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Jinshun Zhao [a] & Vincent Castranova [b]

[a] Public Health Department of Medical School, Ningbo University, Ningbo, Zhejiang, P. R. China

[b] Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

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TOXICOLOGY OF NANOMATERIALS USED IN NANOMEDICINE

Jinshun Zhao[1], Vincent Castranova[2]

[1]Public Health Department of Medical School, Ningbo University, Ningbo, Zhejiang, P. R. China
[2]Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

With the development of nanotechnology, nanomaterials are being widely used in many industries as well as in medicine and pharmacology. Despite the many proposed advantages of nanomaterials, increasing concerns have been expressed on their potential adverse human health effects. In recent years, application of nanotechnology in medicine has been defined as nanomedicine. Techniques in nanomedicine make it possible to deliver therapeutic agents into targeted specific cells, cellular compartments, tissues, and organs by using nanoparticulate carriers. Because nanoparticles possess different physicochemical properties than their fine-sized analogues due to their extremely small size and large surface area, they need to be evaluated separately for toxicity and adverse health effects. In addition, in the field of nanomedicine, intravenous and subcutaneous injections of nanoparticulate carriers deliver exogenous nanoparticles directly into the human body without passing through the normal absorption process. These nanoparticulate carriers themselves may be responsible for toxicity and interaction with biological macromolecules within the human body. Second, insoluble nanoparticulate carriers may accumulate in human tissues or organs. Therefore, it is necessary to address the potential health and safety implications of nanomaterials used in nanomedicine. Toxicological studies for biosafety evaluation of these nanomaterials will be important for the continuous development of nanomedical science. This review summarizes the current knowledge on toxicology of nanomaterials, particularly on those used in nanomedicine.

With the development of nanotechnology, there is a tremendous growth of the application of nanomaterials, which increases the risk of human exposure to these nanomaterials (Kisin et al. 2007). Nanotechnology is an area of science devoted to the construction and use of functional structures at the atomic or molecular scale (Moghimi et al. 2005). The prefix "nano" is derived from the Greek word for "dwarf" and represents one-billionth of a unit, meaning extremely small. In recent years, many new terms related to the development and application of nanotechnology have emerged, including nanoscience, nanomaterials, nanoparticles, nanomedicine, and nanotoxicology. Nanoscience can simply be defined as a branch of science devoted to the study of the unique properties of matter that occur at the nanoscale. Nanomaterials refer to structures composed of nanoparticles. Nanoparticles are particles less than 100 nm in one dimension. Nanoparticles generally possess dramatically different physical, chemical, and biological properties compared to

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Address correspondence to Vincent Castranova, Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA. E-mail: vic1@cdc.gov

fine or coarse particles of the same material. The small feature size of nanoparticles ensures that many atoms will be near interfaces. Therefore, surface properties, such as energy levels, electronic structure, and reactivity, are quite different from interior states. Nanomedicine refers to a concept of application of nanotechnology in medicine. According to BIT's (Bitemics) First Annual World International Congress of Nanomedicine 2010 held in Beijing, the nanomedicine market is expected to increase to $4.8 billion by the year 2012. The National Institutes of Health (NIH) classified nanomedicine as a branch of nanotechnology, which refers to highly specific medical interventions at the molecular scale for treating diseases or repairing damaged tissues, such as bone, muscle, or nerve (Oberdorster 2010). The European Science Foundation (ESF) defined nanomedicine as the science and technology of diagnosing, treating, and preventing disease and traumatic injury, relieving pain, and preserving human health using molecular tools and molecular knowledge of the human body (Foundation 2004). Moghimi et al. (2005) defined nanomedicine as the application of nanotechnology for treating, diagnosing, monitoring, and controlling biological systems. Emergence of nanomedical techniques makes it possible to deliver therapeutic agents into targeted specific cells, cellular compartments, tissues, or organs (Liu et al. 2007). Development of nanocapsules and nanodevices might trigger a revolution in drug delivery, gene therapy, and medical diagnostics. Nanotoxicology is defined as a special branch of toxicology that studies the impact of nanomaterials on living systems and develops the means to prevent such effects. Oberdorster et al. (2005) and Oberdorster (2010) characterized nanotoxicology as a concept of safety assessment and development of industrial and medical applications of nanotechnology.

Nanoparticles can be generated by nature and conventional industrial processes as well as nanomanufacturing technologies. Forest fires and volcanic eruptions are examples of natural events that generate nanoparticles. Incidentally, nanoparticles are also generated from the fumes from the industrial processes of welding or metal smelting. Automobile exhaust also contains nanoparticles (Grose et al. 2006). In this review, engineered nanoparticles refer to man-made nanomaterials produced by nanomanufacturing technologies (Ho et al. 2006).

The potential advantages of nanotechnology in medicine are enormous, ranging from novel approaches of designing artificial organs to nano-robotic biosensors, diagnostic devices, bone grafting, and tiny vehicles for drug delivery (Shvedova and Kagan 2010). The major nanocarriers for drug delivery include liposomes, dendrimers, quantum dots, iron oxide, and carbon nanotubes (Ting et al. 2009). Despite the many proposed advantages of nanomaterials, increasing concerns have been expressed on their potential adverse human health and environmental effects (Gulumian and Vallyathan 2010; Maynard et al. 2004; Price et al. 2010; Tabet et al. 2009; LeBlanc et al. 2009; Scuri et al. 2010; Kim et al. 2010c). In recent years, many government agencies in different countries have begun funding toxicological research to address the potential hazards of nanoparticles. The current research in the field of nanomedicine is mainly focusing on applications of nanotechnology. A systematic toxicological evaluation of these nanomaterials is often not considered during the product development process. Of concern is intravenous or subcutaneous injection of nanoparticulate carriers designed for nanomedical use, since they afford a unique exposure route to deliver exogenous nanoparticles into the human body without passing through the normal absorption process. These nanoparticulate carriers themselves may be responsible for toxicity and interaction with biological macromolecules within the human body. Second, insoluble nanoparticulate carriers may penetrate biological membrane barriers and accumulate in human tissues or organs. Therefore, toxicological evaluation on these nanomaterials is important for the development of nanomedicine (Linkov et al. 2008).

This review summarizes the current knowledge on toxicology of nanomaterials used in

nanomedicine. The subtopics include introduction, classification, and medical use of nanomaterials, toxicokinetics of nanomaterials (including absorption, metabolism, distribution, accumulation, and elimination), acute and chronic toxicity, genotoxicity, carcinogenicity, and pulmonary, cardiovascular, reproductive, and developmental toxicity.

CLASSIFICATION AND MEDICAL USE OF NANOMATERIALS

Nanoparticles display features of particle size in the range of 1 to 100 nm (Teli et al. 2010). There are many types of man-made nanoparticles produced by nanomanufacturing technologies, and a variety of others are expected to appear in the near future. The term *nanoparticle* is perhaps too broad a term to be used in physiological studies, as it covers a hodgepodge of particles with distinct physical and chemical properties. The chemical composition, shape, charge, and size vary among the different particle types (Hagens et al. 2007). Therefore, there is currently no unified method for classification of nanoparticles. Nanomaterials may be composed of many different base materials, such as carbon, metals, material of biological origin, polymeric materials, ceramics, or composite materials. Depending on shape, nanoparticles are classified as nanofibers, nanowires, nanorods, nanoscrolls, nanotubes, and spherical structures. According to the chemical composition, nanomaterials may simply be classified as organic and inorganic nanomaterials. In this review, nanoparticles were classified into the following classes (mainly based on the classification scheme of the U.S. Environmental Protection Agency, U.S. EPA).

Carbon-Based Nanomaterials

These nanoparticles are composed mostly of carbon, most commonly taking the form of hollow spheres, ellipsoids, or tubes. Spherical and ellipsoidal carbon nanoparticles are referred to as fullerenes, while cylindrical ones are nanotubes. Carbon-based particles have many potential applications, including improved films and coatings, electronics and energy storage devices, and nanomedicine. Carbon nanotubes hold great promise for use in biomedical fields, including drug delivery, DNA and protein sensors, bioseparators, biocatalysts, and tissue scaffolds (Koyama et al. 2006). Carbon nanotubes, due to their large surface area, unique surface properties, and needle-like shape, have the potential to deliver large amounts of therapeutic agents, including DNA and siRNAs, to the targeted disease sites (Cheung et al. 2010; Foldvari and Bagonluri 2008; Losic and Simovic 2009). Single-walled carbon nanotubes (SWCNT) are the fibrous analogues of spherical fullerene structures with unique structural and electronic properties. In recent years, SWCNT have been widely investigated as imaging agents for the identification and localization of tumors (Liang and Chen 2010). In addition, functionalized SWCNT were employed as carriers to deliver various anticancer drugs, proteins, and nucleic acids specifically to diseased tissues and to maximize the bioavailability of the drugs by improving solubility and decreasing clearance (Liang and Chen 2010). Multiwalled carbon nanotubes (MWCNT) have also won enormous popularity in nanotechnology in recent years. Due to their unusual fibrous, hollow nanostructure and unique physicochemical properties, MWCNT are highly desirable for use within the commercial, environmental, and medical sectors (Patlolla et al. 2010). Fullerenes are comprised of dozens to hundreds of carbon atoms. The most common and most stable fullerene is C_{60}, a spheroidal molecule resembling a soccer ball, consisting of 60 carbon atoms. The unique structure and properties of fullerenes allow potential uses as superconductors, lubricants, industrial catalysts, and drug-delivery systems (Yudoh et al. 2009).

Metal-Based Nanomaterials

Metal-based nanoparticles or nanometals include quantum dots (QD), nanogold, nanosilver, or metal oxides. Nanometals were used

for a long time for catalysis before the prefix "nano" emerged (Dror et al. 2005). Currently, metal-based nanoparticles have many applications in industry and nanomedicine. Anisotropic metal-based nanoparticles were proposed as targeted contrast agents due to their strong surface plasmon resonance. The field of molecular imaging relies on the development of targeted contrast agents that bind to disease specific biomarkers in vivo and result in good image contrast between labeled and unlabeled tissue (Javier et al. 2008). QD are small closely packed semiconductor devices that emit certain wavelengths of light according to their size. This makes them highly suitable as contrast agents for magnetic resonance imaging (MRI) or positron emission tomography (PET) (Bottrill et al. 2006; Michalet et al. 2005). Similarly, gold nanoparticles (GNP) and QD received significant attention in recent years because their unique physical, chemical, and biological properties are quite different from their bulk counterparts. GNP and QD possess the capability of binding strongly to bio-molecules, such as proteins, peptides, antibodies, oligonucleotides, and pathogens. GNP and QD were investigated as biomarkers to detect diseases and deliver suitable drugs to treat diseases (Nagender et al. 2009). Silver nanoparticles represent another prominent nanoproduct used for medical purposes. Silver nanoparticles are generally smaller than 100 nm and contain 20–15,000 silver atoms. Due to potent antibacterial activity, silver nanoparticles are used for treatment of wounds and burns or as a contraceptive, and are marketed as water disinfectants and room sprays (Chen et al. 2009a; Chen and Schluesener 2008; Shen et al. 2009; Zheng et al. 2008). It is estimated that of all the nanomaterials in the medical and health care sector, silver nanoparticles have the highest number of commercial applications (Chen and Schluesener 2008).

Biological Nanomaterials

Biological nanomaterials are materials of biological origin that are used for nanotechnological applications. Biological nanomaterials include proteins, nucleic acids, and carbohydrates. Applications of these materials are mostly related to nanomedical science. The use of DNA as a structural nanoscale material has opened a new avenue toward the rational design of DNA nanostructures with different polymeric topologies (Campolongo et al. 2010). In recent years, nanotechnology applications in medicine have introduced a number of nanoparticles of various chemistries and architectures for cancer imaging and treatment (Wang and Thanou 2010). Biological nanoparticles made of peroxalate ester polymers with a fluorescent dye encapsulated into the polymer were found to be capable of detecting cancer (Sajja et al. 2009). This is due to the fact that hydrogen peroxide is generated in human cells that are pre-cancerous. The dye in the nanoparticles emits a fluorescent signal when it comes in contact with hydrogen peroxide, which may then be detected as light in imaging equipment. Biological nanomaterials normally are biodegradable and biocompatible when used as drug delivery vehicles. After degradation, their hydrolytical by-products are normally considered to be nontoxic or of low toxicity to the human body (Kim et al. 2010b). Even so, a systematic toxicological study is still necessary to address any possible adverse human health effects of these biological nanomaterials.

Nanopolymers

Polymers are molecules with repeated linked units. Nanopolymers refer to nanostructured polymers. Nanopolymers are defined as self-assembled structures, i.e., lamellar, or non-self assembled structures, such as dendrimers and nanofibers. Nanopolymers have applications in energy, materials science, and medicine. Many synthetic and natural polymers were designed as drug carriers in chemotherapy (Piddubnyak et al. 2004; Williams et al. 2008). Such polymers enable fast drug delivery across cancer cell membranes and being biodegradable release cytotoxic drugs once within cancer cells. Poly(lactic-co-glycolic acid) (PLGA), among

other polymers, were demonstrated to be biodegradable, biocompatible, and safe to the human body (Gref et al. 1994). Gref et al. (1994) used polyethylene glycol (PEG)-coated PLGA nanospheres as injectable carriers to test their application for drug delivery and medical imaging. Results showed that PEG-coated PLGA nanospheres exhibited long circulation times in the bloodstream and low liver accumulation in mice. In addition, they were effective in encapsulating considerable amounts of a test delivery drug within their core in a one-step procedure, and subsequently could be freeze-dried without any added chemicals. These advantages make PLGA nanopolymer ideal as a drug delivery, gene targeting, and medical imaging vehicle (Gref et al. 1994). Polymer thin films and coatings are among the most popular and most successful tools to modulate surface properties of biomaterials, specifically tissue responses and folding behavior. In recent years, deposited polymer thin films were investigated to determine whether they act both as a coating to modulate surface properties and as a reservoir for active therapeutic agents (Zelikin 2010).

Nanoceramics

Nanoceramics are composed of oxide and non-oxide ceramic materials, silicates, or hard metals. Nanoceramics possess superior mechanical properties and physical characteristics compared to conventional engineering ceramics (Xiang et al. 2006), so they can be widely used in architecture and industries. Products of nanoceramics used currently in research and industry include titanium, silica, alumina, tungsten carbide, zirconia, zinc oxide, silicon nitride, magnesia, boron nitride, ferric oxide, ceria, and silicon carbide. In recent years, calcium phosphate nanoceramics have gained regard in the biomedical field due to their superior biological and biomechanical properties. Hydroxyapatite (Hap, $Ca_{10}(PO_4)_6(OH)_2$) is essentially the main calcium phosphate bioceramic material used in the clinics at present. Nano-Hap can be used primarily as bioactive coatings on metallic prosthetics of bioinert materials, such as titanium and its alloys, in bone tissue repair and implants, and for drug delivery (Kalita et al. 2007). After implantation into the body, the nanoceramic materials, consisting of nanometer grains, may also produce health risks because of their in vivo degradability. LeGeros et al. (1993) reported factors involved in the in vivo degradability of calcium phosphate nanoceramics. First is the physical factor. Due to erosion by body fluids and mechanical abrasion, calcium phosphate nanoceramics may break up and form grains. Second is the chemical factor. Chemical reactions may produce free particle release. Finally is the biological process in which osteoclasts and macrophages may resorb the calcium phosphate nanoceramics by releasing acid materials. The degraded nanoceramic nanoparticles are likely to enter cells and blood to produce potential biological risks. Therefore, it is necessary to investigate the potential adverse health effects of free particles of nanoceramics before clinic use.

Nanocomposites

Nanocomposites refer to the combination of nanoparticles with other nanoparticles or with larger, bulk-type materials. Nanocomposites can be widely used in packaging, building and construction, electronics, the automotive industry, and medicine. Nanocomposites, such as nano-sized clays, are already being added to products ranging from auto parts to packaging materials to enhance mechanical, thermal, sound-barrier, and flame-retardant properties. An example of the biomedical use of nanocomposites is manufacturing of artificial bone composites from nanocrystalline calcium phosphates (Schwarz et al. 2006; 2009; Thian et al. 2006). Magnetic, hollow silica nanocomposites (MHSNC) are synthesized using a coating of Fe_3O_4 magnetic nanoparticles and silica on nanosized spherical and nano-needle-like calcium carbonate ($CaCO_3$) surfaces under alkaline conditions. Investigations demonstrated that drug-loaded MHSNC have potential applications in

nanomedicine (Zhou et al. 2005). In addition, carbon nanotube-filled polymer composites, namely, microcatheters, were also investigated for application as medical devices (Koyama et al. 2006).

In summary, nanomaterials are widely used in medical practice. The main applications of nanomaterials in medicine include drug discovery, molecular diagnostics, molecular imaging, implants, active implants, tissue engineering, surgery, genomics, and proteomics. Nanomedicine involves analytical tools, nanoimaging, nanodevices, novel therapeutics, and drug delivery systems. However, the growth of nanomedicine raises clinical, regulatory, and toxicological issues (Oberdorster 2010). Nanomaterials used in medical practice are also classified into several categories, which include biological mimetics, biomaterials, nanoscale devices, sensors, and laboratory diagnostics (Moghimi et al. 2005). Currently, nanoparticles that are frequently used in nanomedicine include titanium dioxide (TiO_2), nanogold, nanosilver, nanotubes, fullerenes, QD, dendrimers, nanoshells, nanocrystals, calcium phosphate vaccines, nanosized electrodes, and metal colloidal particles.

TOXICOKINETICS OF NANOMATERIALS

Toxicokinetics of a chemical include the following steps: absorption, metabolism, distribution, accumulation, and elimination. Nanoparticles possess different physical, chemical, and biological properties compared to fine or coarse particles of the same material. Understanding absorption, metabolism, tissue or organ distribution, accumulation, and elimination of nanoparticles following different routes of exposure is important for health risk assessment.

Absorption

Absorption is the first step in toxicokinetics. Normally, exposure to nanoparticles may occur through inhalation, ingestion, or dermal contact under the conditions of occupational and environmental exposure. The relative amounts of nanoparticles absorbed by the human body are determined not only by the quantities inhaled, or ingested but also by their physical and chemical characteristics. Inhalation is likely the primary route for nanoparticles to enter into the human body in occupational settings. However, in the field of nanomedicine, intravenous and subcutaneous injections of nanoparticulate carriers may represent a unique and more important exposure route than inhalation.

