Green, Vol. **1** (2011), pp. 171–188 DOI 10.1515/GREEN.2011.013

Review

Toxicological Issues of Nanoparticles Employed in Photocatalysis

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Abstract. A huge amount of different nanomaterials is nowadays on the market used for various specific applications. Some nanomaterials such as TiO_2, ZnO as well as several other semiconductors exhibit photocatalytic activity.
Hence these materials are used for many applications, e.g., for self-cleaning and antibacterial coatings on different surfaces and for the purification of wastewater where the cleaning can be induced by simple exposure to sunlight. Because of the frequent use of these nanoparticles it is important to investigate the life cycles of these nanostructured materials as well as their environmental impact and their toxicity to animals and humans.
This review first gives a short overview about nanotechnology and nanotechnological products as well as about photocatalysis and semiconductors used in this field. We then discuss the need for a new technology named nanotoxicology and the problems occurring when investigating the toxic potential of nanomaterials as well as the life cycle of nanomaterials. Furthermore, we focus on the environmental impact of TiO_2 and ZnO nanoparticles including toxic effects to bacteria, water organisms and plants as well as their toxic effects to humans including in vitro and in vivo studies.

Keywords. Nanotoxicology, nanotechnology, TiO_2 ZnO, photocatalysis, environmental impacts, human health risks, in vivo studies, in vitro studies.

PACS®(2010). 87.85.Rs, 79.63.Bd, 78.67.Bf.

1 Nanotechnology

The technological progress allows the production of new tiny materials which have the size of only a few nanometers. Nanotechnology deals with the production, investigation and utilization of these extremely small particles [1]. Nanotechnology is projected to become one of the key technologies of the 21th century [2]. In the nanometer range several physical and chemical properties of materials change which can be specifically used for many applications.

Nanoparticles have an extremely high surface to volume ratio and new effects, properties and functions can be observed in the nanometer scale [3]. Mechanical, optical, magnetic, electrical and chemical properties are not only depending on the material but also on the size and shape of the particles. New phenomena occur which cannot be explained by the laws of classical physics, e.g., quantum effects start playing a role as the particles get very small.

The fabrication of nanomaterials can be achieved employing the top-down or the bottom-up method, respectively. With the top-down process nanoscale materials are produced by miniaturization of any type of source material. The nanoparticles can be build up to more complex systems or can be mixed to other materials to modify their properties. Today this approach dominates in physics and physical techniques with the modern semiconductor industry being just one example. However, the constant development of miniaturization in the semiconductor industry is limited and is not able to follow the increase of the number of transistors [4]. Moreover, the top-down approach is an energy extensive, waste producing and resource wasting process.

Using the bottom-up approach nanomaterials are assembled from atoms or molecules. This approach is frequently used in chemistry and biology. The nanoparticles and their assemblies are, for example, synthesized by chemical vapor deposition or by crystal growth from suitable seeds. One promising approach is the growth by self-assembly [5], [6]. Typical materials synthesized employing the bottom-up process are carbon nanotubes and nanocrystalline zeolites. Since the bottom-up and the self-assembly process can be used in almost every scale these methods are extremely powerful.

Potential applications of nanomaterials can be found in nearly all sectors of industry. In the information and communication sector nanotechnology is specifically important for the production of processors because of the resulting miniaturization. Furthermore, photonic crystals with their potential application in the area of optical circuitry are of great interest for information processing based only on light (photonics).

Nanostructured materials can also be adopted for medical and health care applications [7], e.g., as biocompatible materials produced for the use as implants and for tissue engineering [8], [9]. Moreover, drugs can be transported in the organism and released on target (drug delivery) [10],

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Received: November 30, 2010. Accepted: February 08, 2011.

Industry Sector	Application
Chemistry	Paints [21], Catalysts [22], [23], Hydrogen Storage [24]
Automotive Industry	Wheel Fillers, Antireflection Coatings [25], Scratch-Resistant Paints, De-Mister Coatings, Fuel Cells
Optical Industry	White LEDs, Quantum Dot Laser, Photonic Crystals,
Medicine and Health Care	Implant Materials [8], [9] *Drug Delivery*, [10], [11] *Functional Foods* [26], Cosmetics, Fluorescent Biological Labels [12], [13], [14], Detection of Pathogens [15] Proteins [16], Hyperthermia [18], Antibactericidal Surfaces [21]
Environmental Technology	Water Treatment [27], Solar Energy [28], Conversion and Storage Systems, Pollution Control and Abatement
Information and Communication Technology	Processors

Table 1. Applications of nanoparticles.

[11], and the availability of bioactive substances can be increased (functional foods). Nanoparticles can also be used as fluorescent labels [12], [13], [14], and for the biodetection of pathogens [15] and proteins [16]. Another application of nanotechnology is the separation and purification of biological molecules and cells [17]. A promising and important use in medicine is the selective tumor destruction with nanomaterials via heating called hyperthermia [18].

In the automotive industry nanotechnological products are used as wheel fillers, antireflection coatings, scratch-resistant paints and hydrophilic coatings as de-mister surfaces. Nanoscale materials are used in photovoltaic devices and fuel cell for power supplies. The application of nanotubes in lithium-ion-accumulators can increase the capacity of batteries and mini-accumulators. In the construction industry the application of nanoscale materials in metals enables an essential contribution to light construction. The admixing of silica nanoparticles to construction materials enhances some material properties such as adhesive pull strength and adhesive shear strength between concrete and armoring steel. In environmental technology nanoparticles are used as catalysts for water treatment and for the elimination of pollutants. The underlying mechanism and the employed materials for this purpose will be discussed in more detail in the following section (photocatalysis). It is obvious that nanotechnology products have a wide range of different applications and have already entered their commercial exploration period [19], [20].

Nanostructured materials already exist much longer than the rather recent emergence of nanotechnology. Many long-existing materials are in fact structured on the micro- and nanometer scale and many industrial processes that have been used for decades such as polymer and steel manufacturing exploit nanoscale phenomena. Natural processes have produced nanoparticles for eons for example volcanic eruptions. Nanometer-sized particles are created in countless physical processes like erosion and combustion and the natural world is full of examples of systems with nanoscale structures, e.g., proteins, cells, bacteria, viruses, etc.

Because nanomaterials are used in many different applications and are generated as by-products in technical processes it is important to investigate their potential environmental risks as well as the possible hazards to animals and human beings.

