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Subchronic and chronic toxicity evaluation of inorganic nanoparticles for delivery applications

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ABSTRACT

Inorganic nanoparticles provide the opportunity to localize bioactive agents to the target sites and protect them from degradation. In many cases, acute toxicities of inorganic nanoparticles used for delivery applications have been investigated. However, little information is available regarding the long-term toxicity of such materials. This review focuses on the importance of subchronic and chronic toxicity assessment of inorganic nanoparticles investigated for delivery applications. We have attempted to provide a comprehensive review of the available literature for chronic toxicity assessment of inorganic nanoparticles. Where possible correlations are made between particle composition, physiochemical properties, duration, frequency and route of administration, as well as the sex of animals, with tissue and blood toxicity, immunotoxicity and genotoxicity. A critical gap analysis is provided and important factors that need to be considered for long-term toxicology of inorganic nanoparticles are discussed.

Contents

Abbreviations: Ag, silver; Al, aluminum; Al_2O_3, aluminum oxide; ALP, alkaline phosphatase; ApoE, apolipoprotein E knockout; AST, aspartate aminotransferase; ATC, acute toxic category; Au, gold; Cd, cadmium; CeO_2, cerium oxide; Co, cobalt; Cu, copper; EMA, European Medicine Agency; ERK, extracellular signal-regulated kinase; F, female; FDA, Food and Drug Aadministration; FDP, fixed dose procedure; Fe, iron, Fe_2O_3, iron oxide; Fe_3O_4, magnetite; HMGB1, high mobility group box 1; IFNγ, interferon γ; PEG, poly(ethylene glycol); IIMIs, innate immunity modulating impurities; IL, interleukin; LD50, 50% lethal dose; M, male; MTD, maximum tolerated dose; NCTR, National Center for Toxicological Research; NIEHS, National Institute of Environmental Health Sciences; NIOSH, National Institute for Occupational Safety and Health; NM-202, nanostructured silica; NK, natural killer; NNI, National Nanotechnology Initiative; NOEL, no observable effect limit; NPs, nanoparticles; NTP, National Toxicity Program; OECD, Organization for Economic Cooperation and Development; Pb, lead; rhTNF, recombinant human tumor necrosis factor; ROS, reactive oxygen species; SAS, synthetic amorphous silica nanoparticles; SiO_2, silicon dioxide; SNPs, silica nanoparticles; SPION, superparamagnetic iron oxide nanoparticles; TiO_2, titanium oxide; TLR9, toll-like receptor 9; UDP, up-and-down method; Zn, zinc; ZnO, zinc oxide..

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1. Introduction

Investments in nanotechnology research and development have made an impact on many aspects of medicine, including drug and gene delivery, development of new and improved imaging agents and clinical diagnostics as well as other medical devices. A growing number of these technologies are in the pipeline, and many of them are on the market. The commercialization of nanomaterials is faster than the time it takes to assess their risk on human health and the environment. For this reason, a significant effort is dedicated to research in the area of environmental and health safety of nanotechnology [1]. Several nanoparticle-based delivery systems have reached clinical trials [2]. It takes several years following the creation of new nanoparticles or conceptualization of new uses of existing nanoparticles for the researchers to step back and carefully observe the effect that the physicochemical properties of nanoparticles may have on their applications and to become more realistic regarding the efficacy/toxicity ratio of nanoparticles. While progress has been made in this area, especially in the past decade, the limited information regarding long-term safety of nanomaterials is one of the significant barriers for using nanoparticles in medical applications and as delivery systems [3].

Nanomedicines that have been approved by the regulatory bodies and those that are in clinical trials were reviewed earlier (see Table 1 in [4]). The FDA (Food and Drug Administration) and EMA (European Medicine Agency) approved 51 and 48 nanomedicines or imaging agents based on nanoparticles, respectively, by the year 2016 with some others in clinical trials [5]. The majority of nanoparticles approved by the FDA for medical usage are organic nanomaterials and include liposomal and polymeric systems [4]. Although inorganic nanoparticles have been well represented in a wide range of the fields of research, there are only a few examples of inorganic nanoparticles being investigated in clinical trials and even fewer approved by the FDA [4]. During the second decade of this century, the approval rate for inorganic nanomaterials has significantly dropped. This might be mainly due to the fact that scientists have been more fully characterizing nanomaterials in biological systems and more rigorously analyzing their toxicities.

Despite the promise of inorganic nanomaterials for use in delivery, diagnosis, and therapy, concerns about their long term toxicity need to be addressed [6]. In line with this effort, this review will focus on the available reports on the subchronic and chronic toxicity of inorganic nanoparticles as a function of nanoparticle type and physiochemical properties, route of administration, duration, and frequency of administration and sex of the animals in which these particles were assessed. The information provided in this review does not compare the long-term toxicity of different inorganic nanoparticles or identify these nanoparticles as safe or toxic, but rather is an attempt to provide a realistic overview based on current reports and provide a perspective for the importance of long-term safety evaluations of nanomaterials used in medical applications.

2. Applications of inorganic nanoparticles

A review of the role of inorganic nanoparticles in nanomedicine would not be complete without a few comments about their myriad

Table 1
Examples of companies using inorganic nanoparticles for biomedical applications.[a]

Company	Nanoparticle platform	Application
CytImmune Sciences	Gold Nanoparticles	Cancer
Designer Carbon	Metallofullerenes	Biosensors
MagArray	Magnetic Nanoparticles	Diagnostics
Nanobiotix	Hafnium Oxide Crystallites	Cancer
NanoProbes	Gold Nanoparticles	Cancer
NanoSpectra Biosciences	Gold Nanoparticles	Cancer
Otomagnetics	Magnetic Nanoparticles	Drug delivery to ears
T2 Biosystems	Magnetic Nanoparticles	Diagnostics
Z-Medica	Aluminosilicate Nanoparticles	Wound dressing

[a] The table was prepared based on the individual company's website accessed in March 2019. Each platform is used in a variety of products in various stages of preclinical and clinical development.

of industrial applications in common use. Inorganic nanoparticles come in a wide variety of sizes [7–10] and shapes [11]. Their physical properties can arise from the quantum properties of their core materials [7,12,13]. The small size and large surface area of inorganic nanoparticles have enabled a wide range of applications that are currently being used in hundreds of products within many industries, *e.g.*, electronic, healthcare, chemical, cosmetics, composites, and energy [14–16]. Examples of some of the inorganic nanoparticles for biomedical applications are listed in Table 1. We will only mention here a few of the inorganic nanoparticles including some metals and metal alloys and inorganic non-metallic nanomaterials [14]. The commonly used metals for the synthesis of inorganic nanoparticles are silver (Ag), gold (Au), copper (Cu), iron (Fe), zinc (Zn), aluminum (Al), cadmium (Cd), cobalt (Co) and lead (Pb) [17]. The metal oxide based nanoparticles are also silicon dioxide (SiO_2), zinc oxide (ZnO), titanium oxide (TiO_2), iron oxide (Fe_2O_3), magnetite (Fe_3O_4), aluminum oxide (Al_2O_3) and cerium oxide (CeO_2) [17]. Nanomaterials come in a variety of forms and based on their chemical composition and physicochemical properties, the environmental, health, and safety risks may differ [18,19].

2.1. Gold nanoparticles

Gold nanoparticles have potential applications in immunology, medicine, and biotechnology [20–28]. They can be fabricated in a variety of sizes, and depending on their size can have a large surface area to mass ratio rendering high surface reactivity [20]. For many years researchers have employed gold nanoparticles in medical applications due to their unique tunable size and their thermal and optical properties. Patra et al., reported [29] significant inhibition of pancreatic tumor cell proliferation, and orthotopic pancreatic tumor growth *in vivo* using gold nanoparticles as the delivery vehicle with anti-epidermal growth receptor antibody as the targeting moiety and gemcitabine as the anti-cancer drug [29]. Gold nanorods and nanocages have been investigated as photothermal reagents for prostate tumor hyperthermia [30]. Recombinant human tumor necrosis factor (rhTNF) bound to colloidal gold (CYT-6091), silica-coated gold nanoparticles (Aurolase) and gold particles with silica coat (Sebacia Microparticles) have been investigated in clinical trials [16]. Gold-nanoparticle-based materials (named:NU-0129) are currently in clinical trials for the treatment of patients with recurrent glioblastoma and gliosarcoma [31].

2.2. Iron oxide and magnetic oxide nanoparticles

The size, charge, coating and plasma protein adsorption of iron oxide nanoparticles can be modulated to effectively regulate their *in vivo* pharmacokinetics and biodistribution [32]. Nanostructures of iron, cobalt, and nickel have been shown to have superparamagnetic properties and high magnetic susceptibility. However, iron oxide nanoparticles have been studied more than the others [33]. There are several FDA/EMA approved iron oxide nanomaterials (Ferrlecit®, Venofer®, INFed®, Dexferrum®, and Feraheme®, Feridex I.V®, Resovit®, Gastromark™, Ferumoxtran-10) that are employed to treat iron deficiency in chronic kidney disease, imaging of liver lesions and lymph node metastasis imaging [4,16]. Some of these materials (such as Feridex I.V®, Resovit®, Gastromark™, Ferumoxtran-10) were discontinued or withdrawn [16]. Superparamagnetic iron oxide nanoparticles have also been used for biomedical applications including vascular imaging [32,34–36], drug delivery [37], gene therapy [33], *in vivo* tracking of labeled cells [38], magnetic separation of cells or molecules [39], or as iron supplements for patients with anemia [40]. For example, Nanotherm™ is an aminosilane-coated superparamagnetic iron oxide nanoparticle (SPION) that was approved by the EU in 2010 for glioblastoma treatment and in the US is in its late stage clinical trials [4].