Particulates are determined as solid or liquid matter with aerodynamic diameters ranging from 0.005 to 100 μm. Dusts, smoke, fumes, and organisms, such as bacteria, viruses, pollen grains, and fungal spores, are solid particulate matter, whereas mists and fog can be classified as liquid particulate matter. Particles with aerodynamic diameter above 10 μm are effectively filtered by the human nose and do not reach the remainder of the respiratory tract. Particles with aerodynamic diameter less than 10 μm (called thoracic particles) can be inhaled into the human lung and deposited in the conducting airways or the respiratory zone of the lung, depending on the aerodynamic diameter. For thoracic particles, the smaller the particles, the deeper they can travel into the lung; i.e., particles about 2.5 μm in aerodynamic diameter exhibit a peak deposition in the alveoli, while particles 2.5–10 μm increasingly deposit in the conducting airways. Particles with aerodynamic diameter less than 100 nm are classified as ultrafine particles or nanoparticles and have a high deposition in the alveolar region due to diffusional forces. Indeed, due to the low density of the nanoparticles, Brownian diffusion, the random movement of particles suspended in a fluid or a gas such as air, determines deposition and results in a deep penetration of nanoparticles in the lungs (Hagens et al. 2007). Interestingly, for nanoparticles less than 10 nm, alveolar deposition decreases due to the high deposition in the nasal cavity, before transportation into the deep lung (Hagens et al. 2007). It should be borne in mind that the site of deposition in the respiratory tract determines the nature of the adverse health effects.

In this respect, nanoparticles may not always be more toxic than micrometer particles in the conducting zone (Karlsson et al. 2009). Therefore, toxicology of nanoparticles needs to be evaluated by comparison with fine (or bulk) particles of the same composition. A current issue in the field is the relevance of animal experimental information obtained after intratracheal instillation or aspiration of a nanoparticle suspension (bolus dose) to low-dose-rate exposures that occur in environmental and occupational settings, where exposures occur by chronic inhalation of airborne nanoparticles. There are distinct differences in distribution, clearance, and retention of materials when administered by intratracheal instillation or pharyngeal aspiration compared to chronic and low-dose inhalation. However, bolus exposure is less costly, does not require specialized exposure facilities, and requires smaller quantities of test material. Intratracheal instillation or aspiration can be used as an appropriate substitute for inhalation exposure for hazard identification and evaluation of possible mechanisms of action (Driscoll et al. 2000). Indeed, Shvedova et al. (2008a) reported qualitatively similar pulmonary responses to SWCNT when mice were exposed by pharyngeal aspiration or a 4-d inhalation. Wolfarth et al. (2011) demonstrated that equivalent lung burdens of MWCNT resulted in quantitatively similar levels of lung inflammation and damage 1 d after a bolus aspiration or a 4-d inhalation exposure. Therefore, it appears that bolus exposure is useful for nanotoxicology screening. Fibers are defined as solid particulates with a length >5 μm and an aspect ratio (length:width) of at least 3:1. Their penetration into the lung also depends on their aerodynamic properties, which are dominated by the fiber diameter. Similar to the particles already mentioned, fibers with a small diameter penetrate deeper into the lungs. However, for a given diameter, long fibers exhibit a higher aerodynamic diameter than short fibers (Hoet et al. 2004).

The respiratory system represents the main port of entrance for airborne nanoparticles. Soluble nanoparticles captured or deposited in the lung may dissolve and rapidly enter the systemic circulation. In contrast, insoluble nanoparticles may be phagocytized by alveolar macrophages, migrate to the interstitial space of the alveolar septa, or enter pulmonary lymphatics. In pharmacokinetics, absorption represents the process by which the toxicant proceeds from the site of administration to the systemic blood circulation (Hagens et al. 2007). The chemical form, solubility, particle size, particle charge, and its deposition site in the lungs will affect the extent of absorption. Studies demonstrated that nanoparticles, such as carbon black (14 or 56 nm) (Inoue et al. 2006) and Tc-labeled nanocolloid (less than 80 nm) (Nemmar et al. 2003), produce lung inflammation and tissue damage. Furthermore, nanoparticles enter the lung interstitial tissue and further translocate from the lung into the circulation (Nemmar et al. 2002a; 2002b; 2003), raising the possibility that nanoparticles may not only facilitate lung damage but also induce disorders in systemic organs. Zhu et al. (2009) reported that intratracheal instilled $^{59}Fe_2O_3$ nanoparticles pass through the alveolar–capillary barrier into systemic circulation within 10 min, which is consistent with a one-compartment kinetic model. These nanoparticles that migrated from the lung into the blood were further distributed in different tissues or organs. In contrast, other studies noted that although translocation of nanoparticles from the lung to the blood is possible, it occurs at a relatively slow rate (Kreyling et al. 2002; Nemmar et al. 2003). Several studies demonstrated that the ultrafine particles in polluted air, when inhaled, not only appear to increase the frequency of respiratory diseases as a local effect, but also exert systemic effects resulting in a significant rise in risks in morbidity and mortality associated with cardiovascular diseases (Dominic et al. 2005; Goldberg et al. 2001; LeBlanc et al. 2009; Pope et al. 2002; Samet et al. 200; Vermylen et al. 2005). However, it is not clear whether these effects are a result of nanoparticles themselves or a combination event induced by both nanoparticles and chemical compounds contained in the polluted air. Unfortunately, epidemiological studies of the adverse health effects induced by nanoparticles alone are currently

still lacking. Recently, various drugs were investigated for local or systemic pulmonary delivery (Patton and Byron 2007). These include small molecules, protein/peptide drugs, and genes. Lung exposure of nanoparticles occurs during pulmonary delivery of these nano-enabled drugs as medicinal aerosols through inhalation (Oberdorster et al. 2005). The fate of inhaled nanoparticles includes deposition, retention in the respiratory tract, clearance of nanoparticles out of the respiratory tract through macrophage phagocytosis, and translocation of nanoparticles into blood circulation. If nanoparticles are translocated into the blood circulation, they will be further metabolized in the liver, distributed to secondary targeted organs and tissues, or eliminated out of the body.

The gastrointestinal tract (GIT) may also be an important route for nanoparticle absorption since drug carriers, food products, water, and liquid beverages may contain nanoparticles (Hagens et al. 2007; Lomer et al. 2002). In the field of nanomedicine, the GIT uptake of micro- and nanoparticles has been the subject of recent efforts to develop effective carriers that enhance the oral uptake of drugs and vaccines (Hillyer and Albrecht 2001). Furthermore, inhaled airborne nanoparticles may be cleared via the mucociliary escalator. Such nanoparticles are then swallowed and subsequently ingested into the GIT. Ingested particles enter the body by a process called persorption, the paracellular uptake of nanoparticles from the digestive tract into the lymphatic and blood circulation (Hagens et al. 2007). It is generally agreed that GIT absorption increases with decreasing particle diameter. Studies on polystyrene latex in the range of 50 nm to 3 μm (Jani et al. 1990) revealed that maximal absorption occurred with particles ranging from 50 to 100 nm in diameter, with particles above 1 μm being trapped in the Peyer's patches. These trapped particles did not translocate to the systemic circulation. Polystyrene nanoparticles of 50 and 100 nm were found to be absorbed from the GIT at 34 and 26%, respectively (Jani et al. 1990). Desai et al. (1996) found that the efficiency of absorption of 100-nm polystyrene particles was 250-fold higher compared to larger sized (500-nm) polystyrene particles. Studies in rats demonstrated that nano- and microsized polystyrene microsphere particles (50 nm–20 μm) are absorbed mainly through the Peyer's patches of the small intestine, although microgram-size particles are absorbed at a much lower rate than nanoparticles (Hagens et al. 2007). Hillyer and Albrecht (2001) evaluated the GIT uptake and subsequent tissue/organ distribution of 4-, 10-, 28-, and 58-nm metallic colloidal gold particles following oral administration to mice, and found that colloidal gold uptake is dependent on particle size: Smaller particles cross the GIT more readily. Absorption via intestinal enterocytes was demonstrated also by using polystyrene latex nanoparticles in rats (Jani et al. 1990). In addition, nanoparticle charge also plays an important role in absorption from the GIT. Positively charged nanoparticles are reported to be absorbed more effectively through the GIT than neutral or negative charged particles (Florence 1997; Hussain et al. 2001; Janes et al. 2001).

Dermal absorption of nanoparticles is quantitatively minor compared with absorption from inhalation and ingestion under the conditions of environmental and occupational exposures. Cosmetics containing nanoparticles are directly applied on the skin. Metal oxide nanoparticles, such as zinc oxide nanoparticles, are commonly used in personal-care formulations as protective agents against exposure to ultraviolet radiation. Sadrieh et al. (2010) reported virtually no penetration of titanium through intact skin in a mini pig model. In contrast, Gulson et al. (2010) found that small amounts of zinc (Zn) from zinc oxide (ZnO) nanoparticles in sunscreens applied outdoors are absorbed through human skin. However, it is not known whether Zn had been absorbed as ZnO nanoparticles, soluble Zn, or both. However, dermal exposure might represent an important nanoparticle absorption route in the field of nanomedicine (Hagens et al. 2007). A medical exposure source is textiles or wound dressings, which can contain silver nanoparticles as an antibacterial agent (Lee et al. 2003). Nanoparticles may also be used

as transdermal drug delivery devices. Thus, understanding potential epidermal and dermal penetration, as well as possible toxicity of different nanoparticles, will be important in the development of nanomedicine (Choksi et al. 2010). As the skin is easily accessible, transdermal absorption is well studied in recent drug-delivery research projects (Partidos 2003; Prausnitz et al. 2004). These targeted studies involve delivery of nanoparticles to the dermis by penetration of the epidermis. In recent years, TiO_2 nanoparticles have been used increasingly in many products, such as toothpastes, sunscreens, cosmetics, food products, pharmaceuticals, and nanomedical reagents (Kaida et al. 2004; Paunesku et al. 2008; Wang et al. 2007; Wolf et al. 2003). Such widespread use suggests that TiO_2 nanoparticles pose a potential exposure risk to humans (Long et al. 2007). Fine TiO_2 particles were used traditionally for many years as a "negative control" in many in vitro and in vivo toxicological investigations of dust. However, this view was challenged when lung tumors were found in rats after lifetime exposure to high concentrations of pigment-grade TiO_2 (Lee et al. 1985). Evidence showed that TiO_2 nanoparticles exhibited greater cytotoxicity than fine particles (Zhao et al. 2009a). Churg et al. (1998) reported that TiO_2 nanoparticles enter the epithelium faster and are translocated in greater proportion to the subepithelial space compared with fine particles in a tracheal explant system, while other studies reported that TiO_2 nanoparticles were unable to penetrate the epidermis (Gamer et al. 2006; Sadrieh et al. 2010; Schulz et al. 2002). This is the reason why there is currently no consensus about the ability of nanoparticles to penetrate through the skin. Particles in the micrometer range are generally thought to be unable to penetrate through the skin. The outer skin consists of a tough layer of dead keratinized cells (stratum corneum) that is difficult for particles, ionic compounds, and water-soluble compounds to penetrate. However, dermal absorption of nanoparticles may not be reliably predicted from the properties of the material in bulk form because of their extremely small size (Choksi et al. 2010). In addition, Hagens et al. (2007) suggested that factors such as the movement of skin, the charge of the particle, follicular openings, gender, and age should be considered in all skin penetration studies. Tinkle et al. (2003) reported that penetration of fluorescent beads (500–1000 nm) through the epidermis occurred only after application of a flexing motion. Rouse et al. (2007) showed penetration of fullerene C_{60} amino acid derivatized peptide nanoparticles through the skin after mechanical flexing. These data suggest that migration of nanoparticles through the skin is possible, especially when mechanical flexion is applied. In addition, evidence demonstrated that the charge of nanoparticles is one of the important factors in the transdermal absorption process (Kohli and Alpar 2004). For example, negatively charged latex particles (50 and 500 nm) were found to penetrate the epidermis, but not positively charged or neutral particles (Kohli and Alpar 2004). Another study indicated that QD (spherical 4.6 nm and ellipsoid 12 nm by 6 nm) exhibited penetration through the intact skin (Ryman-Rasmussen et al. 2006). This suggests that the skin is permeable to nanomaterials with distinct physicochemical properties (size, shape, charge, material) (Hagens et al. 2007). In conclusion, nanoparticle absorption through the skin is possible, especially when mechanical flexion is applied to the skin. The absorption of nanoparticles through the dermis suggests that the systemic circulation may be reached. However, quantitative data confirming this absorption process are currently missing (Hagens et al. 2007).

Intravenous and subcutaneous injections of nanoparticulate carriers are unique exposure routes when compared with environmental and occupational exposures (Allen 2002; Allen and Cullis 2004; Fenske et al. 2001; Panyam and Labhasetwar 2003; Sahoo and Labhasetwar 2003; Sudimack and Lee 2000). Nanoparticles may also be injected directly into the body as contrast agents for imaging purposes. In this case, exogenous nanoparticles are delivered into the human body, avoiding normal absorption processes.

Metabolism, Distribution, Accumulation, and Elimination

Currently, there is no evidence that nanoparticles are destroyed in the body after being absorbed into the blood circulation from different exposure routes, but their chemical forms may be altered. Inert nanoparticles, such as gold and silver particles, fullerenes, and carbon nanotubes, are supposedly unable to be metabolized effectively by enzymes in the human body (Hagens et al. 2007). However, it is hypothesized that nanoparticles with functionalized groups may be metabolized. For instance, the protein cap of a functionalized QD can be cut by proteases (Hardman 2006). In addition, the metallic core of QD (and other metal oxides) may be bound by metallothionein and excreted. Enzymes such as metalloproteinases, present in liver and kidneys, bind metal and restore cellular metal homeostasis (Coyle et al. 2002). Evidence also shows that nanoparticle drug-delivery systems consisting of liposomes are able to fuse with cell membranes (Pitard et al. 2004). Recently, Kagan et al. (2010) reported that hypochlorite and reactive radical intermediates of the human neutrophilic enzyme myeloperoxidase catalyze the biodegradation of SWCNT in vitro in neutrophils and to a lesser degree in macrophages. Data suggest that the extent to which carbon nanotubes are biodegraded may be a major determinant of the scale and severity of the associated inflammatory responses in exposed individuals. Therefore, further in vivo studies are necessary to determine the mechanism of metabolism of SWCNT.

Translocation is the way chemicals or particles move from the site of absorption to other parts of the body. After nanoparticles reach the systemic circulation, potentially these particles interact with plasma proteins, coagulation factors, platelets, and red or white blood cells. The binding to plasma components may have a substantial effect on the metabolism, distribution, and excretion of nanoparticles (Hagens et al. 2007). A specific serum component, apolipoprotein-A1 (apo-A1), was shown to bind to silica particles and may be capable of binding to other particles such as asbestos or TiO_2 (Barrett et al. 1999; Hagens et al. 2007). Binding to plasma components might neutralize or inhibit the adverse effects of nanoparticles in the systemic circulation (Hagens et al. 2007). Evidence shows that particles whose diameter is smaller than 200 nm gain access through the blood–brain barrier (Tsuji et al. 2006). In addition, several studies indicate that nanoparticles, such as gold and TiO_2, are able to penetrate human red blood cells (Rothen-Rutishauser et al. 2006). This cellular uptake of nanoparticles might not involve endocytosis (Geiser et al. 2005), since erythrocytes do not have phagocytotic receptors (Rothen-Rutishauser et al. 2006). This implies that nanoparticles might be able to cross the cell membrane by processes other than phagocytosis and endocytosis. Diffusion, transmembrane channels, adhesive interactions, or other, undefined, transmembrane processes may play an important role in this cellular uptake of nanoparticles (Hagens et al. 2007).

Nemmar et al. (2002a) measured the distribution of radioactivity after the inhalation of an aerosol consisting of Tc-labeled carbon nanoparticles (<100 nm) in 5 healthy humans. Studies showed that radioactivity was detected in blood at 1 min, and reached a maximum between 10 and 20 min. In addition, substantial radioactivity was found in the liver and other areas of the body. Several other studies also showed distribution of nanoparticles to different tissues or organs, including liver, spleen, heart, and brain, suggesting distribution of nanoparticles via blood circulation (Oberdorster et al. 2002; 2004). Ogawara et al. (1999) studied the in vivo uptake by hepatocytes and biliary excretion of fluorescein isothiocyanate-labeled polystyrene microsphere (MS) with a particle size of 50 nm (MS-50) after intravenous injection in rats. They found that MS-50 was partially phagocytosed by the hepatocytes, and that MS-50 taken up by the hepatocytes existed exclusively inside the cells 1 h after injection. After intravenous injection of MS-50, about 4% of the dose was excreted into the bile after 24 h. Qualitative

evaluation of the fluorescence detected in the bile revealed that the MS-50 particles were excreted into bile in an intact form. Zinc oxide nanoparticles (ZnONP) may be used for many applications, including cosmetics, cell labeling, and antimicrobial, antibacterial, antibiotic, and antifungal agents (Applerot et al. 2010; John et al. 2010; Kim et al. 2010a; Sharma et al. 2009; Tang et al. 2009; Wahab et al. 2010). Chen et al. (2010) demonstrated that after intravenous administration into mice, radioactive labeled ZnONP exhibited a primary retention in lung (43.6% injected dose/g tissue wet weight) for the first hour and began to be translocated to the intestinal tract for feces excretion at a later stage. Therapeutically used polymeric nanoparticles are composed of biodegradable or biocompatible materials, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), PLGA, alginic acid, gelatin, and chitosan (Mansour et al. 2009). PLGA nanoparticles currently are used as drug delivery systems. In an in vivo study with mice, the extent of tissue distribution and retention following oral administration of PLGA particles was analyzed for 7 d (Semete et al. 2010). The results showed that a mean percentage (40%) of the particles was localized in the liver, 26% in kidneys, and 13% in brain. The lowest percentage was observed in the spleen. After 7 d, the particles remained detectable in the brain, heart, kidneys, liver, lungs, and spleen. Lipid nanoparticles (LN) were found to be an efficient and versatile colloidal drug carrier system exhibiting controlled release behavior and enabling drug protection. Videira et al. (2006) reported that 1 h after endotracheal delivery of LN to the lung, LN began to translocate to stomach, liver, axillary lymph nodes, and inguinal lymph nodes. Para-aortic lymph nodes were the site with the highest accumulation. Lymphatic drainage appeared to be the main route for translocation of endotracheally delivered LN (Videira et al. 2006). Germanium nanoparticles (GN), ranging in size from 60 to 80 nm, were developed as a potential spleen imaging agent. Similar to other metal-based nanoparticles used in nanomedicine, GN may release trace amounts of germanium ions when injected. Metabolic fate and toxicity of GN and other released ions still needs to be evaluated. Sabbioni et al. (2010) reported that at 24 h after intraperitoneal injection of GN (80 mg/kg body weight) in rats, GN was almost equally distributed between plasma and red blood cells in the blood. Further distribution analysis demonstrated that the heart was the organ with the highest GN concentration. GN was poorly retained in kidneys, liver, intestine, femur, and spleen. The excretion of GN was mainly via the urine. Zhu et al. (2009) reported that the $^{59}Fe_2O_3$ nanoparticles intratracheally instilled in the lung in rats were distributed to organs rich in mononuclear phagocytes, including liver, spleen, kidneys, and testes. The plasma elimination half-life of $^{59}Fe_2O_3$ nanoparticles was 22.8 d, and the lung clearance rate was 3.06 mg/d, indicating that systemic accumulation and lung retention occurred. To investigate tissue distribution and clearance of nanomaterials following different routes of exposure, Sarlo et al. (2009) exposed rats to 20-nm, 100-nm, or 1000-nm latex fluorosphere particles by intravenous injection or pharyngeal aspiration. The presence of fluorospheres was detected in tissue up to 90–120 d after the final dose. A small portion of 20-nm particles was detected in kidneys following both acute and repeated pulmonary exposure. The thymus was the largest extrapulmonary depot for the particles; up to 25% of recovered 20-nm particles were in the thymus up to 4 mo after exposure, compared to 6% of 100-nm particles and 1–3% of 1000-nm particles. All three sizes of particles were found in gut and feces 1–7 d after lung exposure. In addition, low numbers of particles were found in the circulation (blood), bone marrow, brain, heart, liver, and spleen, but not in eye, muscle, skin, tongue, ovaries, uterus, or urine. Liu et al. (2009b) studied the potential health impact on mice after nasal instillation of copper nanoparticles and their translocation in mice. They reported that the liver, kidneys, and olfactory bulb are the main site of accumulation for copper nanoparticles. To identify the tissue distribution and excretion of silica nanoparticles in vivo, Cho et al. (2009a) tested fluorescent dye-labeled 50-, 100-, or

200-nm-sized silica particle suspensions after a single intravenous injection in mice, and found that the silica particles of size 50, 100, and 200 nm were cleared via urine and bile (elimination through feces). In addition, silica nanoparticles may be trapped by macrophages in the spleen and liver and remained there as long as 4 wk after a single injection. Kim et al. (2009) studied the tissue distribution of silver nanoparticles via inhalation or oral ingestion and demonstrated a dose-dependent accumulation of silver nanoparticles in all the tissues examined, including testes, kidneys, liver, brain, lungs, and blood.

