32. Nanomaterial Toxicity, Hazards, and Safety

Zuzanna A. Lewicka, Vicki L. Colvin

Manufactured nanoparticles of different chemical compositions are now widely commercially applied. They are found in places as diverse as food packaging and automotive bumpers, where their special nanoscale properties help to lower cost while improving performance. Given these widespread applications, the unintended effects of manufactured nanomaterials on workers, consumers, and the environment have become a focal point for international research. Initially, the human health effects of nanoscale materials were of most interest, but more recently identification of nanoscale particles in wastewater sludge has turned attention towards their environmental impacts. Though the topic of nanomaterial safety has received substantial attention in the literature, many basic questions about nanoparticle transport, fate, and toxicology remain unanswered. A central challenge for researchers has been the definitive characterization of particular manufactured nanomaterials, particularly in commercial products that have significant human or environmental exposure. Careful determination of the physical size, surface chemistry, internal structure, and intermediate stability of manufactured nanomaterials helps investigators compare results, as well as link unwanted biological outcomes to particular material features. This chapter provides an overview of the current exposure and toxicity studies of manufactured (e.g., engineered) nanomaterials. A special emphasis in this chapter is the practice used for nanomaterial characterization as it relates to their biological and environmental properties.

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Nanotechnology has been defined by the American Society for Testing and Materials (ASTM) as [32.1]

> *A term referring to a wide range of technologies that measure, manipulate, or incorporate materials and/or features with at least one dimension between approximately* 1 *and* 100 nm. *Such applications exploit the properties, distinct from bulkmacroscopic systems, of nanoscale components.*

32.1 Engineered Nanomaterials – General Overview

Nanoscale materials (or nanomaterials) have been described as any material having one or more dimension between approximately 1 and 100 nm [32.1, 4]. These materials can be naturally occurring (e.g., volcanic ash), incidentally produced (e.g., diesel exhaust particles) or intentionally manufactured (e.g., carbon nanotubes). Of most relevance to this work is the latter class – manmade (engineered or manufactured) nanoscale materials designed with specific properties (e.g., mechanical, optical, electrical, and/or magnetic) that differ from those of bulk materials. Nanomaterials can exist in various shapes: nanoplates, nanorods, nanotubes or nanoparticles, as presented in Fig. 32.1. According to the British Standards Institution (BSI), nanoplates are objects which have a nanoscale-order thickness while having two other external dimensions significantly larger, nanorods have two similar external dimensions on the nanoscale and the third dimension significantly larger, nanotubes are hollow nanorods, while nanoparticles are defined as particles with all three dimensions within the nanoscale [32.4].

Nanoscale materials are often lighter, stronger, and more reactive than bulk materials and can be applied in industries as diverse as medicine and aerospace [32.2, 5]. One size-dependent property that often motivates incorporation of nanoscale materials is the surface area. As the size of a material decreases, the ratio of surface molecules or atoms to total molecules or atoms increases exponentially [32.6]. Greater surface area means a larger fraction of material is available for chemical reactions, a fact that generally increases nanomaterial overall reactivity as compared with bulk materials (Fig. 32.2) [32.3]. This translates into improved physical, chemical, and biological properties which can be incorporated into many improved applications. Other size-dependent properties may arise from the confinement of electronic excitations, or the enhanced cooperativity of magnetic spins in solids of low dimensions [32.7, 8].

Given their many unique and size-dependent properties, it is not surprising that nanomaterial use in consumer products is increasing rapidly [32.9, 10]. In 2011, the Project on Emerging Nanotechnologies (PEN) inventory identified over 1300 nanotechnology-based consumer products on the market from over 24 countries, including the USA, China, Canada, and Germany [32.10]. The largest group of products (738 products) was within the health and fitness category, which included cosmetics, clothing, personal-care products, sporting goods, sunscreens, and air and water filters. However, the database has not been updated since March 2011. Therefore, the number of consumer

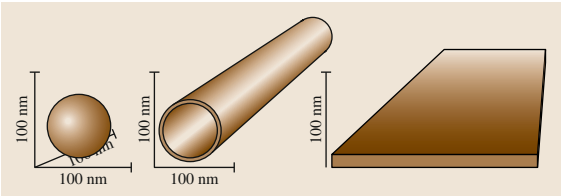

Fig. 32.1 Schematic representations of various forms of nanomaterials, which include nanoparticles (nanoscale in all three dimensions), nanotubes (nanoscale in two dimensions), and nanoplates (nanoscale in only one dimension); nanoscale refers to a dimension between 1 and 100 nm (after [32.2])

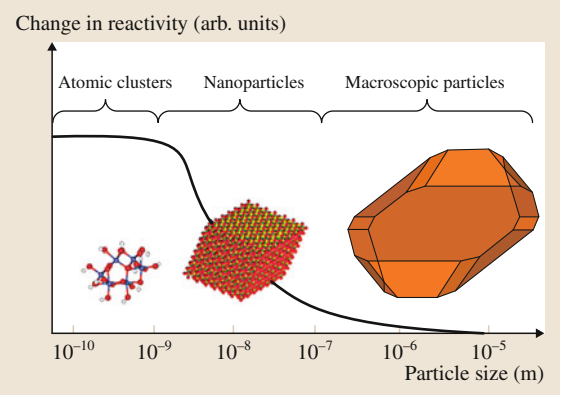

Fig. 32.2 Trend for size-dependent reactivity change of a material as the particle transitions from macroscopic (bulk like) to atomic (after [32.3])

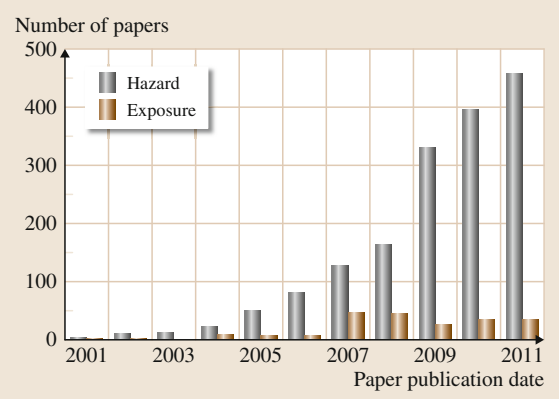

Fig. 32.3 Annual number of articles published in scientific journals, broken down by study topic: nanomaterial hazard versus exposure (after [32.12])

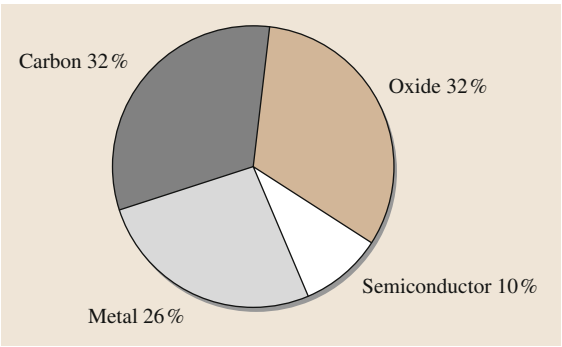

Fig. 32.4 Percentage of scientific papers addressing the hazard and exposure of carbon-based, metal oxide, metal, and semiconductor nanoparticles between 2001 and 2011 (after [32.12])

products having engineered nanomaterials as presented in PEN is only a portion of the nanotechnology-enabled products currently on the market [32.10, 11].

As their use has expanded to include many applications that bring them into contact with people and the environment, significant questions have been raised about their possible biological and environmental interactions [32.13, 14]. As noted by *Simkó* and *Mattsson* [32.15], *A risk can be deduced from exposure data together with the hazard assessment* that results after exposure. As shown in Fig. 32.3, work on hazards has dominated the development of the study of nanomaterial risk, representing 90% of papers published in scientific journals.

Different nanomaterials have been the subject of varying levels of risk-based analysis. These types include carbon-based materials such as fullerenes and carbon nanotubes, metal oxide nanoparticles and rods (iron oxide, cerium oxide, titanium dioxide, silicon dioxide, etc.), metals (gold and silver), as well as semiconductor nanoparticles or so-called quantum dots (typically cadmium sulfide or cadmium selenide) [32.16]. Interestingly, a literature analysis reveals that of these materials the metal oxide and carbon-based systems have been the most examined (Fig. 32.4).

32.2 Occurrence of Engineered Nanoparticles in the Environment

As the number of consumer products containing engineered nanoscale particles grows, so will the chance of environmental exposure of a wide range of organisms [32.17]; For example, it has been estimated that in the USA, based on the reported market size for suncare products, approximately 125 tons of nanoscale TiO_2 and ZnO ultraviolet (UV) blocking agents are used (and released) in commercial products every year [32.18]. Nanoscale TiO_2 and ZnO particles are used in sunscreens because they do not scatter visible light like their larger counterparts and appear transparent when applied to the skin [32.19, 20]. Furthermore, *Hendren* et al. estimated upper and lower bounds for the annual production quantities for five classes of engineered nanomaterials in the USA: titanium dioxide (TiO_2), silver (Ag), cerium oxide (CeO_2), carbon nanotubes (CNTs), and fullerenes, which ranged from 7800–38 000 tons/year for TiO_2, 2.8–20 tons/year for Ag, 35–700 tons/year for CeO_2, 55–1101 tons/year for CNTs, and 2–80 tons/year for fullerenes [32.21]. These estimates were done based on data from academic publications, professional reports, company websites, production process patents, and personal communication with company representatives. The fact that such information was not readily available reflects the lack of labeling and reporting standards for consumer products that contain nanoscale materials.

Nanoparticles can enter the environment during either their manufacture, use, or disposal; studies of nanoparticle fate and transport have concluded that the natural sinks for nanoparticles generally are soil or water [32.22, 23]. So far, no measurements of engineered nanoparticles in the environment have been able to quantify trace concentrations of these mater-

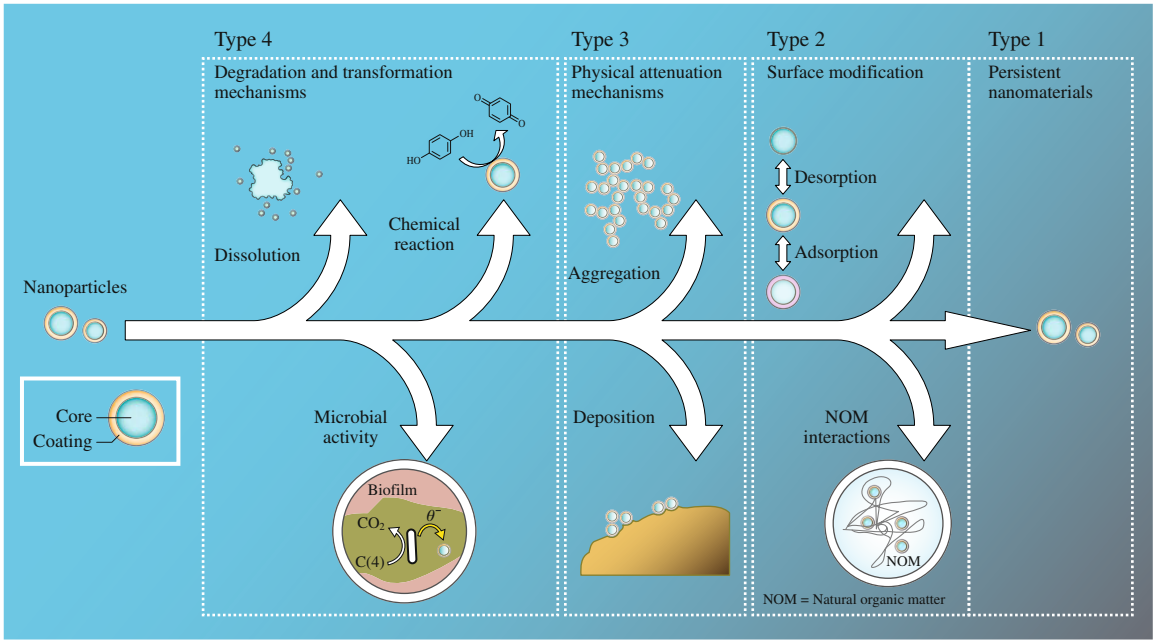

Fig. 32.5 Transformation of nanomaterials as they move through the environment (after [32.28])

ials [32.17]. However, some have found qualitative evidence that nanoscale materials, particularly those from sunscreens, can be detected in the solid waste generated by wastewater treatment plants [32.24]. Due to the lack of quantitative analytical tools for environmental exposures, much of the information about exposure has been derived from relatively coarse-grained environmental exposure models [32.25–27].

To complicate the study of exposure even further, a growing body of research focuses on the fact that nanoparticles can drastically change their behavior and physical characteristics as they move through the environment (Fig. 32.5) [32.28–33].

Changes in physicochemical properties such as surface charge and size can modify environmental fate and transport. As an example, Guzman et al. showed that the mobility of nanoscale materials in aqueous environments is dependent on the size of the nanoparticles. They reported that the point of zero charge (pzc) of TiO_2 nanoparticles changed with their size and that the aggregation state increased as the pH of the solution approached the pzc [32.34]. On the other hand, *Labille* et al. studied the aging and fate of TiO_2 nanoscale particles recommended by the manufacturer (BASF Chemical Company) as sunscreen pigments and discovered that, after contact with water, the TiO_2 nanocomposite dispersed and formed a stable suspension available to microorganisms [32.35]. *Lin* et al. published a thorough review on the fate and transport of nanoscale materials in the environment, which included aggregation and suspension behaviors with emphasis on the influencing factors, including natural colloids, natural organic matter, pH, and ionic strength [32.36]. The authors concluded that there are still many unknowns regarding the environmental fate, transport, exposure, ecotoxicity, and lifecycle of engineered nanomaterials and that future research should focus more on real environments and experiments in the field.