2.3. Silver nanoparticles

Silver nanoparticles are widely used in consumer products by virtue of their antibacterial effect. Silver nanoparticles have been investigated as molecular imaging agents, drug delivery systems, diagnostics, for treatment of vascular diseases and in wound healing [41,42]. The medical uses of silver nanoparticles include therapeutic and diagnostic uses in cancer [43–48], antibacterial activity [49,50], antifungal activity [51–54], antiviral activity [55–61] and in treatment of parasitic infections [62,63]. Silver nanoparticle composites have been used in dentistry [64]. Silver nanoparticles can be taken up by the cells readily and due to their antimicrobial effect, decrease biofilm formation, thereby maintaining better oral health [65,66]. However, there have not been any FDA approved silver based nanocarriers for systemic delivery of other bioactive agents [4].

2.4. Zinc oxide nanoparticles

Zinc oxide nanoparticles have been used extensively as antibacterial agents in the health industry, food storage, textile coatings, and in environmental applications [67]. They have also been investigated for their anticancer [68,69], antibacterial [70–72], antioxidant [73], anti-diabetic [74], and anti-inflammatory [75] properties, and drug delivery applications [76–79]. Also, they have been used in many industrial products such as rubber, paint, coatings, and personal care products such as cosmetics and sunscreen [71,80–85]. In the textile industry, adding zinc oxide to the finished fabrics promotes ultraviolet and visible light resistance and antibacterial and deodorant properties.

2.5. Silica nanoparticles

The potential of silica nanoparticles (SNPs) for drug delivery applications has been reported before [86–93]. Mesoporous silica nanoparticles have a porous honeycomb structure and an internal configuration of a multitude of hollow tubes [94,95]. They have a large surface area, high channel capacity, adjustable size, easy to functionalize surfaces, thermodynamic stability, good biological compatibility, relatively low toxicity, and exert a weak immune response [96]. Various studies have reported that mesoporous silica nanoparticles can adsorb and release drug molecules demonstrating their potential utility in drug delivery and release [97]. Different types of controlled release systems have been designed based on mesoporous silica nanoparticles [98]. Some examples include targeted drug delivery to the nucleus using polypeptide-modified mesoporous silica nanoparticles [99], modified mesoporous material for controlled release of antibiotics [100], fluorescent mesoporous silica nanoparticles to deliver antisense nucleic acids [101], and development of combined gene therapy and chemotherapy for the treatment of cancers that overexpress Bcl-2 protein [102].

2.6. Quantum dots

Quantum dots have great potential in anticancer photodynamic therapy. They can also be used as imaging agents, biosensors, photovoltaic devices, solar cells, light emitting devices, and in catalysis. Valizadeh et al. [103] reported their importance in biological applications, including tracking macromolecules in the cell, tracking various cells in the tissues, labeling organelles and cells, and other applications [104–109]. Quantum dots can be useful in the detection of cancers, which is important for diagnosis, disease stage forecasting, and clinical management [110]. Quantum dots with intense and stable fluorescent properties could enable the detection of tens to hundreds of cancer biomarkers in blood assays, on cancer tissue biopsies, or as contrast agents for medical imaging.

2.7. Copper oxide, titanium oxide, and other nanoparticles

Other inorganic nanoparticles for potential use in drug delivery and therapy include, but are not limited to, copper oxide [111], titanium oxide [112], nickel oxide [113,114], and barium-based nanoparticles [115]. Copper oxide nanoparticles have been studied as antibacterial and disinfectants for wastewater [116], biosensing, and biolabeling [117,118]. Titanium-base nanoparticles have been investigated in photothermal therapy [119,120], photodynamic therapy [121,122], as antimicrobial agents [123,124] and in cosmetic products [125]. Hafnium oxide nanoparticles have been investigated for soft tissue sarcoma therapy, and compounds such as NBTXR3 and PEP503 have been investigated for rectal, and head and neck cancer treatments, respectively [4].

3. The international effort on nanotoxicology

Nanotoxicology has emerged as an important field of study given the emergence of numerous nanomaterials in various industries [126]. This not only raises concerns for potential adverse health effects in humans, but also a worldwide concern for ecotoxicity that includes air and water pollution, and animal and plant health. The main reason for this concern is that the mechanisms of action of nanomaterials and their possible interactions with living systems are not completely understood [126].

In 2005, for the first time, the Organization for Economic Cooperation and Development (OECD) held a special session focused on the human health risk and environmental safety of manufactured nanomaterials. The outcome of this effort was the development of databases listing the benefits and toxicity of nanomaterials and identifying the research gaps. This organization also approved protocols for standards of nanomaterial toxicity testing, including subacute and subchronic toxicity studies for nanomaterials [127]. In the US the National Institute for Occupational Safety and Health (NIOSH), the National Center for Toxicological Research (NCTR), and the National Institute of Environmental Health Sciences (NIEHS) have attempted to coordinate studies of the toxicity of several classes of nanomaterials including inorganic nanoparticles such as silver and titanium oxide nanoparticles [128]. The U.S. National Toxicology Program (NTP) recently released a report alluding to the carcinogenicity of cobalt compounds that release cobalt ions *in vivo* [129]. In the U.K. pharmaceutical companies and contract research organizations have created study designs for acute and chronic toxicology and carcinogenicity studies in rodents and non-rodents [130]. Despite such efforts, there is a huge gap regarding the reports and classified database of long-term toxicity of nanomaterials.

4. Definition and classification of toxicity

Factors influencing toxicity include dose, frequency, and duration of exposure as well as the route of administration [131]. Three common routes of exposure to a toxic substance include skin penetration (*e.g.*, subcutaneous and percutaneous), lung (*e.g.*, inhalation) and gastrointestinal tract (*e.g.*, oral). These same routes are often used to administer drugs. There are a variety of other parenteral routes through which toxic substances can enter the body and which are also used in drug delivery, depending on the indication. According to the FDA guidance for industry [132], when any non-systemic route of administration is used for a drug product, it is necessary to compare the toxicity profile resulting from the exposure via the intended route with that from systemic administration. Toxicity is expected to be higher after systemic, intravenous administration because this route delivers a drug directly into the bloodstream. However, if the same toxicity is observed after drug administration via other routes as that seen after intravenous administration, then the data suggests that the test-compound is absorbed quickly and becomes bioavailable to other organs.

Nanoparticle effects could be desirable or undesirable. The latter may be beneficial under certain conditions; *e.g.*, an immunosuppressive effect of a cytotoxic drug carried by the particle or that of the nanocarrier per se is the undesirable side effect. However, the immunosuppressive properties may benefit when inhibition of the immune response is needed to avoid the overt immune response. The second type of undesirable effects is not beneficial and is considered as an adverse effect. For example, an allergic reaction to a drug or nanocarrier is never desirable. Other adverse conditions include idiosyncratic reactions. Toxicity can be immediate or delayed based on the time required for its manifestation, reversible and non-reversible based on whether the effect is permanent or not, local and systemic based on whether it affects the entire body or manifests only at the site of drug action or administration [133].

Delivery vehicles, including those generated by nanotechnology, can change absorption, distribution, metabolism, and elimination after any route of administration, thereby influencing toxicity. The toxicity observed after a single exposure is often different from that seen after repeated exposure. Single-dose administration, in general, produces acute effects. Long-term effects are often associated with chronic exposure. However, intermediate duration of exposure is also possible. Therefore, depending on the exposure, toxicity can be categorized as acute (observed within less than 24 hours after single administration) or repeated (administration within 24 hours), subacute (observed within less than 1 month following repeated exposure), subchronic (observed within 1-3 months following repeated exposure) and chronic (observed after 3 or more months of chronic exposure). The toxicity observed after a large dose administered as a single dose may be higher than the toxicity observed when the same total amount is administered in smaller aliquots over time [133].

In acute toxicological testing, the goal is to determine the non-observable effect level (NOEL) and maximum tolerated dose (MTD) of nanoparticles up to 14 days. Different methods of acute toxicity evaluation have been reported including 50% lethal dose (LD50), the fixed dose procedure (FDP), the acute toxic category (ATC) method, and the up-and-down (UDP) method, some of which are no longer approved tests (for review see [134,135]). Also, acute and subacute toxicity studies elucidate the organ-specific toxic effects and the mechanism of nanoparticle toxicity [136]. Nanotoxicology also follows the same trend for acute and subacute tests. For this approach the following parameters need to be monitored during the study: immediate hematological, cardiac and neuronal responses to administered dose, weight change, clinical observation such as the effects of nanoparticle administration on cardiovascular system, respiration, locomotor system, vasodilatation and vasoconstriction, ocular sign, gastrointestinal function and the effects on the skin and fur, mortality, gross necropsy, blood toxicity and histopathological evaluation on all vital organs [136]. In addition to these parameters, the toxicokinetic and ADME/PK studies including absorption, distribution, metabolism, excretion, and pharmacokinetics of administered nanoparticles are an essential part of assessing their toxic effects [136,137].

In subchronic and chronic toxicity studies, the long term (one month to 30 months) effect of nanomaterials on the health of animals should be addressed after single and/or repeated chronic exposure. In addition to the parameters for acute toxicity, neurotoxicology, immunotoxicology, cardiovascular function and ophthalmological evaluations should be examined for the long-term safety assessment of nanoparticles (for review see [136,137]). Genotoxicity, carcinogenesis, and developmental toxicity (embryotoxicity) are also required for subchronic and chronic toxicity testing [135,136]. One generation and two-generation reproduction toxicity testing can be performed in chronic studies. In these studies, the nanoparticles will be administrated in both female and male animals at different time points based on the spermatogenic cycle for males and estrous cycles for females or during female pregnancy and nursing. During the study period, the animals will be observed for mortality and morbidity beside any sign of toxicity such as effect on female pregnancy, measurements of estrous cycle length, vaginal cytology evaluations, sperm morphology, parturition,

and number of offspring and their sexes and the number of dead and live pups [135].