In summary, metabolism, tissue distribution, accumulation, and elimination of nanoparticles may be affected by various factors including routes of exposures, the chemical form, particle size, and particle charge. Currently, the most frequently investigated exposure routes in the toxicokinetics studies of nanoparticles include pulmonary (inhalation, intratracheal instillation, aspiration) and intravenous and subcutaneous injections, as well as dermal and oral administration. Distribution of nanoparticles was found in different tissues or organs, such as lung, blood, bone marrow, kidneys, liver, intestine, femur, thymus, gut, heart, spleen, and brain. Elimination of nanoparticles may be through urine, bile and feces, which depends on the unique characteristics of different nanoparticles. As urine is one of the major routes for elimination of nanoparticles, particle concentration in urine may be a useful indicator for evaluation of nanoparticle exposure and metabolism. Interestingly, several studies demonstrated the distribution of nanoparticles in brain following different routes of exposure (Brooking et al. 2001; Hillyer and Albrecht 2001; Lasagna-Reeves et al. 2010; Liu et al. 2009b; Wang et al. 2008), which indicates nanoparticles penetrate the blood–brain barrier. This may be beneficial for the development of nano-sized drug-delivery systems for the brain, despite concerns over potential adverse health risks of nanoparticles to the human central nervous system. Evidence shows nanoparticles, such as silica nanoparticles, remain in the human body for more than 4 wk after a single injection (Cho et al. 2009a). Intravenously injected QD nanoparticles were detectable after 133 d in the lymph nodes and bone marrow of mice, which implies that the half-life of QD nanoparticles might be long (Ballou et al. 2004; Hardman 2006). Though the majority of the absorbed amount of nanoparticles could be excreted rapidly, it is possible that not all nanoparticles will be eliminated from the body. As a result, accumulation of nanoparticles may take place in the human body (Hagens et al. 2007).

Acute and Chronic Toxicity

Nanomaterials or nanoparticles cover a wide range of materials with distinct physical and chemical properties. The chemistry, solubility, degree of agglomeration, size, shape, and charge vary among the different particle types. All these aspects may influence the toxic properties of the particles. Furthermore, nanoparticles are assumed to have a toxicity profile different from that of larger particles of the same chemical composition, due to their extremely small size and high specific surface area (Donaldson et al. 2001; Oberdorster et al. 2005). However, acute toxicity and chronic toxicity of nanoparticles, especially those used in nanomedicine, such as drug delivery carriers, have not yet been studied systematically.

In terms of human health, the acute adverse effects of nickel (Ni) nanoparticles were recently reported after an occupational inhalation exposure. Phillips et al. (2010) reported that a 38-year-old, previously healthy male died of adult respiratory distress syndrome (ARDS) 13 d after inhalation of Ni nanoparticles while spraying Ni onto bushes for turbine bearings using a metal arc process. Ni particles less than 25 nm in diameter were discovered in the victim's lung macrophages. In addition, high levels of Ni were detected in his urine and kidneys, which showed evidence of acute tubular necrosis. As a well-known toxicant, Ni nanoparticles are mainly encountered under occupational settings, but not in nanomedical practice.

Silica (SiO_2) provides an excellent host material for the integration of a wide variety of nanomaterials into multifunctional SiO_2 nanoparticle systems for biomedical applications. SiO_2 engineered nanoparticles are used for drug delivery and as food additives under current regulations (Shi et al. 2010). Mesoporous SiO_2 is a synthetic form of SiO_2 used in nanotechnology. The most common types of mesoporous nanoparticles include Mobile Crystalline Material (MCM-41) and Santa Barbara Amorphous type material (SBA-15) (Katiyar et al. 2006). The large surface area of mesoporous SiO_2 nanoparticles is enhanced by their surface pores. This allows the particles to be filled with a drug. SiO_2 occurs commonly in nature as sandstone, silica sand or quartzite. There are three crystalline forms of SiO_2, including quartz, tridymite, and cristobalite. SiO_2 also exists in an amorphous form (vitreous silica). Crystalline SiO_2 may induce carcinogenesis, as well as produce inflammation, irritation, fibrosis, and other adverse health effects (Castranova et al. 1996; 2002; Castranova and Vallyathan 2000; Driscoll and Guthrie 1997; Shi et al. 1998). Interestingly, mesoporous SiO_2 nanoparticles, a synthetic form of crystalline SiO_2, are currently considered to exhibit low toxicity and are used as carriers for targeted drug delivery to the heart (Galagudza et al. 2010). The concept of targeted drug delivery implies selective accumulation of a drug within a given tissue after systemic administration of the carrier-bound drug with the minimal possible side effects on irrelevant organs and tissues (Galagudza et al. 2010; Ye and Yang 2009). Galagudza et al. (2010) studied the acute hemodynamic effects of SiO_2 nanoparticles in rats and found that intravenous infusion of SiO_2 nanoparticulate carriers (0.07 mg/kg body weight) was associated with mild changes in hemodynamic parameters after 10 min of infusion. However, Galagudza et al. (2010) failed to detect any other acute or chronic toxicity effects in rats. In another study, Nishimori et al. (2009) investigated the relationship between particle size and toxicity using SiO_2 nanoparticles with diameters of 70, 300, and 1000 nm (SP70, SP300, and SP1000) as model materials by intravenous administration in rats. Data showed that SP70 induced liver injury at 30 mg/kg body weight, while SP300 or SP1000 exerted no effect even at 100 mg/kg body weight. Furthermore, administration of SP70 dose-dependently increased serum markers of liver injury, serum aminotransferase and inflammatory cytokines. In addition, repeated administration of SP70 twice a week for 4 wk, even at 10 mg/kg body weight, produced hepatic fibrosis. Based on these results, data suggest that SiO_2 nanoparticles might be hepatotoxic.

In recent years, gold nanoparticles (GNP) received significant attention in biomedicine because their unique physical, chemical and biological properties are quite different from their fine-sized analog (Nagender et al. 2009). However, the use of GNP could be limited because of their toxicity. Studies in vitro demonstrated that GNP induced cytotoxicity in different cells, including apoptosis or necrosis (Goodman et al. 2004; Nagender et al. 2009). Goodman et al. (2004) studied cationic and anionic functionalized GNP toxicity. Results demonstrated that cationic functionalized GNP exerted moderate toxicity when compared with anionic functionalized GNP. Cho et al. (2009b) evaluated the in vivo toxic effects of 13-nm size, PEG-coated GNP on mice and found that the PEG-coated GNP induced acute inflammation and apoptosis in the liver. The PEG-coated GNP accumulated in the liver and spleen for up to 7 d after injection and had long blood circulation times. In addition, transmission electron microscopy examinations revealed numerous cytoplasmic vesicles and lysosomes within liver Kupffer cells and splenic macrophages containing PEG-coated GNP. Chen et al. (2009b) also investigated the toxicity of GNP on mice. Naked GNP ranging from 3 to 100 nm were injected intraperitoneally into mice at a dose of 8 mg/kg body weight. GNP of size 3, 5, 50, or 100 nm did not produce any apparent adverse effects; however, GNP ranging from 8 to 37 nm induced severe distress in mice. In addition, mice injected with GNP in this range showed fatigue, change of fur color, loss of appetite, and

weight loss. Lasagna-Reeves et al. (2010) evaluated the bioaccumulation and adverse effects of different doses (40 200, or 400 μg/kg body weight/d) of 12.5-nm GNP upon intraperitoneal administration in mice every day for 8 d. In this study, tissue accumulation of GNP depended on the doses administered, but no evidence of toxicity was observed in any of the diverse endpoints evaluated, including survival, behavior, animal weight, organ morphology, blood biochemistry, and tissue histology. Therefore, Lasagna-Reeves et al. (2010) suggested that the accumulation of the GNP in mice may not produce subacute physiological damage. In another subacute toxicity study (Pokharkar et al. 2009), GNP were administered orally to rats for a period of 28 d. All animals survived the duration of the study with no significant changes in clinical signs, body weight, food consumption, hematological parameters, organ weights, or histopathological findings. This study suggested that GNP produced no marked treatment-related toxicity in rats following oral administration. The LD50 value of GNP in rats was greater than 2000 mg/kg body weight.

In summary, data are inconsistent with respect to adverse and cytotoxic effects of GNP and indicate that further investigations are required to resolve this issue. Therefore, further systematic studies including in vitro and in vivo experiments are needed for elucidating GNP toxicity (Nagender et al. 2009).

Silver nanoparticles, the new generation of antimicrobial agents, are becoming one of the progressively growing products in nanomedicine. Shavandi et al. (2010) assessed the effect of commercial colloidal nanosilver on murine peritoneal macrophages by the MTT assay in vitro. A significant decrease in cell viability was observed at concentrations of 1 to 25 ppm of nanosilver compared to the control group after 24 h of cell culture. This acute dose-dependent cytotoxicity of silver nanoparticles on peritoneal macrophages urges caution about their use. Shavandi et al. (2010), therefore, recommended carrying out an in vivo investigation to confirm these in vitro results. Initial results of the pulmonary effects of silver nanoparticles in rats exposed by intratracheal instillation indicate inflammatory responses occur (Roberts et al. 2011).

In recent years, fullerenes, a model of carbon-based nanoparticles, have attracted considerable interest due to their unique properties. The potential and the growing use of fullerenes in nanomedicine have raised questions about their health safety. Various groups reported that nanoparticles of pristine fullerene C_{60} have no acute/subacute toxicity or genotoxicity in mice, rats, or guinea pigs (Gharbi et al. 2005; Kolosnjaj et al. 2007; Levi et al. 2006; Mori et al. 2006). However, some fullerene C_{60} derivatives, either covalently or noncovalently modified fullerenes, are highly toxic (Kolosnjaj et al. 2007). The conflicting data on cytotoxic effects of fullerene C_{60} merit attention and require a resolution if these materials are to become useful drug delivery systems (Levi et al. 2006). Chen et al. (1998) investigated the acute and subacute toxicity of polyalkylsulfonated fullerene C_{60}, a highly water-soluble caged fullerene derivative, in rats. No rats died after oral administration, and thus polyalkylsulfonated fullerene C_{60} was considered to be nontoxic if administered orally. In contrast, rats died within 30 h after intraperitoneal injection. The LD50 of polyalkylsulfonated fullerene C_{60} in rats was determined to be approximately 600 mg/kg body weight. In addition, results demonstrated that rats injected with the compound intraperitoneally or intravenously exhibited distinct lysosome-overload nephrosis and phagolysosomal nephropathy, characterized by a tinctorial difference between the outer cortex and the inner cortex and the medulla.

Carbon nanotubes are new members of carbon allotropes similar to fullerenes and graphene (Shvedova et al. 2003). Carbon nanotubes (CNT) including SWCNT and MWCNT recently emerged as delivery vehicles for use in cancer treatment and gene therapy (Firme and Bandaru 2010). These carriers are generally introduced intravenously; however, little is known of their interactions with human cells and tissues (Albini et al. 2010). The potential toxicity of SWCNT and MWCNT in

humans is also not known. Research evidence demonstrates that both SWCNT and MWCNT induce cytotoxicity, oxidative stress, apoptosis, and necrosis in vitro (Casey et al. 2008; De Nicola et al. 2009; Hu et al. 2010; Kim et al. 2010c; Patlolla et al. 2010; Ravichandran et al. 2009). Han et al. (2010) studied the pulmonary response of mice to MWCNT and found that a single treatment of MWCNT is capable of inducing a cytotoxic and inflammatory response in the lungs of mice. Aspiration of MWCNT results in transient inflammation and lung injury, as evidenced by both a rapid and persistent granulomatous response and rapid and progressive interstitial fibrosis in a mouse model (Ma-Hock et al. 2009; Pauluhn 2010; Porter et al. 2010; Mercer et al. 2011). Similarly, aspiration or inhalation of SWCNT in mice induced transient inflammation, granulomas at deposition sites of agglomerates, and progressive interstitial fibrosis due to the rapid migration of smaller SWCNT structures into the alveolar septa (Lam et al. 2004; Mercer et al. 2008; Shvedova et al. 2005 2008a; Warheit et al. 2004). Shvedova et al. (2009) reviewed the literature concerning pulmonary responses to SWCNT or MWCNT exposure. A number of studies in rat or mouse models exposed by inhalation, intratracheal instillation, or pharyngeal aspiration indicate that pulmonary exposure to CNT results in granulomatous inflammation and persistent interstitial fibrosis. Wang et al. (2010) proposed that CNT-induced fibrosis was due to direct stimulation of fibroblast proliferation and collagen production, rather than persistent inflammation as is the case with crystalline SiO_2 or asbestos. Nygaard et al. (2009) investigated the effects of SWCNT and MWCNT on allergic immune responses in a small sample of mice and demonstrated that both SWCNT and MWCNT promote allergic responses in mice. In addition, Shvedova et al. (2008b) reported that aspiration of SWCNT prior to pulmonary exposure to bacteria significantly increased the susceptibility of mice to pulmonary infection. Schipper et al. (2008) studied the toxicology of SWCNT when injected into the bloodstream of mice. Survival as well as clinical and laboratory parameters revealed no evidence of toxicity over 4 mo, but data suggested further confirmation with larger groups of animals was necessary. Therefore, the safety of CNT in drug delivery has been questioned.

Taken together, research on the acute and chronic toxicity of nanoparticles used in nanomedicine has begun, but data are still fragmentary. The literature has demonstrated that some of the nanomaterials used in nanomedicine may possess adverse health potentials on the human body. To translate nanotechnology into nanomedicine, it is important to understand any potential toxicity produced by nanomaterials and to design approaches to mitigate any detrimental effects (Liu et al. 2009a). Therefore, further well-designed and systematic studies including in vitro and in vivo experiments are necessary to clarify the acute and chronic toxicities of nanomaterials used in nanomedicine.

Genotoxicity

Genotoxic substances are known to be potentially mutagenic or carcinogenic, specifically those capable of inducing genetic damage or mutation and contributing to the development of tumors. In recent years, studies examined the genotoxicity of various nanoparticles. The results are summarized in Table 1. As listed in Table 1, toxicological test systems used in the in vivo studies of genotoxicity of nanoparticles include rat and mouse tissues, bone marrow and bronchoalveolar lavage (BAL) cells, and *Drosophilia melanogaster*. Toxicological test systems or endpoints used in studies in vitro include the Comet assay, micronucleus (MN) test, Ames test, mammalian (including human, rat, and mouse) cell gene mutation, sister chromatid exchange (SCE), chromosomal aberrations (CA), and cell transformation. These genotoxic results are summarized in Table 1, which provides useful data not only for genotoxicity evaluation of different nanoparticles but also for their hazard identification.