2 Photocatalysis and Photocatalysts

The fundamental principle of semiconductor photocatalysis is the ability of the employed catalyst particles to absorb photons to create reactive electron-hole-pairs which are capable of oxidizing most organic and inorganic compounds. This phenomenon is the basis for a variety of current and projected applications in different fields, such as surface technology, pollution management and medicine.

Briefly, when a semiconducting material absorbs a photon with an energy equal or higher than its bandgap energy, an electron is excited from the valence to the conduction band. Simultaneously, a positively charged hole (h^+) is created in the valence band [29]. Recombination of these two charge carriers will result in the generation of heat or in the reemission of a photon. However, once these two charge carriers reach the surface of the semiconductor, they can undergo a variety of reactions with surface-adsorbed molecules (see Figure 1). For many applications, the most relevant reaction is the oxidation reaction, as the photogenerated hole in the valence band of, i.e., the very frequently used photocatalyst titanium dioxide (TiO_2), has a sufficiently high redox potential to be able to oxidize most organic compounds. In addition to the direct reaction with the holes, this oxidation reaction can be mediated by several active oxygen species. These mediators include but are not limited to hydroxyl radicals ($^{\bullet}OH$), superoxide radicals ($O_2^{\bullet -}$), and singlet oxygen ($^1O_2$) [30], [31], [23], [32], [33].

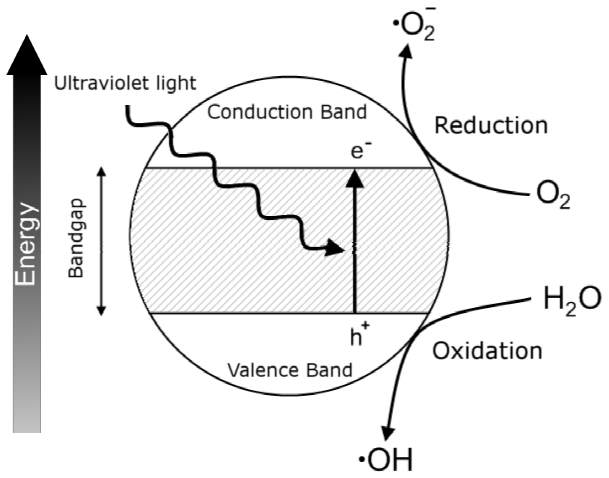

Figure 1. Illustration of the fundamental principle of photocatalysis.

Similarly, the conduction band electron can react with adsorbed molecular oxygen forming superoxide radicals and subsequently hydrogen peroxide (H_2O_2) However, the oxidizing potential of photocatalytic materials is not limited to their surface, as oxidizing species can diffuse away from the photocatalytic surface, inducing redox reactions in the bulk. This effect is usually called remote photocatalysis and is most likely mediated through hydrogen peroxide and singlet oxygen [34], [35], [36], [37], [38], [39], [40], [41], [42], [43], [44], [45] [46], [27]. However, in the case of remote photocatalysis, the reaction rate and thus the product yield is usually one or two orders of magnitude lower than that of the process occurring at the photocatalyst surface. In many cases, only the initial oxidation reaction is photocatalytic, forming a reactive radical that is further oxidized by molecular oxygen, eventually resulting in the formation of carbon dioxide [47]. Besides this exergonic reaction sequence, photocatalytic fuel synthesis, e.g., the cyclic splitting of water into molecular hydrogen and oxygen, presents another very interesting application of photocatalysis that currently receives increased attention and has been the topic of several recently published reviews [48], [49], [50].

Given that photocatalysis is a surface phenomenon, a high surface area potentially contributes to a high photocatalytic activity. Additionally, if the particles are small enough, their light scattering properties become negligible and suspensions or coatings made from these particles will appear optically transparent, which is an important property for many applications [51]. For these reasons, preferably materials exhibiting a high specific surface area and minute dimensions are used in applications, specifically nanoparticles and nanostructured materials [52].

Although sometimes non-oxidic compounds (CdSe, ZnS, GaP) are used as photocatalysts, their instability in aqueous environments and their susceptibility to photocorrosion greatly limits their applications [53], [27], [54]. Therefore, most applications employ the more stable metal oxide semiconductors such as TiO_2, ZnO, and in some cases, Fe_2O_3 and WO_3 as photocatalysts. Unfortunately, due to their rather high bandgap energy of 3.2 eV, the use of TiO_2 and ZnO is limited to light in the ultraviolet region, i.e., below 400 nm. Ultraviolet light, however, only constitutes a small portion of the sunlight. For this reason, the catalysts are sometimes modified by doping, e.g., incorporating N, C, and or S atoms into the bulk material or by surface modification, e.g., depositing noble metal islands as electron transfer catalysts in order to achieve better photocatalytic activity and/or an activity under visible light irradiation.

So far photocatalysis has been proposed for or applied in a variety of applications. Currently, the two most important application fields are surface technology and waste management. In surface technology, surfaces are coated with photocatalytic material to achieve several different properties. One of these properties is the so-called self-cleaning effect: organic pollutants are photocatalytically degraded on surfaces coated with photocatalysts as long as they are illuminated with a sufficient amount of ultraviolet light. This technique is applied to glass surfaces, outdoor building façades, roof tiles, ceramics, plastics and even fabrics and wool [55], [56], [57], [25], [58]. Since the surface can not only be cleaned from organic pollutants but can also be disinfected, the photocatalytic technology can also be used for medical instruments or for surfaces in medical facilities [59], [60], [61].

Another important application of photocatalytic surfaces is the decontamination, deodorization and disinfection of indoor air. Interior facilities are often contaminated with malodorous compounds that reduce the living quality of the residents. Additionally, construction materials and furniture often emit volatile organic compounds (VOCs) in low concentrations. These VOCs may be harmful for the inhabitants and cause diseases such as the *sick building syndrome*. Since almost all airborne pollutants can be degraded by photocatalysis, using this technique to clean the air is more reliable and needs less maintenance than conventional filter-based air-purification systems. Furthermore, since bacteria, fungal spores and viruses are also degraded in this process, they can help to reduce the number of infections transmitted in hospitals and other medical facilities.

Likewise, this method can also be applied for outdoor air purification. The main aim here is, however, to reduce environmental pollutants such as nitrous oxides. Construction materials, roads and pavements are thus modified with photocatalytic material in an attempt to reduce pollutant levels in high-traffic regions [62], [63], [64], [65], [66].