32.3 Effects of Nanoparticles on Organisms

Once released into the environment, nanomaterials will interact with organisms [32.6, 37–39]. Although nanomaterial toxicity is an active area of research, the majority of the published data focus on mammalian toxicity studies using a range of in vitro and in vivo tests to assess the possible toxic behavior of nanoscale materials

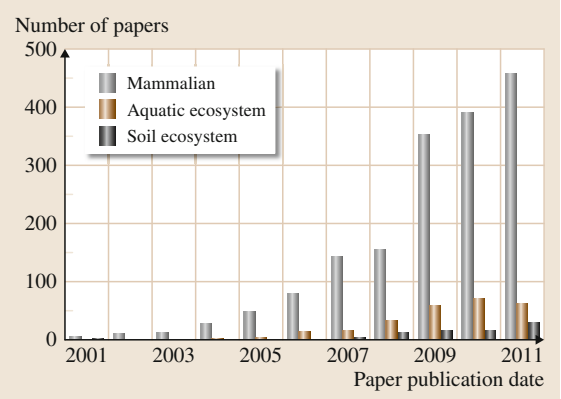

Fig. 32.6 Annual number of articles published in scientific journals by topic: mammalian, aquatic ecosystem, and soil ecosystem toxicity studies (after [32.12])

(Fig. 32.6). On the other hand, study of nanomaterial ecotoxicity, which identifies hazards to the environment in both aquatic and soil ecosystems, is just emerging.

32.3.1 Ecotoxicity of Nanoparticles in Aquatic Ecosystems

In 2004, *Oberdorster* et al. demonstrated for the first time that 0.5 ppm aqueous carbon-based nanoparticles (uncoated fullerenes, C_{60}) can cause oxidative damage and depletion of glutathione (GSH) in vivo in an aquatic species (juvenile largemouth bass) [32.37]. Since that initial report, researchers have studied how nanomaterials affect other freshwater species and marine organisms including trout, fish, waterflea, bacteria, algae, and other aquatic plants [32.40–44]. *Farre* et al. presented a review of ecotoxicological data on carbon-based nanomaterials, metal and metal oxide nanoparticles, as well as quantum dots in the aquatic environment [32.40]. The results indicate that nanoparticles may have ecotoxicological effects which depend sensitively on the physicochemical properties. Factors such as the chemical composition, concentration, size, shape, surface coating, charge, as well as mechanical stability can play a central role in whether a given nanomaterial is toxic or benign [32.45–50]. *Farre* et al. and *Kahru* and *Dubourguier* discussed also challenges in nanoecotoxicological research [32.40, 51]. Both groups agreed that the fate of nanosized materials and in situ investigation of their impact on organisms are of the highest priority for validation of models proposed for environmental risk assessment of nanoparticles. Moreover, physicochemical characteristics of particles before, during, and after experiments were vital to ensure the progress and comparison of research results. Furthermore, a battery of tests with different organisms is recommended to ensure adequate evaluation of the ecological situation; For example, *Kahru* and *Dubourguier* demonstrated that, among organism groups representing the main food-chain levels (bacteria, algae, crustaceans, ciliates, fish, yeasts, and nematodes), algae and crustaceans (daphnids) were the organism groups most sensitive to aquatic exposure of nanoparticles [32.51].

32.3.2 Ecotoxicity of Nanoparticles in Soil Ecosystems

While research on the ecotoxicity of nanoparticles in aquatic ecosystems is not yet complete, it is an extensive dataset compared with the very few studies of nanoparticles in the terrestrial ecosystem (Fig. 32.6). Soil ecosystems are much more complex than aqueous ones. Soils contain a wide variety of colloidal materials, including phyllosilicates, humic acids, iron oxides, and naturally occurring nanosized particles [32.52, 53]. These complexities make it particularly difficult to measure and distinguish between naturally occurring materials and engineered nanoparticles. Moreover, colloidal soil can interact with engineered nanoscale materials and affect their fate, transport, and transformation [32.36, 54]. For this reason, information on the interactions of engineered nanoparticles with soil components is needed to understand the effect of nanoparticles on terrestrial organisms.

Microbes are an important component of the terrestrial ecosystem, yet the antimicrobial activity of nanoparticles largely has been studied with human pathogenic bacteria. There is very little information available on soil microorganisms such as those that promote plant growth (e.g., rhizobacteria, nitrifying and denitrifying bacteria) or those that benefit nutrient cycling in soils [32.55]. Fourteen studies reporting the effects of engineered nanoscale materials on soil microorganisms published between the years 2007 and 2011 were reviewed by *Dinesh* et al. [32.55]. They summarized that fullerenes did not cause any significant toxicity to soil microorganisms while high concentrations (5000 µg/g soil) of multi-walled carbon nanotubes could significantly suppress the activity and biomass of soil microorganisms. Metal nanoparticles such as Al, Si, Pd, Au, and Cu and metal oxides (TiO_2 and ZnO) impacted soil bacterial communities, with silver nanoparticles being highly toxic to these organisms. It is important to note that the mentioned studies were done

using simplified ecosystems, under controlled conditions, far from the actual soil environment. Real-world studies, using components that reflect the complexity of the existing environment, are vital in order to assess the actual risk of manufactured nanomaterials in this environment.

The interactions between engineered nanoparticles and plants are another important area for study. A review of the toxic effects of engineered nanoparticles on plant growth was completed by *Ma* et al. [32.56]. They focused on the toxicity of nanoparticles to plant seedlings and cells, as well as analyzing uptake, translocation, and accumulation of nanoparticles by plants and their interactions with plant cells. Most studies of nanoparticles indicated a certain degree of phytotoxicity to seedlings affecting, e.g., root elongation and seed germination; For instance, *Canas* et al. investigated the effects of the surface properties of single-walled carbon nanotubes on root elongation of six crop species [32.57]. They tested the phytotoxicity of nonfunctionalized and functionalized carbon nanotubes on cucumber, cabbage, carrot, lettuce, tomato, and onion. Nonfunctionalized nanotubes inhibited root elongation in tomato but enhanced that of onion and cucumber. Functionalized nanotubes inhibited root elongation in lettuce, while cabbage and carrots were not affected by either form of nanotubes. On the other hand, *Khodakovskaya* and coworkers did not find any toxic effects of multi-walled carbon nanotubes on root elongation of tomato seedlings up to a concentration of 40 mg/l but observed an increase in seed germination [32.58]. Ma et al. concluded that differences in the toxicity of nanoparticles to plants may arise from the changing physicochemical properties of nanoparticles as they are exposed to the exudates of varying plant species.

Recently, studies have also been performed on terrestrial organisms such as the nematode *Caenorhabditis elegans* [32.59, 60]. Wang et al. did not observe a significant difference in toxicity between nano (TiO_2 of 50 nm, ZnO of 20 nm) and bulk materials (> 200 nm), for example. However, as is the case in many studies, there was a significant difference between the particle compositions as a function of size. On the other hand, Roh et al. demonstrated a relationship between the diameter of TiO_2 nanoparticles and their impact on nematodes. Smaller-sized (7 nm) titania had a more negative impact on *C. elegans* fertility and survival than larger-sized zinc oxide (20 nm). These kinds of inconsistencies in the nanotoxicology literature are more the exception than the rule. *Peralta-Videa* et al. reviewed available literature on the toxicity, fate, and transport of nanoparticles in terrestrial ecosystems for the years between 2008 and 2010 [32.17].

Most analyses of the data suggest that direct comparison between studies is a challenge because the form of the nanomaterials – their size, shape, degree of agglomeration, and composition – was not appropriately defined [32.61]. Thus, studies of nanoscale titania can reach different conclusions because the specific forms of nanoscale titania examined can be different. Furthermore, there are no specific standardized protocols or certified reference materials for nanomaterial testing, which leads to difficulties in comparing results. *Handy* et al. reviewed ecotoxicity test methods for conventional chemicals and presented strategies and modifications to these experimental methods and protocols so they can be applied to nanomaterial testing [32.62].

32.3.3 Routes of Human Exposure to Nanoparticles and Their Translocation in the Body

Human toxicity studies of nanoscale materials are far more common in the research literature (Fig. 32.7). These studies usually start with a perspective about the nanomaterial exposure routes, which include inhalation, skin penetration, and ingestion [32.6, 16, 63, 64]. As shown in Fig. 32.7, inhalation studies are the most prevalent, largely because of the existing work on incidental exposure to exhaust particles.

Respiratory Route

Nanoparticle deposition in the body after inhalation and the factors influencing the fate of inhaled nanomaterials are described in the reviews by *Yang* et al. [32.65] and *Bakand* et al. [32.66]. Once inhaled, these materials will be carried by diffusional motion from the nose or mouth through the various diameters of airways (trachea, bronchi, bronchioles) to the alveoli [32.64, 67]. As a result, particles of different sizes will have different effects in different parts of the lungs [32.6, 63]. Lipid- or water-soluble particles will be dissolved by mucous or serous lining fluid on the walls of the respiratory tract. These soluble components can interact with proteins or subcellular structures and eventually be transferred to the blood. Finally, metabolic products of these solutes may reach other organs and produce toxic effects [32.63]. In case of insoluble particles smaller than 200 nm, neither tracheal mucociliary transport nor digestion by defense cells such as alveolar macrophages would be able to completely remove these particles. Such small materials may be able to translocate through

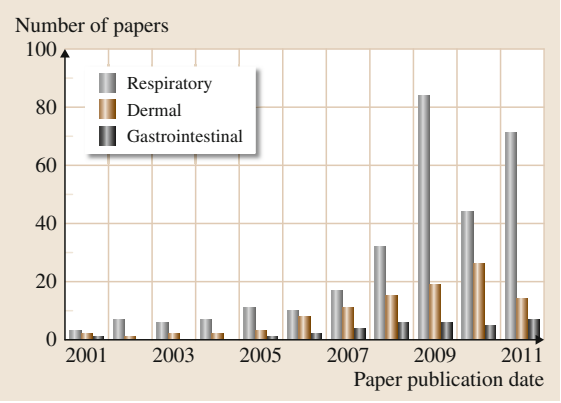

Fig. 32.7 Annual number of articles published in scientific journals by human health topic: respiratory, dermal, and gastrointestinal routes of nanoparticle exposure (after [32.12])

the lymphatic pathways into the blood, resulting in systemic exposure of internal organs [32.6, 63, 64].

For example, in vivo studies by *Kreyling* et al. showed that about 1% of iridium particles (15 and 80 nm) inhaled by rats accessed the systemic circulation and deposited material in organs such as liver, spleen, heart, and brain [32.68]. Moreover, 15 nm particles were deposited at a rate far greater than 80 nm particles. Furthermore, translocation of carbon nanoparticles into the brain via the olfactory nerve system has also been reported [32.69]. Metal oxide nanoparticles such as TiO_2 entered the brain through the olfactory bulb, as well. In the presence of TiO_2 particles, the level of malondialdehyde (MDA), which is a marker of oxidative stress, was elevated and ultrastructural changes of neurons in the hippocampus were observed [32.70]. Wang et al. noticed differences in the responses of the central nervous system to anatase TiO_2 particles versus rutile titania, with lower risk potential for the latter. In summary, different types of inhaled nanoparticles can translocate into the liver, brain, and other organs through blood vessels and the central nervous system. This exposure can produce acute and chronic changes within cells and organisms. Fewer data are available about dermal contact and ingestion exposures [32.2, 71].

Dermal Route

Currently, there is widespread use of nanotechnologies in cosmetic products [32.10, 72, 73]. Most relevant for these topically applied products is the potential for nanoparticles to translocate across the skin. Studies of the skin penetration of cosmetic nanoscale materials have been reviewed by *Nohynek* et al. [32.74, 75]. Researchers found an agreement that such particles can accumulate in hair follicle openings and the stratum corneum but do not reach living cells of the epidermis and dermis. A negligible tendency for penetration into intact and healthy skin, independent of species, has also been reported recently for topically applied nanosized TiO_2, ZnO, and quantum dots [32.76–80].

On the other hand, *Ryman-Rasmussen* et al. reported that spherical quantum dots with diameter 4.6 nm were able to reach the dermis of porcine skin [32.81]. *Baroli* et al. have shown, using human skin pieces, that metallic nanoparticles smaller than 10 nm were able to localize to the epidermal layers [32.82]. *Huand* et al. demonstrated diffusion of 5 nm gold nanoparticles through the stratum corneum of intact mouse skin [32.83]. Other studies performed by *Sonavane* et al. have found that 15, 102, and 198 nm gold nanoparticles penetrated ex vivo rat skin [32.84]. Furthermore, it has been shown that the state of the skin can have a profound effect on its barrier properties; For example, flexed, broken or diseased skin has a greater susceptibility to penetration by nanoparticles [32.78, 80, 85–89].

As noted in prior areas of safety research, these studies are difficult to compare. They differ in methods (for example, tape stripping, scanning or transmission electron microscopy, fluorescent microscopy), type of investigated nanoparticles (elemental composition, size, and shape), and models (e.g., pig, rat, human biopsies). In particular, measuring the penetration of any substance across human skin – whether it is nanoscale or not – is a challenging experiment, and one that is fraught with controversy [32.90–93]. It is not surprising that these unresolved issues in dermal toxicology are reflected in the inconsistent data found in investigations of nanoparticle–skin interactions.

Gastrointestinal Route

Due to the limited number of studies and lack of complete characterization of the nanomaterials studied, there is also no consensus about the behavior of nanomaterials in the gastrointestinal tract [32.2, 94]. Nanomaterials may get into the human body directly via oral ingestion of food, water or drugs that contain nanoscale materials [32.6, 64]. Currently, nanotechnology is mostly used in functional food compounds and delivery systems, food packaging, and food security [32.94, 95]. Food may also be unintentionally contaminated by nanoscale materials through environmental exposure during production [32.94]. Alternatively, exposure to nanoparticles may occur through

hand-to-mouth transfer if products, such as cosmetics, are left on hands [32.64, 96]. Nanoparticles may also be cleaned from the respiratory system through mucociliary transport, where they are swallowed and introduced into the gastrointestinal tract [32.63]. Once in this environment, materials could be eliminated in urine or feces or penetrate the gastrointestinal tract, resulting in translocation to other organs [32.97]; For example, 98% of water-soluble fullerenes when administrated orally to rats were cleared in the feces within 48 h, whereas the rest were eliminated via urine [32.98]. On the other hand, studies by *Jani* et al. have shown that rutile TiO_2 particles of nominal size (500 nm), administered orally as a suspension to female rats, were translocated to systemic organs such as the liver and spleen [32.99]. Furthermore, when mice were exposed to nanoscale particles of copper via he gastrointestinal tract, these materials targeted kidney, liver, and spleen, causing heavy injuries to these organs [32.100].