Table 1 illustrates that nanosized TiO_2, fullerene C_{60}, and silver are the nanoparticles most frequent examined in genotoxic studies in

TABLE 1. Genotoxicity of Nanoparticles In Vivo and In Vitro

	In vivo test system		In vitro test system	
Name of nanoparticle	Positive	Negative	Positive	Negative
Chitosan (CS) and poly(methacrylic acid) (PMAA)				*Allium cepa* chromosome damage test (de Lima et al. 2010), at 1.8 and 180 mg/L (size 60, 82, and 111 nm)
Silicon carbide (SiC)			Comet assay (Barillet et al. 2010), human lung epithelial cells (A549)	
Poly-*N*-isopropylacrylamide (PNIPAM)				Comet assay (Naha et al. 2010), at 12.5 and 800 mg/L, human keratinocyte (HaCaT) and colon cells (SW 480)
Copper oxide (CuO)			DNA damage (Ahamed et al. 2010), human lung epithelial cells (A549); Comet assay (Karlsson et al. 2008), at 0–40 $\mu g/cm^2$, human lung epithelial cells (A549)	
Cerium oxide (CeO_2)			Random amplified polymorphic DNA assay (Lopez-Moreno et al. 2010), at 2000–4000 mg/L, soybean plants	Sister chromatid exchanges (Pierscionek et al. 2010), at 5 and 10 $\mu g/ml$, lens cells
Zinc oxide (ZnO)		Micronucleus test (Landsiedel et al. 2010), mouse bone marrow	Comet assay (Sharma et al. 2009), at 0.8 g/ml, human epidermal cell line (A431) Comet assay (Karlsson et al. 2008), at 0–40 $\mu g/cm^2$, human lung epithelial cells (A549)	Random amplified polymorphic DNA assay (Lopez-Moreno et al. 2010), at 2000–4000 mg/L, soybean plants Ames test (Yoshida et al. 2009), at 0–5000 μg/plate, *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537; Ames test (Landsiedel et al. 2010) Comet assay (Landsiedel et al. 2010), lung cells of rats; V79 micronucleus chromosome mutation test (Landsiedel et al. 2010)
Titanium oxide (TiO_2)		Micronucleus test (Landsiedel et al. 2010), mouse bone marrow	Sister chromatid exchange (SCE), micronucleus test (Di Virgilio et al. 2010), at 0.5 and 1 $\mu g/ml$ Comet assay (Falck et al. 2009), at 40, 60, 80, and 100 $\mu g/cm^2$, in human bronchial epithelial BEAS 2B cells Cell transformation assay (Huang et al. 2009), long-term exposure, cultured fibroblast cells DNA adduct formation (Bhattacharya et al. 2009), at 5 and 10 $\mu g/cm^2$, human lung fibroblasts Gene mutation (Xu et al. 2009), at 10 $\mu g/ml$, gpt delta transgenic mouse primary embryo fibroblasts (MEF) Comet assay (Wang et al. 2007), at 65 $\mu g/ml$ for 6, 24, and 48 h, WIL2-NS cells (a human B lymphoblastoid cell line)	Comet assay (Hackenberg et al. 2010), at 0–100 $\mu g/ml$, human nasal mucosa cells Comet assay (Landsiedel et al. 2010), lung cells from rats Ames test (Landsiedel et al. 2010) V79 micronucleus chromosome mutation test (Landsiedel et al. 2010)

			Comet assay (Karlsson et al. 2008), at 0–40 μg/cm^2, human lung epithelial cells (A549) Comet assay and cytokinesis-block micronucleus (CBMN) assays (Kang et al. 2008), human peripheral blood lymphocytes Micronucleus test (Rahman et al. 2002), at 0–10 μg/cm^2, Syrian hamster embryo (SHE) cells Micronucleus test (Bhattacharya et al. 2008), V79 cells (hamster lung fibroblasts)	
Aluminum oxide (Al_2O_3)			Sister chromatid exchange (SCE) and micronucleus test (Di Virgilio et al. 2010), at 1–25 μg/ml	
Silver		Micronucleus test (Kim et al. 2008), at 30, 300 and 1000 mg/kg body wt for 28 d, bone marrow polychromatic erythrocytes of rats	Chromosomal aberration assay (Wise et al. 2010), at 0–0.3 μg/cm^2, fish cells Allium cepa chromosome damage test (Kumari et al. 2009), at 0–100 ppm Comet assay and cytokinesis blocked micronucleus assay (CBMN) (AshaRani et al. 2009), at 0–400μg/ml, normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251) DNA damage assay (Ahamed et al. 2008), mouse embryonic stem cells and mouse embryonic fibroblasts Micronucleus test (Kawata et al. 2009), human hepatoma cell HepG2	
Platinum			Comet assay (Asharani et al. 2010), at 0–160 μg/ml and incubated at 37°C for 48 h, human lung fibroblasts (IMR-90) Comet assay (Pelka et al. 2009), at 1 ng//cm^2, human colon carcinoma cell line HT29	
Fullerene C(60)	Level of 8-oxodG assay (Folkmann et al. 2009), a single oral administration (0.064 or 0.64 mg/kg body wt) in rats, colon mucosa, liver, and lung tissue;	Micronucleus test (Shinohara et al. 2009), at 88 mg/kg body wt x 2 in mice; Somatic mutation and recombination genotoxicity test (Zakharenko et al. 1997), at 2.46 mg/ml, somatic wing cells and Drosophila melanogaster larvae	Transgenic mouse mutation system (Xu et al. 2009), at 10 μg/ml, gpt delta transgenic mouse primary embryo fibroblasts (MEF) Comet assay (Jacobsen et al. 2008), 24 h at doses between 0 and 200 μg/ml or long-term subculture exposure (576 h) at 100 μg/ml, FE1-mutatrade mark mouse lung epithelial cell line Comet assay (Dhawan et al. 2006), at 2.2 μg/L, human lymphocytes	Ames test and chromosomal aberration test (Shinohara et al. 2009), at 1000μg/plate for Ames test in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2uvrA/pKM101, at 100 and 200 μg/ml in Chinese hamster cells for chromosomal aberration test

(*Continued*)

TABLE 1. (*Continued*)

Name of nanoparticle	In vivo test system		In vitro test system	
	Positive	Negative	Positive	Negative
	Comet assay (Jacobsen et al. 2009), 54 μg/mouse instillation for 3 h, mice bronchoalveolar lavage (BAL) cells			Chromosome aberration assay and the cytokinesis-block micronucleus (CBMN) test (Mrdanovic et al. 2009), at 11–221 μ*M*, Chinese hamster ovary cells (CHO-K1) Prokaryotic in vitro test (Zakharenko et al. 1997), at 2.46 mg/ml, *Escherichia coli* strain PQ37
Cobalt			Micronucleus test and Comet assay (Ponti et al. 2009), at 1–100 μ*M*, Balb/3T3 mouse fibroblasts Comet assay and binucleated micronucleated (BNMN) cells (Colognato et al. 2008), at 10–100 μ*M*, human peripheral blood leukocytes	
SWCNT	Level of 8-oxodG (Folkmann et al. 2009), a single oral administration (0.064 or 0.64 mg/kg body wt) in rats, colon mucosa, liver, and lung tissue; Comet assay (Jacobsen et al. 2009), 54 μg/mouse instillation for 3 h, mice bronchoalveolar lavage (BAL) cells		Comet assay (Jacobsen et al. 2008), at 0–200 μg/ml for 24 h or at 100 μg/ml long-term subculture exposure (576 h), FE1-mutatrade mark mouse lung epithelial cell line Comet assay (Kisin et al. 2007), at 96 μg/cm^2, lung fibroblast (V79) cell DNA damage and MN induction in 77 lung fibroblasts at 48 μg/cm^2 (Kisin et al. 2011) Transformation of bronchial epithelial cells after long-term low dose exposure (Stueckle et al. 2011)	Micronucleus test (Kisin et al. 2007), at 96 μg/cm^2, lung fibroblast (V79) cell Ames test (Kisin et al. 2007), at 0–240 μg/plate, *Salmonella* strains YG1024/YG1029
Quantum dots	Comet assay (Jacobsen et al. 2009), 54 μg/mouse instillation for 3 h, mice bronchoalveolar lavage (BAL) cells			
Gold	Comet assay (Jacobsen et al. 2009), 54 μg/mouse instillation for 3 h, mice bronchoalveolar lavage (BAL) cells			
MWCNT			Comet assay (Karlsson et al. 2008), at 0–40 μg/cm^2, human lung epithelial cells (A549) Transformation of bronchial epithelial cells after long-term low-dose exposure (Stueckle et al. 2011)	Ames test and chromosome aberrations test (Wirnitzer et al. 2009), at 5000 μg/plate for Ames test in *Salmonella typhimurium* (strains TA 1535, TA100, TA1537, TA98, and TA102), at 0–10 μg/ml, 4 h treatment for chromosome aberrations test in V79 cells

Silica		Comet assays (Barnes et al. 2008), at 4 and 40 μg/ml for 3, 6, and 24 h incubations, 3T3-L1 fibroblasts
		Comet assay (Jin et al. 2007), at 0.1–500 μg /ml for 48 h, human lung epithelial cells (A549)
FePt		Ames test (Maenosono et al. 2007), at 0–5000μg/plate, *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 strains and *Escherichia coli* strain WP2uvrA/pKM101, weakly positive in the TA100, but negative in other cases
Carbon black (CB)	Comet assay (Mroz et al. 2007), human lung epithelial cells (A549)	

the past several years. The genotoxicity of other nanoparticles used in nanomedicine has not yet been well evaluated, except for a few studies. The micronucleus test is a test frequently used for screening of potential genotoxicity. This assay is now recognized as one of the most successful and reliable assays for identification of genotoxic carcinogens. There are two major versions of this test, one in vivo and the other in vitro. The in vivo test normally analyzes animal bone marrow or peripheral blood cells (Zhao et al. 2009b). As shown in Table 1, two in vivo MN studies with silver and fullerene C_{60} nanoparticles were negative. The Comet assay, also called single-cell gel electrophoresis (SCGE), is a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells (Rao et al. 1997). As such, the Comet assay is one of the techniques used in the area of cancer research for the evaluation of genotoxicity. As shown in Table 1, most Comet assay results were positive when used to evaluate the genotoxicity of nanoparticles, which implies that nanoparticles possess the potential to induce DNA damage. The Ames test is a biological assay to evaluate the mutagenic potential of chemical compounds (Mortelmans and Zeiger 2000). A positive test result indicates that the compound might act as a carcinogen. As shown in Table 1, all Ames test results in tested nanoparticles were negative. It is possible that Ames tests are not suitable for evaluation of the mutagenic potential of water insoluble nanoparticles.

In summary, research data on the genotoxicity of nanoparticles are still limited and fragmentary. The contradicting results obtained from the studies presented in this review may be attributed to the use of different types, size, charge, agglomeration state, and purity of nanomaterials, and to the different test systems employed. Therefore, as for genotoxicity, the results of studies on nanoparticles are currently inconclusive. Based on the fact that an individual test covers only one definite endpoint of genotoxicity, a battery of tests including in vivo and in vitro experiments evaluating different genetic endpoints is required in further studies for evaluation of the genotoxicity of nanoparticles (Landsiedel et al. 2009).

Carcinogenicity

In recent years, studies related to the carcinogenicity of nanoparticles have begun, but the data are still limited. It has not yet been established whether human exposure to engineered nanoparticles produces cancer because human epidemiological evidence on the carcinogenicity of nanoparticles is still lacking.

TiO_2 nanoparticles are manufactured worldwide in large quantities for use in a wide range of applications including nanomedicine. Carcinogenicity studies in rodents indicate that TiO_2 nanoparticles produced lung tumors when given by inhalation (Heinrich 1995; Rittinghausen et al. 1997) and intratracheal instillation (Pott and Roller 2005) in rats. Heinrich et al. (1995) treated rats by inhalation of TiO_2 nanoparticles at a concentration of 7.5 mg/m^3 for 4 mo, followed by a concentration of 15 mg/ m^3 for 4 mo, and then at a concentration of 10 mg/ m^3 for 16 mo. Rats were sacrificed at mo 30. Results showed that the incidence of lung tumors (19%), including benign and malignant squamous- and alveolar-cell tumors, was significantly increased compared to the control group (0.5%). Rittinghausen et al. (1997) treated rats by inhalation of TiO_2 nanoparticles at a concentration of 11.3 mg/m^3 for 24 mo followed by observation for 6 mo. Incidences of lung tumors, including cystic keratinizing epitheliomas (11.7%) and squamous cell carcinomas (4.8%) were also significantly greater than for the control group (0.5%). Pott and Roller (2005) treated rats with intratracheal instillation of TiO_2 nanoparticles. In this study, several dosing strategies were used, and the instillation was done once weekly. The animals were observed for up to 30 wk. The incidence of lung tumors, including adenomas/carcinomas and squamous-cell epitheliomas/carcinomas, in rats receiving both hydrophilic TiO_2 nanoparticles (52–69.6%) and anatase TiO_2 nanoparticles (29.5–63.6%) were significantly increased compared with untreated controls

(0%). Based on the studies outlined here, the International Agency for Research on Cancer (IARC) concluded there is sufficient evidence for the carcinogenicity of TiO_2 nanoparticles in experimental animals (Roller 2009; Tsuda et al. 2009). However, human epidemiological data on the carcinogenicity of TiO_2 nanoparticles are still lacking. Therefore, TiO_2 nanoparticles are classified by WHO/IARC as a Group 2B compound (possibly carcinogenic to humans) (Baan 2007). The National Institute for Occupational Safety and Health (NIOSH) recently conducted a risk assessment of the available data comparing the carcinogenicity of fine versus ultrafine TiO_2 (NIOSH 2011). This analysis resulted in publication of recommended exposure levels (REL) of 0.3 mg/m^3 and 2.4 mg/m^3 for ultrafine and fine TiO_2, respectively, in the workplace.

Carbon black nanoparticles (CBN) are also carcinogenic to the lung of rats (Tsuda et al. 2009). Heinrich et al. (1995) treated rats with CBN at a concentration of 7.5 mg/m^3 for 4 mo, then at a concentration of 12 mg/m^3 for 20 mo, followed by clean air for 6 mo. The incidence of lung tumors (39%), including benign and malignant squamous-cell tumors and bronchioalveolar-cell tumors, was significantly increased compared to the clean air group (0.5%). Dasenbrock et al. (1996) treated rats with CBN by intratracheal instillation once a week for 3 wk at a dose of 0.66 mg/rat, and then once a week for 13 wk at a dose of 1 mg/rat. Animals were observed for up to 800 d from the beginning of the study. The incidence of lung tumors, including cystic keratinizing epitheliomas and bronchioalveolar-cell tumors (21%), was significantly elevated compared to the control group (0%). Rittinghausen et al. (1997) obtained similar results when they treated rats with CBN by intratracheal instillation at a concentration of 11.3 mg/rat for 4 mo, and then at a concentration of 12.2 mg/rat for the following 20 mo. The total incidence (20%) of lung tumors was significantly raised compared to control (0%). In another study, Pott and Roller (2005) treated rats by intratracheal instillation with CBN at 1.5 to 6 mg/rat from 5 to 20 times. Animals were observed for up to 30 mo. Total lung tumor incidence (56–80%) was significantly elevated compared to the control (2%). Based on the studies just described, CBN are classified by WHO/IARC as a Group 2B compound (possibly carcinogenic to humans) (Baan 2007).

Carbon nanotubes (CNT) are allotropes of carbon with a cylindrical nanostructure. There are two types of carbon nanotubes: SWCNT are tube structures with a diameter of close to 1 nm and composed of a 1-atom-thick layer of graphite, and MWCNT are tube structures with diameters of tens of nanometers and composed of 2 or more layers of graphene atoms (Tsuda et al. 2009). In short- to medium-term (6 mo or less) pulmonary exposure studies in rodents after intratracheal instillation, aspiration, or inhalation of SWCNT or MWCNT, no tumors were reported (Lam et al. 2004; Ma-Hock et al. 2009; Mercer et al. 2008; Pauluhn 2010; Porter et al. 2010; Shvedova et al. 2005; 2008; Warheit et al. 2004). However, cellular atopia, characterized by hypertrophied bronchiolar and alveolar epithelial cells and multinucleated alveolar macrophages, was observed (Porter et al. 2010; Shvedova et al. 2005). In addition, inhalation (4 d at 5 mg/m^3) of SWCNT in mice produced in the k-ras gene significant mutations that were associated with lung cancer (Shvedova et al. 2008a). MWCNT have been reported to induce mesothelioma after intraperitoneal injection. Takagi et al. (2008) administered MWCNT (3500 ppm iron [Fe] content; diameter 100 nm; approximate length 1–5 μm) to male p53 (+/-) mice with a C57BL/6 background by intraperitoneal injection at a dose of 3 mg/mouse. The animals were then observed for 25 wk. The incidence of mesothelioma in the peritoneal cavity (87.5%) in the MWCNT group was significantly elevated compared to the control. Sakamoto et al. (2009) treated male rats with MWCNT (3500 ppm Fe content; diameter 70–100 nm; approximate length 1–4 μm) by a single intrascrotal injection of 20 mg/rat. The animals were then observed until wk 104. The incidence of disseminated mesothelioma in the peritoneal cavity was 86% in MWCNT groups, which was higher than asbestos control. In

contrast, Muller et al. (2009) treated rats with MWCNT (11.3 nm in mean diameter, approximate length 0.7 μm) by a single intraperitoneal injection at a dose of 20 mg/rat. The animals were then observed for up to wk 104. The incidence of mesothelioma in the MWCNT groups (up to 6%) was also not statistically higher than the incidence (3.8%) in the vehicle control group. This negative finding appears due to the use of short MWCNT in the Muller et al. (2008) study. Indeed, Poland et al. (2009) found that abdominal injection of long MWCNT or asbestos produced inflammatory granulomatous lesions on the diaphragm, but short MWCNT did not appear to be active. In summary, studies of pulmonary response to SWCNT or MWCNT in mice or rats have been relatively short in duration and are not adequate to evaluate the possibility of lung cancer. . However, intraperitoneal or intrascrotal injections of MWCNT produced mesothelioma in rodents. Of interest, Mercer et al. (2010) reported that aspirated MWCNT migrate to the subpleural tissue, penetrate the visceral pleura, and enter the intrapleural space. Further studies are needed to confirm the pulmonary carcinogenic potential of CNT in rodents.

The available in vivo data concerning the carcinogenic effects of TiO_2 nanoparticles, CBN, SWCNT, and MWCNT have been reviewed. Of these, TiO_2 nanoparticles and CBN exhibited carcinogenicity in experimental animals. No carcinogenic activity of SWCNT was found in the relatively short-term (<6 mo) in vivo studies conducted to date. Some results reported carcinogenicity for MWCNT after intraperitoneal or intrascrotal injection in rodents. No data are available to confirm the carcinogenicity of other nanoparticles (Tsuda et al. 2009).

Reproductive and Developmental Toxicity

While research into the potential adverse properties of nanomaterials is now increasing, the area of reproductive and developmental toxicity has remained relatively uninvestigated (Park et al. 2009). It has not yet been established whether human exposure to nanoparticles produces adverse reproductive or developmental effects. In this review, in vivo and in vitro studies on the reproductive and developmental toxicity of nanosized TiO_2, carbon black, SWCNT, MWCNT, fullerene C_{60}, silica, gold, and silver are reviewed.