Photocatalysis can also play an important role in industrial and communal waste water management. The advantage of using photocatalysis for waste water treatment is that, unlike in other advanced oxidation techniques, besides

air no external reagent (e.g., O_3, H_2O_2) is needed for the reaction. This makes photocatalysis a potentially cheaper and easier to operate alternative to conventional advanced oxidation techniques for the decontamination of waste water [27], [67], [68], [69], [70], [71]. However, besides the engineering of appropriate reactors the main current drawback for a wider application of this technology for water treatment is the lack of photocatalysts exhibiting sufficient and long-lasting activity and/or being able to utilize also visible light.

3 Nanotoxicology

Currently, a wide range of nanoparticles of different types and exhibiting different properties are being synthesized which are going to be tested for their suitability for various industrial applications. As more and more technological products are becoming available to the customers, concerns have been raised regarding the harmlessness and the potential toxicity of these materials [72], [73], [74]. Every technology or designed product requires careful investigations regarding its sustainability and the associated risk potential before being introduced into the market. For this reason, a new subdiscipline of nanotechnology namely nanotoxicology emerged [75], [76]. Nanotoxicology was initially defined as the science dealing with the effect of engineered nanomaterials and nanostructures on living organisms emerging from the toxicology of ultrafine airborne particles [75]. If the definition of toxicology is adapted to nanomaterials, nanotoxicology can be described as the science dealing with the environmental impact and the hazard of nanomaterials for living organisms as well as the interaction of these materials with biological and environmental systems, including the prevention and minimization of adverse effects caused by nanomaterials [74].

Various new difficulties and subsequent new approaches result from the investigation of the toxicity of nanostructured materials, because compared to their bulk counterparts nanomaterials exhibit a different behavior resulting from property changes in the nanoscale regime.

One of the important factors in nanotoxicology is the possible exposure to nanoparticles and the life cycle of nanostructured materials. The main problem in the determination of the exposure is the lack of labeling of products containing nanomaterials and the lack of knowledge of the concentration of these materials in the environment. Appropriate measurements of particle concentrations and doses and the knowledge of produced materials and their disposal in the environment therefore constitute the first perrequisite for this new discipline. Natural airborne particles in the environment add to the extremely complicated task of the determination of anthropogenic nanoparticles. There are some approaches to determine, to calculate and, or to measure the number of particles in a given environment [77], [78], [79], [80], [81]. Furthermore, since workers and consumers have to be protected, a labeling of nanomaterials will be needed.

Toxicity testings of nanomaterials in vivo or in vitro by establishing dose-response relationships have been developed to identify a potential hazard. Because risk is a function of hazard and exposure [risk $= f$ (hazard, exposure)], the approach is to incorporate both components into a paradigm. Therefore, hazard identification has to be achieved and consequently dose-response and exposure assessment must be carried out. Following these steps the risk assessment and finally the risk management can executed [83], [84].

The characterization of nanomaterials is very important and several nanoparticle classes have to be identified. The physiochemical characteristics and the biological reactivity of particles play important roles. The chemical structure alone is not sufficient to characterize nanomaterials, but rather shape, size (ferret/hydrodynamic), size distribution, agglomeration/aggregation, stability, solubility in different media, surface properties (area/porosity, charge, reactivity, coatings/contaminants, defects), composition and crystal structure have to be determined to enable a judgment concerning the possible hazards of these materials. These properties can change with the method of production, the preparation process, the storage of materials and the introduction into physiological media and the organism. It is not possible to compare the toxicity of nanomaterials to that of their bulk counterparts, because as described in Section 1 the reactivity and the material properties are not identical. Size is an important parameter to predict entry routes, efficiency and translocation of nanoparticles in the organism. Their surface charge may contribute to possible molecular interactions.

Due to their small size, the particles can usually be easily incorporated into the human body. The most important and most investigated adsorption pathway is the assimilation via the lung. Several studies highlight the possible hazard of lung diseases caused by nanomaterials reaching the respiratory tract [85], [86], [87], [88]. Exposition and inhalation studies show the incorporation of particles in the lung and the localization and possible elimination of particles depending on their size, shape, aggregation and surface area [89], [90], [91], [92]. A considerable amount of research demonstrates the toxicity of combustion-derived particles such as diesel soot [90], [91], welding fume [92], carbon black [93], and coal fly-ash [94]. However, there are also studies concerning the incorporation of nanoparticles via the skin [95]. Until now, there is no evidence that the nanoparticles can enter the organism via skin adsorption. Usually the particles are located in the horny layers while only a very small fraction of nanoparticles such as TiO_2 and ZnO used in sunscreens was found to penetrate into deeper tissue layers but only inside single follicle channels. [96], [97], [98], [99]. Luke et al., however, showed that Quantum Dots are able to penetrate the skin [100].

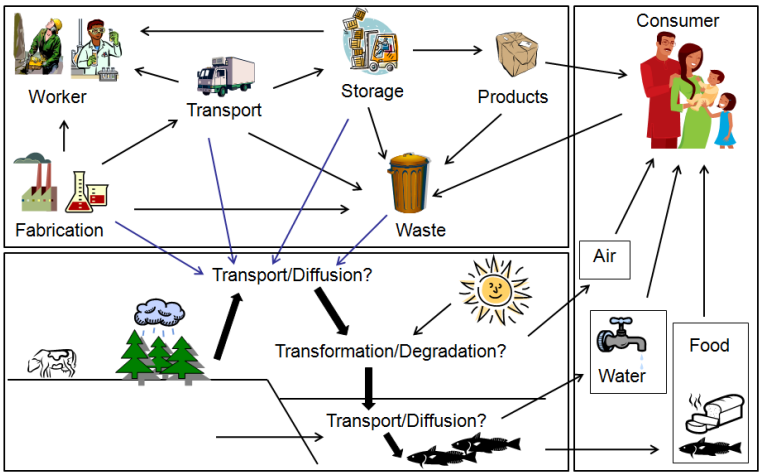

Figure 2. : Scheme of the life cycle of nanomaterials [82].

Besides these "natural" or accidental adsorption pathways of nanoparticles into the organism, intended insertion of nanoparticles into the body occurs for medical applications either intravenously [101] or when present as a layer on bone tissue implants [8], [9].

After entering the organism, nanoparticles will most likely be able to move freely because of their small size thus reaching almost every part of the body. Various studies have shown that the localization of particles in the body depends on their adsorption path in the organism [102] as well as on their size and shape [103]. The effective clearance mechanism of particles that had entered the tissues was also found to depend on their size [104], [105]. Translocation and toxicity are also regulated by specific cellular interactions and by specific nanomaterial factors, such as their biochemical stability and their potential to generate reactive oxygen species (ROS) [106]. ROS can cause oxidative stress which has been linked to increased cytotoxicity [107], [108], [109], [110].

