge	Uptake, interaction with biomolecules
Conductibility	Interference with transport processes or signaling
Surface area	Interaction with and binding of biomolecules
Solubility	Release of potentially toxic ions, translocation, elimination
Strength/biopersistance	Clearance

these properties are expected to change depending on the particular biological environment.

The most important exposure routes of humans to be considered for nanoparticles are skin penetration, ingestion, and inhalation [70]. The skin represents an organ with large surface (about 1.5–2 m^2 for adults), and its architecture reveals kind of complex due to stratification. So, the outermost part is made by the stratum corneum barrier, which consists of dead cells and which is followed by the living layers in the epidermis and dermis. The epidermis does not contain blood vessels but is composed of keratinocytes, melanocytes, Langerhans cells, and Merckel cells. The basement membrane then connects the epidermis with the dermis beneath, which contains blood vessels, neurons, and hair roots and which is made of fibroblasts, adipose cells, macrophages, and dendritic cells. Since nanoparticles are also applied in various cosmetic products, dermal exposure and dermal penetration have been extensively studied in several *in vivo* and *in vitro* studies [112, 113]. Most *in vivo* studies have been performed with titanium dioxide (TiO_2) and zinc oxide (ZnO), which is reasonable as these metal oxides are currently widely applied in nanoparticulate configuration in sunscreens, typically with particle sizes of 20–50 nm. So far, there is no evidence that the stratum corneum barrier can be penetrated by such particles so that viable tissue will be reached [114–116]. Sometimes, it was observed that the nanoparticles entered hair follicles, and from this, it was concluded that the hair follicles might act as a reservoir that could facilitate dermal penetration [117]. However, since conclusive data were not presented, there is currently wide agreement that these particles tested do not reach viable cell layers in the skin, neither directly nor via hair follicles. Based on this, there was broad consensus that the dermal route is to be considered safe for nanoparticulate TiO_2 [118]. However, the range of nanoparticle types studied with regard to their dermal uptake is still very low, and—as noted above—the field was mainly concentrated on metal oxides. By contrast, investigation of other types of nanoparticles such as silver provided evidence that it well might be possible that such species penetrate into living skin [119]. Similar results were observed for fluorescent particles (fluorospheres) of 0.5 and 1 μm diameter that could reach viable epidermal and dermal layers [120]. It also should be taken into account that all of these data refer to intact and undamaged skin tissue. By contrast, damaged skin (e.g., UV burned) or skin from susceptible individuals afflicted by

skin diseases such as atopic eczema (neurodermitis) usually is not been considered. One study with hairless mice demonstrated that susceptible skin might be penetrated by nanoparticles quite easily [121]. This clearly shows that further work is needed also for the dermal route.

The second exposure route, that is, the oral or ingestion route, is only poorly addressed so far [122]. Most of the published studies are related to nanomedicine and drug delivery issues. It has been shown that microfold or M cells of the Peyer's patches in the small intestine, which can ingest particulate matter, might represent the major route of intestinal uptake of nanoparticles [122]. One study with nanogold demonstrated a size dependency of the uptake in the small intestine [123]. Investigation of 56-nm silver particles in a subchronic study revealed gastrointestinal absorption and subsequent systemic distribution toward a wide range of different organs including bladder, heart, lungs, prostate, kidneys, spleen, liver, brain, *etc*. [124]. In addition, signs of liver toxicity at medium and high doses of nanosilver were observed in this study. Other studies were performed with polystyrene or latex particles. Recently, one study showed that upon oral administration, 300 nm poly-D,L-lactide-co-glycolide (PLGA) particles could be detected in liver, kidney, brain, and other organs [125]. In general, however, the number of available *in vivo* and *in vitro* studies addressing the intestinal absorption of nanoparticles is as yet not sufficient enough to draw any health-related conclusions from it.

The inhalation route has gained great attention in recent years, and in fact, most *in vivo* studies in the field of nanotoxicology published so far relate to inhalation exposure [70]. It is considered the most relevant exposure route especially for workplace exposures [70]. Furthermore, a significant hazard was expected based on experiences with asbestos or crystalline silica (i.e., quartz, cristobalite), both of which are among classified carcinogens [126, 127]. Also, many experiences exist from studies with ambient air particulate matter (PM), which is traditionally classified according to its respective size as PM_{10}, $PM_{2.5}$, and $PM_{0.1}$ (see chapter on *Toxicology of Ambient Particulate Matter*, authored by van Berlo *et al*.). In terms of size, the $PM_{0.1}$ fraction would pertain to nanoparticles as it covers particulate matter in a size range below 100 nm. For ambient particulate matter, it was deduced that it is mainly the ultrafine fraction ($PM_{0.1}$) within many kinds of (environmental) pollution which causes adverse effects such as cardiovascular dysfunction [128].