In summary, current in vivo studies indicate that the target organ for nanomaterial exposure depends on the experimental conditions and nanoparticle physicochemical properties. However, a central and still unanswered question is the rate at which nanoparticles are cleared or degraded. A review discussing the interaction of nanoparticles with living systems has been carried out recently by *Lu* et al. [32.101]. One model anticipates that, should nanoparticles find their way into the body, they would be marked with proteins as foreign agents and consumed by phagocytes (immune cells) [32.102]. After delivery to the lymph nodes, they could be biodegraded into biologically safe components if their constituent material is itself nontoxic. Phagocytosis is a basic defense mechanism against exogenous substances. However, if particles are very stable and difficult to metabolize within the body, they could reside in the body for long time periods and unknown health effects may occur.

32.3.4 In Vitro Toxicity of Nanoparticles

In the past few years many investigators have been developing in vitro model systems using both human and animal cells to simplify the study of the cellular response to nanoparticles [32.103, 104]. While in vitro testing provides a limited view of the responses of only the cell types being tested, it is quick and relatively inexpensive, and allows evaluation of specific mechanisms of action [32.105]. Although nanoparticle-induced cytotoxicity has been reported by several groups, the exact mechanism for toxicity is not well understood [32.106–109]. Nonetheless, studies point to nanoparticle-enhanced generation of reactive oxygen species (ROS) that may result in oxidative stress, inflammation, and consequent damage to proteins, membranes, and DNA [32.110, 111].

Lewinski et al., who reviewed the cytotoxicity of carbon-, metal-, and semiconductor-based nanoparticles, concluded that different data have been published about cytotoxicity due to differences in experimental procedures as well as differing nanoparticle properties [32.106]. Incomplete characterization and lack of protocols and calibration standards in nanoparticle characterization will result in inconsistent and unreliable in vitro toxicity data; For example, in one study, multiwalled carbon nanotubes were reported to be toxic and cause a decrease in mitochondrial membrane potential [32.112], whereas another study reported that these nanotubes were nontoxic [32.113]. Such uncertainties and inconsistencies are likely caused by variations in nanoparticle characteristics and experimental procedures [32.106, 114–116].

Because the physicochemical properties of nanomaterials can influence toxicological endpoints, thorough characterization is vital to this community [32.29, 105, 117, 118]. There is no doubt that nanoscale particles can in some forms be biologically reactive and lead to cell damage; what is less clear is whether these cell culture effects are apparent in animal systems [32.119]. In addition, the risk due to any potentially toxic substance is not only a function of the hazard but also of the chance of exposure. However, lack of exposure data for humans and the environment limits the effective risk assessment of commercially available nanomaterials [32.9, 120].

32.4 Nanoparticle Physicochemical Characteristics of Relevance for Toxicology

The examples provided in human and ecological toxicity studies of engineered nanomaterials highlight that nanomaterial structure is essential for determining biological outcomes. This broad hypothesis certainly

What does the material look like?

- Particle size/size distribution
- Agglomeration state/aggregation
- Shape

What is the material made of?

- Overall composition (including chemical composition and crystal structure)
- Surface composition
- Purity (including levels of impurities)

What factors affect how a material interacts with its surroundings?

- Surface area
- Surface chemistry including reactivity, hydrophobicity
- Surface charge

Overarching considerations

- *Stability*—how do material properties change with time (dynamic stability), storage, handling, preparation, delivery, etc.? Include solubility, and the rate of material release through dissolution.
- *Context/media*—how do material properties change in different media, i.e. from the bulk material to dispersions to material in various biological matrices? ("as administered" characterization is considered to be particularly important)
- *Where possible, materials should be characterized sufficiently to interpret the response to the amount of material against a range of potentially relevant dose metrics, including mass, surface area and number concentration.*

Fig. 32.8 Recommended minimum physical and chemical parameters for characterizing nanomaterials in toxicology studies (after [32.123])

needs refinement, with a specific focus on which physicochemical properties of nanomaterials are the most important in defining their hazard [32.119, 121, 122].

To date, no specific regulation for nanomaterials at the international level exists. The International Standards Organization (ISO) published nanotechnology terminology in 2008 as an outcome of ISO/TC 229 Nanotechnologies: ISO/TS 27687 *Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofiber and nanoplate* [32.124]. This international organization continues its work in developing effective regulation for nanotechnology-related products, including *Guidance on physicochemical characterization of engineered nanoscale materials for toxicologic assessment* (ISO/DTR 13014), but writing new rules takes time; hence it will take some time before new standards are completed [32.124].

A minimum set of parameters that accounts for the most important and identifiable nanomaterial characterization in nanotoxicity studies was discussed and proposed at the Woodrow Wilson International Center for Scholars in Washington,DC in 2008 [32.123]. Although there is some variability across the list of ideal properties, most authors agree with the minimum characterization set presented in Fig. 32.8 [32.29, 121, 123, 125, 126]. Definitions of nanoparticle characteristics and techniques suitable for their measurements were described in [32.29, 127].

Characterization of nanoscale particles should begin with the study of as-received samples, which are often shipped as dry powders [32.127]. This primary characterization is useful, particularly for ensuring batch similarity, however study of these powders is not sufficient for safety studies. It is vital to investigate the properties of nanomaterials when they are dispersed in the media used for toxicity studies. Some material properties, such as primary particle size and shape, chemical composition, and crystal structure, are the same in both dry and dispersed phases. However, the composition of the fluid in which nanoparticles are dispersed may affect their agglomeration state, surface charge, and reactivity [32.119, 125]. Also, many of these changes can be slow to occur, and thus samples dispersed in fluids may change over a period of time. Ideally, physicochemical studies should be completed at various time points which reflect the times relevant to the biological questions [32.29].

Technique	Information
Scanning electron microscopy (SEM)	Particle size distributions, shape, agglomeration/aggregation state
Transmission electron microscopy (TEM) Atomic force microscopy (AFM)	Particle size distributions, shape, agglomeration/aggregation state, surface texture
Brunauer–Emmett–Teller (BET) surface area analysis	Specific surface area, porosity, average diameter
X-ray diffraction (XRD)	Crystal structure, crystallite size
Energy-dispersive spectroscopy (EDS)	Overall elemental composition
X-ray photoelectron spectroscopy (XPS)	Surface elemental composition
Inductively coupled plasma mass spectrometry (ICP-MS)	Overall elemental composition
Dynamic light scattering (DLS)	Hydrodynamic size, aggregation/agglomeration state, charge on a particle surface

Table 32.1 Common techniques for investigating nanomaterial properties

32.4.1 Characterization Methods

Physicochemical characterization of nanoparticles generally requires advanced instrumentation. Scanning and transmission electron microscopies can produce high-resolution two-dimensional images of nanoparticles [32.128], while atomic force microscopy (AFM) gives information about nanoparticles in three dimensions [32.129, 130]. Another approach to characterization of nanoparticles involves Brunauer–Emmett–Teller (BET) surface area analysis and x-ray diffraction (XRD). The BET method can analyze dry powders and provide the specific surface area of the nanoparticles [32.131], while XRD yields the atomic structure [32.132]. Energy-dispersive x-ray spectroscopy identifies the elemental composition of the sample [32.128], and x-ray photoelectron spectroscopy (XPS) can be employed to determine the chemical composition at the nanoparticle surface [32.133]. Inductively coupled plasma mass spectrometry (ICP-MS) is a type of MS which is capable of detecting nanoparticles in environmental samples [32.134, 135]. Furthermore, measurement of the nanoparticle hydrodynamic size and zeta potential is useful for getting information about the stability of nanoparticle suspensions with respect to time and medium [32.136, 137]. Common techniques capable of investigating nanomaterial properties are summarized in Table 32.1.

32.4.2 Electron and Atomic Force Microscopy

In vitro and in vivo studies, using carbon, metal oxide, and metal nanomaterials, have all noted various size- and surface-dependent toxicity effects [32.106, 138–144]. These parameters are critical determinants of cellular uptake, distribution through the body, and accumulation in organs.

Direct visualization of nanoparticles by electron or atomic force microscopy allows exact determination of primary particle size and shape. They can also indicate if aggregation or agglomeration is a factor in the sample, an essential issue for safety studies. The agglomeration/aggregation state is likely to differ depending on whether it is determined in powder form or in the experimental media [32.145]; For example, the tendency for airborne nanoparticles to form clusters may limit inhalation exposure to free nanoscale particles, but on the other hand promote locally high concentrations in sediments. Furthermore, depending on the interparticle bond strength, such agglomerates could still undergo deagglomeration once reaching biological fluids. In this case, the primary particles will be of interest for toxicity studies. Therefore, characterization of the size, shape, and agglomeration/aggregation state of nanoparticles in the context of the experimental exposure media (cell culture media, dosing solution, aerosol, etc.) is necessary for informative nanotoxicity studies.

Scanning electron microscopy (SEM) offers the ability to image an area of the order of square millimeters and can be used to observe the formation and arrangement of nanoparticle clusters. However, whether the clusters are formed by nanoparticles in an agglomerated state (weak van der Waals forces between the particles) or aggregated state (strong attractive interactions between the particles) is difficult to establish. Quantitative methods to measure the strength of nanoparticle associations are not routinely available [32.146]. Additionally, it can sometimes be a challenge to fully evaluate aggregated or agglomerated nanomaterials. In the example shown in Fig. 32.9a, some of the individual nanoparticles forming the clusters appear to be visible in higher-magnification SEM

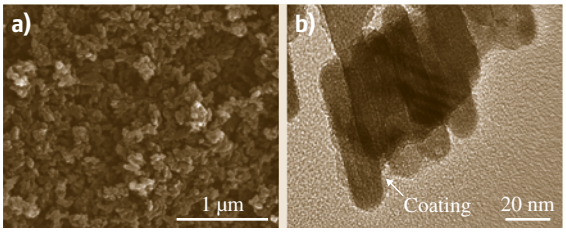

Fig. 32.9a,b Electron microscopy images of TiO_2 nanoparticles coated with SiO_2: **(a)** SEM image showing degree of agglomeration/aggregation in the sample and **(b)** high-magnification TEM image showing surface coating

images. However, given the 20 nm resolution limit of SEM, it is hard to conclude that nanoscale particles are made of one crystallite or several smaller nanoparticles.

Transmission electron microscopy (TEM) analysis provides improved spatial resolution and can, under some circumstances, confirm the presence of nanoscale primary particles in clusters and even measure their size distribution [32.128,147,148]. Moreover, high-resolution TEM can reveal a coating layer on nanoparticle surfaces (Fig. 32.9b) [32.149]. Furthermore, TEM of a cryogenically fresh-frozen sample (cryo-TEM) can produce micrographs of what a biological system, e.g., cell, encounters when exposed to nanoparticles [32.150]. Electron microscopy analysis can be time consuming and expensive, but it provides valuable information in toxicological testing regarding the formation of clusters, primary particle size/size distribution, shapes, and surface coating.

Unlike electron microscopies, AFM does not rely on electron beams to create an image. AFM offers three-dimensional visualization of nanoparticles distributed on a flat surface by measuring the small force between a sharp probe, which is supported on a flexible cantilever, and the surface [32.129]. Therefore, unlike electron microscopies, AFM does not require a vacuum environment or special sample preparation. Such versatility makes it very useful for many questions relevant for nanomaterial safety testing, but it lacks the intrinsic structural sensitivity of electron microscopies [32.130]. Nevertheless, with the appropriate protocols, it can provide information about nanoparticle size, shape, surface texture, and roughness [32.129].

32.4.3 Brunauer–Emmett–Teller Analysis and X-Ray Diffraction

Size-dependent toxic effects of nanoparticles have been correlated with the increased surface-to-volume ratio

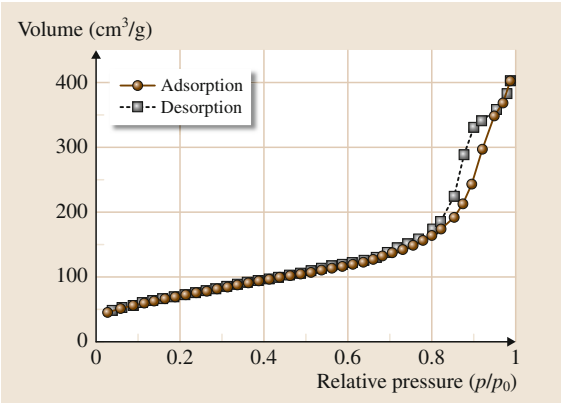

Fig. 32.10 Adsorption–desorption isotherms of nitrogen measured on TiO_2 nanoparticles with surface area of 261 m^2/g that at high relative pressures exhibit a hysteresis loop attributed to the interparticle spaces

of small particles [32.6]. Therefore, the surface area is a physicochemical parameter often considered to be of central importance to nanoparticle toxicity. When the surface area-to-volume ratio increases, there is a greater portion of nanoparticle surface atoms or molecules available to react, and reactivity is generally thought to be a prelude to toxicity [32.127]. This principle has been demonstrated explicitly in several instances where the surface area rather than the particle mass was found to be the best measure of nanomaterial dose [32.151–154]. However, there are also studies that defined the hypothesis of increased toxicity for smaller-surface-area nanoparticles because nanomaterial toxicity depends on several physicochemical parameters that include surface area [32.116,155].

Brunauer–Emmett–Teller (BET) analysis provides specific surface area (SSA) evaluation of nanoscale materials by nitrogen multilayer adsorption measured as a function of relative pressure. Furthermore, the surface area can be related to primary particle size [32.131]. BET analysis can also be used for an indication about aggregation/agglomeration state; For example, if the primary particles form strong aggregates (due to strong bonds between particles), they have lower surface areas than that calculated from TEM images [32.156, 157]. The aggregation/agglomeration state of nanoparticles can also be determined from adsorption–desorption isotherms of nitrogen measured on nanoparticle surfaces (Fig. 32.10), as well as the pore shape, area, and specific pore volume [32.158–160].