Nanoparticles can interact with various biological molecules such as proteins [111], [112] and are able to penetrate the cell membrane interacting with DNA and causing DNA damage [113]. Evidence has also been presented that nanoparticles can break through the blood-brain-barrier [95], [114]. The surface charge will influence nonspecific adhesion of proteins on the nanomaterials surface as well as its permeation through junctions and its cellular uptake [104].

As mentioned before, particles can react with proteins. Therefore, the particle size, the aggregation and the agglomeration state in the biological media used for cell culture tests has to be determined. It is important to verify that during any test nanoparticles and not only aggregates of particles are still in the culture media [115].

Several studies show that particles exhibit a different behavior in the culture medium as compared to aqueous suspensions. Some particles are stabilized by the proteins in the cell culture media while some are aggregated by salts [116], [117], [118], [119], [120].

This overview concerning nanotoxicology research illustrates the difficulties in applying generalized concepts for the prediction of nanomaterial permeation, of cellular uptake and of toxicity in biological systems. Nanotoxicology is an emerging field with a lot of antagonisms existing in the published literature since standardized tests do currently not exist.

4 Environmental Impacts of Nanoparticles

The environmental impact caused by nanomaterials is presently being extensively discussed in particular in correlation with the regulations concerning airborne particulate matter. To be able to estimate the potential risk of nanomaterials informations concerning their mobility and the life cycle are of crucial importance.

Furthermore, a clear differentiation between particles generated by technical processes and those unintended released is definitely required. Most of the latter particles are generated by combustion processes (flow ash, diesel soot, catalyst dust, carbon black).

Naturally occurring nanoparticles vary in size, composition and shape, whereas artificial nanomaterials are more uniform because they are synthesized for special applications. With the progressive development of traffic a drastic increase of ultrafine particles in the air is noticeable. The life cycle of nanomaterials is not obvious, cf. Section 3. Depending on production and use, nanomaterials can be emitted into air or water and can thus be found on the ground or in ground or surface water, respectively.

Investigations of combustion-derived ultrafine particles can assess the behavior and the impact of nanoparticles

in the environment. The ultrafine particles produced by diesel engines have the property to agglomerate and to subsequently sediment within a few days, hence, these dusts are eliminated from the air after a certain period of time. Synthesized, artificial particles frequently do not agglomerate because they are often stabilized with, e.g., core-shell-systems for specific applications; consequently, they stay in the air for a longer period of time and can travel over long distances.

Because of their large surface area nanoparticles can adsorb more pollutants and bigger amounts of toxic substances such as pesticides, fertilizer, pollutants and heavy metals, which can thus be transported over long distances and can be widely distributed [121]. Due to their high reactivity nanoparticles may react with natural substances possibly forming toxic compounds or composites. Furthermore, the bactericidal effect of some materials could change the microbial composition in the water or on the ground. To assess the environmental hazard, most studies investigate the impact and toxic effects of nanomaterials to organisms living in water. The first step in an investigation of this exposure route is the characterization of the form and the concentration of engineered nanomaterials in water since this is where many substances exhibit their most significant environmental impact. Materials present in water can be degraded, transformed and/or accumulated in a variety of ways.

In the following we will focus on the environmental effects caused by semiconductors such as TiO_2 and ZnO used in photocatalysis. The life cycle of these materials is long. They can be released to the environment from laboratories and factories during their production processes. Because of the wide use as photocatalysts release to the environment is also possible from products containing these materials, such as photocatalytic wall paints and coated surfaces, e.g., roof tiles. In some products the photocatalyst particles are present in solutions, e.g., during the treatment of wastewater. In others they are present as fixed particles in coatings. TiO_2 and ZnO nanoparticles are being prepared in large quantities and in many different varieties; some are even stabilized with proteins and other reagents. As mentioned before, the bactericidal effect of materials can also have an environmental impact.

Bactericidal Effect of TiO_2 and ZnO Nanoparticles

The toxic effect of illuminated TiO_2 and ZnO nanoparticles is being thoroughly investigated and many papers have been published on this topic with the majority of these studies being performed with TiO_2 because this is by far the most used photocatalyst.

When exposed to near-UV light, titanium dioxide exhibits a strong bactericidal activity. However, the underlying killing mechanism of this photocatalytic reaction is not yet well understood. Recently, total oxidation of *Escherichia coli* cells has been demonstrated [122]. The reactive oxygen species (ROS) generated by the TiO_2 photocatalytic reactions cause various damages to living organisms. This is not surprising since the latter are composed of organic compounds. In 1985, Matsunaga and coworkers reported the microbiocidal effect of TiO_2 photocatalytic reactions for the first time [123].

E. coli has been chosen in many studies to investigate the toxic effect of photocatalysis [124], [125], [126]. Coleman et al. examined the photocatalytic degradation of *E. coli* in water comparing various catalysts and showed that TiO_2 (P25) was the most efficient catalyst. Non-buffered water samples displayed a greater bactericidal efficiency, which was attributed to a decrease in the electrostatic repulsion between TiO_2 and *E. coli* and to elevated stress on *E. coli* at acidic pH. Buffered samples showed a decrease in bactericidal efficiency which was attributed to the presence of HCO_3^--ions competing for oxidizing species and blocking the surface of the TiO_2-particles [124].

It has also been shown that TiO_2 photocatalyst particles illuminated with UV-light exhibit high disinfection rates against other microorganisms besides *E. coli* such as *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* [123], *Lactobacillus helveticus* [127], *Pseudomonas aeruginosa* [128] *Pseudomonas stutzeri* [129] and *Bacillus pumilus* [130].

The toxic effect of photocatalytic systems to bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium* and *Candida albicans* which are relevant for hygiene has also been investigated [131]. Kuhn et al. observed a high mortality for all these bacteria on UV-illuminated surfaces coated with TiO_2 (P25) and proposed that $^{\bullet}OH$-radicals formed on the catalyst surface are causing this effects [131].