Large numbers of studies exist on the size-dependent deposition and clearing of particles in the lungs [70, 129]. The upper airway and lower respiratory tract, down to the bronchial tubes (bronchi) and bronchioles, are covered by an epithelium and a small lining of tiny cilia which act as filter and motor to move the epithelial mucus upward. So, the main clearance mechanism in this ciliated region of the respiratory tract enables to trap the particles in the mucus and to subsequently push them upward toward the trachea via the so-called mucociliary escalator mechanism. By contrast, the most proximal endings of the tracheobronchial tree, that is, the gas-exchanging alveoli, are neither covered with cilia nor possess any mucus layer; instead, they produce surfactant [129]. Particle clearance in this part of the lungs is only feasible via cellular phagocytosis, mainly executed by pulmonary (i.e., alveolar) macrophages or by dissolution and subsequent removal via bloodstream.

Particles below 2.5 μm can reach even these lowest parts of the pulmonary tract and are being potentially eliminated via both kinds of mechanisms [70]. In this context, it should be mentioned that the International Commission of Radiological Protection (ICRP) proposed a prediction model which also enables for estimation of the amounts of particles deposited in each lung compartment depending on the particle size [130]. According to this, more than 90% of very small nanoparticles (1 nm) retain in the nasopharyngeal region, while the remaining fraction distributes in the tracheobronchial tract without reaching the alveoli. By contrast, about 50% of 20-nm particles would spread far down and populate the pulmonary alveoli [70].

It has been proposed that the higher inhalation toxicity of nanoparticles compared to their corresponding bulk particles not solely results from the facilitated and deeper penetration of the smaller particles into the region of pulmonary alveoli. To prove this, Oberdörster and coworkers used TiO_2 particles of two different primary sizes (25 nm and 250 nm) but with the same overall hydrodynamic radius (1 μm), thus leading to the assumption that the deposition behavior in the lungs most likely would be comparable for both. The results of the studies performed, however, showed that the 250-nm particles failed to induce pulmonary inflammation, while the 25-nm particles did [131, 132]. Similar studies have been performed with other types of nanoparticles such as carbon black [133, 134] or polystyrene beads [135]. The current conclusion drawn from the data obtained is that the increased surface area of nanoparticles, compared to corresponding bulk particles, which is also partially retained in agglomerates, will cause oxidative stress and inflammation in the distal pulmonary tract. An additional major concern derived from these *in vivo* studies comes from the observed translocation of particles to extrapulmonary tissues [70, 136]. It is thus highly likely that nanoparticles may reach secondary target organs through the pulmonary exposure route. These secondary organs and tissues can then be adversely affected, for instance, the cardiovascular system, the spleen, or the brain [137–139].

Due to time constraints, monetary aspects, and—first and foremost—animal welfare considerations, for sure it is inconceivable to characterize all kinds of different nanoparticles and nanomaterials regarding their safety and toxicological behavior via long-term *in vivo* studies. Instead, robust and reliable non-animal testing approaches based on *in vitro* and *in silico* methods need to be developed. As another objection against any full-range *in vivo* testing philosophy, the knowledge gained through whole animal studies into the *molecular mechanisms* that may underlie any kind of nanotoxicological effect and its transferability to the human system is likely to be limited. To date, a large amount of *in vitro* studies are published, which all report some adverse effects—at least in high concentration ranges—of a large variety of different nanoparticles (cf. above). For instance, the available data for titanium dioxide, nanosilver, and nanogold are nicely summarized in several reviews [140–142]. Most of the *in vitro* studies report on oxidative stress, cytotoxicity, and inflammatory responses. For several types of nanoparticles, it has been shown in a range of different cell lines that reactive oxygen species (ROS) are being formed and/or the levels of glutathione become depleted [143]. In addition, it could be demonstrated that certain signaling pathways like NFκB or

AP-1 are being activated [144]. In terms of genotoxicity, the data are still contradictory and inconsistent [106]. Currently, there is much discussion about how to harmonize such studies, how intense the characterization needs to be done in advance, and which readouts are suitable and meaningful enough [107, 108]. Finally, besides harmonization, validation is necessary as well. At OECD WPMN [108], one working package is commissioned to particularly focus on the further development of *in vitro* assays. Beyond that there is intense discussion on how to correlate data obtained *in vitro* to the *in vivo* situation expected to be present in all kinds of organs and tissues in the body [145, 146]. A general problem is the issue of dosimetry. Traditionally also nanoparticle dosages are reported as mass doses (e.g., mg/L). However, there is evidence that other dose metrics, such as particle number or surface, might be better suited and more meaningful [70]. Although it seems that—to a good part—data might be calculationally converted from one dose metric to another, there can be severe limitations as the shape or the agglomeration grade might vary from batch to batch or from study to study. So, it still looks that several unresolved issues pave the road toward reliable and resilient risk assessment approaches required for safe nanotechnology applications in the years ahead.