Wiench et al. (2009) investigated reproductive effects of TiO_2 nanoparticles using *Daphnia magna* as a model organism. It was concluded that TiO_2 nanoparticles produced adverse reproductive effects in *Daphnia magna*. Zhu et al. (2010) investigated the potential ecotoxicity of TiO_2 nanoparticles on *Daphnia magna* and found that TiO_2 nanoparticles exerted minimal toxicity to *Daphnia* within 48 h of exposure, but produced marked toxicity when the exposure time was extended to 72 h. After chronic exposure to TiO_2 nanoparticles for 21 d, *Daphnia magna* displayed severe growth retardation and mortality, as well as reproductive defects. In addition, a significant amount of TiO_2 nanoparticles accumulated in *Daphnia magna*. Shimizu et al. (2009) reported alterations of gene expression related to brain development in the mouse after maternal exposure to TiO_2 nanoparticles during the prenatal period. TiO_2 nanoparticles (100 μl of suspension at 1 μg/μl) were injected subcutaneously into pregnant mice on gestational days 6, 9, 12, and 15 in the exposed group. It was found that maternal exposure of mice to TiO_2 nanoparticles may affect the expression of genes related to the development and function of the central nervous system. Ken et al. (2009) showed that TiO_2 nanoparticles administered subcutaneously to pregnant mice were transferred to the offspring and produced effects on the genital and cranial nervous systems of the male offspring. In addition, TiO_2 nanoparticles were found in testes and brain of exposed 6-wk-old male mice. In the offspring of TiO_2 nanoparticle-injected mice, various functional and pathologic disorders, such as reduced daily sperm production, were observed. Chen et al. (2011) observed the ecological toxicity of TiO_2 nanoparticles using zebrafish in a chronic toxicity test and found TiO_2 nanoparticles inhibit growth of zebrafish

in a dose-dependent manner. In addition, TiO_2 nanoparticles penetrate the blood–brain barrier after a long-term exposure, demonstrated by their accumulation in zebrafish brain.

Yoshida et al. (2010) investigated the reproductive function of male offspring after a 200-μg/mouse intratracheal instillation of CBN on d 7 and 14 of gestation. Reproductive function of male offspring was determined at ages 5, 10, and 15 wk after birth. Daily sperm production (DSP) was significantly decreased in fetal CBN-exposed mice. In fetal CBN-treated mice, the DSP decreased by 47% at the age of 5 wk, by 34% at the age of 10 wk, and by 32% at the age of 15 wk. These findings suggest that fetal CBN exposure affects the reproductive function of male offspring. Ema et al. (2010) tested the reproductive effects of three sizes (14, 56, and 95 nm) of CBN by intratracheal instillation of 0.1 mg/mouse once a week for 10 wk. The serum testosterone levels were elevated significantly in the 14- and 56-nm CBN-exposed groups. Histological examination showed partial vacuolation of the seminiferous tubules. These results suggest that CBN exerted adverse effects on the mouse male reproductive function. Furthermore, it was found that the effects of CBN on the male reproductive system depended on particle mass rather than particle number.

Developmental toxicity of SWCNT and MWCNT was determined using developing zebrafish embryos by Cheng et al. (2007) under several environmental conditions. Exposure to SWCNT induced a significant hatching delay in zebrafish embryos at concentrations of greater than 120 mg/L. MWCNT also induced a hatching delay at concentrations of greater than 240 mg/L. The hatching delay observed in this study likely was induced by the Co and Ni catalysts used in the production of SWCNT that remained at trace concentrations after purification. This study suggests that materials associated with raw SWCNT (perhaps metal contaminants) have the potential to affect aquatic life when released into the environment. Scott-Fordsmand et al. (2008) investigated the reproductive toxicity of MWCNT on *Eisenia veneta* earthworms. Reproduction of *Eisenia veneta* earthworms was affected by MWCNT administered through food at concentrations above 37 mg/kg food. The most sensitive toxicological parameter was reproduction (cocoon production), with no marked effect on hatchability, survival, or mortality at up to 495 mg MWCNT/kg food.

Sumner et al. (2010) observed the distribution of carbon-14-labeled fullerene C_{60} in the pregnant rat and fetuses and in the lactating rat and offspring. Pregnant rats were treated on gestation day 15 and lactating rats were treated on postnatal day 8 via tail-vein injection with a suspension of approximately 0.3 mg/kg body weight of fullerene C_{60} prepared in polyvinylpyrrolidone (PVP), or with PVP alone. Tissues were collected at 24 and 48 h after dosing. Results showed that fullerene C_{60} nanoparticles crossed the placenta and were transmitted to offspring via the dam's milk and subsequently systemically absorbed. Tsuchiya et al. (1996) investigated reproductive effects of fullerene C_{60} on mouse embryos in vivo and in vitro. After the injection of fullerene C_{60}, all embryos died at 137 mg/kg body weight in the in vivo experiment. At 50 mg/kg body weight, fullerene C_{60} was clearly distributed into the yolk sac and embryos, and 50% of the embryos were abnormal in shape predominantly in the head and tail regions. In the in vitro test, fullerene C_{60} in the medium was incorporated into the midbrain culture of embryos of pregnant mice, and further cultured for 6 d. Differentiation was inhibited as cytotoxicity increased. Fullerene C_{60} was assumed to decrease cell proliferation via reactive oxygen species. The IC50 values of fullerene C_{60} for cell differentiation and proliferation were 0.43 and 0.47 mg/ml, respectively. Therefore, data suggested that fullerene C_{60} nanoparticles exert a harmful effect on development of mice.

The embryonic stem cell test is an in vitro screening assay that is used to investigate the embryotoxic potential of chemicals by determining their ability to inhibit differentiation of embryonic stem cells into spontaneously contracting cardiomyocytes. Park et al. (2009) treated mouse embryonic stem cells with SiO_2 nanoparticles at concentrations 1 to

100 μg/ml. A concentration-dependent inhibition of differentiation of stem cells into contracting cardiomyocytes by two SiO_2 nanoparticles of primary size 10 and 30 nm was observed, while two other SiO_2 particles of primary size 80 and 400 nm had no effect up to the highest concentration tested. The inhibition of differentiation of stem cells occurred below cytotoxic concentrations, suggesting a specific effect of the nanoparticles on the differentiation of the embryonic stem cells. Park et al. (2009) suggested further investigation into the potential of SiO_2 nanoparticles to migrate into the uterus, placenta, and embryo and their possible effects on embryogenesis.

Wiwanitkit et al. (2009) investigated the in vitro toxic effects of 9-nm-size gold nanoparticles (GNP) on human spermatozoa. A fresh semen sample was cultured with GNP and motility and morphological changes were studied after 15 min by microscopy. Microscopic examinations revealed that 25% of sperm cells were not motile, and penetration of GNP into the sperm head and tail was observed. In addition, fragmentation of sperm was observed.

Braydich-Stolle et al. (2005) investigated the suitability of a mouse spermatogonial stem cell line as a model to assess toxicity of silver nanoparticles in the male germ cell line in vitro. The germ cells (C18-4 cells) were incubated with silver nanoparticles at final concentrations of 5, 10, 25, 50, or 100 μg/ml for 48 h. Results showed that silver nanoparticles were toxic to mouse spermatogonia stem cells by inducing necrosis and apoptosis at 10μg/ml and above. Lee et al. (2007) observed the transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos using in vivo imaging and found single silver nanoparticles (5–46 nm) are transported into and out of embryos through chorionic pore canals (CPC) and exhibit Brownian diffusion (not active transport), with the diffusion coefficient inside the chorionic space approximately 26-fold lower than in egg water. Further analysis indicated that rates of passive diffusion and accumulation of silver nanoparticles in embryos are likely responsible for the dose-dependent abnormalities. Asharani et al. (2011) compared the toxicity of silver, gold, and platinum nanoparticles in developing zebrafish embryos and found silver nanoparticles to be the most toxic. Xia et al. (2011) found that exposure of zebrafish embryos to ZnO resulted in a decrease in hatching. Doping ZnO with Fe decreased the dissolution rate of ZnO nanoparticles and significantly improved hatching rate to a near normal level. The adverse effect of ZnO on hatching is postulate to involve blockage by the addition of a metal chelator (DTPA), supporting the conclusion that ionic Zn was responsible for this adverse effect.

Taken together, chronic exposure to TiO_2 nanoparticles produced reproductive defects on *Daphnia magna*. After maternal subcutaneous injection to pregnant mice, TiO_2 nanoparticles are transferred to the offspring and affect the genital and cranial nervous systems of the male offspring. TiO_2 nanoparticles may penetrate blood–testes barriers and reduce the daily sperm production in the offspring of TiO_2 nanoparticle-treated mice. TiO_2 nanoparticles may also affect the expression of genes related to the development and function of the central nervous system in the offspring. Intratracheal instillation of carbon black nanoparticles in mice might produce adverse effects on the mouse male reproductive function. Exposure to SWCNT and MWCNT induced a significant developmental toxicity in zebrafish embryos. Death and morphological abnormalities in mouse embryos were observed after maternal intraperitoneal injection of fullerene C_{60}. SiO2 nanoparticles produced inhibition of differentiation of stem cells in the embryonic stem cell test. Gold nanoparticles reduced the motility of human sperm and induced fragmentation of the sperm. Silver nanoparticles were found to be toxic to mouse spermatogonia stem cells. ZnO nanoparticles adversely affected hatching of zebrafish embryos. No data are available to confirm induction of reproductive or developmental toxicity among other nanoparticles (Ema et al. 2010). Based on the fact of the accumulation of TiO_2 nanoparticles in testes and brain, caution needs to be taken when considering the reproductive and developmental

toxicity of nanoparticles. Future studies comparing the reproductive and developmental toxicity between fine and nanosized materials are necessary. In addition, studies evaluating the potential for nanoparticles to cross the fetal–placental barrier are warranted.

SUMMARY

Despite the fact that nanomaterial applications are being developed increasingly in industry, medicine, and pharmacology, there is still much remaining to be elucidated concerning their possible adverse health effects. Nanoparticles consist of a wide variety of particles with distinct physical and chemical properties. At present, there is no unified method for the classification of nanoparticles and prediction of their potential toxicity. The main applications of nanoparticles in medicine are drug delivery, molecular diagnostics, molecular imaging, dental implants, bone grafting, active implants, tissue engineering, surgery, genomics, and proteomics. Nanoparticles enter the human body through the lungs, GIT, or skin. Inhalation of nanoparticles is normally the primary route of entry into the human body under conditions of occupational and environmental exposures. Intravenous and subcutaneous injections of nanoparticulate carriers represent unique exposure routes in nanomedical practice. The relative amounts of nanoparticles absorbed by the human body are determined not only by the quantities inhaled or ingested, but also by their physical and chemical characteristics. Metabolism, tissue distribution, accumulation, and elimination of nanoparticles may be affected by various factors including routes of exposures, chemical form, agglomeration state, particle size, shape, solubility, and charge. Distribution of nanoparticles was found in different tissues or organs, such as lung, blood, bone marrow, kidneys, liver, intestine, femur, thymus, gut, heart, spleen, and brain. Nanoparticles may penetrate blood–testis, –placenta, or –brain barriers. Elimination of nanoparticles is mainly through urine, bile, and feces. Studies report that some of the nanoparticles, such as TiO_2 nanoparticles, penetrate blood–testes and blood–brain barriers. In addition, there may also be an accumulation of nanoparticles in humans.

Research data on the acute and chronic toxicity of nanoparticles are expanding but are not yet complete. Animal experiments suggest that some of the nanoparticles may exhibit adverse health potential on the human body. Research reports related to the genotoxicity of nanoparticles are still limited. A battery of tests for evaluation of the genotoxicity of nanoparticles is essential in future studies because individual tests each cover only one aspect of genotoxicity. Whether human exposure to nanoparticles designed for medical use induces cancer remains unclear. TiO_2 nanoparticles and carbon black nanoparticles are classified as possibly carcinogenic to humans by WHO/IARC based solely on carcinogenicity data in experimental animals. Further studies are necessary to elucidate possible mechanisms of the carcinogenicity of these nanoparticles. No carcinogenic activity of SWCNT was found in the studies conducted to date. Limited results are reported for the carcinogenicity of MWCNT in animal experiments after intraperitoneal injection, but no data are available for pulmonary exposure. In addition, no data are available to confirm carcinogenicity among other nanoparticles. TiO_2 nanoparticles produce reproductive and developmental toxicity in experimental animals. Carbon black nanoparticles, SWCNT and MWCNT, fullerene C_{60}, SiO_2, gold nanoparticles, and silver nanoparticles may also exhibit certain reproductive and/or developmental toxicity.

In conclusion, with the increasing applications of nanomaterials in medicine, further systematic and well-designed toxicological studies are urgently needed. Due to intravenous and subcutaneous injections, nanoparticulate carriers for drug-delivery application need to undergo biosafety evaluation. Evaluation of the toxicology of nanoparticles by extrapolation from data with parent fine (or bulk) particles may not be appropriate, considering the unique physicochemical properties of selected nanoparticles. Therefore, specific,

no-observed-effect concentration (NOEC) levels need to be determined from toxicological studies with nanoparticles, especially for those nanoparticles designed for use in nanomedicine.

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REFERENCES

Ahamed, M., Karns, M., Goodson, M., Rowe, J., Hussain, S. M., Schlager, J. J., and Hong, Y. 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. *Toxicol. Appl. Pharmacol.* 233: 404–10.

Ahamed, M., Siddiqui, M. A., Akhtar, M. J., Ahmad, I., Pant, A. B., and Alhadlaq, H. A. 2010. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. *Biochem. Biophys. Res. Commun.* 396: 578–83.

Albini, A., Mussi, V., Parodi, A., Ventura, A., Principi, E., Tegami, S., Rocchia, M., Francheschi, E., Sogno, I., Cammarota, R., Cammarota, R., Finzi, G., Sessa, F., Noonan, D. M., and Valbusa, U. 2010. Interactions of single-wall carbon nanotubes with endothelial cells. *Nanomedicine* 6: 277–88.

Allen, T. M. 2002. Ligand-targeted therapeutics in anticancer therapy. *Nat. Rev. Cancer* 2: 750–63.

Allen, T. M., and Cullis, P. R. 2004. Drug delivery systems: Entering the mainstream. *Science* 303: 1818–22.

Applerot, G., Abu-Mukh, R., Irzh, A., Charmet, J., Keppner, H., Laux, E., Guibert, G., and Gedanken, A. 2010. Decorating parylene-coated glass with ZnO nanoparticles for antibacterial applications: A comparative study of sonochemical, microwave, and microwave-plasma coating routes. *ACS Appl. Mater. Interfaces* 2: 1052–59.

Asharani, P. V., Lianwu,Y., Gong, Z., and Valiyaveettil, S. 2011. Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. *Nanotoxicology* 5: 43–54.

Asharani, P. V., Low Kah Mun, G., Hande, M. P., and Valiyaveettil, S. 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3: 279–90.

Asharani, P. V., Xinyi, N., Hande, M. P., and Valiyaveettil, S. 2010. DNA damage and p53-mediated growth arrest in human cells treated with platinum nanoparticles. *Nanomedicine (Lond.)* 5: 51–64.

Baan, R. A. 2007. Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: Recent evaluations by an IARC Monographs Working Group. *Inhal. Toxicol.* 19(suppl. 1): 213–28.

Ballou, B., Lagerholm, B. C., Ernst, L. A., Bruchez, M. P., and Waggoner, A. S. 2004. Noninvasive imaging of quantum dots in mice. *Bioconj. Chem.* 15: 79–86.

Barillet, S., Jugan, M. L., Laye, M., Leconte, Y., Herlin-Boime, N., Reynaud, C., and Carriere, M. 2010. In vitro evaluation of SiC nanoparticles impact on A549 pulmonary cells: Cyto-, genotoxicity and oxidative stress. *Toxicol. Lett.* 198: 324–30.

Barnes, C. A., Elsaesser, A., Arkusz, J., Smok, A., Palus, J., Lesniak, A., Salvati, A., Hanrahan, J. P., Jong, W. H., Dziubaltowska, E., Stepnik, M., Rydzynski, K., McKerr, G., Lynch, I, Dawson, K. A., and Howard, C. V. 2008. Reproducible comet assay of amorphous silica nanoparticles detects no genotoxicity. *Nano Lett.* 8: 3069–74.

Barrett, E. G., Johnston, C., Oberdorster, G., and Finkelstein, J. N. 1999. Silica binds serum proteins resulting in a shift of the

dose-response for silica-induced chemokine expression in an alveolar type II cell line. *Toxicol. Appl. Pharmacol.* 161: 111–22.

Bhattacharya, K., Cramer, H., Albrecht, C., Schins, R., Rahman, Q., Zimmermann, U., and Dopp, E. 2008. Vanadium pentoxide-coated ultrafine titanium dioxide particles induce cellular damage and micronucleus formation in V79 cells. *J. Toxicol. Environ. Health A* 71: 976–80.

Bhattacharya, K., Davoren, M., Boertz, J., Schins, R. P., Hoffmann, E., and Dopp, E. 2009. Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells. *Part. Fibre Toxicol.* 6: 17.

Bottrill, M., Kwok, L., and Long, N. J. 2006. Lanthanides in magnetic resonance imaging. *Chem. Soc. Rev.* 35: 557–71.

Braydich-Stolle, L., Hussain, S., Schlager, J. J., and Hofmann, M. C. 2005. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicol. Sci.* 88: 412–19.

Brooking, J., Davis, S. S., and Illum, L. 2001. Transport of nanoparticles across the rat nasal mucosa. *J. Drug Target* 9: 267–79.

Campolongo, M. J., Tan, S. J., Xu, J., and Luo, D. 2010. DNA nanomedicine: Engineering DNA as a polymer for therapeutic and diagnostic applications. *Adv. Drug Deliv. Rev.* 62: 606–16.

Casey, A., Herzog, E., Lyng, F. M., Byrne, H. J., Chambers, G., and Davoren, M. 2008. Single walled carbon nanotubes induce indirect cytotoxicity by medium depletion in A549 lung cells. *Toxicol. Lett.* 179: 78–84.

Castranova, V., Porter, D, Millecchia, L, Ma, J. Y. C., Hubbs, A. F., and Teass, A. (2002). Effect of inhaled crystalline silica in a rat model: Time course of pulmonary reactions. *Mol. Cell Biochem.* 234/235: 177–84.

Castranova, V., Vallyathan, V., and Wallace, W. E. 1996. *Silica and silica-induced lung diseases*. Boca Raton, FL: CRC Press.

Castranova, V., and Vallyathan, V. 2000. Silicosis and coal workers' pneumeconiosis. *Environ. Health Perspect.* 108(suppl 4): 675–84.