Detailed protocols for acute, subchronic, and chronic toxicity studies of nanomaterials based on the route of exposure were reviewed earlier [6]. It was suggested that testing to determine NOEL doses in oral, dermal, and inhalation subchronic studies, should be performed over 10% of the animal's life span. Moreover, there should be at least 10 animals/sex/dose with at least 3 doses of nanoparticles and the exposure time should be 7 days/week (dermal and oral) and 6 hrs/day for 7 days/week (inhalation) [6,130]. Power analysis would need to be done since the sample size, including the number of animals and dose and frequency of injection depends on the significant level of the effects. Owing to morbidity and mortality concerns, assessment should be twice per day. All of the toxicology observations (hematology, clinical biochemistry, urinalysis and necropsy analyses of organs of interest) are crucial for preclinical evaluation of inorganic nanomaterials intended for use in medical treatment (Table 1 in [6]). In chronic studies, at least 50 animals/dose/sex should be used in each treatment group [6]. Nonetheless, this number of animals cannot enable detection of an adverse effect of less than 2% of the population. Carcinogenicity of chronically injected nanoparticles is one of the major concerns for long-term toxicity. Evaluation of the carcinogenicity of nanomaterials should involve *in vivo* treatment for 24 months in rodents [6].

5. Common toxicity reports for inorganic nanoparticles

An accurate comparison of the toxicity of inorganic nanoparticles is often challenging due to the differences in dose level, route, purity, and frequency of administration between published studies. Moreover, besides common properties such as particle size, surface area, and charge, each class of nanomaterials may have properties that contribute to toxicity via unique mechanisms. While inflammation and induction of oxidative stress are among the common mechanisms of toxicities reported for inorganic nanomaterials, some mechanisms are rather unique and specific to the type of nanomaterials. Below we will review some examples to highlight the types of toxicities, and the biomarkers useful in their detection.

Chronic exposure to inorganic particles has been associated with impaired clearance, inflammation, and fibrosis [138]. The exposure to magnetite iron oxide nanoparticles increases endothelial barrier permeability through the induction of oxidative stress [139]. Intratracheal instillation of superparamagnetic iron oxide and titanium oxide and oral administration of silver and silica nanoparticles have led to local inflammation [140–143]. Chronic inflammatory response accompanied by microgranulomatous changes, increases in B- and T-cell counts along with histopathological changes in lungs were reported in mice exposed to a single-dose of magnetite iron oxide [141]. Phagocytosis of these particles by the cells of the mononuclear phagocytic system (MPS) was accompanied by the production of cytokines (TNFα, IL-1β, IL-6, IL-12, IL-4, IL-5, and IL-2) [141]. While pro-inflammatory cytokines, like IL-6, peaked within earlier time points (first 24 hours after exposure), the regulatory cytokines, such as IL-4 and IL-5, were observed up to day 28 [141]. Titanium dioxide nanoparticles of anatase structure are more toxic and induce higher levels of reactive oxygen species (ROS) than rutile TiO_2 [144,145].

The accumulation of nanoparticles in the body may correlate with carcinogenesis or cardiovascular disease. For example, recently the potential risk of gold nanoparticles for causing human vascular disease was reported by Miller et al. [146] They exposed healthy male non-smoking volunteers to 5 nm and 30 nm gold nanoparticles for 2 hours. Gold was detectable in the blood and urine of 86% of the studied men 24 hrs post-administration where smaller nanoparticles were detected to a greater extent than larger nanoparticles. Even 3 months after exposure, gold nanoparticles were still detected in the blood and urine. Also, the gold nanoparticles were evaluated at sites of atherosclerosis in patients with cerebrovascular disease and risk of stroke. These data showed the selective accumulation of these nanoparticles at sites of vascular inflammation in both human and animal models of disease [146].

Among the safety concerns regarding inorganic nanoparticles are their stability and a potential to saturate the mononuclear phagocytic system. Such accumulation might alter MPS function and lead to a diminished response to pathogens. A recent study by the FDA reported that repeated administration (once/week for 8 weeks) of silica (5 mg.kg^{-1}), gold (10 mg.kg^{-1}) and silver (5 mg.kg^{-1}) nanoparticles to mice did not saturate the MPS [147]. Toxicity was not observed with gold and silver nanoparticles during the entire duration of the study, which took two months [147]. However, changes in the blood chemistry were noticed in animals exposed to silica nanoparticles [147]. Since silica-based nanomaterials may considerably vary in their toxicity profile due to differences in porosity [148] more repeated-dose studies using different types of silica-based nanomaterials are needed to fully understand the safety of these materials. Even though gold nanoparticles are generally reported as biocompatible, a variety of opinions exist regarding their potential harm, especially after long-term exposure, due to their non-biodegradable nature [149]. Particularly, a recent FDA position paper raised a concern that traditional preclinical safety models may overlook secondary effects which may occur due to the durable nature of gold and other similar nanomaterials that retain their particulate state during administration, distribution and accumulation in the body [149].

6. Challenges and considerations for subchronic and chronic studies of inorganic nanoparticles

6.1. Why do we care about subchronic and chronic toxicity of inorganic nanoparticles?

Knowledge of acute toxicity effects of inorganic nanomaterials is limited but is rapidly growing. Limited data are available regarding subchronic and chronic toxicity assessment of new inorganic nanomaterials compared to the increasing rate of research and patents introducing new inorganic nanoparticles for different applications, including drug delivery. Acute and subacute toxicity observations of nanoparticles are insufficient to evaluate their safety because: a) dissolution and/or degradation of many of the nanomaterials, including inorganic nanomaterials, may take a longer time, b) the dissolution products of the nanoparticles could be toxic by themselves, c) biodistribution of the nanoparticles may change during the period of study, d) different rates of accumulation and excretion of nanomaterials from the body may occur, and e) clinical effects of nanoparticles may need time to develop and be detected. Although there are some reports regarding the duration of, or after exposure for subchronic and chronic

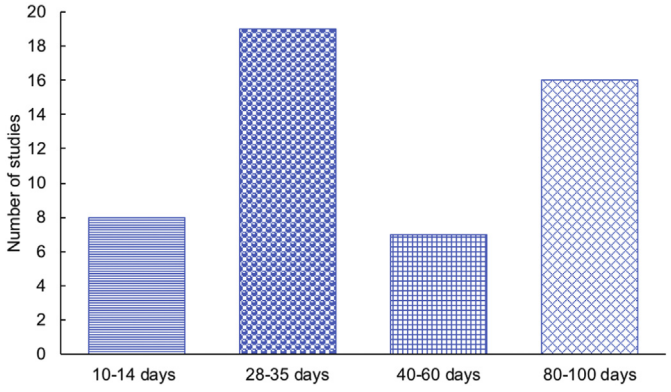

Fig. 1. Number of subchronic and chronic toxicity studies of inorganic nanoparticles based on duration of exposure to inorganic nanoparticles (as of April 2019; search in Google Scholar, Scopus and PubMed with search terms in the title or abstract or keywords for nanomaterials, including "subchronic", "chronic", "toxicity/safety evaluation" and "28 days, 90 days, and 13 weeks).

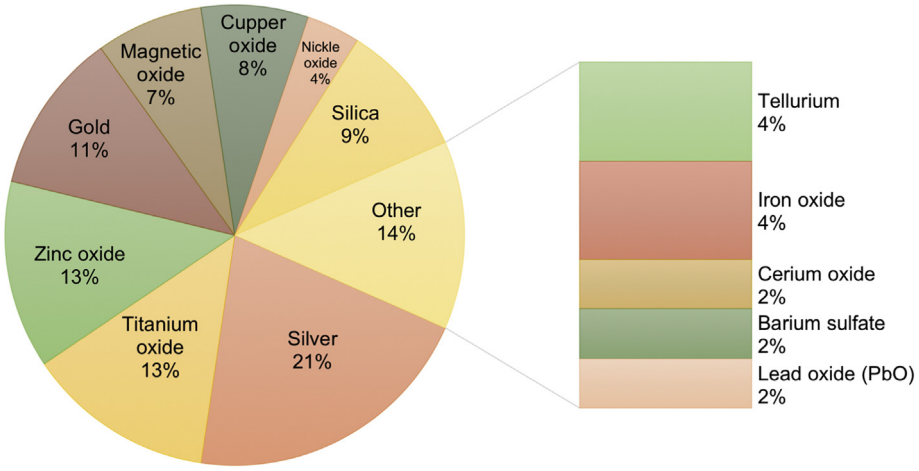

Fig. 2. Relative percentage of subchronic toxicity reports based on different types of inorganic nanoparticles (as of April 2019; search in Google Scholar, Scopus and PubMed with search terms in the title or abstract or keywords for nanomaterials, including "subchronic", "chronic", "toxicity/safety evaluation" and "28 days, 90 days, and 13 weeks).

administration, the toxic effects or the time needed to recover from injuries are not predictable. These potential toxic effects require subchronic and chronic safety evaluation before human clinical trials. However, the lack of attention to this area is all the more alarming because few published studies have been reported to date. Fig. 1 shows that there are only a few reports on the subchronic and chronic toxicity of inorganic materials. In our review of the literature, we used a number of search terms in the title or abstract or keywords for nanomaterials, including "subchronic", "chronic", "toxicity/safety evaluation", and "28 days, 90 days, and 13 weeks". It is possible that in some reports, these special terms might not have been included. Most of the long-term toxicity studies we reviewed focused on subchronic evaluation with a maximum of 13-14 weeks exposure time. About 50% of these were focused on titanium oxide, zinc oxide, and silver oxide subchronic toxicity evaluation, probably because of their approved use in medical products (Fig. 2).