The interior crystal structure also determines the toxicity of nanoscale materials; For example, the two

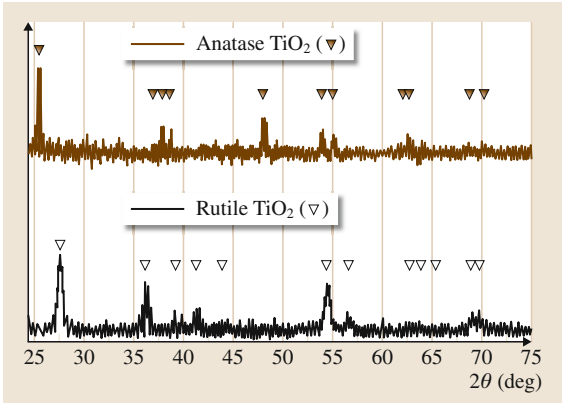

Fig. 32.11 X-ray diffraction patterns of anatase and rutile TiO_2 nanoparticles

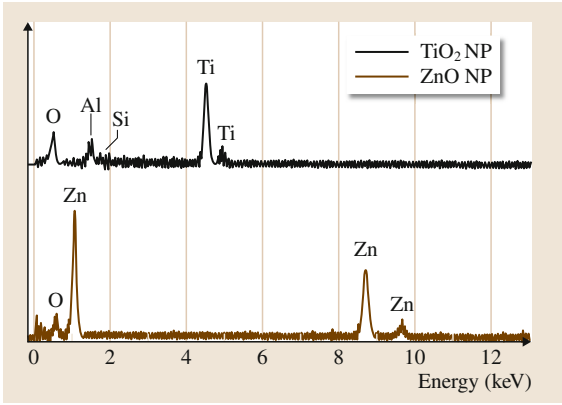

Fig. 32.12 EDS spectra of TiO_2 nanoscale particles (NPs) coated with alumina plus simethicone and uncoated ZnO particles (after [32.18])

most important polymorphs of titania, rutile and anatase (Fig. 32.11), display different photocatalytic activities, with anatase acting as a stronger photocatalyst than rutile [32.161]. Studies have shown that this chemical activity of anatase TiO_2 translates into an increase in biological activity [32.70, 126]. The crystalline structure of nanoparticles can be identified by x-ray diffraction, which is based on the constructive interference of monochromatic x-rays and a crystalline sample. The crystallite size of nanopowders can be determined from the most intense peaks of the XRD patterns according to the Scherrer equation [32.132].

32.4.4 Overall and Surface Elemental Analysis

Several studies have shown that nanomaterials of similar size but differing chemical composition can have varying biological effects [32.115, 116]; For example, *Lanone* et al. demonstrated that, among 24 nanoparticles of similar shape and size but various elemental composition, copper- and zinc-based nanomaterials had the greatest toxicity to human pulmonary cells [32.116]. Furthermore, in addition to the primary material structure, impurities in nanomaterials may also be responsible for biological effects; For example, *Pulskamp* et al. showed that impurities associated with commercial nanotubes caused toxicity in cells [32.112]. Nanomaterials may be contaminated during the preparation process by more toxic, surface-adsorbed surfactants used, for example, to control the size and shape of the particles [32.162]. Unfortunately, manufacturers are not willing to share the details of their proprietary manufacturing methods; hence, in nanotoxicity studies, researchers need to investigate the purity of nanoparticles independently.

For environmental and health studies, the nanoparticles should be free from reactants used in the synthetic steps. Otherwise, toxic impurities can drastically change the results of the toxicity study; For example, for nickel ferrite particles coated with oleic acid prepared by the polyol method, the cytotoxicity significantly increased when one or two layers of oleic acid were deposited as compared with particles without oleic acid prepared by ball milling [32.163].

Moreover, particle surface coatings intentionally designed to optimize use in applications may end up defining the overall toxicity of the nanomaterial. Studies on a number of commercial formulations of TiO_2 particles indicate that different surface treatments can influence the pulmonary toxicity [32.164]. Surface coatings can render noxious particles nontoxic, while less harmful particles can be made highly toxic; For example, in the case of TiO_2 nanoparticles, silica is often used to block access to the titania surface in a solution environment, and consequently reduce its reactivity [32.165, 166].

The overall chemical composition of nanoparticles can be analyzed by energy-dispersive x-ray spectroscopy (EDS) to evaluate impurities; for more surface-specific information, x-ray photoelectron spectroscopy (XPS) is an ideal tool. EDS is commonly coupled with SEM or TEM, where an electron beam scans the surface of the sample and causes emission of x-rays characteristic of the elements present [32.128]. By analyzing the energy of the x-rays from the elements, qualitative analysis of the sample can be

performed in a few minutes (Fig. 32.12). However, EDS has some limitations; For example, elements lighter than sodium are not detectable, and closely spaced lines may not be resolved due to poor energy resolution of the detector [32.128]. Furthermore, EDS does not provide information about chemical bonding. To obtain such detailed chemical characterization of the nanoparticle surface, XPS is often performed.

XPS uses x-rays of sufficient energy to eject core electrons from the sample's various atoms and then measures the energy of the ejected electrons. In addition to chemical state information, XPS spectra can be quantified to provide additional semiquantitative elemental analysis, offering far greater surface sensitivity than EDS or conventional elemental techniques [32.133]. XPS is among the most common analytical methods used in determining the chemical composition of a nanoscale particle's surface. Alternative surface chemical analysis tools for characterization of nanoparticles have been presented recently by *Bear* et al. [32.129].

Analyses by EDS and XPS can provide important information in terms of the chemistry of nanoparticles. Unfortunately, in these cases the samples are analyzed under a vacuum, and thus the results are difficult to apply to the complex environments relevant to nanomaterial safety. ICP-MS has been used as a detection method for nanoparticles in environmental materials [32.134, 135]. It offers sensitive and accurate determination of chemical elements in aqueous media as well as biological samples. A common approach for quantification of nanoparticles by ICP-MS is based on the determination of the total elemental concentrations in a nanoparticle suspension after sample digestion by acid. In an ICP-MS system, a high-temperature argon plasma creates elemental ions, which are separated according to their mass-to-charge ratios, enabling identification and quantification of unknown materials. Another common technique for nanoparticle characterization in solution is dynamic light scattering, which determines the hydrodynamic size.

32.4.5 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) involves monochromatic light that interacts with particles undergoing Brownian motion in a fluid. The motion is size dependent (e.g., larger particles move more slowly through the fluid), and this causes a shift in the frequency of the scattered light (Doppler shift). Larger particles, which have lower velocities, cause lower frequency shifts, whereas smaller particles that move rapidly through the fluid cause higher frequency shifts. Therefore, measuring the frequency shift provides the movement of the particles and their hydrodynamic size distribution. Moreover, DLS measurements of nanoparticle hydrodynamic diameter can be used as a method for monitoring the stability of nanoparticle solutions [32.136]. When the stability of the nanoparticles is changing and aggregates/agglomerates form, the DLS spectrum of hydrodynamic diameter increases. DLS can also be used for determining the quality of nanoparticle dispersion by directly measuring the zeta potential, which is a measure of particle interaction. The particles will repel each other and resist the formation of clusters when their zeta potential is large, above +30 mV or below −30 mV [32.137].

Well-characterized hydrodynamic size and zeta potential of nanoparticle dispersions are imperative for toxicological studies. As a result, The International Alliance for NanoEHS (environment, health, safety) Harmonization (IANH) decided to perform round-robin tests on the hydrodynamic size and zeta potential of monodisperse gold, silica, polystyrene, and agglomerated/aggregated ceria nanoparticles [32.167]. *Roebben* et al. concluded that, for highly uniform nanoparticles, DLS provided an excellent measure of hydrodynamic diameter. Therefore, the IANH DLS test protocol can be applied to the characterization of nearly monodisperse nanoparticle dispersions in ecotoxicity studies. However, polydisperse samples, such as ceria that consisted of nanoparticle aggregates, showed large lab-to-lab variations in measured hydrodynamic diameters. Sonication was a difficult process to standardize, and without this preparation the highly aggregated materials sedimented to the bottom of solution vials, yielding nonuniform solutions for DLS analysis. Methods to produce homogeneous dispersions of nanomaterials should use sonication cautiously and with detailed procedures to ensure reproducibility across laboratories [32.167]. There was also a significant difference between round-robin participants in reported zeta potential data. Therefore, IANH protocols for zeta potential require further improvement to enable accurate and precise measurements.

The surface charge of nanoparticles, which can be approximated by zeta potential measurement, and surface composition are not the only surface chemistry properties that need to be measured for safety research. According to *Powers* et al. surface chemistry includes also surface energy (or wettability), solubility, catalytic properties, surface adsorption and desorption of molecules from solution, etc., and quantification of

these characteristics can be difficult [32.127]. Powers et al. listed several common methods for analysis of nanoparticle surfaces that included, for example, contact angle and microcalorimetric measurements for surface energy and reactivity.

In summary, currently, the research literature on nanomaterial safety is inconclusive due to the lack of sufficient detail about characterization and the dearth of standardized laboratory methods for characterization. The IANH developed protocols for measuring nanoparticle hydrodynamic diameter and performed round-robin experiments to ensure that the protocols are reproducible. This one example needs to be replicated and expanded to more critical physiochemical parameters. With such standard tools, it would be possible to integrate the vast amount of safety data to provide a correlation of nanoparticle properties to their toxicological effects.

One topic not highlighted yet is the importance of completing characterization studies directly on the most relevant engineered nanomaterials, i. e., those found directly in consumer products [32.31]. In the next section, an overview of the challenges in risk evaluation of sunscreen nanoparticles is presented as a case study for nanomaterial characterization within consumer products.

32.5 Special Case – Sunscreens

In sunscreens, the size-dependent optical properties of TiO_2 and ZnO nanoparticles make them transparent at visible wavelengths; thus, when applied to the skin, they form clear films as opposed to white, cloudy applications. Moreover, both materials have very strong absorbance and scattering at UVB and UVA wavelengths (290–320 and 320–400 nm, respectively), which makes them effective at protecting skin from the ultraviolet radiation present in sunlight [32.20, 168–170]. SEM and TEM images of TiO_2 and ZnO sunscreen particles are presented in Fig. 32.13.

32.5.1 Regulatory Policy as Related to Sunscreens

In the USA, product labeling regulations have specifically addressed the issue of pigment size in the list of suncare ingredients. Briefly, manufacturers need only list the chemical composition rather than the diameter, form, or crystalline structure of the pigment. Historically, micronized titania and zinc oxide particles have been used in sunscreens for decades. Because of their large size, they scatter visible light and produce a white and chalky appearance when applied. When manufacturers began to shrink the particle size of these inorganic pigments, the US Food and Drug Administration (FDA) reviewed their requirements for labeling of personal care product ingredients. They ruled in 1999 that *micronized* titania and zinc oxide was an appropriate terminology for sunscreen pigments and that it was not necessary to specify whether pigments were truly nanoscale [32.171]. As a result, consumers do not know from the label when the products they are using contain nanoscale materials.

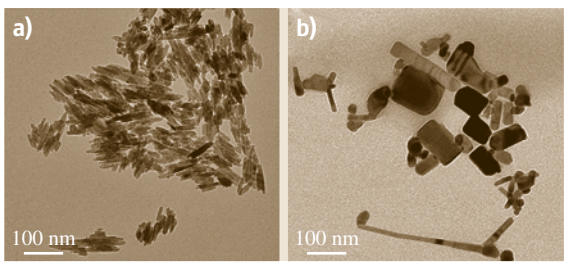

Fig. 32.13a,b TEM images of **(a)** TiO_2 and **(b)** ZnO sunscreen nanoparticles (after [32.18])

32.5.2 Photocatalytic Activity of TiO_2 and ZnO Nanoscale Particles

While the label may not convey the particle size of sunscreen pigments, this does not mean that such information is irrelevant. One specific area of concern has been the native photocatalytic activity of sunscreen pigments. While they are effective at absorbing ultraviolet light, some forms of both titania and zinc oxide are also effective at transforming their photoexcitations into surface-reactive species that generate highly oxidizing products. As a result, in addition to suncare products, nanoscale TiO_2 is also of interest in the ultraviolet-mediated oxidation of organic pollutants and wastewater contaminants [32.172–176]. These applications rely on the ability of TiO_2 nanoparticles to form reactive oxygen species (ROS) such as hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), and hydroperoxy ($HOO^{\bullet}$) radicals and hydrogen peroxide (H_2O_2) when excited with UV light [32.177–180].

Critical for these remediation examples is the observation that the generation of ROS by certain forms of nanoscale titania is catalytic: a single particle of titania can generate many hundreds of ROS species under constant ultraviolet illumination [32.108]. While less studied, nanoscale ZnO materials can also catalyze the photooxidation of organic species in water, and may be more efficient photocatalysts than TiO_2 [32.181–188]. However, since zinc oxide is more soluble than titania, the lifetime of these materials is more limited than titania for some applications. However, since zinc oxide is more soluble than titania, the lifetime of these materials is more limited than titania for some applications.

32.5.3 Health Effects of Nanoscale Titania and Zinc Oxide

Reactive oxygen species are generally acutely toxic to living systems because they rapidly react with many cell components (e.g., DNA, proteins, and lipids) and lead to cell damage [32.189–191]. In light of their potential as photocatalytic materials, it is not surprising that toxicology studies have shown that TiO_2 and ZnO nanoparticles have an adverse effect on cellular function when illuminated by UV light. Some studies have shown that TiO_2 nanoparticles are toxic only in the presence of UV light [32.192, 193], while other studies have shown that in the presence of UV irradiation the toxicity is higher than in the dark [32.194–198]. ZnO nanoparticles have also shown photocatalytic effect on cells [32.197, 199–201].

Whether these acute cell effects are relevant to a product applied to skin depends in part on whether the particles translocate across the dermis; studies of TiO_2 and ZnO pigments appear to indicate that, for healthy and intact skin, these nanoscale materials do not penetrate the dermis but can end up in hair follicles, sweat glands, or skin folds [32.74, 75, 202–205]. However, at the seaside, where sunscreens are more likely to be used, the action of sun, water, and sand can irritate or even harm the skin by hydration, infrared (IR) irradiation, peeling or inflammation, consequently enhancing its permeability [32.206]. Furthermore, recent studies demonstrated that the state of skin can have significant effects on its barrier performance [32.78, 80, 85–89]. Therefore, there is still uncertainty regarding skin penetration of sunscreen zinc oxide and titanium dioxide nanoparticles under a variety of real-life conditions. Moreover, to fully assess the toxicological impact of sunscreen nanoparticles, future studies will need to focus on the potential for ROS generation by TiO_2 and ZnO nanoparticles lodged in hair follicles [32.204]. In addition to the potential photocatalytic effect on cells, ZnO and TiO_2 nanoparticles may cause loss of the ultraviolet protection efficacy of sunscreens, due to the enhanced photooxidative degradation of organic sunscreen components [32.165].