Chen, H. H., Yu, C., Ueng, T. H., Chen, S., Chen, B. J., Huang, K. J., and Chiang, L. Y. 1998. Acute and subacute toxicity study of water-soluble polyalkylsulfonated C60 in rats. *Toxicol. Pathol.* 26: 143–51.

Chen, H. Q., Luo, F. B., Liu, Y., Liang, A. N., Lin, B., and Wang, L. 2009a. Application of functional CdS nanoparticles in determination of silver ion by resonance light-scattering technique. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 71: 1701–3.

Chen, J., Dong, X., Xin, Y., and Zhao, M. 2011. Effects of titanium dioxide nanoparticles on growth and some histological parameters of zebrafish (*Danio rerio*) after a long-term exposure. *Aquat. Toxicol.* 101: 493–99.

Chen, J. K., Shih, M. H., Peir, J. J., Liu, C. H., Chou, F. I., Lai, W. H., Chang, L. W., Lin, P., Wang, M. Y., Yang, M. H., and Yang, C. S. 2010. The use of radioactive zinc oxide nanoparticles in determination of their tissue concentrations following intravenous administration in mice. *Analyst* 135: 1742–46.

Chen, X., and Schluesener, H. J. 2008. Nanosilver: A nanoproduct in medical application. *Toxicol. Lett.* 176: 1–12.

Chen, Y. S., Hung, Y. C., Liau, I., and Huang, G. S. 2009b. Assessment of the *in vivo* toxicity of gold nanoparticles. *Nanoscale Res. Lett.* 4: 858–64.

Cheng, J., Flahaut, E., and Cheng, S. H. 2007. Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. *Environ. Toxicol. Chem.* 26: 708–16.

Cheung, W., Pontoriero, F., Taratula, O., Chen, A. M., and He, H. 2010. DNA and carbon nanotubes as medicine. *Adv. Drug Deliv. Rev.* 62: 633–49.

Cho, M., Cho, W. S., Choi, M., Kim, S. J., Han, B. S., Kim, S. H., Kim, H. O., Sheen, Y. Y., and Jeong, J. 2009a. The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. *Toxicol. Lett.* 189: 177–83.

Cho, W. S., Cho, M., Jeong, J., Choi, M., Cho, H. Y., Han, B. S., Kim, S. H., Kim, H. O., Lim, Y. T., and Chung, B. H. 2009b. Acute toxicity and pharmacokinetics of 13

nm-sized PEG-coated gold nanoparticles. *Toxicol. Appl. Pharmacol.* 236: 16–24.

Choksi, A. N., Poonawalla, T., and Wilkerson, M. G. 2010. Nanoparticles: A closer look at their dermal effects. *J. Drugs Dermatol.* 9: 475–81.

Churg, A., Stevens, B., and Wright, J. L. 1998. Comparison of the uptake of fine and ultrafine TiO_2 in a tracheal explant system. *Am. J. Physiol* 274: L81–L86.

Colognato, R., Bonelli, A., Ponti, J., Farina, M., Bergamaschi, E., Sabbioni, E., and Migliore, L. 2008. Comparative genotoxicity of cobalt nanoparticles and ions on human peripheral leukocytes in vitro. *Mutagenesis* 23: 377–82.

Coyle, P., Philcox, J. C., Carey, L. C., and Rofe, A. M. 2002. Metallothionein: the multipurpose protein. *Cell. Mol. Life Sci.* 59: 627–47.

Dasenbrock, C., Peters, L., Creutzenberg, O., and Heinrich, U. 1996. The carcinogenic potency of carbon particles with and without PAH after repeated intratracheal administration in the rat. *Toxicol. Lett.* 88: 15–21.

de Lima, R., Feitosa, L., Pereira, A. D., de Moura, M. R., Aouada, F. A., Mattoso, L. H., and Fraceto, L. F. 2010. Evaluation of the genotoxicity of Chitosan nanoparticles for use in food packaging films. *J. Food Sci.* 75: N89–N96.

De Nicola, M., Nuccitelli, S., Gattia, D. M., Traversa, E., Magrini, A., Bergamaschi, A., and Ghibelli, L. 2009. Effects of carbon nanotubes on human monocytes. *Ann. NY Acad. Sci.* 1171: 600–5.

Desai, M. P., Labhasetwar, V., Amidon, G. L., and Levy, R. J. 1996. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharm. Res.* 13: 1838–45.

Dhawan, A., Taurozzi, J. S., Pandey, A. K., Shan, W., Miller, S. M., Hashsham, S. A., and Tarabara, V. V. 2006. Stable colloidal dispersions of C_{60} fullerenes in water: evidence for genotoxicity. *Environ. Sci. Technol.* 40: 7394–401.

Di Virgilio, A. L., Reigosa, M., Arnal, P. M., and Fernandez Lorenzo de Mele, M. 2010. Comparative study of the cytotoxic and genotoxic effects of titanium oxide and aluminium oxide nanoparticles in Chinese hamster ovary (CHO-K1) cells. *J. Hazard. Mater.* 177: 711–18.

Dominic, F., McDermott, A., Daniels, M., Zeger, S. L, and Samet, J. M. 2005. Revised analysis of the national mortality, morbidity and air pollution study: Mortality among residents of 90 cities. *J. Toxicol. Environ. Health A* 68: 1071–92.

Donaldson, K., Stone, V., Clouter, A., Renwick, L., and MacNee, W. 2001. Ultrafine particles. *Occup. Environ. Med.* 58: 211–16.

Driscoll, K. E., Costa, D. L., Hatch, G., Henderson, R., Oberdorster, G., Salem, H., and Schlesinger, R. B. 2000. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: Uses and limitations. *Toxicol. Sci.* 55: 24–35.

Driscoll, K. E., and Guthrie, G. D. 1997. Crystalline silica and silicosis. In *Comprehensive toxicology, Vol. 8, Toxicology of the respiratory system*, ed. G. Sipes, C. A. Mc Queen, and A. J. Gandolf, vol. ed. R. A. Roth, 373–391. New York: Pergamon.

Dror, I., Baram, D., and Berkowitz, B. 2005. Use of nanosized catalysts for transformation of chloro-organic pollutants. *Environ. Sci. Technol.* 39: 1283–90.

Ema, M., Kobayashi, N., Naya, M., Hanai, S., and Nakanishi, J. 2010. Reproductive and developmental toxicity studies of manufactured nanomaterials. *Reprod. Toxicol.* 30: 343–52.

Falck, G. C., Lindberg, H. K., Suhonen, S., Vippola, M., Vanhala, E., Catalan, J., Savolainen, K., and Norppa, H. 2009. Genotoxic effects of nanosized and fine TiO_2. *Hum. Exp. Toxicol.* 28: 339–52.

Fenske, D. B., MacLachlan, I., and Cullis, P. R. 2001. Long-circulating vectors for the systemic delivery of genes. *Curr. Opin. Mol. Ther.* 3: 153–58.

Firme, C. P. 3rd, and Bandaru, P. R. 2010. Toxicity issues in the application of carbon nanotubes to biological systems. *Nanomedicine* 6: 245–56.

Florence, A. T. 1997. The oral absorption of micro- and nanoparticulates: neither

exceptional nor unusual. *Pharm. Res*. 14: 259–66.

Foldvari, M., and Bagonluri, M. 2008. Carbon nanotubes as functional excipients for nanomedicines: II. Drug delivery and biocompatibility issues. *Nanomedicine* 4: 183–200.

Folkmann, J. K., Risom, L., Jacobsen, N. R., Wallin, H., Loft, S., and Moller, P. 2009. Oxidatively damaged DNA in rats exposed by oral gavage to C_{60} fullerenes and single-walled carbon nanotubes. *Environ. Health Perspect*. 117: 703–8.

Foundation, E. E. S. 2004. *Nanomedicine, An ESF–European Medical Research Councils (EMRC) forward look report*. Strasbourg Cedex, France: ESF.

Galagudza, M. M., Korolev, D. V., Sonin, D. L., Postnov, V. N., Papayan, G. V., Uskov, I. S., Belozertseva, A. V., and Shlyakhto, E. V. 2010. Targeted drug delivery into reversibly injured myocardium with silica nanoparticles: Surface functionalization, natural biodistribution, and acute toxicity. *Int. J. Nanomedicine* 5: 231–37.

Gamer, A. O., Leibold, E., and van Ravenzwaay, B. 2006. The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin. *Toxicol. In Vitro* 20: 301–7.

Geiser, M., Rothen-Rutishauser, B., Kapp, N., Schurch, S., Kreyling, W., Schulz, H., Semmler, M., Im Hof, V., Heyder, J., and Gehr, P. 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ. Health Perspect*. 113: 1555–60.

Gharbi, N., Pressac, M., Hadchouel, M., Szwarc, H., Wilson, S. R., and Moussa, F. 2005. [60]Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett*. 5: 2578–85.

Goldberg, M. S., Burnett, R. T., Bailar, J. C. III, Tamblyn, R., Ernst, R., Flegel, K., Brook, J., Bonvalot, J., Singh, R., Valois, M. F., and Vincent, R. 2001. Identification of persons with cardiorespiratory conditions who are at risk of dying from the acute effects of ambient air particles. *Environ. Health Perspect*. 109(suppl. 4): 487–94.

Goodman, C. M., McCusker, C. D., Yilmaz, T., and Rotello, V. M. 2004. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconj. Chem*. 15: 897–900.

Gref, R., Minamitake, Y., Peracchia, M. T., Trubetskoy, V., Torchilin, V., and Langer, R. 1994. Biodegradable long-circulating polymeric nanospheres. *Science* 263: 1600–3.

Grose, M., Sakurai, H., Savstrom, J., Stolzenburg, M. R., Watts, W. F., Jr., Morgan, C. G., Murray, I. P., Twigg, M. V., Kittelson, D. B., and McMurry, P. H. 2006. Chemical and physical properties of ultrafine diesel exhaust particles sampled downstream of a catalytic trap. *Environ. Sci. Technol*. 40: 5502–7.

Gulson, B., McCall,M., Korsch, M., Gomez, L., Casey, P., Oytam,Y., Taylor, A., McCulloch, M., Trotter, J., Kinsley, L., and Greenoak, G. 2010. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. *Toxicol. Sci*. 118: 140–49.

Gulumian, M., and Vallyathan, V. 2010. Nanoparticles and potential human health implications: Past and future directions. Preface. *J. Toxicol. Environ. Health A* 73: 339–40.

Hackenberg, S., Friehs, G., Froelich, K., Ginzkey, C., Koehler, C., Scherzed, A., Burghartz, M., Hagen, R., and Kleinsasser, N. 2010. Intracellular distribution, geno- and cytotoxic effects of nanosized titanium dioxide particles in the anatase crystal phase on human nasal mucosa cells. *Toxicol. Lett*. 195: 9–14.

Hagens, W. I., Oomen, A. G., de Jong, W. H., Cassee, F. R., and Sips, A. J. 2007. What do we (need to) know about the kinetic properties of nanoparticles in the body? *Regul. Toxicol. Pharmacol*. 49: 217–29.

Han, S. G., Andrews, R., and Gairola, C. G. 2010. Acute pulmonary response of mice to multi-wall carbon nanotubes. *Inhal. Toxicol*. 22: 340–47.

Hardman, R. 2006. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect*. 114: 165–72.

Heinrich, U. F. R., Rittinghausen, S., Creutzenberg, O., Bellmann, B., Koch, W., and Levsen, K. 1995. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. *Inhal. Toxicol*. 7: 533–56.

Hillyer, J. F., and Albrecht, R. M. 2001. Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. *J. Pharm. Sci*. 90: 1927–36.

Ho, D., Garcia, D., and Ho, C. M. 2006. Nanomanufacturing and characterization modalities for bio-nano-informatics systems. *J. Nanosci. Nanotechnol*. 6: 875–91.

Hoet, P. H., Bruske-Hohlfeld, I., and Salata, O. V. 2004. Nanoparticles—Known and unknown health risks. *J. Nanobiotechnol*. 2: 12.

Hu, X., Cook, S., Wang, P., Hwang, H. M., Liu, X., and Williams, Q. L. 2010. In vitro evaluation of cytotoxicity of engineered carbon nanotubes in selected human cell lines. *Sci. Total Environ*. 408: 1812–17.

Huang, S., Chueh, P. J., Lin, Y. W., Shih, T. S., and Chuang, S. M. 2009. Disturbed mitotic progression and genome segregation are involved in cell transformation mediated by nano-TiO_2 long-term exposure. *Toxicol. Appl. Pharmacol*. 241: 182–94.

Hussain, N., Jaitley, V., and Florence, A. T. 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Adv. Drug Deliv. Rev*. 50: 107–42.

Inoue, K., Takano, H., Yanagisawa, R., Hirano, S., Sakurai, M., Shimada, A., and Yoshikawa, T. 2006. Effects of airway exposure to nanoparticles on lung inflammation induced by bacterial endotoxin in mice. *Environ. Health Perspect*. 114: 1325–30.

Jacobsen, N. R., Moller, P., Jensen, K. A., Vogel, U., Ladefoged, O., Loft, S., and Wallin, H. 2009. Lung inflammation and genotoxicity following pulmonary exposure to nanoparticles in ApoE-/- mice. *Part. Fibre Toxicol*. 6: 2.

Jacobsen, N. R., Pojana, G., White, P., Moller, P., Cohn, C. A., Korsholm, K. S., Vogel, U., Marcomini, A., Loft, S., and Wallin, H. 2008. Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and $C^{(60)}$ fullerenes in the FE1-Mutatrade markMouse lung epithelial cells. *Environ. Mol. Mutagen*. 49: 476–87.

Janes, K. A., Fresneau, M. P., Marazuela, A., Fabra, A., and Alonso, M. J. 2001. Chitosan nanoparticles as delivery systems for doxorubicin. *J. Controlled Release* 73: 255–67.

Jani, P., Halbert, G. W., Langridge, J., and Florence, A. T. 1990. Nanoparticle uptake by the rat gastrointestinal mucosa: Quantitation and particle size dependency. *J. Pharm. Pharmacol*. 42: 821–26.

Javier, D., Nitin, N., Roblye, D., and Richards-Kortumd, R. 2008. Metal-based nanorods as molecule-specific contrast agents for reflectance imaging in 3D tissues. *Nanophotonics* 2: 23506.

Jin, Y., Kannan, S., Wu, M., and Zhao, J. X. 2007. Toxicity of luminescent silica nanoparticles to living cells. *Chem. Res. Toxicol*. 20: 1126–33.

John, S., Marpu, S., Li, J., Omary, M., Hu, Z., Fujita, Y., and Neogi, A. 2010. Hybrid zinc oxide nanoparticles for biophotonics. *J. Nanosci. Nanotechnol*. 10: 1707–12.

Kagan, V. E., Konduru, N. V., Feng, W., Allen, B. L., Conroy, J., Volkov, Y., Vlasova, II, Belikova, N. A., Yanamala, N., Kapralov, A., Tyurina, Y. Y., Shi, J., Kisin, E. R., Murray, A. R„ Franks, J., Stolz, D., Gou, P., Klein-Seetharaman, J. Fadeel, B., Star, A., and Shvedova, A. A. 2010. Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. *Nat. Nanotechnol*. 5: 354–59.

Kaida, T., Kobayashi, K., Adachi, M., and Suzuki, F. 2004. Optical characteristics of titanium oxide interference film and the film laminated with oxides and their applications for cosmetics. *J. Cosmet. Sci*. 55: 219–20.

Kalita., S. J., Bhardwaja., A., and Bhatta, H. A. 2007. Nanocrystalline calcium phosphate

ceramics in biomedical engineering. *Mater. Sci. Eng. C* 27: 441–49.

Kang, S. J., Kim, B. M., Lee, Y. J., and Chung, H. W. 2008. Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. *Environ. Mol. Mutagen.* 49: 399–405.

Karlsson, H. L., Cronholm, P., Gustafsson, J., and Moller, L. 2008. Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. *Chem. Res. Toxicol.* 21: 1726–32.

Karlsson, H. L., Gustafsson, J., Cronholm, P., and Moller, L. 2009. Size-dependent toxicity of metal oxide particles—A comparison between nano- and micrometer size. *Toxicol. Lett.* 188: 112–18.

Katiyar, A., Yadav, S., Smirniotis, P. G., and Pinto, N. G. 2006. Synthesis of ordered large pore SBA-15 spherical particles for adsorption of biomolecules. *J. Chromatogr. A* 1122: 13–20.

Kawata, K., Osawa, M., and Okabe, S. 2009. In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. *Environ. Sci. Technol.* 43: 6046–51.

Ken, T., Ken-ichiro, S., Aki, I., Miyoko, K.-I., Rie, F., Masako, T., Shigeru, O., Yoshimasa, N., Tomomi, I., and Masao, S. 2009. Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. *Health Sci.* 55: 95–102.

Kim, I. S., Baek, M., and Choi, S. J. 2010a. Comparative cytotoxicity of Al_2O_3, CeO_2, TiO_2 and ZnO nanoparticles to human lung cells. *J. Nanosci. Nanotechnol.* 10: 3453–58.

Kim, S., Seong, K., Kim, O., Seo, H., Lee, M., Khang, G., and Lee, D. 2010b. Polyoxalate nanoparticles as a biodegradable and biocompatible drug delivery vehicle. *Biomacromolecules* 11: 555–60.

Kim, J.- E., Lim, H.-T., Minai -Tehrani, A., Kwon, J.-T., Shin, J.-Y., Woo, C.- G., Choi, M., Back, J. Jeong, D. J., Ha, Y-C., Chae, C-H., Song, K.- S., Ahn, K.-H., Lee, J.,- H., Sung, H.- J., Yu, I.- J., Beck, G. R, and Cho, M.- H. 2010c. Toxicity and clearence of intratracheally administrered multiwalled carbon nanotubes from muine lung. *J. Toxicol Environ. Health A* 73: 1530–43.

Kim, W. Y., Kim, J., Park, J. D., Ryu, H. Y., and Yu, I. J. 2009. Histological study of gender differences in accumulation of silver nanoparticles in kidneys of Fischer 344 rats. *J. Toxicol. Environ. Health A* 72: 1279–84.

Kim, Y. S., Kim, J. S., Cho, H. S., Rha, D. S., Kim, J. M., Park, J. D., Choi, B. S., Lim, R., Chang, H. K., Chung, Y. H., Kwon, I. H., Jeong, J., Han, B. S., and Yu, I. J. 2008. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal. Toxicol.* 20: 575–83.