6.2. Which parameters are important in in vivo subchronic and chronic toxicity assessment of nanoparticles?

There have been many studies on the toxicity and efficacy of nanoparticles as a function of their physiochemical properties. Similarly, knowledge of the properties and purity of nanoparticles is crucial prior to their toxicity evaluation. One of the essential factors in the evaluation of the toxicity of nanomaterials is their careful characterization. Publications from different groups [150–152] are available to guide researchers to fully characterize nanomaterials before addressing any application. In addition to the physicochemical properties of nanomaterials including composition, shape, size, charge, aggregation, agglomeration, degradation and dispersion [126,153], the route of exposure, dose, duration, and frequency of exposure, animal age, strain and sex affect the toxicity of nanomaterials. Further to the physicochemical characterization, biological interaction could significantly alter the behavior and effect of nanoparticles. Significant research is required to understand the physiochemical changes that nanomaterials undergo in the biological systems and how these changes can affect their biological fate. Here we will review the subchronic and chronic reports of inorganic nanomaterials that have been generated as a function of their physiochemical properties.

6.2.1. Physiochemical properties

The biological fate of nanomaterials is affected by their size, shape, geometry, hydrophobicity, and their charge since these surface-chemical properties influence their cellular interaction and uptake. It has been found that the shape, charge, size, and geometry of inorganic nanoparticles influence their *in vitro* and *in vivo* acute toxicity [154–157]. There are a few reports focused on investigating if the acute toxicity of inorganic nanoparticles is the same as their subchronic and chronic toxicity. For example, in subchronic studies, zinc oxide nanoparticles showed the same effect on tissue toxicity regardless of their size [158]. However, size dependence of subchronic immunotoxicity of silver nanoparticles after intravenous administration [159], size dependence of kidney and liver subchronic toxicity, hematotoxicity and DNA damage of gold nanoparticles [160,161], and size dependence of systemic toxicity caused by iron oxide nanoparticles [162] post-intraperitoneal administration have been reported after subchronic exposure. Kim et al., studied the 90-day subchronic toxicity of 100 nm zinc oxide nanoparticles with two different charges following oral gavage [158]. They reported that the charge of the inorganic nanoparticles did not influence the severity of lesions in the eye, stomach, pancreas, and prostate gland tissues [158]. Jon et al., also demonstrated that the size of silver nanoparticles (20 and 100nm), rather than the dose, affected the animal weights, immunotoxicity with the expression of different interleukins and on the microscopic toxicity of spleen when the particles were administered intravenously to rats for 28 days [159]. However, other studies on subchronic toxicity evaluation of silver nanoparticles for 28 days and 90 days upon inhalation and oral exposure, showed the distribution and toxicity of these nanoparticles did not depend on their size and route of administration in the tested dosage range [163,164]. It was hypothesized that the effects of silver nanoparticles could be due to both ionization of nanoparticles and also as a direct effect of nanoparticles [163].

There are also different reports on the influence of silica and gold nanoparticle size on subchronic toxicity. PEGylated coated spherical gold nanoparticles (4.4 nm) showed a lower subchronic hepatotoxicity and lower rate of infection when compared with gold nanoparticles of different sizes (22.5, 29.3, 36.1 nm). The larger particles had significant renal and hepatic subchronic toxicity following intraperitoneal administration to C57 BL/6 female and male mice every two days for 28 days [160]. Also, two of these nanoparticles (22.5, 36.1 nm) showed subchronic hematotoxicity in male animals [160]. The size-dependent acute and subchronic DNA damage of gold nanoparticles (10 nm and 30 nm) was studied by another group [161]. These data confirmed that larger gold nanoparticles caused higher levels of DNA damage compared to smaller particles [161]. Different sizes of gold nanoparticles (2, 5, 10, 30, or 200 nm) were administrated by the pulmonary route to male apolipoprotein E knockout ($ApoE^{-/-}$) mice twice per week for 5 weeks. The size-dependent translocation of gold nanoparticles from the lung to the circulation was demonstrated to be greater for nanoparticles<10nm [146]. The size of Stöber silica nanoparticles influences the recovery time needed for BALB/c mice from blood and tissue

toxicity and inflammation induced symptoms by 50 and 500 nm SNPs at MTD dose [165].

Therefore, it seems that long-term toxicity of inorganic nanoparticles, as well as their acute toxicity, are significantly affected by their size. Since the source of nanoparticles and the animal strain as well as experimental conditions are different in these studies, it cannot be concluded if there is a linear correlation or any specific size range that shows higher or lower toxicity. For a better understanding of this effect, the experiments should be conducted with the same nanoparticles over time (acute to chronic) and to include *in vitro* and *in vivo* mechanistic studies with the same batch of nanoparticles. It must be noted that the physicochemical properties of nanoparticles may change upon administration *in vivo*, and these changes (such as protein adsorption, potential aggregation, degradation, etc.) may affect toxicity. Monitoring of the physicochemical properties of nanoparticles during long-term exposure in simulated physiological conditions or *in vivo* would provide data concerning the effect of the real shape, size, charge, and geometry of nanoparticles *in vivo* on accumulation and toxicity. In many studies, one batch of nanoparticles is synthesized and evaluated for the entire study (*e.g.*, repeated injection for subchronic studies). In such cases, the physicochemical properties of stored nanoparticles and the endotoxin level should be monitored over time to ensure colloidal stability and consistency [147]. Conducting parallel studies on the stability of nanoparticles, their shelf-life and half-life will be important for long-term safety evaluations involving repeated exposure.

There are some efforts in the community to compare the efficacy and toxicity of microparticles with their nanoparticle forms. Size-dependent biodistribution of magnetic nanoparticles was reported in 5-week subchronic exposure to female rats at 500 mg.kg^{-1} [162]. The concentration of total iron in the spleen and liver following subchronic intraperitoneal injections of Fe_3O_4 nanoparticles was reported to be much higher than in the case of microparticles [162]. Another study of magnetic oxide micro and nanoparticles showed more bioaccumulation of nanosized particles as compared with the microparticles at the same dose level after 28 days of repeated oral dose administration in male and female rats [166]. Together, this data demonstrates that studies incorporating both size and charge of inorganic nanoparticles are beneficial for subchronic toxicity evaluations. To the best of our knowledge, there are no comprehensive studies on the effects of shape and geometry of nanoparticles on their subchronic and chronic toxicity. Such studies should be undertaken since we know that there can be significant effects of shape and geometry of nanoparticles on their acute toxicity [154,167].

6.2.2. Dose, frequency, duration, and route of administration

One of the key parameters in nanotoxicology is calculating the appropriate dose for administration. Finding the realistic dose for administration depends on the exposure route and the intended application of nanoparticles. For example, as we discussed before in acute toxicity of pure nanoparticles, the goal is to evaluate the dosage in terms of MTD and NOEL doses. In delivery applications of nanomaterials, the relative doses of cargo and carrier are important based on the efficacy and toxicity of both. In any unintentional exposure to nanoparticles, the toxicity of the relevant exposure dose is important. However, chronic low dose exposure or the chronic effect after single high dose exposure is the main public health concern [126]. Monitoring the alteration in biodistribution of nanoparticles in the body is extremely important in subchronic and chronic toxicity evaluation of inorganic nanoparticles. Most of the subchronic studies have been done on different doses of inorganic nanoparticles, such as: a) zinc oxide nanoparticles after repeated oral administration [158,168] and dermal [169,170] exposure, b) silver nanoparticles following oral [163,171–173], inhalation [164,174,175], dermal [176], and intravenous [159] administration, c) iron oxide nanoparticles after intravenous injection [177,178], and d) titanium oxide nanoparticles [179–182], copper nanoparticles [183], and silica nanoparticles [143] following intragastric or oral gavage. Recently, there has been considerable discussion regarding the appropriate metric for the dosage of nanoparticles in toxicology [126,184]. However, classic toxicology studies have been based on the particle mass, but, more recently some hypotheses are focused on the possible dominant role of surface area and number of particles that can predict a realistic metric for their harmful effects, as well as biological effects since these parameters may more accurately explain the reactivity of nanoparticles with blood components and cells. Therefore, reporting the *in vitro* and *in vivo* toxic or safe dose of nanoparticles based on mass, number, and surface area would be more helpful to compare different reports and plan for future studies.

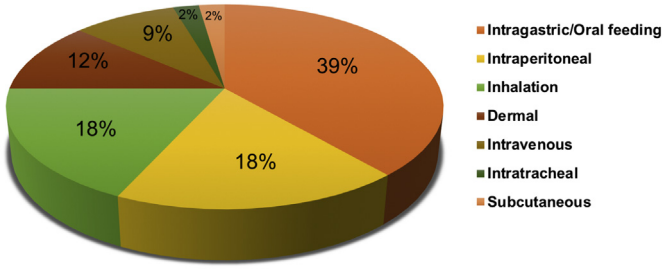

Fig. 3. Relative percentages of subchronic and chronic toxicity studies based on the route of administration of inorganic nanoparticles. Search in Google Scholar, Scopus, and PubMed with search terms in the title or abstract or keywords for nanomaterials, including "subchronic", "chronic", "toxicity/safety evaluation" and "28 days, 90 days, and 13 weeks, as of April 2019.