Also important in understanding the connection between chemical and biological reactivity are the physicochemical characteristics such as particle size and surface area, crystal structure, surface chemistry, and particle aggregation/agglomeration tendency; For example, photochemical reactivity is quite sensitive to the phase composition of TiO_2, with rutile materials being orders of magnitude less chemically reactive than equivalently sized anatase systems. This trend in chemical reactivity parallels the results of acute in vitro cell toxicology studies, which find anatase to be more toxic than rutile for a range of diameters [32.126]. Additionally, the chemical reactivity of nanoscale oxides can be reduced by modifying their surface with inert inorganic materials such as silica (SiO_2) or alumina (Al_2O_3), and/or by doping with manganese or vanadium [32.165, 207–211]. Due to the great variety of different nanoparticle forms of TiO_2 and ZnO that exhibit different chemical behaviors, it is difficult to generalize the potential ROS generation capacity of inorganic pigments used in sunscreens [32.212].

32.5.4 Materials Derived from Consumer Products and Their Photochemical Behavior

FDA regulations do not require that labels provide information about the diameter, form or photoactivity of inorganic sunscreen ingredients [32.171]; therefore, consumers and researchers have little information about what people are exposed to. Compared with studies of model titania and zinc oxide pigments, there has been comparatively little systematic study of the properties of pigments derived directly from commercial products.

Hidaka et al. showed that ZnO pigments extracted from sunscreen products commercially available in Japan, when illuminated by UV, caused DNA plasmid strand breaks via the generation of ROS [32.199]. The same pigments were also photoactive toward degradation of phenol. A similar effect of rapid photodegradation of methylene blue dye by sunscreen-isolated zinc oxide pigments (uncoated, dimethicone coated, and mixtures of ZnO and TiO_2 particles) was presented by *Rampaul* et al. [32.209]. In the area of the poten-

tial hazard of sunscreen pigments, ZnO has been less studied than TiO_2 [32.213]. Titania is a more commonly used photocatalyst than ZnO, and as a result there has been more concern regarding this material. DNA damage induced by TiO_2 nanoparticles extracted form sunscreens was first noted in 1997 by *Dunford* et al. [32.214]. Then, *Rampaul* et al. found that some TiO_2 particles isolated from sunscreens caused significant cellular damage to cultured human skin and other animal epithelium cells [32.209]. Recently, *Buchalska* et al. tested the photoreactivity of TiO_2 sunscreen components toward degradation of azur B and oxidation of α-terpinene and showed high efficiency of singlet oxygen formation [32.215].

These studies largely relied on *pure* pigments; it is possible that the other constituents in sunscreens may modify or augment the nanoparticle effects. Full sunscreen, including TiO_2 pigments, has also been tested toward the formation of oxygen- and carbon-centered radicals using spin-trap electron paramagnetic resonance (EPR) spectroscopy by Brezova et al. They concluded that some sunscreens generated ROS [32.178]. These observations were confirmed by *Barker* and *Branch*, who showed that sunscreens containing titanium dioxide and zinc oxide nanoparticles were the primary cause of the rapid corrosion of paint surfaces on steel roofing via the production of reactive oxygen species [32.216]. Furthermore, *Lewicka* and *Colvin*, who evaluated the photochemical properties of whole sunscreen emulsions that contained nanoscale components and the inorganic particles derived from these sunscreens using several assays such as dichlorofluorescein fluorescence, decolorization of Congo red dye, and 5,5-dimethyl-pyrroline *N*-oxide (DMPO) spin-trap electron paramagnetic resonance spectroscopy, showed that samples with nanoscale ZnO materials were more photoactive than the samples that contained TiO_2 nanoparticles [32.217].

32.5.5 Physicochemical Characteristics of Sunscreen Nanoscale Materials

A central challenge in assessing the risk of inorganic pigments used in sunscreens has been the great diversity of material types present: both titania and zinc oxide are used, often with different sizes and forms, with different surface coatings and possibly distinct crystal structures [32.18]. Recently, *Lewicka* et al. characterized the inorganic pigments derived from nine commercial products purchased in the USA [32.18]. Nanoscale pigments were apparent in evaporated sunscreen residues from all items that listed TiO_2 or ZnO as active ingredients, and these materials could be isolated further for analysis via water or alcohol washes. Their dimensions, shape, phase, and elemental composition were determined using a suite of methods including TEM, SEM, XRD, EDS, and inductively coupled plasma optical emission spectroscopy. Wurtzite zinc oxide pigments were rod-like in shape with short axes under 40 nm and longer dimension ranging from tens to hundreds of nanometers. TiO_2 materials were generally rutile and exhibited needle-like or near-spherical shapes; they were consistently smaller than the zinc oxide materials, with average length of 25 nm and widths ranging from 7 to 16 nm. The physical and chemical features of pigments derived from commercial sunscreens were notably similar to two commercial sources of TiO_2 and ZnO nanoparticle powders obtained from EMD and BASF [32.18]. Therefore, in some cases, these pure materials may serve as surrogates for ongoing research evaluating the transport, fate, and toxicology of these widely applied engineered nanomaterials.

32.6 Conclusions

Adequate characterization of manufactured nanomaterials in toxicity, ecotoxicity, and exposure studies is central to clear definition and management of their risks. Diameter, shape, aggregation state, and surface area are some of the basic parameters that should be measured, ideally on the most relevant samples in media that reflect the biological or environmental questions of interest. Typically, such characterization should utilize a variety of techniques that provide complementary information about nanoparticles before in vitro or in vivo testing. Moreover, standardized tests and protocols for nanoparticle characterization need to be established for accurate assessment of their physiochemical properties. Ultimately, such data could be held in a public-access *nanoparticle safety database*, which in addition to listing the material composition (e.g., ZnO) could also include its characteristics (e.g., size, shape) and the results of toxicity tests. Then, by asking a specific question (e.g., "How hazardous are ZnO nanoparticles of a certain size and form?"),

the database would provide the result of an experiment that was done on the safety of this specific material [32.11]. Even though characterization of nanomaterials can be complicated, time consuming, and expensive, it is vital in order to define exactly what manufacturers, consumers, and the environment are exposed to during the production, application, and release of nanomaterials.

References

32.1 ASTM (2006) Terminology for Nanotechnology. E2456-06 American Society for Testing and Materials, available online at http://www.astm.org/Standards/E2456.htm (last accessed 7 February 2011)

32.2 H.F. Krug, P. Wick: Nanotoxicology: An interdisciplinary challenge, Angew. Chem. Int. Ed. **50**(6), 1260–1278 (2011)

32.3 N.S. Wigginton, K.L. Haus, M.F. Hochella: Aquatic environmental nanoparticles, J. Environ. Monitoring **9**(12), 1306–1316 (2007)

32.4 BSI (2007) Terminology for nanomaterials. PAS 136: 2007 British Standards Institution, available online at www.bsigroup.com (last accessed 15 February 2010)

32.5 A.D. Maynard: Nanotechnology: The next big thing, or much ado about nothing?, Ann. Occup. Hyg. **51**(1), 1–12 (2007)

32.6 G. Oberdörster, E. Oberdörster, J. Oberdörster: Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles, Environ. Health Perspect **113**(7), 823–839 (2005)

32.7 R. Rossetti, S. Nakahara, L.E. Brus: Quantum size effects in the redox potentials, resonance Raman-spectra, and electronic-spectra of Cds crystallites in aqueous-solution, J. Chem. Phys. **79**(2), 1086–1088 (1983)

32.8 E.A. Schutz-Sikma, H.M. Joshi, Q. Ma, K.W. MacRenaris, A.L. Eckermann, V.P. Dravid, T.J. Meade: Probing the chemical stability of mixed ferrites: Implications for magnetic resonance contrast agent design, Chem. Mater. **23**(10), 2657–2664 (2011)

32.9 S.F. Hansen, E.S. Michelson, A. Kamper, P. Borling, F. Stuer-Lauridsen, A. Baun: Categorization framework to aid exposure assessment of nanomaterials in consumer products, Ecotoxicology **17**(5), 438–447 (2008)

32.10 PEN (2009): The Project on Emerging Nanotechnologies *Woodrow Wilson International Center for Scholars* (Washington 2011), available online from www.nanotechproject. org (last accesses 08 February 2011)

32.11 R. Kessler: Engineered nanoparticles in consumer products: Understanding a new ingredient, Environ. Health Perspect **119**(3), 120–125 (2011)

32.12 ICON (2012) Nano-EHS Database Analysis Tool. International Council on Nanotechnology, Rice University, Houston (2012), available online from http://icon.rice.edu/ report.cfm (last accessed 05 March 2012)

32.13 A.D. Maynard, R.J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdörster, M.A. Philbert, J. Ryan, A. Seaton, V. Stone, S.S. Tinkle, L. Tran, N.J. Walker, D.B. Warheit: Safe handling of nanotechnology, Nature **444**(7117), 267–269 (2006)

32.14 E. Nielsen: *Human Health and Nanomaterials in Consumer Products. Literature Review for the Region of Peel's Public Health Unit* (EBN Consulting, Richmond 2008)

32.15 M. Simkó, M.O. Mattsson: Risks from accidental exposures to engineered nanoparticles and neurological health effects: A critical review, Part. Fibre Toxicol. **7**, 42 (2010)

32.16 P.J.A. Borm, D. Robbins, S. Haubold, T. Kuhlbusch, H. Fissan, K. Donaldson, R. Schins, V. Stone, W. Kreyling, J. Lademann, J. Krutmann, D. Warheit, E. Oberdorster: The potential risks of nanomaterials: A review carried out for ECETOC, Part. Fibre Toxicol. **3**, 11 (2006)

32.17 J.R. Peralta-Videa, L.J. Zhao, M.L. Lopez-Moreno, G. de la Rosa, J. Hong, J.L. Gardea-Torresdey: Nanomaterials and the environment: A review for the biennium 2008–2010, J. Hazard. Mater. **186**(1), 1–15 (2011)

32.18 Z.A. Lewicka, A.F. Benedetto, D.N. Benoit, W.W. Yu, J.D. Fortner, V.L. Colvin: The structure, composition and dimensions of TiO_2 and ZnO nanomaterials in commercial sunscreens, J. Nanopart. Res. **12**(4), 3607–3617 (2011)

32.19 B.D. More: Physical sunscreens: On the comeback trail, Indian J. Dermatol. Venereol. Leprol. **73**(2), 80–85 (2007)

32.20 A.P. Popov, A.V. Priezzhev, J. Lademann, R. Myllya: The effect of nanometer particles of titanium oxide on the protective properties of skin in the UV region, J. Opt. Technol. **73**, 208–211 (2005)

32.21 C.O. Hendren, X. Mesnard, J. Dröge, M.R. Wiesner: Estimating production data for five engineered nanomaterials as a basis for exposure assessment, Environ. Sci. Technol. **45**(7), 2562–2568 (2011)

32.22 B. Nowack, T.D. Bucheli: Occurrence, behavior and effects of nanoparticles in the environment, Environ. Pollut. **150**, 5–22 (2007)

32.23 N. Musee: Nanowastes and the environment: Potential new waste management paradigm, Environ. Int. **37**(1), 112–128 (2011)

32.24 M.A. Kiser, P. Westerhoff, T. Benn, Y. Wang, J. Perez-Rivera, K. Hristovski: Titanium nanomaterial removal and release from wastewater

treatment plants, Environ. Sci. Technol. **43**(17), 6757–6763 (2009)
32.25 F. Gottschalk, T. Sonderer, R.W. Scholz, B. Nowack: Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis, Environ. Toxicol. Chem. **29**(5), 1036–1048 (2010)
32.26 F. Gottschalk, T. Sonderer, R.W. Scholz, B. Nowack: Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions, Environ. Sci. Technol. **43**(24), 9216–9222 (2009)
32.27 B. Nowack, J.F. Ranville, S. Diamond, J.A. Gallego-Urrea, C. Metcalfe, J. Rose, N. Horne, A.A. Koelmans, S.J. Klaine: Potential scenarios for nanomaterial release and subsequent alteration in the environment, Environ. Toxicol. Chem. **31**(1), 50–59 (2012)
32.28 P.J.J. Alvarez, V. Colvin, J. Lead, V. Stone: Research priorities to advance eco-responsible nanotechnology, ACS Nano **3**(7), 1616–1619 (2009)
32.29 V. Stone, B. Nowack, A. Baun, N. van den Brink, F. von der Kammer, M. Dusinska, R. Handy, S. Hankin, M. Hassellov, E. Joner, T.F. Fernandes: Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisation, Sci. Total Environ. **408**(7), 1745–1754 (2010)
32.30 S.K. Brar, M. Verma, R.D. Tyagi, R.Y. Surampalli: Engineered nanoparticles in wastewater and wastewater sludge – Evidence and impacts, Waste Manag. **30**(3), 504–520 (2010)
32.31 M.J. McCall: Environmental, Health and Safety Issues; Nanoparticles in the real world, Nat. Nanotechnol. **6**(10), 613–614 (2011)
32.32 J.R. Lead, K.J. Wilkinson: Aquatic colloids and nanoparticles: Current knowledge and future trends, Environ. Chem. **3**(3), 159–171 (2006)
32.33 M. Delay, F.H. Frimmel: Nanoparticles in aquatic systems, Anal. Bioanal. Chem. **402**(2), 583–592 (2012)
32.34 K.A.D. Guzman, M.P. Finnegan, J.F. Banfield: Influence of surface potential on aggregation and transport of titania nanoparticles, Environ. Sci. Technol. **40**(24), 7688–7693 (2006)
32.35 J. Labille, J.H. Feng, C. Botta, D. Borschneck, M. Sammut, M. Cabie, M. Auffan, J. Rose, J.Y. Bottero: Aging of TiO_2 nanocomposites used in sunscreen. Dispersion and fate of the degradation products in aqueous environment, Environ. Pollut. **158**(12), 3482–3489 (2010)
32.36 D.H. Lin, X.L. Tian, F.C. Wu, B.S. Xing: Fate and transport of engineered nanomaterials in the environment, J. Environ. Qual. **39**(6), 1896–1908 (2010)
32.37 E. Oberdörster: Manufactured nanomaterials (Fullerenes, C-60) induce oxidative stress in the brain of juvenile largemouth bass, Environ. Health Perspect **112**(10), 1058–1062 (2004)
32.38 V.L. Colvin: The potential environmental impact of engineered nanomaterials, Nat. Biotechnol. **21**(10), 1166–1170 (2003)
32.39 E. Bergamaschi: Occupational exposure to nanomaterials: Present knowledge and future development, Nanotoxicology **3**(3), 194–201 (2009)
32.40 M. Farre, K. Gajda-Schrantz, L. Kantiani, D. Barcelo: Ecotoxicity and analysis of nanomaterials in the aquatic environment, Anal. Bioanal. Chem. **393**(1), 81–95 (2009)
32.41 V. Matranga, I. Corsi: Toxic effects of engineered nanoparticles in the marine environment: Model organisms and molecular approaches, Mar. Environ. Res. **76**, 32–40 (2012)
32.42 S.J. Klaine, P.J.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy, D.Y. Lyon, S. Mahendra, M.J. McLaughlin, J.R. Lead: Nanomaterials in the environment: Behavior, fate, bioavailability, and effects, Environ. Toxicol. Chem. **27**(9), 1825–1851 (2008)
32.43 R.D. Handy, F. von der Kammer, J.R. Lead, M. Hassellov, R. Owen, M. Crane: The ecotoxicology and chemistry of manufactured nanoparticles, Ecotoxicology **17**(4), 287–314 (2008)
32.44 A. Kahru, K. Savolainen: Potential hazard of nanoparticles: From properties to biological and environmental effects, Toxicology **269**(2/3), 89–91 (2010)
32.45 J.P. Cheng, E. Flahaut, S.H. Cheng: Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos, Environ. Toxicol. Chem. **26**(4), 708–716 (2007)
32.46 L.K. Adams, D.Y. Lyon, P.J.J. Alvarez: Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions, Water Res. **40**(19), 3527–3532 (2006)
32.47 R.J. Griffitt, R. Weil, K.A. Hyndman, N.D. Denslow, K. Powers, D. Taylor, D.S. Barber: Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*), Environ. Sci. Technol. **41**(23), 8178–8186 (2007)
32.48 M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier, A. Kahru: Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, Chemosphere **71**(7), 1308–1316 (2008)
32.49 F. Gagne, J. Auclair, P. Turcotte, M. Fournier, C. Gagnon, S. Sauve, C. Blaise: Ecotoxicity of CdTe quantum dots to freshwater mussels: Impacts on immune system, oxidative stress and genotoxicity, Aquat. Toxicol. **86**(3), 333–340 (2008)
32.50 M.K. Yeo, M. Kang: Effects of nanometer sized silver materials on biological toxicity during zebrafish embryogenesis, Bull. Korean Chem. Soc. **29**(6), 1179–1184 (2008)
32.51 A. Kahru, H.C. Dubourguier: From ecotoxicology to nanoecotoxicology, Toxicology **269**(2/3), 105–119 (2010)