Gogniat et al. analyzed the bactericidal effect of UV-illuminated TiO_2 in NaCl-KCl or sodium phosphate solutions to an *E. coli* strain. They found that the adsorption of bacteria on the catalyst occurred immediately in NaCl-KCl solution, whereas it was delayed in the sodium phosphate solution. Moreover, the adsorption rate of the cells onto the catalyst was found to be positively correlated with its bactericidal effect. The authors suggest that the photocatalytic bactericidal effect is related to the loss of membrane integrity of the cells aggregated on the TiO_2 surface [132].

The toxic effect of TiO_2 photocatalysis to phytophatogenic bacteria is not only a chance to alternatively control plant diseases rather than using pesticides, but it can also present a hazard which currently cannot yet be estimated [133], [134].

The photocatalytic activity of ZnO nanoparticles on different microorganisms has also been reported [135], [136], [137], [138]. Upon UV(A)-illumination ZnO causes a significant growth inhibition to *E. coli* [136], [137], [138], *Bacillus subtilis* [139], *Streptococcus agalactiae*, and *Staphylococcus aureus*, with the latter two being patho-

genetic agents causing several infective diseases [135]. The antibactericidal activity of ZnO to *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis* [139] has also been investigated. Jones et al. found the antibacterial activity of ZnO nanoparticles to depend on their size, with smaller particles exhibiting a greater efficacy for the bacteria growth inhibition.

While in the absence of UV illumination TiO_2 is not able to inhibit the growth of, e.g., *Staphylococcus aureus* significantly, ZnO particles are found to also exhibit bactericidal activity in the dark [140], [141].

Brayner et al. observed that synthesized ZnO nanoparticles with average particle diameters of 12 nm are able to inhibit the bacterial growth (100% at concentrations of 3 mM) and suggested this to be due to the disorganization of the *E. coli* membranes. As a consequence the membrane permeability increases leading to the accumulation of nanoparticles in the bacterial membrane and the cytoplasm of the cells [142].

Roselli et al. proposed that the toxic effect of ZnO to *E. coli* is related to zinc ions which are metabolized as an oligo-element [138]. Certain bacteria have developed mechanisms to regulate the influx and efflux processes to maintain a steady intracellular concentration of metal ions, including the Zn^{2+} ion. The genes responsible for the transport of zinc ions have also been characterized in several bacteria, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *E. coli*, and *Bacillus subtilis* [143], [144].

Impacts of TiO_2 and ZnO Nanoparticles on Water Organisms and Plants

The effect of nanoparticles on water organisms and plants also plays an important role concerning the environmental impact of nanotechnological products, because nanoparticles may be released into freshwater systems as a result of their use for the treatment of waste from industry and medicine. There are only a few studies investigating these interactions. Free radicals generated by the irradiation of photocatalysts may cause a risk for organisms living in water.

Hundt-Rinke et al. investigated the effect of two different nanoparticle suspensions under appropriate illumination to induce their photocatalytic activity. The growth inhibition of the green alga *Desmodesmus subspicatus* and the immobilization of *Daphnia magna* were selected as test reaction, respectively. It was shown that nanoparticles may induce ecotoxicological effects depending upon the nature of these particles. TiO_2 particles with a size of 25 nm mainly consisting of anatase exhibited a harmful effect on algae (EC_{50}-values 44 mg/L), whereas pure anatase with a size of 100 nm show no toxic effect to the algae (maximum investigated concentration 50 mg/L). In the tests performed with daphnia, toxicity was observed for both TiO_2 nanoparticles [145]. Lovern and coworkers also investigated the toxic effect of TiO_2 to daphnia and reported an increase in mortality with an increase of the nanoparticle concentration [146].

Velzeboer et al. investigated the ecotoxic effect of some nanomaterials employing different methods and ecotoxicity endpoints. Four different ecotoxicity tests were performed: the Microtox® test, the pulse-amplitude modulation (PAM) test, the Chydotox test, and the Biolog® test [147]. The Microtox® test uses bioluminescent bacteria, specifically, the strain *Vibrio fischeri* [148], the pulse-amplitude modulation (PAM) uses the green alga *Pseudokirchneriella subcapitata* [149], the Chydotox is based on the survival of *Chydorus sphaericus*, a small, benthic cladoceran [150], and the Biolog® test uses a mix of soil bacteria to determine toxicity via multivariate analysis [151]. No appreciable effects were observed at concentrations up to 100 mg/L for tested particles (TiO_2, ZrO_2, Al_2O_3, CeO_2, fullerene (C_{60}), single-walled carbon nanotubes, and polymethylmethacrylate). It was suggested that colloid (in)stability is of primary importance in explaining ecotoxic effects of nanoparticles in the natural environment [147].

The toxicity of TiO_2 nanoparticles to the alga *Pseudokirchneriella subcapitata* was also investigated with bulk TiO_2 (EC_{50} = 35.9 mg/L / NOAEC = 10,1 mg/L) showing a lower toxicity as compared to their nano formulations (EC_{50} = 5.83 mg/L / NOAEC = 0,98 mg/L). Nano TiO_2 formed characteristic aggregates entrapping algal cells which may contribute to the toxic effect of nanostructured TiO_2 to algae [152].

On the other hand, suspensions of nano and bulk TiO_2 were found to be non toxic even at loadings at 20 g/L to bacteria *Vibrio fischeri* and to crustaceans *Daphnia magna*, and to *Thamnocephalus platyurus* [153].This study did not include any measurements of the TiO_2 particle size in water. Commercially available TiO_2 nanoparticles e.g., Evonik-Degussa Aeroxide P25 showed no measurable effect on the growth rates of four different phytoplankton species: *Thalassiosira pseudonana*, *Skeletonema marinoi*, *Dunaliella tertiolecta*, and *Isochrysis galbana*, up to concentrations of 1000 μg/L (ppb) [154].

Zinc oxides were found to be equally toxic to the alga *Pseudokirchneriella subcapitata* in their bulk and in their nano formulations [151]. The toxicity of ZnO was attributed to soluble zinc ions originating from the metal oxide particles. This study by Aruoja et al. again demonstrates that solubility appears to be a key issue governing the toxicity of metal containing nanoparticles, at least for organisms that a priori are not internalizing the particles [152].