Another important factor influencing the toxicity of inorganic nanoparticles is the route of administration. For example, the subacute and subchronic toxicity studies on gold nanoparticles by different administration routes to mice showed that following single oral and intraperitoneal exposures of gold nanoparticles, toxic effects are much higher than following tail vein injections [178]. Fig. 3 indicates the classification of subchronic or chronic toxicity studies involving inorganic nanoparticles based on the route of administration (Fig. 3). Most of the studies in this area were conducted using intragastric and intraperitoneal administration of inorganic nanoparticles.

Therefore, route of nanoparticle administration, frequency and duration of exposure and doses are important parameters in subchronic administration of inorganic nanoparticles. In addition, there are some unwanted exposures during delivery of nanoparticles that could induce toxicity. For example, when the nanoparticles are delivered through oral feeding, the inhalation or dermal exposure of nanoparticles could cause side effects. These unintended procedural effects might be multiplied in long-term exposure studies, especially following repeated dose administration, rather than acute toxicity. Thus, having greater control of the experimental conditions would be advantageous for future subchronic and chronic studies.

6.2.3. Animal sex

There are many observations that confirm the sex of the animal does influence toxicodynamic and toxicokinetic evaluation of drugs. Adverse drug reactions have been reported to be sex-related for many pharmaceutical compounds that are in the clinic (to review see [185]), and it might be of concern for nanomaterials as well. It has been shown that certain innate and adaptive immunological responses to foreign and self-antigens are influenced by sex. [186,187] In some studies, to reduce the number of used animals, females are chosen more often than males due to their higher sensitivity to the toxicity in question. For example, the autoimmune response is more prevalent in females than in males; both in humans and in mice, the male-to-female ratio of developing lupus is 1-to-9 injection [188]. However, there are reports that show that the immune response in females is much stronger than in males [189]. In this case the safety evaluation in male animals would be of greater concern. Subchronic safety evaluations of inorganic nanoparticles have been done on both sexes (see Table 2). Among these, a few inorganic nanoparticles including iron [160], silver [175], and silica

Table 2
List of subchronic and chronic toxicity reports for inorganic nanoparticles.

Nanoparticle (s)	Characterization	Route of administration	Frequency of administration	Dose	Animal strain	Exposure time	Sex(s)	Ref
Zinc oxide NPs	100 nm, positive and negative charge	Intragastric	Daily	14 days: 500, 1000, 2000 mg/kg 90 days: 500, 125, 31.25 mg/kg	Sprague Dawley rat	14 days, 90 days	F & M	[158]
	15 ± 4 and 26 ±11 nm	Inhalation	4 hrs/day 5 days/week up to 3 weeks	3.5 mg/m^3	C57Bl/6 Mice	2 weeks, 13 weeks	M	[221]
	Primary size: 40 nm Surface area: 60 ± 10 m^{2}g^{-1} Hydrodynamic size: 201.75 ± 17.15 nm	Oral	Daily	Single dose, 24hrs: 2147 mg/kg 14-days, repeated dose: 536.75, 1073.5 or 2147 mg/kg 13-weeks, repeated dose: 67.1, 134.2, 268.4 or 536.8 mg/kg	Sprague Dawley rat	1day, 14 days,13 weeks	F & M	[168]
	20 nm; -30.9 mv	Dermal	5 days/week	75, 180, and 360 mg/kg; 10% of the total body surface area	Sprague Dawley rat	28 days	F & M	[169]
	29 ± 3 nm -44.4 ± 1.0 mV	Dermal	Daily	250, 500, and 1,000 mg/kg	Sprague Dawley rat	90 days	F & M	[170]
Silver NPs	60 nm	Oral	Daily	30-1000 mg/kg	Sprague Dawley rat	28 days	F & M	[171]
	64.9 nm	Inhalation	6 h/day 5 days/week	Low-dose group (1.73 × 10^4/cm^3), Middle-dose group (1.27 × 10^5/cm^3), High-dose group (1.32 × 10^6 particles/cm^3 ,61 μg/m^3)	Sprague-Dawley rat	28 days	F & M	[171]
	20-30 nm	Intragastric	Daily	50, 100, 200 mg/kg	Sprague Dawley rat	90 days	M	[172]
	20 and 100 nm, negative charge	Intravenously	Daily	0.0082 - 6 mg/kg	Wistar derived WU rat	28 days	F & M	[159]
	100 nm	Dermal	Once	(100, 1000 and 10000 ppm) applied to 10% of the body surface area	Hartley-albino guinea pigs	13 weeks	M	[176]
	18-19 nm	Inhalation	6h/day, 5day/week	Low dose (0.6 ×10^6 particle/cm^3, 49 mg/m^3), middle dose (1.4 ×10^6 particle/cm^3, 133 mg/m^3), high dose (3.0 × 10^6 particle/cm^3, 515 mg/m^3)	Sprague-Dawley rat	13 weeks	F & M	[164]
	53 nm	Intragastric	Daily	Low-dose (30 mg/kg), middle-dose (125 mg/kg), high-dose (500 mg/kg)	F344 rats	13 weeks	F & M	[163]
	18 nm	Inhalation	Daily, 6h/day	0.7 × 10^6 particle/cm^3 (low dose), 1.4 × 10^6 particle/cm^3 (middle dose), 2.9 × 10^6 particle/cm^3 (high dose)	Sprague-Dawley rat	90 days	F & M	[175]
	7.9 ± 0.95 nm	Oral	Daily	62.5, 125 and 250 mg/kg	Sprague-Dawley rat	42 days for males, 52 days for females	F & M	[173]
	50 nm, spherical, negative charge	Intravenous	Weekly	5 mg/kg	BALB/c mice	8 weeks	F	[147]
Copper NPs	Cu NPs: 25 nm; Cu MPs: 14–25 μm	Oral	Once/day for 2 weeks	100, 200, 400 mg/kg	Sprague–Dawley rat	28 days	M	[183]
	20 nm	Intraperitoneal	Three times/week up to 19 injections	10 mg/kg	Outbred White rat	30 days	F	[249]
Copper oxide, lead oxide and zinc oxide NPs	PbO-NPs and CuO-NPs; virtually spherical; 47±16 nm CuO-NPs; virtually spherical, 24.5 ± 4.8 nm ZnO-NPs; rod-like, 83 ± 20 and 30 ± 11 nm.	Intraperitoneal	18 times during 6 weeks	Different combination of NPs 0.5 mg in 1.0 mL of the suspension plus 2.0 mL of de-ionized water	Outbred White rat	6 weeks	M	[250]
Metal oxide NPS: CeO_2 NPs, NiO NPs, ZnO NPs, CuO NPs,	CeO2: 20-30 nm, −32.4 ± 5.6 NiO: 10-20 nm, −26.0 ± 5.0 ZnO: < 10 nm, −27.1 ± 1.4 CuO: < 50 nm, −26.7 ± 12.5	Intratracheal	Daily	50 and 150 cm^2/rat	Wistar rat	4 weeks repeated dose	F	[223]
Gold NPs	4.4, 22.5, 29.3, 36.1 nm (PEG coated)	Intraperitoneal	Every 2 days	4000 μg/kg	C57/BL6 mice	28 days	F & M	[160]
	13.5 nm	Oral, intraperitoneal, Intravenous	Single injection	137.5–2200 μg/kg	ICR mice	14 days, 28 days	M	[178]
	14 nm	Intravenous	Once/week	90 μg, 9 μg, 0.9 μg	Sprague Dawley rats	14 days, 7 weeks	M	[177]
	10 and 30 nm	Intraperitoneal injection	Daily	70 μg/kg	Wistar rats	28 days	M	[161]
	10 nm, spherical, negative charge	Intravenously	Weekly	10 mg/kg	BALB/c mice	8 weeks	F	[147]
	2, 5, 10, 30, 200 nm	Inhalation	Twice/week	50 μL of 2.3 mg/mL	Apolipoprotein E knockout (ApoE−/−) mice	5 weeks	M	[146]

(continued on next page)

Table 2 (*continued*)