32.52 M.A. Wilson, N.H. Tran, A.S. Milev, G.S.K. Kannangara, H. Volk, G.Q.M. Lu: Nanomaterials in soils, Geoderma **146**(1/2), 291–302 (2008)

32.53 B.K.G. Theng, G. Yuan: Nanoparticles in the soil environment, Elements **4**(6), 395–399 (2008)

32.54 G.V. Lowry, E.M. Hotze, E.S. Bernhardt, D.D. Dionysiou, J.A. Pedersen, M.R. Wiesner, B.S. Xing: Environmental occurrences, behavior, fate, and ecological effects of nanomaterials: An introduction to the special series, J. Environ. Qual. **39**(6), 1867–1874 (2010)

32.55 R. Dinesh, M. Anandaraj, V. Srinivasan, S. Hamza: Engineered nanoparticles in the soil and their potential implications to microbial activity, Geoderma **173**, 19–27 (2012)

32.56 X. Ma, J. Geiser-Lee, Y. Deng, A. Kolmakov: Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation, Sci. Total Environ. **408**(16), 3053–3061 (2010)

32.57 J.E. Canas, M. Long, S. Nations, R. Vadan, L. Dai, M. Luo, R. Ambikapathi, E.H. Lee, D. Olszyk: Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species, Environ. Toxicol. Chem. **27**(9), 1922–1931 (2008)

32.58 M. Khodakovskaya, E. Dervishi, M. Mahmood, Y. Xu, Z. Li, F. Watanabe, A.S. Biris: Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth, Acs Nano **3**(10), 3221–3227 (2009)

32.59 J.-Y. Roh, Y.-K. Park, K. Park, J. Choi: Ecotoxicological investigation of CeO_2 and TiO_2 nanoparticles on the soil nematode Caenorhabditis elegans using gene expression, growth, fertility, and survival as endpoints, Environ. Toxicol. Pharmacol. **29**(2), 167–172 (2010)

32.60 H.H. Wang, R.L. Wick, B.S. Xing: Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*, Environ. Pollut. **157**(4), 1171–1177 (2009)

32.61 V.K. Sharma: Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment – A Review, J. Environ. Sci. Health Pt. A **44**(14), 1485–1495 (2009)

32.62 R.D. Handy, N. van den Brink, M. Chappell, M. Mühling, R. Behra, M. Dušinská, P. Simpson, J. Ahtiainen, A.N. Jha, J. Seiter, A. Bednar, A. Kennedy, T.F. Fernandes, M. Riediker: Practical considerations for conducting ecotoxicity test methods with manufactured nanomaterials: What have we learnt so far?, Ecotoxicology **21**(4), 933–972 (2012)

32.63 W.G. Kreyling, M. Semmler-Behnke, W. Moeller: Health implications of nanoparticles, J. Nanopart. Res. **8**(5), 543–562 (2006)

32.64 C.S. Yah, G.S. Simate, S.E. Iyuke: Nanoparticles toxicity and their routes of exposures, Pak. J. Pharm. Sci. **25**(2), 477–491 (2012)

32.65 W. Yang, J.I. Peters, R.O. Williams: Inhaled nanoparticles – A current review, Int. J. Pharm. **356**(1/2), 239–247 (2008)

32.66 S. Bakand, A. Hayes, F. Dechsakulthorn: Nanoparticles: A review of particle toxicology following inhalation exposure, Inhal. Toxicol. **24**(2), 125–135 (2012)

32.67 W. Hofmann: Modelling inhaled particle deposition in the human lung–A review, J. Aerosol. Sci. **42**(10), 693–724 (2011)

32.68 W.G. Kreyling, M. Semmler, F. Erbe, P. Mayer, S. Takenaka, H. Schulz, G. Oberdörster, A. Ziesenis: Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low, J. Toxicol. Env. Health Pt. A **65**(20), 1513–1530 (2002)

32.69 G. Oberdörster, Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling, C. Cox: Translocation of inhaled ultrafine particles to the brain, Inhal. Toxicol. **16**(6/7), 437–445 (2004)

32.70 J.X. Wang, Y. Liu, F. Jiao, F. Lao, W. Li, Y.Q. Gu, Y.F. Li, C.C. Ge, G.Q. Zhou, B. Li, Y.L. Zhao, Z.F. Chai, C.Y. Chen: Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles, Toxicology **254**(1/2), 82–90 (2008)

32.71 T.G.M. Smijs, J.A. Bouwstra: Focus on skin as a possible port of entry for solid nanoparticles and the toxicological impact, J. Biomed. Nanotechnol. **6**(5), 469–484 (2010)

32.72 C. Smith (Feature Ed): Nanotechnology in cosmetics analysed, Personal Care Mag. (Nov. 2010), available online from http://www.personalcaremagazine.com (last accessed 08 June 2012)

32.73 L.A. DeLouise: Applications of nanotechnology in dermatology, J. Investig. Dermatol. **132**(3), 964–975 (2012)

32.74 G.J. Nohynek, E. Antignac, T. Re, H. Toutain: Safety assessment of personal care products/cosmetics and their ingredients, Toxicol. Appl. Pharmacol. **243**(2), 239–259 (2009)

32.75 G.J. Nohynek, J. Lademann, C. Ribaud, M.S. Roberts: Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety, Crit. Rev. Toxicol. **37**(3), 251–277 (2007)

32.76 P. Filipe, J.N. Silva, R. Silva, J.L.C. de Castro, M.M. Gomes, L.C. Alves, R. Santus, T. Pinheiro: Stratum corneum is an effective barrier to TiO_2 and ZnO nanoparticle percutaneous absorption, Skin. Pharmacol. Physiol. **22**(5), 266–275 (2009)

32.77 R.F.V. Lopez, J.E. Seto, D. Blankschtein, R. Langer: Enhancing the transdermal delivery of rigid nanoparticles using the simultaneous application of ultrasound and sodium lauryl sulfate, Biomaterials **32**(3), 933–941 (2011)

32.78 N.A. Monteiro-Riviere, K. Wiench, R. Landsiedel, S. Schulte, A.O. Inman, J.E. Riviere: Safety evaluation of sunscreen formulations containing titanium

ioxide and zinc oxide nanoparticles in UVB sunburned skin: An in vitro and in vivo study, Toxicol. Sci. **123**(1), 264–280 (2011)
32.79 T.W. Prow, J.E. Grice, L.L. Lin, R. Faye, M. Butler, W. Becker, E.M.T. Wurm, C. Yoong, T.A. Robertson, H.P. Soyer, M.S. Roberts: Nanoparticles and microparticles for skin drug delivery, Adv. Drug. Deliv. Rev. **63**(6), 470–491 (2011)
32.80 N.V. Gopee, D.W. Roberts, P. Webb, C.R. Cozart, P.H. Siitonen, J.R. Latendresse, A.R. Warbitton, W.W. Yu, V.L. Colvin, N.J. Walker, P.C. Howard: Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not Intact SKH-1 hairless mouse skin, Toxicol. Sci. **111**(1), 37–48 (2009)
32.81 J.P. Ryman-Rasmussen, J.E. Riviere, N.A. Monteiro-Riviere: Penetration of intact skin by quantum dots with diverse physicochemical properties, Toxicol. Sci. **91**(1), 159–165 (2006)
32.82 B. Baroli, M.G. Ennas, F. Loffredo, M. Isola, R. Pinna, M.A. Lopez-Quintela: Penetration of metallic nanoparticles in human full-thickness skin, J. Investig. Dermatol. **127**(7), 1701–1712 (2007)
32.83 Y.Z. Huang, F.Q. Yu, Y.S. Park, J.X. Wang, M.C. Shin, H.S. Chung, V.C. Yang: Co-administration of protein drugs with gold nanoparticles to enable percutaneous delivery, Biomaterials **31**(34), 9086–9091 (2010)
32.84 G. Sonavane, K. Tomoda, A. Sano, H. Ohshima, H. Terada, K. Makino: In vitro permeation of gold nanoparticles through rat skin and rat intestine: Effect of particle size, Colloid Surf. B – Biointerfaces **65**(1), 1–10 (2008)
32.85 S.S. Tinkle, J.M. Antonini, B.A. Rich, J.R. Roberts, R. Salmen, K. DePree, E.J. Adkins: Skin as a route of exposure and sensitization in chronic beryllium disease, Environ. Health Perspect. **111**, 1202–1208 (2003)
32.86 J.G. Rouse, J. Yang, J.P. Ryman-Rasmussen, A.R. Barron, N.A. Monteiro-Riviere: Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin, Nano Lett. **7**, 155–160 (2007)
32.87 L.J. Mortensen, G. Oberdörster, A.P. Pentland, L.A. DeLouise: In vivo skin penetration of quantum dot nanoparticles in the murine model: The effect of UVR, Nano Lett. **8**(9), 2779–2787 (2008)
32.88 L.W. Zhang, N.A. Monteiro-Riviere: Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin, Skin. Pharmacol. Physiol. **21**(3), 166–180 (2008)
32.89 S. Ravichandran, L.J. Mortensen, L.A. Delouise: Quantification of human skin barrier function and susceptibility to quantum dot skin penetration, Nanotoxicology **5**(4), 675–686 (2010)
32.90 J.R. Bond, B.W. Barry: Limitations of hairless mouse skin as a model for invitro permeation studies through human-skin – hydration damage, J. Investig. Dermatol. **90**(4), 486–489 (1988)
32.91 G.A. Simon, H.I. Maibach: Relevance of hairless mouse as an experimental model of percutaneous penetration in man, Skin. Pharmacol. Appl. Skin. Physiol. **11**(2), 80–86 (1998)
32.92 R.L. Bronaugh, R.F. Stewart, E.R. Congdon: Methods for invitro percutaneous-absorption studies II. Animal-models for human-skin, Toxicol. Appl. Pharmacol. **62**(3), 481–488 (1982)
32.93 N. Otberg, H. Richter, H. Schaefer, U. Blume-Peytavi, W. Sterry, J. Lademann: Variations of hair follicle size and distribution in different body sites, J. Investig. Dermatol. **122**(1), 14–19 (2004)
32.94 B.A. Magnuson, T.S. Jonaitis, J.W. Card: A brief review of the occurrence, use, and safety of food-related nanomaterials, J. Food Sci. **76**(6), R126–R133 (2011)
32.95 C.-Y. Kuan, W. Yee-Fung, K.-H. Yuen, M.-T. Liong: Nanotech: Propensity in foods and bioactives, Crit. Revi. Food Sci. Nutr. **52**(1–3), 55–71 (2012)
32.96 E. Frohlich, E. Roblegg: Models for oral uptake of nanoparticles in consumer products, Toxicology **291**(1–3), 10–17 (2012)
32.97 K.R. Vega-Villa, J.K. Takemoto, J.A. Yanez, C.M. Remsberg, M.L. Forrest, N.M. Davies: Clinical toxicities of nanocarrier systems, Adv. Drug. Deliv. Rev. **60**(8), 929–938 (2008)
32.98 S. Yamago, H. Tokuyama, E. Nakamura, K. Kikuchi, S. Kananishi, K. Sueki, H. Nakahara, S. Enomoto, F. Ambe: In-vivo biological behavior of a water-miscible fullerene – C-14 labeling, absorption, distribution, excretion and acute toxicity, Chem. Biol. **2**(6), 385–389 (1995)
32.99 P.U. Jani, D.E. McCarthy, A.T. Florence: Titanium-dioxide (rutile) particle uptake from the rat GI tract and translocation to systemic organs after oral-administration, Int. J. Pharm. **105**(2), 157–168 (1994)
32.100 Z. Chen, H.A. Meng, G.M. Xing, C.Y. Chen, Y.L. Zhao, G.A. Jia, T.C. Wang, H. Yuan, C. Ye, F. Zhao, Z.F. Chai, C.F. Zhu, X.H. Fang, B.C. Ma, L.J. Wan: Acute toxicological effects of copper nanoparticles in vivo, Toxicol. Lett. **163**(2), 109–120 (2006)
32.101 M. Lu, K.T. Al-Jamal, K. Kostarelos, J. Reineke: Physiologically based pharmacokinetic modeling of nanoparticles, Acs Nano **4**(11), 6303–6317 (2010)
32.102 M. Bartneck, H.A. Keul, G. Zwadlo-Klarwasser, J. Groll: Phagocytosis independent extracellular nanoparticle clearance by human immune cells, Nano Lett. **10**(1), 59–63 (2010)
32.103 V. Stone, H. Johnston, R.P.F. Schins: Development of in vitro systems for nanotoxicology: Methodological considerations, Crit. Rev. Toxicol. **39**(7), 613–626 (2009)
32.104 B. Kong, J.H. Seog, L.M. Graham, S.B. Lee: Experimental considerations on the cytotoxicity of nanoparticles, Nanomedicine **6**(5), 929–941 (2011)