Heinlaan and coworkers investigated the toxic effect of nano and bulk ZnO as well as of Zn^{2+} ions to bacteria *Vibrio fischeri*, to crustaceans *Daphnia magna* and *Thamnocephalus platyurus* [153]. All Zn formulations were found to be very toxic with the EC_{50} values determined for *Vibrio fischeri* being 1,8 mg/L for bulk ZnO, 1,9 mg/L for

nano ZnO, and 1,1 mg/L for $ZnSO_4$ $7H_2O$, for *Daphnia magna* 8,8 mg/L for bulk ZnO, 3,2 mg/L for nano ZnO and 6,1 mg/L for $ZnSO_4 \cdot 7\ H_2O$, and for *Thamnocephalus platyurus* 0,24 mg/L for bulk ZnO, 0,18 mg/L for nano ZnO, and 0,98 mg/L for $ZnSO_4 \cdot 7H_2O$, respectively. The toxicity was found to be caused by solubilized Zn ions as proved by tests performed with recombinant Zn-sensor bacteria [153].

Miller et al. showed that ZnO nanoparticles significantly inhibited the growth rate of four different phytoplankton species: *Thalassiosira pseudonana*, *Skeletonema marinoi*, *Dunaliella tertiolecta*, and *Isochrysis galbana*. The ZnO particles aggregated rapidly in seawater forming particles with hydrodynamic diameters exceeding 400 nm [154]. The toxicity of ZnO nanoparticles to phytoplankton was likely due to dissolution, release, and uptake of free zinc ions, with specific nanoparticulate effects being difficult to distinguish from effects due to free zinc ions [154], [155].

The toxicity of Zn and other trace metals to phytoplankton moreover depends on the concentration of other limiting trace metals and nutrients [155]. The mechanism of Zn^{2+}-ion toxicity in phytoplankton can be explained by an antagonism between the toxic metal, in this case zinc, and the nutrient metal. Excessive free Zn^{2+}-ions competitively inhibit manganese uptake, causing Mn deficiency [156]. Zn^{2+} has also been shown to cause increased ATP production in diatoms, which may be linked to increased thiol and glutathione production [157]. Zn-thiol binding may act as a detoxification mechanism, but the energy required for this reaction may result in a decrease of the cell division rate and therefore of the population growth rate [157].

Mortimer and coworkers investigated the toxic effect of ZnO nanoparticles on the model organism protozoa *Tetrahymena thermophila* and reported the EC_{50} values after 4 h exposure to be 3,7 mg/L for bulk ZnO, 3,9 mg/L for nano ZnO, and 4,9 mg/L for Zn^{2+}, respectively. The toxicity of the zinc compounds was found to be about 1.5 times lower after 24 h of exposure as compared with an exposure time of 4 h, probably due to adaptation of the organisms [158].

The dissolution of ZnO nanoparticles and its contribution to the toxicity on ryegrass was also investigated. Zn^{2+} ions were used to compare and verify the root uptake and phytotoxicity of ZnO nanoparticles in these hydroponic culture systems. The root uptake and the phytotoxicity were visualized by light, scanning electron, and transmission electron microscopy. In the presence of ZnO nanoparticles, ryegrass biomass was significantly reduced, root tips were shrunk, and root epidermal and cortical cells highly vacuolated or collapsed. ZnO nanoparticles were found to be able to concentrate in the rhizosphere, to enter the root cells and to inhibit seedling growth of ryegrass. The phytotoxicity of ZnO nanoparticles could not primarily be explained by their dissolution in the bulk nutrient solution or in the rhizosphere [159].

Because of the entry of nanotechnological products to the air, the water, and the ground nanoparticles can be adsorbed by plants and mammalian organisms. Hence, they enter the food chain and can cause possible hazards and risks to animals and humans. The toxicological aspects to animals and human beings will be discussed in the following section.

5 Human health risks of Nanomaterials

As discussed in Section 4, toxic effects of metal oxide nanomaterials to different bacteria, water organisms and plants have been detected. The potential hazardous effect of semiconductor particles employed in photocatalysis will summarized in this section. Nanoparticles are able to reach the organism via the lung, via the gastrointestinal tract and partly via the skin. Within the organism they can move freely, and should be able to break through the blood-brain barrier and through cell membranes. Via the blood vessels they can reach and eventually accumulate in different organs such as kidney, heart, and liver. In the body nanoparticles are able to interact with different proteins and cell components as described in Section 3. Therefore, various studies have investigated the toxic effect of TiO_2 and ZnO nanoparticles to different cell lines and animals.

Picatonotto et al. investigated the photocatalytic activity of pigments grade titanium dioxide (TiO_2), used, e.g., in sunscreens and observed a photocatalytic degradation of the organic additives of protective creams and the generation of active species inducing the transformation of biological molecules present on the human skin. Some sunscreens have been photo-degraded after UV-irradiation with the potential risk to act as photosensitizers able to initiate harmful reactions for the skin, especially photo-induced mutagenicity [160]. Hidaka et al. examined the fate of DNA, RNA and their corresponding pyrimidine and purine bases in presence of TiO_2 under UV illumination. Although these in vitro studies may not reflect actual in vivo cases it has been demonstrated that illuminated TiO_2 interacts with DNA or RNA components with serious damage to these substrates being possible [161]. Lu and coworkers have demonstrated that UV-illuminated pure anatase TiO_2 as well as the mixed anatase-rutile Evonik-Degussa Aeroxide P25 can promote the protein tyrosine nitration. The latter is known to occur as a result of oxidative and nitrative stress and is directly involved in the onset or the progression of diseases. On the other hand, the photocatalytic effect of rutile nanoparticles resulting in protein tyrosine nitration was reported to be marginal. Considering the existence of nanostructured TiO_2 in the environment and in cosmetics, e.g., sunscreen products along with the high content of nitrite in sweat, UV-exposed skin may be a significant target for the photosensitized damage [162] and for photo-oxidative injuries

[163], [164], [165]. The potential physiological significance of nano TiO_2 induced photocatalytic protein nitration was also demonstrated in mouse skin homogenate. The relationship between photocatalytic protein tyrosine nitration and chronic cutaneous diseases still needs further investigations [162].

In vitro Studies of TiO_2 and ZnO Nanomaterials

The effect of TiO_2 nanoparticles in different sizes, shapes and surface coatings to various cell lines such as HaCaT cells, an immortalized keratinocyte cell line [166], L929 cells, a mouse fibroblast cell line [167], HeLa cells, an ovarian cell line [168], [169], PC-12 cells, a rat pheochromocytoma cell line [162], [170], [171], A-549, a lung cell line [170], [171], NIH-3T3 cells, a mouse fibroblast cell line [170], [171], and to HEP-G2 cells [170], [171] has been investigated in great detail.