Nanoparticle (s)	Characterization	Route of administration	Frequency of administration	Dose	Animal strain	Exposure time	Sex(s)	Ref
Tellurium nanorods	Te NRs: rod shape and hexagonal 85 nm length by less than 22 nm	Intragastric	Daily	Te NRs once daily at the doses of 1/50, 1/25 and 1/10 LD50 1/4 60 mg/kg (1.2, 2.4 and 6 mg/kg), respectively	NMRI mice	14 days	M	[217]
Cerium oxide and barium sulfate NPs	CeO_2: 28.4 nm $BaSO_4$: 37.5 nm	Inhalation	6 h/day, 5 days/week	CeO_2: 0.1, 0.3, 1.0 and 3.0 mg/m^3, $BaSO_4$:50.0 mg/m^3	Wistar rat	28 days, 90 days	F	[224]
Titanium dioxide NPs	19 nm	Intragastric	Daily	10, 30, 50,100, 200 mg/kg	Wistar rat	60 days	M	[179]
	Antase TiO_2 NPs, 5-10 nm	Intragastric	Daily	50, 100, 200 mg/kg	Wistar rat	60 days	M	[180]
	17 nm	Dermal	Daily	0.4 mg/cm^2	Hairless Wistar Yagi rat	2,4,8 weeks	M	[244]
	TiO_2 powders in the forms of anatase (4 and 10 nm), rutile (25, 60 and 90 nm) and Degussa P25 (21 nm)	Dermal	Daily	24 mg of test formulation containing 5% titanium dioxide (4 nm or 60 nm) was topically applied in dorsal surface of both ears, areas of 3 cm^2	Pig and mutant *hairless mice* (BALB/c *nu/nu*)	30 days (pig), 60 days (mice)	M	[50]
	90-day: surface-coated pigment-grade TiO_2 test particles (d50 1/4 145 nm, 21% NPs by particle number criteria) 28-day: uncoated, pigment-grade TiO_2 test particles (d50 1/4 173 nm by number) 14-day: surface-treated rutile/anatase nanoscale TiO_2 particles (d50 1/4 73 nm by number)	Intragastric	Single dose for 14 days Daily for 28 and 90 days	Different doses based on time of study 24,000 mg/kg 5,000 mg/kg	Crl: CD(SD) rats	14 day 28 days 90 day	F & M	[182]
	15–20 nm	Intragastric	Daily	10 mg/kg	CD-1 (ICR) mice	90 days	F	[248]
	hydrodynamic diameter: 208–330 nm	Peroral Nasal	Daily	2.5, 5, and 10 mg/kg	CD-1 mice	90 days	F	[181]
Iron oxide (Fe_3O_4) NPs	10, 50 nm and 1μm	Intraperitoneal	3 times/week	500 mg/kg	Outbred Wight rat	5 weeks	F	[162]
NiO and Mn_3O_4 NPs	NiO of 16.7 ± 8.2 nm Mn_3O_4 18.4 ± 5.4 nm	Intraperitoneal	3 times/week up to 18 injections	0.50 mg or 0.25 mg per rat (separately or in different combinations)	Outbred Wight rat	6 weeks	F	[251]
Magnetic oxide NPs	MnO_2 NPs: < 30 nm MnO_2 MPs: < 5 μm	Intragastric	Daily	MnO_2 NPs at 1,000, 300 mg/kg MnO_2 MPs at 1,000 mg/kg	Wistar rat	28 days	F & M	[166]
	30 nm	Inhalation	6 hr/day, 5 days/week	500 μg/m^3; 18·10^6 particles/cm^3	Fischer 344 rats	11 and 12 days	M	[222]
Silica NPs	Mesoporous SNP: 98 ± 6 nm Colloidal silica : 102 ± 6 nm	Intraperitoneal	Daily	2, 20, and 50 mg/kg	BALB/c mice	4 weeks	F	[252]
	Synthetic amorphous silica:7 nm NM:10-25 nm	Oral feed	Daily	100, 1000 or 2500 mg/kg of SAS; 100, 500 or 1000 mg/kg of NM-202	Sprague–Dawley rat	28 days 84 days	M	[143]
	10 nm, spherical, negative charge	Intravenous	Weekly	5 mg/kg	BALB/c mice	8 weeks	F	[147]
	Large Stöber SNPs and Mesoporous SNPs (432 ± 18.7 nm, -53.1 ± 1.5 mV) Small Stöber SNPs (46 ± 4.9 nm; -40.3 ± 1.9 mV)	Intravenous	Once	Maximum tolerated dose (100 mg/kg for small SNPs and large MSNPs; 300 mg/kg for large SNPs)	BALB/c mice	10 days, 60 days, 180 days	F & M	[165]
Silicon dioxide, Silver and Iron oxide NPs	SiO2: spherical, 12 nm, -44.37 mv Ag: spherical, 11 nm, -21.13 mv Fe2O3: α form, 60 nm, +13.60 mv	Oral	Daily	975.9, 1030.5 and 1000 mg/kg	Sprague–Dawley rat	13 weeks	F & M	[216]

nanoparticles [165] have shown sex-related subchronic toxicity. For example, a sex-related difference in the accumulation of silver nanoparticles was noted in the kidneys after subchronic exposure [175,190]. Female Sprague-Dawley rats showed two to three times more accumulation of 18 nm and 53 nm silver nanoparticles in the kidney after inhalation and oral gavage administration, respectively [175,190]. PEGylated gold nanoparticles also showed significant liver toxicity, inflammation, and infection in male C57/BL6 mice, whereas female mice presented high renal toxicity at day 28 post-intraperitoneal administration [160]. Our recent study on mesoporous silica nanoparticles showed considerable systemic sex-related toxicity, with MTDs ranging from 40 ± 2 mg.kg^{-1} to 95 ± 2 mg.kg^{-1} for male and female mice, respectively. This toxicity was influenced by the porosity of SNPs, whereas the same sized Stöber SNPs did not show the same effect. The reason for these sex-related toxicity or distribution of inorganic nanoparticles is not clear yet. Of course, differences or rates of change in the physiology, anatomy, hormonal, behavior, biochemistry and genetics between females and males, especially rodents which have a shorter life span than humans, make it necessary to do the sex-related long-term toxicity assays. This will fill an important gap in current nanotoxicology studies.

6.2.4. Importance of endotoxin screening

Endotoxin or LPS is a large, heat-stable molecule found on the outer membrane of gram-negative bacteria and commonly reported in engineered nanomaterials. Endotoxin contamination can mask the true biological effects of nanoparticles and change the distribution and clearance of nanoparticles in the body [191–196].

The acute toxicity due to endotoxin and endotoxin-contaminated nanoparticles is primarily manifested by the cytokine release syndrome. The exaggeration of endotoxin-mediated inflammation by certain

nanomaterials is a well-documented problem [197–200]. Some inorganic nanomaterials, for example, titanium oxide nanobelts, do not activate the cytokine response per se but exaggerate endotoxin-mediated responses via a mechanism involving inflammasome activation [201]. Such properties create a regulatory challenge for the biomedical application of nanomaterials [192]. All drugs coming into contact with human blood are required to contain no more than 5 EU/kg of endotoxin [202,203]. This threshold pyrogenic dose was established in clinical trials evaluating the safety and efficacy of systemically administered endotoxin for cancer therapy [204–207]. However, the threshold pyrogenic dose of endotoxin in the presence of mesoporous silica, titanium nanobelts, or any other nanomaterial capable of exaggerating the endotoxin-mediated inflammation is currently unknown.

Also, the influence of chronic exposure to endotoxin and nanoparticles is not well understood. In this context, it is important to recognize that prolonged exposure to endotoxin is known to induce so-called endotoxin tolerance [208–211], a condition, wherein an initial acute exposure to a small dose of endotoxin prevents the development of inflammation after subsequent exposure to a high endotoxin dose. While acute exposure to the endotoxin, especially in the presence of nanomaterials capable of enhancing its inflammatory properties, represents a threat of cytokine storm, septic-shock syndrome and associated infusion reactions, the prolonged exposure may blunt the immune response to the opportunistic flora, thereby representing a threat of opportunistic infections and the body's inability to provide adequate immune defense [208–211]. Both scenarios are potentially fatal. We hypothesize that similar to the threat of opportunistic infections among the survivors of sepsis [209,210], the prolonged exposure to endotoxin contaminated nanomaterials is capable of exaggerating endotoxin-mediated responses and may create similar conditions and associated health risks. Clearly, more research is needed in this area.

While endotoxin levels in pharmaceutical products are strictly regulated, there are other microbial cell components that may contribute to the immune response of a drug product. Bacterial flagellin, microbial DNA, RNA, lipoproteins, and beta-glucans are among common innate immunity modulating impurities (IIMIs), that may contaminate drug products. The levels of these contaminants are monitored and considered in the context of immunogenicity [212]. The relationship between these IIMIs and nanoparticles is not well understood. However, a potential contribution of these impurities to the immunogenicity of a protein-based component of a nano-formulation is possible. The consequences of such response could vary from the increased clearance of nanoparticles and their cargo to anaphylaxis and would depend on the type of particle, its ability to enhance or inhibit the immune response and the function of the protein component (*i.e.*, API *vs.* structural component of the nanocarrier).

7. Reports on *in vivo* subchronic and chronic toxicity of inorganic nanoparticles

Important questions in the design and development of nanoparticles for delivery applications may arise as follows: How long does it take for the nanoparticles or their resulted products to accumulate and then be cleared, degraded or dissolved in the body? Does the biodistribution of those nanoparticles change over time? Are there any long-term, direct or indirect, toxicities, or side effects caused by the nanoparticles in different organs? If yes, is the toxicity reversible? How long does the body need to recover? What is the mechanism of long-term toxicity of nanoparticles and does the mechanism change with time? Do the toxic effects transfer to the next generation? Do the physiochemical properties of inorganic nanoparticles influence their long-term effects? Thus, there are some particularly common toxicity concerns following nanoparticle administration that need to be addressed after subchronic and chronic exposure, including hepatotoxicity, mesothelioma, thrombosis, pseudoallergy, pulmonary fibrosis and carcinogenicity that were reviewed earlier [6]. Addressing these questions will help investigators create less toxic nanoparticles using selected administration routes, frequency, and doses. Table 2 shows a list of subchronic and chronic toxicity studies of inorganic nanomaterials focused on route and frequency of administration, dose, strain, and sex of animals and time of exposure. We have summarized the subchronic and chronic toxicity effects of these inorganic nanoparticles in major tissues including liver, lung, kidney, spleen, brain, skin, pancreas, reproductive organs, blood and bone marrow with subchronic and chronic reports on the immunotoxicity and genotoxicity of inorganic nanoparticles.

7.1. Specific tissue toxicity

7.1.1. Hepatotoxicity

Administered nanoparticles in the bloodstream go to the liver (30-99%), which contains 80-90% of total body macrophage population [213]. Hepatic processing and biliary excretion of nanoparticles are usually slow and may take months [214,215]. High accumulation of nanoparticles, including inorganic nanoparticles in the liver with the prolonged retention from these relatively slow clearance pathways increase the chronic liver toxicity of these nanoparticles [213].