32.105 P. Rivera Gil, G. Oberdörster, A. Elder, V. Puntes, W.J. Parak: Correlating physico-chemical with toxicological properties of nanoparticles: The present and the future, Acs Nano **4**(10), 5527–5531 (2010)

32.106 N. Lewinski, V. Colvin, R. Drezek: Cytotoxicity of nanoparticles, Small **4**(1), 26–49 (2008)

32.107 N. Khlebtsov, L. Dykman: Biodistribution and toxicity of engineered gold nanoparticles: A review of in vitro and in vivo studies, Chem. Soc. Rev. **40**(3), 1647–1671 (2010)

32.108 R. Landsiedel, L. Ma-Hock, A. Kroll, D. Hahn, J. Schnekenburger, K. Wiench, W. Wohlleben: Testing metal-oxide nanomaterials for human safety, Adv. Mater. **22**(24), 2601–2627 (2010)

32.109 A. Dhawan, V. Sharma: Toxicity assessment of nanomaterials: Methods and challenges, Anal. Bioanal. Chem. **398**(2), 589–605 (2010)

32.110 A. Nel, T. Xia, L. Madler, N. Li: Toxic potential of materials at the nanolevel, Science **311**(5761), 622–627 (2006)

32.111 A. Seaton, L. Tran, R. Aitken, K. Donaldson: Nanoparticles, human health hazard and regulation, J. R. Soc. Interface **7**, S119–S129 (2011)

32.112 K. Pulskamp, S. Diabate, H.F. Krug: Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants, Toxicol. Lett. **168**(1), 58–74 (2007)

32.113 J. Chlopek, B. Czajkowska, B. Szaraniec, E. Frackowiak, K. Szostak, F. Béguin: In vitro studies of carbon nanotubes biocompatibility, Carbon **44**(6), 1106–1111 (2006)

32.114 S.J. Soenen, P. Rivera-Gil, J.-M. Montenegro, W.J. Parak, S.C. De Smedt, K. Braeckmans: Cellular toxicity of inorganic nanoparticles: Common aspects and guidelines for improved nanotoxicity evaluation, Nano Today **6**(5), 446–465 (2011)

32.115 S.K. Sohaebuddin, P.T. Thevenot, D. Baker, J.W. Eaton, L. Tang: Nanomaterial cytotoxicity is composition, size, and cell type dependent, Part. Fibre Toxicol. **7**, 22 (2010)

32.116 S. Lanone, F. Rogerieux, J. Geys, A. Dupont, E. Maillot-Marechal, J. Boczkowski, G. Lacroix, P. Hoet: Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines, Part. Fibre Toxicol. **6**, 14 (2009)

32.117 C.R. Thomas, S. George, A.M. Horst, Z.X. Ji, R.J. Miller, J.R. Peralta-Videa, T.A. Xia, S. Pokhrel, L. Madler, J.L. Gardea-Torresdey, P.A. Holden, A.A. Keller, H.S. Lenihan, A.E. Nel, J.I. Zink: Nanomaterials in the environment: From materials to high-throughput screening to organisms, Acs Nano **5**(1), 13–20 (2011)

32.118 D.B. Warheit: How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization?, Toxicol. Sci. **101**(2), 183–185 (2008)

32.119 G. Oberdörster: Safety assessment for nanotechnology and nanomedicine: Concepts of nanotoxicology, J. Int. Med. **267**(1), 89–105 (2009)

32.120 K.D. Grieger, A. Baun, R. Owen: Redefining risk research priorities for nanomaterials, J. Nanopart. Res. **12**(2), 383–392 (2010)

32.121 J.W. Card, B.A. Magnuson: A method to assess the quality of studies that examine the toxicity of engineered nanomaterials, Int. J. Toxicol. **29**(4), 402–410 (2011)

32.122 D.R. Boverhof, R.M. David: Nanomaterial characterization: Considerations and needs for hazard assessment and safety evaluation, Anal. Bioanal. Chem. **396**(3), 953 (2010)

32.123 A. Maynard: MINChar Initiative, Recommended Minimum Physical and Chemical Parameters for Characterizing Nanomaterials on Toxicology Studies (2008), available online from http://characterizationmatters.org/parameters/ (last accessed 21 May 2011)

32.124 D. Hyde: Technical Committee, TC 229 Nanotechnologies, ISO International Standards for Business, Government and Society (ISO, Geneva 2011), available online from http://www.iso.org/iso/iso_technical_committee?commid=381983 (last accessed 18 May 2011)

32.125 A.D. Maynard, D.B. Warheit, M.A. Philbert: The new toxicology of sophisticated materials: Nanotoxicology and beyond, Toxicol. Sci. **120**, S109–S129 (2011)

32.126 C.M. Sayes, R. Wahi, P.A. Kurian, Y. Liu, J.L. West, K.D. Ausman, D.B. Warheit, V.L. Colvin: Correlating nanoscale titania structure with toxicity: A cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells, Toxicol. Sci. **92**, 174–185 (2006)

32.127 K.W. Powers, S.C. Brown, V.B. Krishna, S.C. Wasdo, B.M. Moudgil, S.M. Roberts: Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation, Toxicol. Sci. **90**(2), 296–303 (2006)

32.128 P.J. Goodhew, J. Humphreys, R. Beanland (Eds.): *Electron Microscopy and Analysis*, 3rd edn. (Taylor Francis, London 2001)

32.129 D.R. Baer, D.J. Gaspar, P. Nachimuthu, S.D. Techane, D.G. Castner: Application of surface chemical analysis tools for characterization of nanoparticles, Anal. Bioanal. Chem. **396**(3), 983–1002 (2010)

32.130 S. Gupta, P. Brouwer, S. Bandyopadhyay, S. Patil, R. Briggs, J. Jain, S. Seal: TEM/AFM investigation of size and surface properties of nanocrystalline ceria, J. Nanosci. Nanotechnol. **5**(7), 1101–1107 (2005)

32.131 S. Brunauer, P.H. Emmett, E. Teller: Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. **60**, 309–319 (1938)

32.132 B.D. Cullity, S.R. Stock (Eds.): *Elements of x-ray Diffraction* (Prentice Hall, Upper Saddle River 2001)

32.133 J.F. Watts, J. Wolstenholme (Eds.): *An Introduction to Surface Analysis by XPS and AES* (Wiley, Chichester 2003)

32.134 A. Scheffer, C. Engelhard, M. Sperling, W. Buscher: ICP-MS as a new tool for the determination of gold nanoparticles in bioanalytical applications, Anal. Bioanal. Chem. **390**(1), 249–252 (2008)

32.135 B. Fernandez, J.M. Costa, R. Pereiro, A. Sanz-Medel: Inorganic mass spectrometry as a tool for characterisation at the nanoscale, Anal. Bioanal. Chem. **396**(1), 15–29 (2010)

32.136 R.C. Murdock, L. Braydich-Stolle, A.M. Schrand, J.J. Schlager, S.M. Hussain: Characterization of nanomaterial dispersion in solution prior to In vitro exposure using dynamic light scattering technique, Toxicol. Sci. **101**(2), 239–253 (2008)

32.137 Malvern Inc.: Zeta Potential: An Introduction in 30 Minutes, Malvern Tech. Note MRK654-01, available online from http://www.nbtc.cornell.edu/facilities/downloads/Zeta%20potential%20-%20An%20introduction%20in%2030%20minutes.pdf. (last accessed 29 July 2012)

32.138 B.D. Chithrani, A.A. Ghazani, W.C.W. Chan: Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells, Nano Lett. **6**(4), 662–668 (2006)

32.139 B.D. Chithrani, W.C.W. Chan: Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes, Nano Lett. **7**(6), 1542–1550 (2007)

32.140 K.W. Powers, M. Palazuelos, B.M. Moudgil, S.M. Roberts: Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies, Nanotoxicology **1**(1), 42–51 (2007)

32.141 A. Magrez, S. Kasas, V. Salicio, N. Pasquier, J.W. Seo, M. Celio, S. Catsicas, B. Schwaller, L. Forro: Cellular toxicity of carbon-based nanomaterials, Nano Lett. **6**(6), 1121–1125 (2006)

32.142 H.L. Karlsson, J. Gustafsson, P. Cronholm, L. Moller: Size-dependent toxicity of metal oxide particles – A comparison between nano- and micrometer size, Toxicol. Lett. **188**(2), 112–118 (2009)

32.143 A.M. Schrand, M.F. Rahman, S.M. Hussain, J.J. Schlager, D.A. Smith, A.F. Syed: Metal-based nanoparticles and their toxicity assessment, Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol. **2**(5), 544–568 (2010)

32.144 S. Wang, W. Lu, O. Tovmachenko, U.S. Rai, H. Yu, P.C. Ray: Challenge in understanding size and shape dependent toxicity of gold nanomaterials in human skin keratinocytes, Chem. Phys. Lett. **463**(1–3), 145–149 (2008)

32.145 I. Montes-Burgos, D. Walczyk, P. Hole, J. Smith, I. Lynch, K. Dawson: Characterisation of nanoparticle size and state prior to nanotoxicological studies, J. Nanopart. Res. **12**(1), 47–53 (2010)

32.146 G. Nichols, S. Byard, M.J. Bloxham, J. Botterill, N.J. Dawson, A. Dennis, V. Diart, N.C. North, J.D. Sherwood: A review of the terms agglomerate and aggregate with a recommendation for nomenclature used in powder and particle characterization, J. Pharm. Sci. **91**(10), 2103–2109 (2002)

32.147 E. Vigneau, C. Loisel, M.F. Devaux, P. Cantoni: Number of particles for the determination of size distribution from microscopic images, Powder Technol. **107**(3), 243–250 (2000)

32.148 A.J. Paine: Error-estimates in the sampling from particle-size distributions, Part. Part. Syst. Charact. **10**(1), 26–32 (1993)

32.149 J.T. Nurmi, P.G. Tratnyek, V. Sarathy, D.R. Baer, J.E. Amonette, K. Pecher, C.M. Wang, J.C. Linehan, D.W. Matson, R.L. Penn, M.D. Driessen: Characterization and properties of metallic iron nanoparticles: Spectroscopy, electrochemistry, and kinetics, Environ. Sci. Technol. **39**(5), 1221–1230 (2005)

32.150 S.C. Sahu, D.A. Casciano (Eds.): *Nanotoxicity: From In Vivo and In Vitro Models to Health Risks* (Wiley, Chichester 2009)

32.151 T. Stoeger, C. Reinhard, S. Takenaka, A. Schroeppel, E. Karg, B. Ritter, J. Heyder, H. Schulz: Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice, Environ. Health Perspect. **114**(3), 328–333 (2006)

32.152 D.M. Brown, M.R. Wilson, W. MacNee, V. Stone, K. Donaldson: Size-dependent proinflammatory effects of ultrafine polystyrene particles: A role for surface area and oxidative stress in the enhanced activity of ultrafines, Toxicol. Appl. Pharmacol. **175**(3), 191–199 (2001)

32.153 R. Duffin, L. Tran, D. Brown, V. Stone, K. Donaldson: Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: Highlighting the role of particle surface area and surface reactivity, Inhal. Toxicol. **19**(10), 849–856 (2007)

32.154 D. Napierska, L.C.J. Thomassen, V. Rabolli, D. Lison, L. Gonzalez, M. Kirsch-Volders, J.A. Martens, P.H. Hoet: Size-dependent cytotoxicity of monodisperse silica nanoparticles in human endothelial cells, Small **5**(7), 846–853 (2009)

32.155 M.A. Maurer-Jones, Y.S. Lin, C.L. Haynes: Functional assessment of metal oxide nanoparticle toxicity in immune cells, ACS Nano **4**(6), 3363–3373 (2010)

32.156 A.L. Ortiz, W. Osborn, T. Markmaitree, L.L. Shaw: Crystallite sizes of LiH before and after ball milling and thermal exposure, J. Alloy. Compound. **454**(1/2), 297–305 (2008)

32.157 M.K. Naskar: Soft solution processing for the synthesis of alumina nanoparticles in the presence of glucose, J. Am. Ceram. Soc. **93**(5), 1260–1263 (2010)