Kisin, E. R., Murray, A. R., Keane, M. J., Shi, X. C., Schwegler-Berry, D., Gorelik, O., Arepalli, S., Castranova, V., Wallace, W. E., Kagan, V. E., and Shvedova, A. A. 2007. Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells. *J. Toxicol. Environ. Health A* 70: 2071–79.

Kisin, E. R., Murray, A. R., Sargent, L., Lowry, D., Chirila, M., Siegrist, K. J., Schwegler-Berry, D., Leonard, S., Castranova, V., Fadeel, B., Kagan, V. E., and Shvedova, A. A. 2011. Genotoxicity of carbon nanofibers: Are they potentially more or less dangerous than carbon nanotubes or asbestos? *Toxicol. Appl. Pharmacol.* 252: 1–10.

Kohli, A. K., and Alpar, H. O. 2004. Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge. *Int. J. Pharm.* 275: 13–17.

Kolosnjaj, J., Szwarc, H., and Moussa, F. 2007. Toxicity studies of fullerenes and derivatives. *Adv. Exp. Med. Biol.* 620: 168–80.

Koyama, S., Haniu, H., Osaka, K., Koyama, H., Kuroiwa, N., Endo, M., Kim, Y. A., and Hayashi, T. 2006. Medical application of carbon-nanotube-filled nanocomposites: The microcatheter. *Small* 2: 1406–11.

Kreyling, W. G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Oberdorster, G., and Ziesenis, A. 2002. Minute translocation of inhaled ultrafine insoluble iridium particles from

lung epithelium to extrapulmonary tissues. *J. Toxicol. Environ. Health A* 65: 1513–30.

Kumari, M., Mukherjee, A., and Chandrasekaran, N. 2009. Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci. Total Environ.* 407: 5243–46.

Landsiedel, R., Kapp, M. D., Schulz, M., Wiench, K., and Oesch, F. 2009. Genotoxicity investigations on nanomaterials: Methods, preparation and characterization of test material, potential artifacts and limitations—Many questions, some answers. *Mutat. Res.* 681: 241–58.

Landsiedel, R., Ma-Hock, L., Van Ravenzwaay, B., Schulz, M., Wiench, K., Champ, S., Schulte, S., Wohlleben, W., and Oesch, F. 2010. Gene toxicity studies on titanium dioxide and zinc oxide nanomaterials used for UV-protection in cosmetic formulations. *Nanotoxicology* 4: 364–81.

Lam, C. W., James, J. T., McCluskey, R., and Hunter, R. L. 2004. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol. Sci.* 77: 126–134.

Lasagna-Reeves, C., Gonzalez-Romero, D., Barria, M. A., Olmedo, I., Clos, A., Sadagopa Ramanujam, V. M., Urayama, A., Vergara, L., Kogan, M. J., and Soto, C. 2010. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. *Biochem. Biophys. Res. Commun.* 393: 649–55.

LeBlanc, A. J., Crumpston, J. L., Chen, B. T., Frazer, D., Castranova, V., and Nurkiewicz, T. R. 2009. Nanoparticle inhalation impairs endothelium-dependent vasodilation in subepicardial arterioles. *J. Toxicol. Environ. Health A* 72: 1576–84.

Lee, H. J., Yeo, S. Y., and Jeong, S. H. 2003. Antibacterial effect of nanosized silver colloidal solution on textile fabrics. *J. Mater. Sci.* 38: 2199–204.

Lee, K. J., Nallathamby, P. D., Browning, L. M., Osgood, C. J., and Xu, X. H. 2007. *In vivo* imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS Nano* 1: 133–43.

Lee, K. P., Trochimowicz, H. J., and Reinhardt, C. F. 1985. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. *Toxicol. Appl. Pharmacol.* 79: 179–92.

LeGeros, R. Z. 1993. Biodegradation and bioresorption of calcium phosphate ceramics. *Clin. Mater.* 14: 65–88.

Levi, N., Hantgan, R. R., Lively, M. O., Carroll, D. L., and Prasad, G. L. 2006. C60-fullerenes: Detection of intracellular photoluminescence and lack of cytotoxic effects. *J. Nanobiotechnol.* 4: 14.

Liang, F., and Chen, B. 2010. A review on biomedical applications of single-walled carbon nanotubes. *Curr. Med. Chem.* 17: 10–24.

Linkov, I., Satterstrom, F. K., and Corey, L. M. 2008. Nanotoxicology and nanomedicine: making hard decisions. *Nanomedicine* 4: 167–71.

Liu, M., Zhang, H., and Slutsky, A. S. 2009a. Acute lung injury: A yellow card for engineered nanoparticles? *J. Mol. Cell Biol.* 1: 6–7.

Liu, Y., Gao, Y. X., Zhang, L. L., Wang, T. C., Wang, J. X., Jiao, F., Li, W., Liu, Y., Li, Y. F., Li, B., Chai, Z. F., Wu, G., and Chen, C. Y. 2009b. Potential health impact on mice after nasal instillation of nano-sized copper particles and their translocation in mice. *J. Nanosci. Nanotechnol.* 9: 6335–43.

Liu, Y., Miyoshi, H., and Nakamura, M. 2007. Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *Int. J. Cancer* 120: 2527–37.

Lomer, M. C., Thompson, R. P., and Powell, J. J. 2002. Fine and ultrafine particles of the diet: Influence on the mucosal immune response and association with Crohn's disease. *Proc. Nutr. Soc.* 61: 123–30.

Long, T. C., Tajuba, J., Sama, P., Saleh, N., Swartz, C., Parker, J., Hester, S., Lowry, G. V., and Veronesi, B. 2007. Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons in vitro. *Environ. Health Perspect.* 115: 1631–37.

Lopez-Moreno, M. L., de la Rosa, G., Hernandez-Viezcas, J. A., Castillo-Michel, H., Botez, C. E., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max)* plants. *Environ. Sci. Technol*. 44: 7315–20.

Losic, D., and Simovic, S. 2009. Self-ordered nanopore and nanotube platforms for drug delivery applications. *Expert Opin. Drug Deliv*. 6: 1363–81.

Maenosono, S., Suzuki, T., and Saita, S. 2007. Mutagenicity of water-soluble FePt nanoparticles in Ames test. *J. Toxicol. Sci*. 32: 575–79.

Ma-Hock, L., Treumann, S., Strauss, V., Brill, S., Luizi, F., Mertler, M., Wiench, K., Gamer, A. O., van Ravenzwaay, B., and Landsiedel, R. 2009. Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. *Toxicol. Sci*. 112: 468–81.

Mansour, H. M., Rhee, Y. S., and Wu, X. 2009. Nanomedicine in pulmonary delivery. *Int. J. Nanomedicine* 4: 299–319.

Maynard, A. D., Baron, P. A., Foley, M., Shvedova, A. A., Kisin, E. R., and Castranova, V. 2004. Exposure to carbon nanotube material: Aerosol release during the handling of unrefined single-walled carbon nanotube material. *J. Toxicol. Environ. Health A* 67: 87–107.

Mercer, R. R., Scabilloni, J., Wang, L., Kisin, E., Murray, A. R., Schwegler-Berry, D., Shvedova, A. A., and Castranova, V. 2008. Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single walled carbon nantoubes in a mouse model. *Am. J. Physiol. Lung Cell Mol. Physiol*. 294: L87–L97.

Mercer, R. R., Hubbs, A. F., Scabilloni, J. F., Wang, L., Battelli, L. A., Friend, S., Castranova, V., and Porter, D. W. 2011. Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes. *Part. Fibre Toxicol*. 8: 21.

Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., Sundaresan, G., Wu, A. M., Gambhir, S. S., and Weiss, S. 2005. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* 307: 538–44.

Moghimi, S. M., Hunter, A. C., and Murray, J. C. 2005. Nanomedicine: Current status and future prospects. *FASEB J*. 19: 311–30.

Mori, T., Takada, H., Ito, S., Matsubayashi, K., Miwa, N., and Sawaguchi, T. 2006. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. *Toxicology* 225: 48–54.

Mortelmans, K., and Zeiger, E. 2000. The Ames *Salmonella*/microsome mutagenicity assay. *Mutat. Res*. 455: 29–60.

Mrdanovic, J., Solajic, S., Bogdanovic, V., Stankov, K., Bogdanovic, G., and Djordjevic, A. 2009. Effects of fullerenol $C_{60}(OH)_{24}$ on the frequency of micronuclei and chromosome aberrations in CHO-K1 cells. *Mutat. Res*. 680: 25–30.

Mroz, R. M., Schins, R. P., Li, H., Drost, E. M., Macnee, W., and Donaldson, K. 2007. Nanoparticle carbon black driven DNA damage induces growth arrest and AP-1 and NFkappaB DNA binding in lung epithelial A549 cell line. *J. Physiol. Pharmacol*. 58(suppl. 5): 461–70.

Muller, J., Delos, M., Panin, N., Rabolli, V., Huaux, F., and Lison, D. 2009. Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. *Toxicol. Sci*. 110: 442–48.

Nagender, R. P., Pena-Mendez, E. M., and Havel, J. 2009. Gold and nano-gold in medicine: Overview, toxicology and perspectives. *J. Appl. Biomed*. 7: 75–91.

Naha, P. C., Bhattacharya, K., Tenuta, T., Dawson, K. A., Lynch, I., Gracia, A., Lyng, F. M., and Byrne, H. J. 2010. Intracellular localisation, geno- and cytotoxic response of polyN-isopropylacrylamide (PNIPAM) nanoparticles to human keratinocyte (HaCaT) and colon cells (SW 480). *Toxicol Lett*. 19: 134–43.

Nemmar, A., Hoet, P. H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M. F., Vanbilloen, H., Mortelmans, L., and Nemery, B. 2002a. Passage of inhaled

particles into the blood circulation in humans. *Circulation* 105: 411–14.

Nemmar, A., Hoylaerts, M. F., Hoet, P. H., Dinsdale, D., Smith, T., Xu, H., Vermylen, J., and Nemery, B. 2002b. Ultrafine particles affect experimental thrombosis in an in vivo hamster model. *Am. J. Respir. Crit. Care Med* 166: 998–1004.

Nemmar, A., Hoylaerts, M. F., Hoet, P. H., Vermylen, J., and Nemery, B. 2003. Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis. *Toxicol. Appl. Pharmacol.* 186: 38–45.

Nemmar, A., Vanbilloen, H., Hoylaerts, M. F., Hoet, P. H., Verbruggen, A., and Nemery, B. 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am. J. Respir. Crit. Care Med* 164: 1665–68.

Nishimori, H., Kondoh, M., Isoda, K., Tsunoda, S., Tsutsumi, Y., and Yagi, K. 2009. Silica nanoparticles as hepatotoxicants. *Eur. J. Pharm. Biopharm.* 72: 496–501.

National Insitute for Occupational Safety and Health. 2011. *Current intelligence bulletin 63: Occupational exposure to titanium dioxide*. DHHS (NIOSH) Publication number 2011-160. http://www.cdc.gov/niosh/docs/2011-160/

Nygaard, U. C., Hansen, J. S., Samuelsen, M., Alberg, T., Marioara, C. D., and Lovik, M. 2009. Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. *Toxicol. Sci.* 109: 113–23.

Oberdorster, G. 2010. Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. *J. Intern. Med.* 267: 89–105.

Oberdorster, G., Oberdorster, E., and Oberdorster, J. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.* 113: 823–39.

Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C. 2004. Translocation of inhaled ultrafine particles to the brain. *Inhal. Toxicol.* 16: 437–45.

Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., and Cox, C. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J. Toxicol. Environ. Health A* 65: 1531–43.

Ogawara, K., Yoshida, M., Furumoto, K., Takakura, Y., Hashida, M., Higaki, K., and Kimura, T. 1999. Uptake by hepatocytes and biliary excretion of intravenously administered polystyrene microspheres in rats. *J. Drug Target.* 7: 213–21.

Pauluhn, J. 2010. Multi-walled carbon nanotubes (Baytubes): Approach for derivation of occupational exposure limit. *Regul. Toxicol. Pharmacol.* 57: 78–89.

Panyam, J., and Labhasetwar, V. 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Deliv. Rev.* 55: 329–47.

Park, M. V., Annema, W., Salvati, A., Lesniak, A., Elsaesser, A., Barnes, C., McKerr, G., Howard, C. V., Lynch, I., Dawson, K. A., Piersma, A. H., and de Jong, W. H. 2009. In vitro developmental toxicity test detects inhibition of stem cell differentiation by silica nanoparticles. *Toxicol. Appl. Pharmacol.* 240: 108–16.

Partidos, C. D. 2003. Delivering vaccines into the skin without needles and syringes. *Expert Rev. Vaccines* 2: 753–61.

Patlolla, A., Knighten, B., and Tchounwou, P. 2010. Multi-walled carbon nanotubes induce cytotoxicity, genotoxicity and apoptosis in normal human dermal fibroblast cells. *Ethnicity Dis.* 20: S1-65–72.

Patton, J. S., and Byron, P. R. 2007. Inhaling medicines: delivering drugs to the body through the lungs. *Nat. Rev. Drug Discov.* 6: 67–74.

Paunesku, T., Ke, T., Dharmakumar, R., Mascheri, N., Wu, A., Lai, B., Vogt, S., Maser, J., Thurn, K., Szolc-Kowalska, B., Omary, R., Li, D., Lu, Z. R., and Woloschak, G. E. 2008. Gadolinium-conjugated TiO2-DNA oligonucleotide nanoconjugates show prolonged intracellular retention period and T1-weighted contrast enhancement in magnetic resonance images. *Nanomedicine* 4: 201–7.

Pelka, J., Gehrke, H., Esselen, M., Turk, M., Crone, M., Brase, S., Muller, T., Blank, H., Send, W., Zibat, V., Brenner, P., Schneider, R., Gerthsen, D., and Marko, D. 2009. Cellular uptake of platinum nanoparticles in human colon carcinoma cells and their impact on cellular redox systems and DNA integrity. *Chem. Res. Toxicol.* 22: 649–59.

Phillips, J. I., Green, F. Y., Davies, J. C., and Murray, J. 2010. Pulmonary and systemic toxicity following exposure to nickel nanoparticles. *Am. J. Ind. Med.* 53: 763–67.

Piddubnyak, V., Kurcok, P., Matuszowicz, A., Glowala, M., Fiszer-Kierzkowska, A., Jedlinski, Z., Juzwa, M., and Krawczyk, Z. 2004. Oligo-3-hydroxybutyrates as potential carriers for drug delivery. *Biomaterials* 25: 5271–79.

Pierscionek, B. K., Li, Y., Yasseen, A. A., Colhoun, L. M., Schachar, R. A., and Chen, W. 2010. Nanoceria have no genotoxic effect on human lens epithelial cells. *Nanotechnology* 21: 035102.

Pitard, B., Bello-Roufai, M., Lambert, O., Richard, P., Desigaux, L., Fernandes, S., Lanctin, C., Pollard, H., Zeghal, M., Rescan, P. Y., and Escande, D. 2004. Negatively charged self-assembling DNA/poloxamine nanospheres for in vivo gene transfer. *Nucleic Acids Res.* 32: e159.

Pokharkar, V., Dhar, S., Bhumkar, D., Mali, V., Bodhankar, S., and Prasad, B. L. 2009. Acute and subacute toxicity studies of chitosan reduced gold nanoparticles: a novel carrier for therapeutic agents. *J. Biomed. Nanotechnol.* 5: 233–39.

Ponti, J., Sabbioni, E., Munaro, B., Broggi, F., Marmorato, P., Franchini, F., Colognato, R., and Rossi, F. 2009. Genotoxicity and morphological transformation induced by cobalt nanoparticles and cobalt chloride: An in vitro study in Balb/3T3 mouse fibroblasts. *Mutagenesis* 24: 439–45.

Pope, C. A. III, Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., and Thurston, G. D. 2002. Lung cancer, cardiopulmonary motality, and long-term exposure to fine particulate air pollution. *J. Am. Med. Assoc.* 287: 1132–41.

Porter, D. W., Hubbs, A., Mercer, R., Wu, N., Wolfarth, M. G., Sriram, K., Leonard, S., Battelli, L., Schwegler-Berry, D., Friend, S., Andrew, M., Chen, B. T., Tsuruoko, S., Endo, M., and Castranova, V. 2010. Mouse pulmonary dose- and time course-response induced by exposure to multi-walled carbon nanotubes. *Toxicology* 269: 136–147.

Pott, F., and Roller, M. 2005. Carcinogenicity study with nineteen granular dusts in rats. *Eur. J. Oncol.* 10: 249–82.

Prausnitz, M. R., Mitragotri, S., and Langer, R. 2004. Current status and future potential of transdermal drug delivery. *Nat. Rev. Drug Discov.* 3: 115–24.

Price, H., Arthur, R., Sexton, K., Gregory, C., Hoogendoorn, B., Matthews, I., Jones, T., and BeruBe, K. 2010. Airborne particles in Swansea, UK: Their collection and characterization. *J. Toxicol. Environ. Health A* 73: 355–67.

Rahman, Q., Lohani, M., Dopp, E., Pemsel, H., Jonas, L., Weiss, D. G., and Schiffmann, D. 2002. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. *Environ. Health Perspect.* 110: 797–800.

Rao, G. V., Kumar, G. S., and Ahuja, Y. R. 1997. Single cell gel electrophoresis on peripheral blood leukocytes of patients with oral squamous cell carcinoma. *J. Oral Pathol. Med.* 26: 377–80.

Ravichandran, P., Periyakaruppan, A., Sadanandan, B., Ramesh, V., Hall, J. C., Jejelowo, O., and Ramesh, G. T. 2009. Induction of apoptosis in rat lung epithelial cells by multiwalled carbon nanotubes. *J. Biochem. Mol. Toxicol.* 23: 333–44.

Rittinghausen, S., Mohr, U., and Dungworth, D. L. 1997. Pulmonary cystic keratinizing squamous cell lesions of rats after inhalation/instillation of different particles. *Exp. Toxicol. Pathol.* 49: 433–46.

Roberts, J. R., Chapman, R. S., Young, S., Kenyon, A., Schwegler-Berry, D., Stefaniak, A. B., Chen, B. T., and Antonini, J. M. 2011. Pulmonary toxicity following intratracheal instillation of dispersed silver nanoparticles in rats. *Toxicologist* 120: A1759.

Roller, M. 2009. Carcinogenicity of inhaled nanoparticles. *Inhal. Toxicol.* 21(suppl. 1): 144–57.

Rothen-Rutishauser, B. M., Schurch, S., Haenni, B., Kapp, N., and Gehr, P. 2006. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. *Environ. Sci. Technol.* 40: 4353–59.