The applications of silver nanoparticles are widespread. Their subchronic liver damage was reported after oral, inhalation, intravenous, and dermal administration (for references see Table 2). The histopathology examination of female and male rat livers from animals that had received repeated oral doses of 60 nm silver nanoparticles for 28-days showed infiltration of inflammatory cells in the portal triad and hepatic lobules beside significant dose-dependent changes in alkaline phosphatase and cholesterol values that indicate some subchronic liver damage [171]. Kim et al., also reported that almost the same size of silver nanoparticles (53 nm) caused slight subchronic liver damage at the dose of 125 $mg.kg^{-1}$ after 90 days exposure in F344 rats with clear changes in alkaline phosphatase and cholesterol level in blood [163]. On the other hand, accumulation in the liver with no harmful effects was noted following 90 days daily oral gavage administration of smaller silver nanoparticles (20-30 nm) at different doses in Sprague-Dawley rats [172]. This demonstrates that subchronic liver toxicity caused by silver nanoparticles in rodents is dependent on their size, dosage, and animal strain.

Liver toxicity caused by silver nanoparticles was demonstrated after 28 days and 90 days following repeated inhalation exposure. Inhalation of relatively high doses (1.32×10^6 particles/cm^3, 61 $\mu g/m^3$) of 64.9 nm silver nanoparticles, showed significant increases in liver Ag concentration in female and male rats [174]. Besides lung, the liver was considered to be the target organ for 13 weeks subchronic exposure to 18 nm silver nanoparticles in both sexes of Sprague-Dawley rats [175]. Size-dependent subchronic hepatotoxicity of silver nanoparticles was also reported [159]. Yun and coworkers compared the 13-week subchronic liver toxicity of three different inorganic nanoparticles, silicon dioxide, silver, and iron oxide nanoparticles [216]. They demonstrated that daily oral dosing of Ag nanoparticles resulted in increases in serum ALP and calcium as well as exerting an increase in the incidence of lymphocyte infiltration in the liver, raising the possibility of liver toxicity induced by these nanoparticles [216]. Thirteen-week liver accumulation and damage were observed after dermal application of 100 nm silver nanoparticles to Hartley-albino guinea pigs [176]. Altogether, these reports show silver nanoparticles have been found in the liver months following administration with signs of inflammation and toxicity that are time- and physicochemical properties-dependent. Understanding the mechanism(s) of liver toxicity and inflammation as a function of physicochemical characteristics of silver nanoparticles is an area that needs to be investigated in more detail.

Long-term liver accumulation and toxicity were also reported for other types of inorganic nanoparticles. Blood chemistry analysis confirmed changes in liver enzymes after oral or intraperitoneal administration of gold nanoparticles and tellurium nanorods, respectively [160,217]. Chen et al., suggests that different sizes of PEG-coated gold

nanoparticles (10-30 nm) are still not sufficiently safe to use because of more serious liver toxicity, infarction, and inflammation in male C57/BL6 mice, 28 days post-intraperitoneal injection at a dose of 4 $mg.kg^{-1}$ [160]. On the other hand, Rambanapasi et al., reported that liver had the highest accumulation per gram of tissue of 14 nm gold nanoparticles at 56 days following repeated intravenous administration to rats, though they did not observe any subchronic toxicity that might be attributed to AuNPs accumulation [177]. Therefore, based on these reports, it can be concluded that long-term toxicity of gold nanoparticles depends on the route of administration. Chronic toxicity studies will clarify the duration by which such gold nanoparticles are cleared from the liver. Detailed investigation of the effect of size, geometry, and charge of gold nanoparticles on subchronic liver toxicity as well as understanding the mechanisms of liver inflammation provide for future avenues of research.

Titanium oxide nanoparticles are some of the most widely used inorganic nanoparticles that have various applications in the food industry and the medical field. Prolonged and repetitive exposure of TiNPs can result in liver injury since it is a major organ for the accumulation of nanoparticles. Biochemical and histological analyses demonstrated that oral intragastric administration of 19 nm TiNPs (100 $mg.kg^{-1}$) for 60 continuous days caused hepatotoxicity in male Wistar rats [179]. Due to the application of TiNPs in the cosmetic industry such as sunscreen formulations, skin penetration, and subchronic safety evaluation of these NPs on internal organs after dermal exposure have received greater attention. Recently, Wu et al., reported 30-day and 60-day subchronic toxicity of TiNPs in different forms (anatase, rutile, and degussa) in pig and mice [50]. They reported that TiNPs could penetrate and reach major organs such as the liver and cause pathological changes in this organ that were identified as focal and liquefaction necrosis [50]. However, Warheit et al., have observed little or no toxicity in female or male rats following oral exposure to different doses of titanium dioxide particles based on OECD test-guideline acute (14-day) (OECD TG 425), subacute (28-day) (OECD TG 407) and subchronic (90-day) (OECD TG 408) studies [182]. These different observations of the long-term toxicity of titanium oxide nanoparticles could be because of different physicochemical properties of studied nanoparticles (characteristics summarized in Table 2) and also due to the effect of route of administration (dermal versus intragastric) on long term liver toxicity.

Evaluation of functional and biochemical indicators beside histopathological examination following intraperitoneal exposure of different sizes of iron oxide nanoparticles (10 nm, 50 nm, and 1 μm) was also reported by Katsnelson et al., who demonstrated a subchronic effect of this inorganic nanoparticle on rat liver tissue [162]. In this study, histological data showed more liver pathological changes in response to 10 nm rather than 50 nm Fe_3O_4 (500 $mg.kg^{-1}$) nanoparticles 5 weeks post-injection to female rats. These subchronic changes were mostly changes in the structure of the lobules, discomplexation of hepatic tubules, and vacuolar degeneration in hepatocytes. The iron-containing pigment was found in periportal zones, intralobularly and, occasionally, centrilobularly, sinusoids and also in the cytoplasm of Kupffer cells [162]. The subchronic liver accumulation and toxicity were reported for other types of inorganic nanoparticles. Repeated oral gavage of magnetic oxide nanoparticles significantly increased AST and ALT activity in the liver that was one of the tissues with the maximum amount of Mn at day 28 [166]. This toxic effect was followed by hepatic fibrosis and expanded portal tract with periportal inflammation [166]. Lee et al., also reported the 28-day subchronic toxicity effects of copper nanoparticles in Sprague-Dawley rats [183]. They found that higher dietary doses caused necrotic lesions and high-grade hydropic degeneration in the livers [183].

Long-term accumulation of silica nanoparticles and the ensuing immune response can raise concerns about the safety of these particles depending on the duration of exposure and physicochemical properties. The liver is the main organ for spherical Stöber and mesoporous silica nanoparticles accumulation 10 days and 60 days following single dose intravenous administration at MTD which can be cleared at the 180th day [165]. Our results show that at 180 days post single dose intravenous administration of the nanoparticles, subchronic tissue toxicity of SNPs is influenced by the size of the particles. Liver and spleen recovery in mice following administration of the larger Stöber SNPs (432 ± 18.7 nm in diameter with -53.1 ± 1.5 mV zeta potential) took a longer period (180 days) compared to administration of the smaller Stöber SNPs (46 ± 4.9 nm; -40.3 ± 1.9 mV). The accumulation of SNPs in these organs correlated with the histology lesions that were mostly small foci of lobular inflammation and ceroid laden macrophages in the liver [165]. Liver and spleen have been reported as the major organs for the toxicity of SNPs (70 nm) at 4 weeks in male BALB/c mice when administered intravenously [218]. Yu and coworkers have also seen liver, lung, and spleen toxicity in female and male ICR mice 14 days after single intravenous injection of lethal doses of amorphous SNPs [219]. The time-dependent accumulation of silica nanoparticles was confirmed in our study in which the amount of silicon in the liver was more on day 10 than that on day 60 and much more than on day 180 following single dose intravenous administration. However, severe inflammatory response occurred in our subchronic studies [165]. Zande et al., have demonstrated that oral gavage of 25nm NM-202 (nanostructured silica) to rats resulted in pathological effects on liver tissue after 84 days of exposure, whereas exposure to 7 nm SAS (synthetic amorphous silica nanoparticles) did not [143]. Histopathological analysis, showed an increased incidence of liver fibrosis that only reached significance in the NM-202 treated animals. This observation was accompanied by a moderate, but significant, increase in the expression of fibrosis-related genes in liver samples [143]. Altogether, these studies showed that hepatotoxicity is one of the main subchronic toxic effects of inorganic nanomaterials that needs to be taken into consideration in safety evaluations of inorganic nanoparticles.

7.1.2. Pulmonary toxicity

Based on the potential therapeutic applications, inorganic nanoparticles may reach the lung via inhalation or systemic delivery. Furthermore, inhalation of inorganic nanoparticles due to occupational exposure to some nanoparticles produced in industrial scale can cause toxic effects. Inflammation arising as a result of nanoparticle accumulation in the lung could lead to lung disorders including pulmonary fibrosis, pneumoconiosis, and exacerbation of existing lung disorders such as asthma [220]. The prolonged accumulation of nanoparticles in the lung has been reported for silver [164], gold [146,177], zinc oxide [221], magnetic nanoparticles [166,222] and titanium oxide [50] nanoparticles (Table 2). For example, subchronic evaluation of inhaled zinc oxide nanoparticles (3.5 mg/m^3) for 13 weeks, significantly enhanced recruitment of white blood cells, especially macrophages, that caused histopathological changes and inflammation in the lungs of C57BI/6 mice [221]. Subchronic 90-day silver nanoparticle inhalation toxicity study also indicated that lung is one of the major target tissues for prolonged silver nanoparticle accumulation [164,175]. In this report, a clear dose-dependent silver concentration in the lung tissue has been reported in Sprague-Dawley rats that received 18 nm silver nanoparticles for 90 days. This treatment caused some chronic alveolar inflammation, a mixed cell perivascular infiltrate, and alveolar macrophage accumulation [164].