32.158 M. Khalfaoui, S. Knani, M.A. Hachicha, A. Ben Lamine: New theoretical expressions for the five adsorption type isotherms classified by BET based

on statistical physics treatment, J. Colloid Interface Sci. **263**(2), 350–356 (2003)
32.159 Y.H. Tan, J.A. Davis, K. Fujikawa, N.V. Ganesh, A.V. Demchenko, K.J. Stine: Surface area and pore size characteristics of nanoporous gold subjected to thermal, mechanical, or surface modification studied using gas adsorption isotherms, cyclic voltammetry, thermogravimetric analysis, and scanning electron microscopy, J. Mater. Chem. **22**(14), 6733–6745 (2012)
32.160 S. Lowell, J.E. Shields, M.A. Thomas, M. Thommes (Eds.): *Characterization of Porous Solids and Powders: Surface Area, Pore Size and Density* (Kluwer Academic, Dordrecht 2004)
32.161 P. Bouras, E. Stathatos, P. Lianos: Pure versus metal-ion-doped nanocrystalline titania for photocatalysis, Appl. Catal. B-Environ. **73**(1/2), 51–59 (2007)
32.162 S.G. Wang, W.T. Lu, O. Tovmachenko, U.S. Rai, H.T. Yu, P.C. Ray: Challenge in understanding size and shape dependent toxicity of gold nanomaterials in human skin keratinocytes, Chem. Phys. Lett. **463**(1–3), 145–149 (2008)
32.163 H. Yin, H.P. Too, G.M. Chow: The effects of particle size and surface coating on the cytotoxicity of nickel ferrite, Biomaterials **26**(29), 5818–5826 (2005)
32.164 D.B. Warheit, W.J. Brock, K.P. Lee, T.R. Webb, K.L. Reed: Comparative pulmonary toxicity inhalation and instillation studies with different TiO_2 particle formulations: Impact of surface treatments on particle toxicity, Toxicol. Sci. **88**(2), 514–524 (2005)
32.165 T.A. Egerton, N.J. Everall, J.A. Mattinson, L.M. Kessell, I.R. Tooley: Interaction of TiO_2 nano-particles with organic UV absorbers, J. Photochem. Photobiol. A **193**(1), 10–17 (2008)
32.166 J.-E. Otterstedt, D.A. Brandreth (Eds.): *Small Particles Technology* (Plenum, New York 1998)
32.167 G. Roebben, S. Ramirez-Garcia, V.A. Hackley, M. Roesslein, F. Klaessig, V. Kestens, I. Lynch, C.M. Garner, A. Rawle, A. Elder, V.L. Colvin, W. Kreyling, H.F. Krug, Z.A. Lewicka, S. McNeil, A. Nel, A. Patri, P. Wick, M. Wiesner, T. Xia, G. Oberdörster, K.A. Dawson: Interlaboratory comparison of size and surface charge measurements on nanoparticles prior to biological impact assessment, J. Nanopart. Res. **13**(7), 2675–2687 (2011)
32.168 D. Fairhurst, M.A. Mitchnick: Particulate sun blocks: General principles. In: *Sunscreens*, ed. by N.J. Lowe, N.A. Shaath, M.A. Pathak (Marcel Dekker, New York 1997) p. 313
32.169 N. Serpone, D. Dondi, A. Albini: Inorganic and organic UV filters: Their role and efficacy in sunscreens and suncare product, Inorg. Chim. Acta **360**(3), 794–802 (2007)
32.170 P. Stamatakis, B.R. Palmer, G.C. Salzman, C.F. Bohren, T.B. Allen: Optimum particle-size of titanium-dioxide and zinc-oxide for attenuation of ultraviolet-radiation, J. Coat. Technol. **62**(789), 95–98 (1990)
32.171 FDA: *Sunscreen Drug Products for Over-The-Counter Human Use*, Final Monograph Federal Register **48**(98) (US Food and Drug Administration, Washington 1999)
32.172 J. Cunningham, P. Sedlak: Interrelationships between pollutant concentration, extent of adsorption, TiO_2-sensitized removal, photon flux and levels of electron or hole trapping additives. 1. Aqueous monochlorophenol-TiO_2 (P25) suspensions, J. Photochem. Photobiol. A **77**(2/3), 255–263 (1994)
32.173 J.C. Doliveira, G. Alsayyed, P. Pichat: Photodegradation of 2-chlorophenol and 3-chlorophenol in TiO_2 aqueous suspensions, Environ. Sci. Technol. **24**(7), 990–996 (1990)
32.174 A.L. Linsebigler, G.Q. Lu, J.T. Yates: Photocatalysis on TiO_2 surfaces – principles, mechanisms, and selected results, Chem. Rev. **95**(3), 735–758 (1995)
32.175 G. Mills, M.R. Hoffmann: Photocatalytic degradation of pentachlorophenol on TiO_2 particles – Identification of intermediates and mechanism of reaction, Environ. Sci. Technol. **27**(8), 1681–1689 (1993)
32.176 U. Stafford, K.A. Gray, P.V. Kamat: Radiolytic and TiO_2-assisted photocatalytic degradation of 4-chlorophenol – a comparative-study, J. Phys. Chem. **98**(25), 6343–6351 (1994)
32.177 V. Brezova, D. Dvoranova, A. Stasko: Characterization of titanium dioxide photoactivity following the formation of radicals by EPR spectroscopy, Res. Chem. Intermed. **33**(3–5), 251–268 (2007)
32.178 V. Brezova, S. Gabcova, D. Dvoranova, A. Stasko: Reactive oxygen species produced upon photoexcitation of sunscreens containing titanium dioxide (an EPR study), J. Photochem. Photobiol. B **79**(2), 121–134 (2005)
32.179 M.R. Hoffmann, S.T. Martin, W.Y. Choi, D.W. Bahnemann: Environmental applications of semiconductor photocatalysis, Chem. Rev. **95**(1), 69–96 (1995)
32.180 G. Riegel, J.R. Bolton: Photocatalytic efficiency variability in TiO_2 particles, J. Phys. Chem. **99**(12), 4215–4224 (1995)
32.181 N. Daneshvar, D. Salari, A.R. Khataee: Photocatalytic degradation of azo dye acid red 14 in water on ZnO as an alternative catalyst to TiO_2, J. Photochem. Photobiol. A **162**(2/3), 317–322 (2004)
32.182 C.A.K. Gouvea, F. Wypych, S.G. Moraes, N. Duran, N. Nagata, P. Peralta-Zamora: Semiconductor-assisted photocatalytic degradation of reactive dyes in aqueous solution, Chemosphere **40**(4), 433–440 (2000)
32.183 J.R. Harbour, M.L. Hair: Radical intermediates in the photosynthetic generation of H_2O_2 with aque-

ous ZnO dispersions, J. Phys. Chem. **83**(6), 652–656 (1979)
32.184 V. Kandavelu, H. Kastien, K.R. Thampi: Photocatalytic degradation of isothiazolin-3-ones in water and emulsion paints containing nanocrystalline TiO_2 and ZnO catalysts, Appl. Catal. B **48**(2), 101–111 (2004)
32.185 C. Kormann, D.W. Bahnemann, M.R. Hoffmann: Photocatalytic production of H_2O_2 and organic peroxides in aqueous suspensions of TiO_2, ZnO, and desert sand, Environ. Sci. Technol. **22**(7), 798–806 (1988)
32.186 A. Lipovsky, Z. Tzitrinovich, H. Friedmann, G. Applerot, A. Gedanken, R. Lubart: EPR study of visible light-induced ROS generation by nanoparticles of ZnO, J. Phys. Chem. C **113**(36), 15997–16001 (2009)
32.187 A. Shafaei, M. Nikazar, M. Arami: Photocatalytic degradation of terephthalic acid using titania and zinc oxide photocatalysts: Comparative study, Desalination **252**(1–3), 8–16 (2010)
32.188 J. Villasenor, P. Reyes, G. Pecchi: Photodegradation of pentachlorophenol on ZnO, J. Chem. Technol. Biotechnol. **72**(2), 105–110 (1998)
32.189 B. Balasubramanian, W.K. Pogozelski, T.D. Tullius: DNA strand breaking by the hydroxyl radical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone, Proc. Natl. Acad. Sci. USA **95**, 9738–9743 (1998)
32.190 K.J.A. Davies: Protein damage and degradation by oxygen radicals I. General-aspects, J. Biol. Chem. **262**(20), 9895–9901 (1987)
32.191 I. Kruk: Environmental toxicology and chemistry of oxygen species: Reactions and processes. In: *The Handbook of Environmental Chemistry*, 2nd edn., ed. by O. Hyutzinger (Springer, Berlin, Heidelberg 1998) p. 261
32.192 Y. Nakagawa, S. Wakuri, K. Sakamoto, N. Tanaka: The photogenotoxicity of titanium dioxide particles, Mutat. Res. Genet. Toxicol. Environ. Mutagen **394**(1–3), 125–132 (1997)
32.193 J.F. Reeves, S.J. Davies, N.J.F. Dodd, A.N. Jha: Hydroxyl radicals ($^{\bullet}OH$) are associated with titanium dioxide (TiO_2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells, Mutat. Res. Fundam. Mol. Mech. Mutagen **640**(1/2), 113–122 (2008)
32.194 T. Uchino, H. Tokunaga, M. Ando, H. Utsumi: Quantitative determination of OH radical generation and its cytotoxicity induced by TiO_2-UVA treatment, Toxicol. Vitro **16**(5), 629–635 (2002)
32.195 C. Wang, S.Q. Cao, X.X. Tie, B. Qiu, A.H. Wu, Z.H. Zheng: Induction of cytotoxicity by photoexcitation of TiO_2 can prolong survival in glioma-bearing mice, Mol. Biol. Rep. **38**(1), 523–530 (2011)
32.196 J. Petkovic, T. Kuzma, K. Rade, S. Novak, M. Filipic: Pre-irradiation of anatase TiO_2 particles with UV enhances their cytotoxic and genotoxic potential in human hepatoma HepG2 cells, J. Hazard. Mater. **196**, 145–152 (2011)
32.197 R.C. Gopalan, I.F. Osman, A. Amani, M. De Matas, D. Anderson: The effect of zinc oxide and titanium dioxide nanoparticles in the Comet assay with UVA photoactivation of human sperm and lymphocytes, Nanotoxicology **3**(1), 33–39 (2009)
32.198 K. Hirakawa, M. Mori, M. Yoshida, S. Oikawa, S. Kawanishi: Photo-irradiated titanium dioxide catalyzes site specific DNA damage via generation of hydrogen peroxide, Free Radic. Res. **38**(5), 439–447 (2004)
32.199 H. Hidaka, H. Kobaysahi, T. Koike, T. Sato, N. Serpone: DNA damage photoinduced by cosmetic pigments and sunscreen agents under solar exposure and artifical UV illumination, J. Oleo Sci. **55**, 205–1212 (2006)
32.200 TGA: A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens, Australian Governemt (Therapeutic Goods Administration, Woden 2009), available online from http://www.tga.gov.au/pdf/review-sunscreens-060220.pdf (last accessed 20 July 2012)
32.201 D.T. Tran, R. Salmon: Potential photocarcinogenic effects of nanoparticle sunscreens, Australas. J. Dermatol. **52**, 1–6 (2010)
32.202 M. Crosera, M. Bovenzi, G. Maina, G. Adami, C. Zanette, C. Florio, F.F. Larese: Nanoparticle dermal absorption and toxicity: A review of the literature, Int. Arch. Occup. Environ. Health **82**(9), 1043–1055 (2009)
32.203 M.D. Newman, M. Stotland, J.I. Ellis: The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens, J. Am. Acad. Dermatol. **61**(4), 685–692 (2009)
32.204 M.J. Osmond, M.J. McCall: Zinc oxide nanoparticles in modern sunscreens: An analysis of potential exposure and hazard, Nanotoxicology **4**(1), 15–41 (2010)
32.205 N. Sadrieh, A.M. Wokovich, N.V. Gopee, J.W. Zheng, D. Haines, D. Parmiter, P.H. Siitonen, C.R. Cozart, A.K. Patri, S.E. McNeil, P.C. Howard, W.H. Doub, L.F. Buhse: Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO_2 particles, Toxicol. Sci. **115**(1), 156–166 (2010)
32.206 B. Baroli: Penetration of nanoplarticles and nanomaterials in the skin: Fiction or reality?, J. Pharm. Sci. **99**(1), 21–50 (2009)
32.207 M.E. Carlotti, E. Ugazio, S. Sapino, I. Fenoglio, G. Greco, B. Fubini: Role of particle coating in controlling skin damage photoinduced by titania nanoparticles, Free Radic. Res. **43**(3), 312–322 (2009)
32.208 L.F. Hakim, D.M. King, Y. Zhou, C.J. Gump, S.M. George, A.W. Weimer: Nanoparticle coating for advanced optical, mechanical and rheological properties, Adv. Funct. Mater. **17**, 3175–3181 (2007)

32.209 A. Rampaul, I.P. Parkin, L.P. Cramer: Damaging and protective properties of inorganic components of sunscreens applied to cultured human skin cells, J. Photochem. Photobiol. A **191**(2/3), 138–148 (2007)
32.210 G. Wakefield, M. Green, S. Lipscomb, B. Flutter: Modified titania nanomaterials for sunscreen applications – reducing free radical generation and DNA damage, Mater. Sci. Technol. **20**(8), 985–988 (2004)
32.211 G. Wakefield, S. Lipscomb, E. Holland, J. Knowland: The effects of manganese doping on UVA absorption and free radical generation of micronised titanium dioxide and its consequences for the photostability of UVA absorbing organic sunscreen components, Photochem. Photobiol. Sci. **3**(7), 648–652 (2004)
32.212 H.J. Johnston, G.R. Hutchison, F.M. Christensen, S. Peters, S. Hankin, V. Stone: Identification of the mechanisms that drive the toxicity of TiO_2 particulates: The contribution of physicochemical characteristics, Part. Fibre Toxicol. **6**, 33 (2009)
32.213 K. Schilling, B. Bradford, D. Castelli, E. Dufour, J.F. Nash, W. Pape, S. Schulte, I. Tooley, J. van den Bosch, F. Schellauf: Human safety review of "nano" titanium dioxide and zinc oxide, Photochem. Photobiol. Sci. **9**(4), 495–509 (2010)
32.214 R. Dunford, A. Salinaro, L. Cai, N. Serpone, S. Horikohi, H. Hidaka, J. Knowland: Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients, FEBS Lett. **418**, 87–90 (1997)
32.215 M. Buchalska, G. Kras, M. Oszajca, W. Lasocha, W. Macyk: Singlet oxygen generation in the presence of titanium dioxide materials used as sunscreens in suntan lotions, J. Photochem. Photobiol. A **213**(2/3), 158–163 (2010)
32.216 P.J. Barker, A. Branch: The interaction of modern sunscreen formulations with surface coatings, Prog. Org. Coat. **62**(3), 313–320 (2008)
32.217 Z.A. Lewicka, V.L. Colvin: Photoactivity tests of TiO_2 and ZnO sunscreen ingredients, Mater. Res. Soc. Symp. Proc. **1413**, mrsf11–1413–gg03–03 (2012)