First studies investigating the possible cytotoxic effect of TiO_2 nanoparticles to mammalian cells were carried out by Cai and coworkers in 1992 [169]. They observed that HeLa cells showed no more viability after cultivation with photoactivated (10 min/ 550 W Hg lamp) TiO_2 nanoparticles (P25) at a loading of 50 µg/ml (50 ppm), whereas the treatment with TiO_2 particles in the absence of irradiation showed only a slight decrease of the cell viability [169].

PC-12 cells treated with different concentrations (1, 10, 50, 100 µg/mL) of nanostructured TiO_2 (P25) showed a significant decrease of their viability in periods of 6, 12, 24, and 48 h evincing an explicit dose effect and time dependence. It was suggested that TiO_2 nanoparticles induce intracellular accumulation of reactive oxygen species and the apoptosis of PC-12 cells both of which increase with increasing concentration of TiO_2 [172].

Chen and coworkers tested the cytotoxicity of various anatase nanostructures, possessing three different morphological structures to HeLa cells. 0D anatase nanoparticles at a concentration of 125 µg/mL were found to decrease the cell viability to 80 %, whereas the viability of the cells was close to 100 % when adding the 2D and 3D nanostructures at the same concentration.

By irradiating the treated cells with 8 mW/cm^2 UV light for 1 min their mortality increased. These differences in toxicity may be related to the different uptake ability of unmodified structures of varying geometry into cells [168]. The studies of Jin et al. showed that weakly aggregated anatase nanoparticles with an average size of less than 100 nm induced significant toxicity in L929 cells at concentrations between 30 µg/mL and 600 µg/mL. The cell shape became spherical accompanied with a cell shrinkage as the concentration of TiO_2 nanoparticles increased. The thus treated cells were found to be necrotic and a significant increase in oxidative stress at higher TiO_2 concentrations (>60 $\mu g/mL$) was observed [167].

Oxidative stress and the formation of reactive oxygen species (ROS) were shown to be integral parts of the key mechanisms of cellular defense after particle uptake. TiO_2 nanoparticles evidently induce intracellular oxidative stress by disturbing the balance between oxidant and antioxidant processes [173].

However, other studies have shown that TiO_2 nanomaterials do not exhibit any toxic effect to cells [170], [171]. Nine different powders and three different suspensions, all consisting of TiO_2 nanoparticles have been tested concerning their toxicity to A-549, HEP-G2, PC-12, and NIH-3T3 cells. The particles differed in crystal structure, size and BET-surface area. In one of these studies, none of the tested particles showed any toxic effect on the cells in a concentration range between 100 and 1000 ppm, with the mitochondrial activity of the cells being the only parameter that was determined [170]. Another study investigating the toxic effect of suspensions of different TiO_2 nanoparticles showed that some cells are more sensitive to particle exposure than others. The sensitivity of the employed cell lines was determined to be NIH-3T3 > A-549 > PC-12 > HEP-G2, with the NIH-3T3 being the most sensitive cell line. The highest applied TiO_2 loading of 3125 $\mu g/cm^2$ decreases the viability to 33% (NIH-3T3 cells cultivated with TiO_2 particles with a hydrodynamic diameter of 144.0 ± 23.7 nm) in comparison to untreated cells. However, this study also showed that only very high particle doses exceeding 625 $\mu g/cm^2$ yield a significant decrease in viability. No additional decrease in the viability of cells was observed upon UV(A)-illumination [171].

Several studies have investigated the toxic effect of ZnO nanoparticles to MSTO cells and 3T3-cells [174], to SMMC-7721-cells, a hepatocellular cancer cell line [175], to HeLa and L929 cells [176], to PBMC (peripheral blood mononuclear) cells [177], to human skin fibroblasts [178] to primary mouse embryo fibroblasts, and to A-549 cells. Brunner et al. investigated the toxic effect of ZnO nanoparticles to MSTO- and fibroblasts (3T3-cells) [174] and found that the cells were no longer viable after 3 days exposure to zinc oxide (particle diameter 40 nm) at concentrations exceeding 15 ppm. The authors assumed that the toxic effect of the ZnO nanoparticles is related to the solved Zn^{2+}-ions. Furthermore, Lin et al. investigated the toxic effect of ZnO nanoparticles to A-549 cells at two different particle sizes (70 nm and 420 nm). They conclude that the exposure to both sizes of ZnO particles leads to dose- and time-dependent cytotoxicity reflected in oxidative stress, lipid peroxidation, cell membrane damage, and oxidative DNA damage. Neither free Zn^{2+} ions nor metal impurities appear to be major contributors of ROS induction [179] in contrast to the results of Brunner et al. [174].

Decksakulthorn and coworkers determined the IC_{50} values of ZnO particles (average particle sizes 70 nm) to be 50 ppm for A-549 cells in contrast to the IC_{50} values of TiO_2

particles (average particle size 50 nm) being around 2700 ppm [178].

Studies of Hanley and coworkers demonstrate that ZnO nanoparticles induce toxicity in a cell-type specific manner that depends on the degree of particle-cellular membrane association, the phagocytic ability, and the inherent cellular capacities for ROS production. Monocytic cells displayed the greatest susceptibility and intracellular ROS production upon exposure to ZnO nanoparticle, followed by NK cells, and by lymphocytes, which displayed the highest resistance. Hanley et al. assumed that ROS formation is the major mechanism of ZnO nanoparticle-induced toxicity, with the generation of ROS and the cytotoxic effect occurring in a particle size-dependent manner, i.e., smaller particles display the greatest effect [177].

When studying ZnO particle sizes ranging from 20-100 nm Li et al. did not find any particle size dependent toxic effect to SMMC-7721 cells [175].

The proliferation activity of L929 and HeLa cells has been found to be strongly deteriorated upon cultivation in the presence different doses of ZnO nanoparticle (20 nm) suspensions after 24 h and 48 h exposure. Concurrently, an increase in necrotic and apoptotic cells was observed after cultivation with ZnO particles [176].

In vivo Studies of TiO_2 and ZnO Nanoparticles

The effect of various TiO_2 particles in mice, rats, and hamsters after oral exposure and instillation [180], [181], [182], [183], [184], [185], [186], injection [187], [188] and inhalation [189], [181], [190], [191] has been studied in detail.