Perspectives

To further develop nanotoxicological assays, we need to better understand which kinds of physicochemical properties of the particles may exert an influence on the overall adversity in cells and tissues and by which mechanisms. This set of characteristics is likely to encompass intrinsic material properties such as size or shape, but also dynamic properties, such as the corona, that may change over the life cycle of particles. Currently existing data gaps are huge, which does not really come as surprise given that nanotechnology is still a young and heavily developing branch of science. These data gaps pertain to both core elements of any risk assessment, that is, the quantitative characterization of external and internal exposures as well as the dose dependency of toxicological effects [147, 148]. As for exposure assessments, there is still the issue awaiting to be resolved how to measure nanoparticles in their natural environments. High background levels from natural (nano)particles exist and, thus, currently available techniques usually reach their limits. Ideally a measurement method would be able to quantitatively assess the number of particles in a certain size range and—at the same time—provide information about their chemical composition. Since such analytical instrumentation is currently unavailable, at the present state we can only obtain estimates on the levels of exposures in the general environment or under consumer conditions. On the other hand, this situation might be different for workplaces, where the type of material and the putative emission source are usually known. Therefore, currently serious strategies for the assessment of exposures to nanoparticles only exist for occupational environments [149].

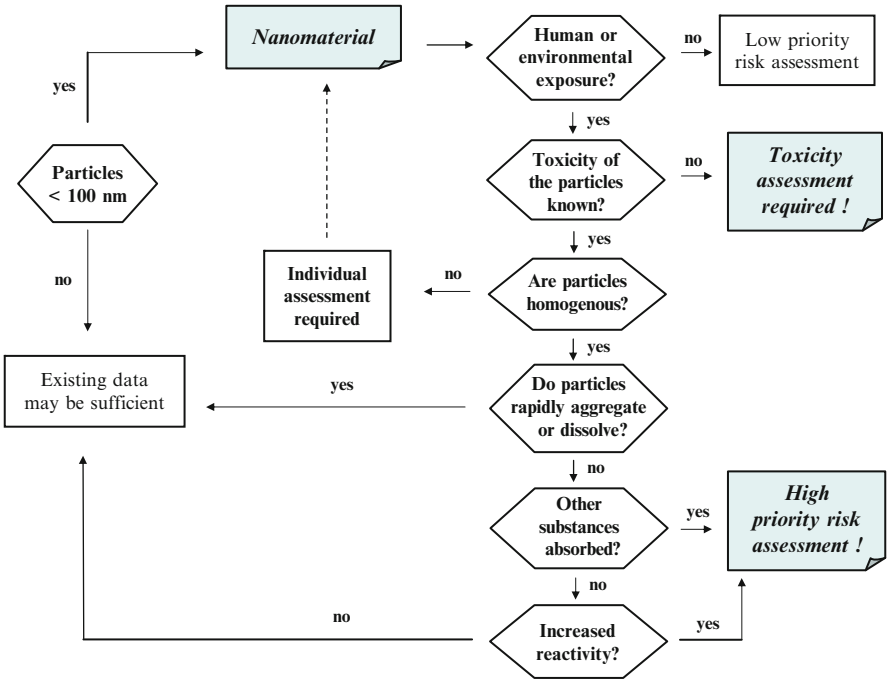

Fig. 5 Flowchart of a possible risk assessment decision tree applicable for nanoparticles and nanomaterials (adapted from [150])

Important for the toxicological assessment of nanomaterials, uncertainties also exist on which and how abiotic factors might contribute to the time-dependent alteration of nanoparticle's properties and thus how they would influence its toxicity. We almost know nothing about how mixtures or formulations may contribute or alter inherent properties and the environmental behavior of nanoparticles. As it is not even conceivable to introduce each different type of nanomaterial into a conventional testing strategy, it would be completely beyond any scope to test all kinds of possible mixtures or formulations individually. Hence, we have to find alternative ways to deal with and to successfully address these issues. Another major lack of knowledge relates to low and repeated dose exposures and to chronic endpoints. Given all of this, it is obvious that significant work needs to be done before regulators will be in a more comfortable situation with regard to health safety considerations.

Several authors made suggestions how currently a decision tree for risk assessment of nanomaterials could look like. An example is given in Fig. 5.

Risk assessment of chemicals in general and of nanoparticles or nanomaterials in particular should be performed in an evidence-based, robust, and transparent way, and the final conclusions drawn need to be comprehensive, reasonable, and logic. As explained in detail above, the evidence base for nanomaterials is still extremely holey and incomplete. It seems reasonable that new technologies may need some

time until all methods required for characterization and testing are sufficiently developed and in place. In the field of nanotechnology, however, the closing of data gaps will be crucial for the development of risk assessment strategies and for establishing regulatory measures [151]. Moreover, it even will be key for no less than the further development and the general acceptance of this technology in the public. To reach this goal, we need to be as soon as possible in a situation where all of the following questions can be answered by a "yes":

- Are the existing methods of testing (exposure and toxicology) sufficiently suited for nanomaterials?
- Are our risk assessment strategies reliably applicable and sufficiently suited for nanomaterials?
- Is our current legislation sufficient to cover also nanomaterials and all application fields adequately?

Certainly, there is still a long way to go in the safety field pertaining to nanotechnology. The past years have taught us, however, that thanks to worldwide efforts we can gain much progress even in such complicated areas in short term. In light of the most current developments in analytical instrumentation, exposure monitoring measures, and non-animal testing procedures, now, much more than 5 or 10 years ago, it becomes conceivable to reach a point in the future where the continuing evolution of nanotechnology can be accompanied, backed up, and supported by adequate safety assessments of and decision-making on the materials produced [151].

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