Rouse, J. G., Yang, J., Ryman-Rasmussen, J. P., Barron, A. R., and Monteiro-Riviere, N. A. 2007. Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. *Nano Lett.* 7: 155–60.

Ryman-Rasmussen, J. P., Riviere, J. E., and Monteiro-Riviere, N. A. 2006. Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicol. Sci.* 91: 159–65.

Sabbioni, E., Fortaner, S., Bosisio, S., Farina, M., Del Torchio, R., Edel, J., and Fischbach, M. 2010. Metabolic fate of ultratrace levels of GeCl(4) in the rat and in vitro studies on its basal cytotoxicity and carcinogenic potential in Balb/3T3 and HaCaT cell lines dagger. *J. Appl. Toxicol.* 30: 34–41.

Sadrieh, N., Wokovich, A. M., Gopee, N. V., Zheng, J., Haines, D., Parmiter, D., Siitonen, P. H., Cozart, C. R., Patri, A. K., McNeil, S. E., Howard, P. C., Doub, W. H., and Buhse, L. F. 2010. Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO_2 particles. *Toxicol. Sci.* 115: 156–66.

Sahoo, S. K., and Labhasetwar, V. 2003. Nanotech approaches to drug delivery and imaging. *Drug Discov. Today* 8: 1112–1120.

Sajja, H. K., East, M. P., Mao, H., Wang, Y. A., Nie, S., and Yang, L. 2009. Development of multifunctional nanoparticles for targeted drug delivery and noninvasive imaging of therapeutic effect. *Curr. Drug Discov. Technol.* 6: 43–51.

Sakamoto, Y., Nakae, D., Fukumori, N., Tayama, K., Maekawa, A., Imai, K., Hirose, A., Nishimura, T., Ohashi, N., and Ogata, A. 2009. Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. *J. Toxicol. Sci.* 34: 65–76.

Samet, J. M., Dominici, F., Carriero, F. C., Coursac, I., and Zeger, S. L. 2000. Fine particulate air pollution and mortality in 20 U.S. Cities, 1987–1994. *N. Engl. J. Med.* 343: 1742–1749.

Sarlo, K., Blackburn, K. L., Clark, E. D., Grothaus, J., Chaney, J., Neu, S., Flood, J., Abbott, D., Bohne, C., Casey, K., et al. 2009. Tissue distribution of 20 nm, 100 nm and 1000 nm fluorescent polystyrene latex nanospheres following acute systemic or acute and repeat airway exposure in the rat. *Toxicology* 263: 117–26.

Schipper, M. L., Nakayama-Ratchford, N., Davis, C. R., Kam, N. W., Chu, P., Liu, Z., Sun, X., Dai, H., and Gambhir, S. S. 2008. A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. *Nat. Nanotechnol.* 3: 216–21.

Schulz, J., Hohenberg, H., Pflucker, F., Gartner, E., Will, T., Pfeiffer, S., Wepf, R., Wendel, V., Gers-Barlag, H., and Wittern, K. P. 2002. Distribution of sunscreens on skin. *Adv. Drug Deliv. Rev.* 54(suppl. 1): S157–S163.

Schwarz, F., Bieling, K., Latz, T., Nuesry, E., and Becker, J. 2006. Healing of intrabony periimplantitis defects following application of a nanocrystalline hydroxyapatite (Ostim) or a bovine-derived xenograft (Bio-Oss) in combination with a collagen membrane (Bio-Gide). A case series. *J. Clin. Periodontol.* 33: 491–99.

Schwarz, F., Sahm, N., Bieling, K., and Becker, J. 2009. Surgical regenerative treatment of peri-implantitis lesions using a nanocrystalline hydroxyapatite or a natural bone mineral in combination with a collagen membrane: A four-year clinical follow-up report. *J. Clin. Periodontol.* 36: 807–14.

Scott-Fordsmand, J. J., Krogh, P. H., Schaefer, M., and Johansen, A. 2008. The toxicity testing of double-walled nanotubes-contaminated food to *Eisenia veneta* earthworms. *Ecotoxicol. Environ. Safety* 71: 616–19.

Scuri, M., Chen, B. T., Castranova, V., Reynolds, J. S., Johnson, V. J., Samsell, L., Watson, C.,

and Piedimonte, G. 2010. Effects of titanium dioxide nanoparticle exposure on neuro immune responses in rat airways. *J. Toxicol Enviorn Health A* 73: 1353–69.

Semete, B., Booysen, L., Lemmer, Y., Kalombo, L., Katata, L., Verschoor, J., and Swai, H. S. 2010. In vivo evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems. *Nanomedicine* 6: 662–71.

Sharma, V., Shukla, R. K., Saxena, N., Parmar, D., Das, M., and Dhawan, A. 2009. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicol. Lett.* 185: 211–18.

Shavandi, Z., Ghazanfari, T., and Moghaddam, K. N. 2010. In vitro toxicity of silver nanoparticles on murine peritoneal macrophages. *Immunopharmacol. Immunotoxicol.* doi: 10.3109/08923973.2010.487489.

Shen, X. S., Wang, G. Z., Hong, X., and Zhu, W. 2009. Nanospheres of silver nanoparticles: Agglomeration, surface morphology control and application as SERS substrates. *Phys. Chem. Chem. Phys.* 11: 7450–54.

Shi, X., Castranova, V., Halliwell, B, and Vallyathan, V. 1998. Reactive oxygen species and silica - induced carcinogenesis. *J. Toxicol. Environ. Health B* 1: 181–97.

Shi, Y., Yadav, S., Wang, F., and Wang, H. 2010. Endotoxin promotes adverse effects of amorphous silica nanoparticles on lung epithelial cells in vitro. *J. Toxicol. Environ. Health A* 73: 748–56.

Shimizu, M., Tainaka, H., Oba, T., Mizuo, K., Umezawa, M., and Takeda, K. 2009. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. *Part. Fibre Toxicol.* 6: 20.

Shinohara, N., Matsumoto, K., Endoh, S., Maru, J., and Nakanishi, J. 2009. In vitro and in vivo genotoxicity tests on fullerene C^{60} nanoparticles. *Toxicol. Lett.* 191: 289–96.

Shvedova, A. A., Castranova, V., Kisin, E. R., Schwegler-Berry, D., Murray, A. R., Gandelsman, V. Z., Maynard, A., and Baron, P. 2003. Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J. Toxicol. Environ. Health A* 66: 1909–1926.

Shvedova, A. A., Kisin, E. R. Mercer, R., Murray, A. R., Johnson, V. J., Potapovich, A. F., Tyurina, Y. Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A. F., Antonini, J., Evans, D. E., Ku, B.-K., Ramsey, D., Maynard, A., Kagan, V. E., Castranova, V., and Baron, P. 2005. Unusual inflammatory and fibrogenic pulmonary, response to single walled carbon nanotubes in mice. *Am. J. Physiol. Lung Cell Mol. Physiol.* 289: L698–L708.

Shvedova, A. A., and Kagan, V. E. 2010. The role of nanotoxicology in realizing the 'helping without harm' paradigm of nanomedicine: Lessons from studies of pulmonary effects of single-walled carbon nanotubes. *J. Intern. Med.* 267: 106–18.

Shvedova, A. A., Kisin, E., Murray, A. R., Johnson, V. J., Gorelik, O., Arepalli, S., Hubbs, A. F., Mercer, R. R., Keohavong, P., Sussman, N. Jin, J., Yin, J., Stone, S., Chen, B., T., Deye, G., Maynard, A., Castranova, V., Baron, P. A., and Kagan, V. E. 2008a. Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: Inflammation, fibrosis, oxidative stress, and mutagenesis. *Am. J. Physiol. Lung Cell Mol. Physiol.* 295: L552–L565.

Shvedova, A. A., Fabisiak, J. P., Kisin, E. R., Murray, A. R., Roberts, J. R., Antonini, J. A., Kommineni, C., Reynolds, J., Barchowsky, A., Castranova, V., and Kagan, V. 2008b. Sequential exposure to carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. *Am. J. Respir. Cell Mol. Biol.* 38: 579–90.

Shvedova, A. A., Kisin, E. R., Porter, D., Schulte, P., Kagan, V. E., Fadeel, B., and Castranova, V. 2009. Mechanisms of toxicity and medical applications of carbon nanotubes: Two faces of Janus? *J. Pharmacol. Ther.* 121: 192–204.

Stueckle, T. A., Mishra, A., Derk, R., Rojanasakul, Y., Castranova, V., and Wang, L. 2011. In vitro assessment of potential tumorgenicity of chronic SWCNT and

MWCNT exposure to lung epithelium. *Toxicologist* 120: A1182.

Sudimack, J., and Lee, R. J. 2000. Targeted drug delivery via the folate receptor. *Adv. Drug Deliv. Rev.* 41: 147–62.

Sumner, S. C., Fennell, T. R., Snyder, R. W., Taylor, G. F., and Lewin, A. H. 2010. Distribution of carbon-14 labeled C_{60} ($[^{14}C]C_{60}$) in the pregnant and in the lactating dam and the effect of C_{60} exposure on the biochemical profile of urine. *J. Appl. Toxicol.* 30: 354–60.

Tabet, L., Bussy, C., Amara, N., Setyan, A., Grodet, A., Rossi, M. J., Pairon, J. C., Boczkowski, J., and Lanone, S. 2009. Adverse effects of industrial multiwalled carbon nanotubes on human pulmonary cells. *J. Toxicol. Environ. Health A* 72: 60–73.

Takagi, A., Hirose, A., Nishimura, T., Fukumori, N., Ogata, A., Ohashi, N., Kitajima, S., and Kanno, J. 2008. Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. *J. Toxicol. Sci.* 33: 105–16.

Tang, X., Choo, E. S., Li, L., Ding, J., and Xue, J. 2009. One-pot synthesis of water-stable ZnO nanoparticles via a polyol hydrolysis route and their cell labeling applications. *Langmuir* 25: 5271–75.

Teli, M. K., Mutalik, S., and Rajanikant, G. K. 2010. Nanotechnology and nanomedicine: Going small means aiming big. *Curr. Pharm. Des.* 16: 1882–92.

Thian, E. S., Huang, J., Best, S. M., Barber, Z. H., Brooks, R. A., Rushton, N., and Bonfield, W. 2006. The response of osteoblasts to nanocrystalline silicon-substituted hydroxyapatite thin films. *Biomaterials* 27: 2692–98.

Ting, G., Chang, C. H., and Wang, H. E. 2009. Cancer nanotargeted radiopharmaceuticals for tumor imaging and therapy. *Anticancer Res.* 29: 4107–18.

Tinkle, S. S., Antonini, J. M., Rich, B. A., Roberts, J. R., Salmen, R., DePree, K., and Adkins, E. J. 2003. Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ. Health Perspect.* 111: 1202–8.

Tsuchiya, T., Oguri, I., Yamakoshi, Y. N., and Miyata, N. 1996. Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo. *FEBS Lett.* 393: 139–45.

Tsuda, H., Xu, J., Sakai, Y., Futakuchi, M., and Fukamachi, K. 2009. Toxicology of engineered nanomaterials—A review of carcinogenic potential. *Asian Pacific J. Cancer Prev.* 10: 975–80.

Tsuji, J. S., Maynard, A. D., Howard, P. C., James, J. T., Lam, C. W., Warheit, D. B., and Santamaria, A. B. 2006. Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles. *Toxicol. Sci.* 89: 42–50.

Vermylen, J., Nemmar, A., Nemery, B., and Hoylaerts, M. F. 2005. Ambient air pollution and acute myocardial infarction. *J. Thromb. Haemost.* 3: 1955–61.

Videira, M. A., Gano, L., Santos, C., Neves, M., and Almeida, A. J. 2006. Lymphatic uptake of lipid nanoparticles following endotracheal administration. *J. Microencapsul.* 23: 855–62.

Wahab, R., Mishra, A., Yun, S. I., Kim, Y. S., and Shin, H. S. 2010. Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. *Appl. Microbiol. Biotechnol.* 87: 1917–25.

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

Wang, J. J., Sanderson, B. J., and Wang, H. 2007. Cyto- and genotoxicity of ultrafine TiO2 particles in cultured human lymphoblastoid cells. *Mutat. Res.* 628: 99–106.

Wang, L., Mercer, R. R., Rojanasakul, Y., Qiu, A., Lu, Y., Scabilloni, J. F., Wu, N., and Castranova, V. 2010. Direct fibrogenic effects of dispersed single-walled carbon nanotubes on human lung fibroblasts. *J. Toxicol. Environ. Health A* 73: 410–22.

Wang, M., and Thanou, M. 2010. Targeting nanoparticles to cancer. *Pharmacol. Res.* 62: 90–99.

Warheit, D. B., Laurence, B. R., Reed, K. L., Roach, D. H., Reynolds, G. A., and Webb, T. R. 2004. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol. Sci.* 77: 117–25.

Wiench, K., Wohlleben, W., Hisgen, V., Radke, K., Salinas, E., Zok, S., and Landsiedel, R. 2009. Acute and chronic effects of nano- and non-nano-scale TiO(2) and ZnO particles on mobility and reproduction of the freshwater invertebrate *Daphnia magna*. *Chemosphere* 76: 1356–65.

Williams, J. H., Schray, R. C., Sirsi, S. R., and Lutz, G. J. 2008. Nanopolymers improve delivery of exon skipping oligonucleotides and concomitant dystrophin expression in skeletal muscle of mdx mice. *BMC Biotechnol.* 8: 35.

Wirnitzer, U., Herbold, B., Voetz, M., and Ragot, J. 2009. Studies on the in vitro genotoxicity of baytubes, agglomerates of engineered multi-walled carbon-nanotubes (MWCNT). *Toxicol. Lett.* 186: 160–65.

Wise, J. P., Sr., Goodale, B. C., Wise, S. S., Craig, G. A., Pongan, A. F., Walter, R. B., Thompson, W. D., Ng, A. K., Aboueissa, A. M., Mitani, H., Spalding, M. J., and Mason, M. D. 2010. Silver nanospheres are cytotoxic and genotoxic to fish cells. *Aquat. Toxicol.* 97: 34–41.

Wiwanitkit, V., Sereemaspun, A., and Rojanathanes, R. 2009. Effect of gold nanoparticles on spermatozoa: The first world report. *Fertil. Steril.* 91: e7–e8.

Wolf, R., Matz, H., Orion, E., and Lipozencic, J. 2003. Sunscreens—The ultimate cosmetic. *Acta Dermatovenerol. Croat.* 11: 158–62.

Wolfarth, M. G., McKinney, W., Chen, B. T., Castranova, V., and Porter, D. W. 2011. Acute pulmonary responses to MWCNT inhalation. *Toxicologist* 120: A53.

Xia, T., Zhao, Y., Sager, T., George, S., Pokhrel, S., Li, N., Schoenfield, D., Meng, H., Lin, S., Wang, X., Wang, M., Ji, Z., Zink, J. I., Madler, L., Castranova, V., and Nel, A. E. 2011. Decreased dissolution of ZnO by iron doping yields nanoparticles with reduced toxicity in the rodent lung and zebrafish embryos. *ACS Nano* 5: 1223–35.

Xiang, D., Ma, Y., Zhao, B., and Chen, M. 2006. Study on surface quality of nano ZrO2 ceramics in grinding by the aid of ultrasonic vibration. *Key Eng. Mater.* 190: 315–16.

Xu, A., Chai, Y., Nohmi, T., and Hei, T. K. 2009. Genotoxic responses to titanium dioxide nanoparticles and fullerene in gpt delta transgenic MEF cells. *Part. Fibre Toxicol.* 6: 3.

Ye, X., and Yang, D. 2009. Recent advances in biological strategies for targeted drug delivery. *Cardiovasc. Hematol. Disord. Drug Targets* 9: 206–21.

Yoshida, R., Kitamura, D., and Maenosono, S. 2009. Mutagenicity of water-soluble ZnO nanoparticles in Ames test. *J. Toxicol. Sci.* 34: 119–22.

Yoshida, S., Hiyoshi, K., Oshio, S., Takano, H., Takeda, K., and Ichinose, T. 2010. Effects of fetal exposure to carbon nanoparticles on reproductive function in male offspring. *Fertil. Steril.* 93: 1695–99.

Yudoh, K., Karasawa, R., Masuko, K., and Kato, T. 2009. Water-soluble fullerene (C60) inhibits the osteoclast differentiation and bone destruction in arthritis. *Int. J. Nanomed.* 4: 233–39.

Zakharenko, L. P., Zakharov, I. K., Vasiunina, E. A., Karamysheva, T. V., Danilenko, A. M., and Nikiforov, A. A. 1997. Determination of the genotoxicity of fullerene C60 and fullerol using the method of somatic mosaics on cells of *Drosophila melanogaster* wing and SOS-chromotest. *Genetika* 33: 405–9.

Zelikin, A. N. 2010. Drug releasing polymer thin films: new era of surface-mediated drug delivery. *ACS Nano* 4: 2494–509.

Zhao, J., Bowman, L., Zhang, X., Vallyathan, V., Young, S. H., Castranova, V., and Ding, M. 2009a. Titanium dioxide (TiO_2) nanoparticles induce JB6 cell apoptosis through activation of the caspase-8/Bid and mitochondrial pathways. *J. Toxicol. Environ. Health A* 72: 1141–49.

Zhao, J., Shi, X., Castranova, V., and Ding, M. 2009b. Occupational toxicology of nickel and nickel compounds. *J. Environ. Pathol. Toxicol. Oncol*. 28: 177–208.

Zheng, J., Wu, X., Wang, M., Ran, D., Xu, W., and Yang, J. 2008. Study on the interaction between silver nanoparticles and nucleic acids in the presence of cetyltrimethylammonium bromide and its analytical application. *Talanta* 74: 526–32.

Zhou, W., Gao, P., Shao, L., Caruntu, D., Yu, M., Chen, J., and O'Connor, C. J. 2005. Drug-loaded, magnetic, hollow silica nanocomposites for nanomedicine. *Nanomedicine* 1: 233–37.

Zhu, M. T., Feng, W. Y., Wang, Y., Wang, B., Wang, M., Ouyang, H., Zhao, Y. L., and Chai, Z. F. 2009. Particokinetics and extrapulmonary translocation of intratracheally instilled ferric oxide nanoparticles in rats and the potential health risk assessment. *Toxicol. Sci.* 107: 342–51.

Zhu, X., Chang, Y., and Chen, Y. 2010. Toxicity and bioaccumulation of TiO_2 nanoparticle aggregates in *Daphnia magna*. *Chemosphere* 78: 209–15.