The inhalation studies with rats showed that a possible hazard and an inflammatory response do exist depending upon the particle characteristics. Lee and coworkers exposed rats to TiO_2 by inhalation exposure with concentrations of 0, 10, 50, and 250 mg/m^3 for 6 h/day, 5 days/week for 2 years and found no abnormal clinical signs, no body weight changes, and no excess mortality in any exposed group. However, the exposed groups showed slight increases in the incidence of pneumonia, tracheitis, and rhinitis, and obvious hepatic damage and renal lesion in female mice [188]. TiO_2 particles were found to be mainly retained in liver, kidney, spleen and lung [189]. Based on the excessive dust loading and overwhelmed clearance mechanisms in the lungs of rats exposed chronically at 250 mg/m^3, the biological relevance of lung tumors to man appears to be negligible. Furthermore, no evidence exists to suggest that TiO_2 causes lung tumors in humans [189], [192].

Warheit et al. showed in their inhalation studies with rats that the composition and the surface treatment of the nanoparticles can influence the toxicity of TiO_2 particles in the lung. TiO_2 formulations containing 7 % Al_2O_3 and 11 % amorphous silica were found to produce adverse lung effects as compared to pure TiO_2 [181].

Toxicological inhalation studies of Hext et al., with rats, mice, and hamsters being exposed to pigment grade TiO_2 and ultrafine TiO_2 (P25) for 6h/day, 5 days/week for 13 weeks at concentrations of 0, 10, 50, and 250 mg/m^3 of TiO_2 were performed to investigate whether rats are oversensitive to TiO_2 compared to mice or hamsters. These epidemiology studies have been carried out to investigate whether a link exists between increased incidence of lung cancer and exposure to TiO_2. The results of these studies do, however, not suggest that TiO_2 nanoparticles exhibit any carcinogenic effect on the human lung [190]. In contrast to the study of Hext et al., Bermudez and coworkers reported that TiO_2 exposure to rats in concentrations of 250 mg/m^3 can induce epithelial and fibroproliferative lesions as well as alveolar cell metaplasia using the pure rutile form of TiO_2 for their inhalation studies [191].

Biodistribution experiments performed after oral administration of TiO_2 nanoparticles to rats showed that the particles were retained in liver, spleen, kidney, and lung tissue indicating that TiO_2 nanoparticles can be transported to other tissues and organs after uptake by the gastrointestinal tract [180], [184]. Following inhalation exposure the distribution of the particles was found to be the same [189]. Cui et al. reported that liver damage can be caused by oxidative stress after oral exposure of TiO_2 (100 % anatase) nanoparticles to rats [182].

Hohr and coworkers investigated the acute inflammatory response and the cell damage induced by the intratracheal instillation of surface modified (hydrophilic and hydrophobic) fine (diameter 180 nm) and ultrafine (20 ± 5 nm) TiO_2 particles for 16 h at equivalent mass (1 or 6 mg) and surface doses (100, 500, 600 and 3000 cm^2) in rats. The results of Hohr et al. suggest that the surface area rather than the hydrophobicity of the surface determines the acute, pulmonary inflammation induced by both fine and ultrafine TiO_2 [183], whereas Warheit et al. postulated in their studies that nanoscale particles do not exhibit any cytotoxicity or inflammation to the lung as compared to larger sized particles of similar chemical composition [186].

After intraperitoneal injection of high doses of TiO_2 in rats (150 mg/kg) the production of ROS ($O^{-\bullet}$ and H_2O_2) occurred in the liver indicating that this organ underwent oxidative stress which was found to be higher for nano anatase TiO_2 than for bulk TiO_2 [188]. Liu et al. observed that there was a serious damage of liver, kidney, and myocardium, an inflammatory response, and a metabolism imbalance of blood sugar and lipid after abdominal cavity injection of high doses of TiO_2 to mice [187].

Acute toxicity to male mice after intratracheal injection of ZnO particles of different sizes (10, 30, 100 nm) at doses between 0.05 and 0.5 g/kg body weight were also observed. Pathological examination showed the accumulation of ZnO particles after intratracheal injection in lung, spleen, pancreas, bone, and liver. At low doses, 30 and 100 nm sized ZnO particles induced a slight damage, whereas 10 nm

sized particles caused a more serious lung and liver damage. At high doses, the 30 nm sized ZnO particles induced worse lung, liver, and pancreas injury [193].

Wang and coworkers investigated the acute oral toxicity of ZnO particles with different sizes (20 nm and 120 nm) at doses of 1, 2, 3, 4 and 5 g/kg body weight. This study showed that following oral administration ZnO was mainly retained in bone, kidney, and pancreas. The results of blood measurements suggest that the increase in blood viscosity could be induced by high doses of 120 nm sized ZnO nanoparticles. For smaller particles (20 nm) lower concentrations caused the same effect [194].

6 Conclusions

Nanostructured materials are widely used in almost every sector of industry with photocatalysis being one important application field. Suitable semiconductors irradiated with UV- or visible light exhibit photocatalytic activity and can therefore be used for various applications, including the treatment of wastewater and the production of self-cleaning surfaces. Because of the common use of these materials it is important to investigate the possible effects of nanoparticulate photocatalysts to the environment and to human health.

Nanotoxicology is an emerging new subdiscipline of nanotechnology focusing on the toxic potential of nanostructured substances. Particularly difficult tasks within the field of nanotoxicology are the definition of the life cycle of these materials, the handling of the huge amount of natural nanoparticles and the lack of any logical labeling of nanomaterials. Nanoparticles exhibit other physical and chemical properties compared to their bulk counterparts. Therefore, a detailed characterization of these materials is certainly required. Shape, size, size distribution, agglomeration/aggregation, stability, solubility in different media, surface chemistry, composition, charge, crystal structure, zeta potential etc. of the particles have to be determined as a basis for the interpretation of the toxicity studies. To assess their environmental hazards the investigation of the toxic effect of nanomaterials to organisms living in water and to bacteria has to be carried out. For the assessment of the possible hazard to human health, in vitro and in vivo studies need to be performed.

The results of a wide selection of publications investigating the toxic effect of TiO_2 and ZnO, i.e., the two most commonly used photocatalysts, to different bacteria, water organisms, algae and plants as well as in vitro and in vivo studies with different sized and shaped TiO_2 and ZnO particles are presented and discussed. This review shows that considerable differences in the published toxicity results do exist which can mainly be attributed to differences in the types of employed TiO_2 and ZnO nanoparticles (size, shape, etc.) and the treatment methods demonstrating the importance of a suitable characterization of the particles.

In conclusion, it is obvious that the toxic effect of TiO_2 nanoparticles can mainly be attributed to the production of reactive oxygen species, whereas the toxic effect caused by ZnO nanoparticles may be attributed to free Zn^{2+} ions.

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