Cho et al., studied the subchronic toxicity and inflammation of a panel of metal oxide nanoparticles after instillation in the lung of female rats [223]. The histology and immunohistochemistry analyses of the lung tissue that was extracted for CeO_2, NiO, ZnO, and CuO nanoparticles showed inflammatory effects after 4 weeks at the high dose used (Table 2) [223]. Recently, Schwotzer and coworkers administered cerium oxide (28.4 nm) and barium sulfate (37.5 nm) nanoparticles by inhalation to female Wistar rats, 6 hrs/day, 5 days/week in different doses (Table 2) for 28 and 90 days [224]. This subchronic exposure induced pulmonary inflammation in alveolar space for CeO_2 nanoparticles at the NOEL below 1.0 mg/m^3. Because of the rapid clearance of $BaSO_4$,

inflammatory effects of these nanoparticles were mainly restricted to the nasal cavity, were less severe and persistent and most likely related to the high dose level compared to CeO_2 [224]. Therefore, these subchronic pulmonary accumulation and toxicities for some of the inorganic nanoparticles after inhalation exposure again show the significance of long-term studies for risk assessment of inorganic nanoproducts. Also, understanding the rate of clearance of nanoparticles from the body long term after administration following repeated dose-inhalation, helps to predict and design safer doses and frequency of administration and develop occupational safety guidelines.

7.1.3. Mononuclear phagocytic system (MPS)

This section reviews the toxicity to spleen, bone marrow, and phagocytic cells. It is clear that the spleen plays a significant role in the accumulation and pharmacokinetics of nanoparticles since splenic macrophages have a significant role in their uptake and metabolism [225]. The accumulation of nanoparticles in the spleen may trigger an inflammatory response that can be responsible for unwanted effects and toxicity. After 90 days of oral treatment of Sprague Dawley rats with PVP-AgNPs, the spleen was one of the major tissues with significantly higher levels of Ag [172,176]. Different sizes of silver nanoparticles also caused severe increases in spleen size and weight at day 28 post-intravenous administration to Wistar rats [159]. This subchronic toxic effect was confirmed by suppression of the natural killer (NK) cell activity in the spleen at the higher doses. The authors suggest that the increase in the number of B, T, and NK cells might be the reason for the weight increase in the spleen [159]. Although these effects were the same for both sizes of the studied silver nanoparticles, it seems the mechanism of their immunotoxicity was size dependent, since the data showed different expression patterns of INF-γ, TNF-α, IL-6 and IL-10 cytokines for different sizes of nanoparticles [159].

As mentioned before (Section 6.2.3) long term toxicity of inorganic nanoparticles in spleen has been reported to be sex-dependent. Chen et al., demonstrated obvious spleen and thymus indices in female C57/BL6 mice that represented the immune response activation upon subchronic (28 days) intraperitoneal administration of PEGylated gold nanoparticles [160]. However, Zang et al., had seen significant decreases in spleen size when gold nanoparticles (13.5 nm) were administrated orally to male ICR mice for 28 days [178]. After the liver, the spleen was the main organ for same size gold nanoparticle (14 nm) accumulation during 56 days of intravenous injection (once weekly), without any obvious subchronic toxicity [177]. These observations of subchronic accumulation and toxicity of silver and gold nanoparticles upon different routes of exposure such as dermal, intragastric and intravenous administrations show that the phagocytic system needs months to clear the nanoparticles or their remnants from the body. The mechanism of this clearance process for specific nanoparticles is a subject of ongoing and future investigations.

Irrespective of the route of administration, the subchronic accumulation of other groups of inorganic nanoparticles in spleen have also been reported (Table 2), such as titanium dioxide nanoparticles [50], iron oxide nanoparticles [162], magnetic nanoparticles [166], copper oxide nanoparticles [183] and silica nanoparticles [143,165]. Our subchronic (60 days and 180 days) toxicity evaluation of silica nanoparticles showed accumulation of these nanoparticles in spleen up to 60 days after intravenous injection that was size and porosity dependent [165]. This was followed by aggregation of foamy macrophages and overexpression of inflammatory genes [165]. Also, the spleen injury was reported up to 60 days after chronic intravenous administration of 13 nm mesoporous silica nanoparticles and Stöber silica nanoparticles (70 nm) to male Wistar rats and male BALB/c mice, respectively [218,226]. Exposure to SAS or NM-202 silica nanoparticles by other researchers did not result in clearly elevated tissue silica levels after 28-days of oral feeding exposure. However, silica accumulated in the spleen after 84-days of exposure to SAS, but not to NM-202 [143]. This delayed accumulation of silica nanoparticles in the spleen was also seen at 60 days after intravenous exposure to larger (about 500 nm) silica nanoparticles [165].

These reports refer to the accumulation of different inorganic nanoparticles in the body after subchronic exposure that might result in toxicity. A question arises if the potential degradation products of the same nanomaterials can be cleared faster from the body and therefore, might be safer than their non-degradable counterparts. However, fast degradation or dissolution of inorganic nanoparticles may or may not correlate with their lower subacute, subchronic, or chronic toxicity. The toxicity of dissolution and degradation products of inorganic nanoparticles needs to be established. Degradation, dissolution, eruption, or breakage of nanoparticles in the body will decrease their size, and smaller products might be taken up to a greater extent by cells. On the other hand, smaller nanoparticles can have a different distribution pattern than their larger counterparts and throughout acute, subchronic, or chronic exposure. Also, there is evidence that metal ions, as the products of inorganic nanoparticle dissolution and/or degradation, can be toxic and carcinogenic [227]. Metal ions can induce reactive oxygen and nitrogen species, hydrogen peroxide and hydroxyl radicals, induce DNA damage, and enhance lipid peroxidation [227,228]. Phagocytes, which contain these metal ions after nanoparticle engulfment, might be a major source of reactive oxygen species in the body [228]. Therefore, comparative long-term toxicity studies are needed for investigating the dissolution and degradation products of nanoparticles. Also, studying the re-distribution of inorganic nanoparticles or their products in the body over time could help for a better understanding of organ-specific toxicity.

The concern that chronic toxicity of nanoparticles may be greater than that of microparticles of similar composition has been raised [162]. The subchronic effect of Fe_3O_4 in nano and micro size range was studied [162]. Results show considerable deposits of lumps of iron-containing pigment in the red pulp, and infrequently in compressed follicles of the white pulp of the spleen. This histologic change has been observed for both sizes (10 and 50 nm) of iron oxide nanoparticles that were considerably more marked in comparison with the effect of micrometric ones (1 μm) [162].

7.1.4. Renal toxicity

The kidney receives 20% of the entire cardiac output and has a critical role in regulating blood circulation and the removal of waste products from the body [229]. It has been reported that small nanoparticles (15 nm <) can be filtered by the kidney, larger than 15 nm and smaller than 200 nm are captured by Kupffer cells and splenic macrophages, and large nanoparticles (> 200 nm) can be retained in the red pulp of the spleen [225,230]. Renal clearance of nanoparticles through glomerular filtration is time-dependent and dependent on physiochemical properties, particularly size [229]. Particles above 7 nm usually cannot pass through the glomerular endothelial cells, and hence are not cleared. However, in passing through the special architecture of the renal vessels, nanoparticles tend to accumulate in renal blood vessels. Peritubular renal capillaries are special for their dendritic shape, with sharp angles that could possibly entrap nonelastic large organic particles. Silver nanoparticles have shown subchronic accumulation toxicity in kidneys after inhalation [164,175], oral [171,172,216] and dermal [176] exposure. Following 13-week dermal delivery of these inorganic nanoparticles to guinea pigs, the kidney of the animals showed the highest accumulation of Ag. This caused chronic toxicity in kidney including glomerular adhesion to Bowman's capsule, capsular thickening, membranous thickening and increased mesangial cells, proximal convoluted tubule degeneration, and Inflammation [176]. Lymphocyte infiltration and slight inflammation in the kidney have also been reported following oral treatment of the small size (11 nm) Ag nanoparticles [216]. On the other hand, as described before, the kidney has been reported as the only organ with sex-dependent accumulation of silver nanoparticles after prolonged exposure [163,164,171,190]. Iron oxide nanoparticles have not been excluded from this sex-dependent toxicity.

As discussed in Section 6.2.1, the size-dependent significant kidney toxicity of iron oxide nanoparticles at day 28 has been reported. The observed toxicity was more severe in female C57/BL6 mice compared to male based on blood chemistry analysis [160]. Sing, et al., have observed a large fraction of MnO_2 nanoparticles and microparticles can be cleared by urine and feces at 28 days upon oral delivery to Wistar rats. Only MnO_2 nanoparticles can cause focal tubular damage in histopathological and kidney AST and ALT alteration in blood chemistry analyses [166]. Histopathological changes in the kidney of rats treated with copper nanoparticles (administered once a day for 2 weeks) showed subchronic toxic signs including diluted tubules, pink- or purple-colored casts in tubules, cell debris in tubules, degenerated tubular cells, and inflammatory cell infiltration 28 days post oral delivery [183].

In some instances, delivery of inorganic nanoparticles is needed as a high single-dose administration. This exposure to nanoparticles might have acute or subacute toxicity, but it is necessary to understand how long it takes for the patient to recover from the damage. With this information, the best frequency of administration of the drug with minimum toxicity effects can be predicted. Subchronic time after single administration of inorganic nanoparticles could be the time for recovering from acute and subacute toxicity injuries. For example, intravenous delivery of silica nanoparticles with different sizes, porosity and geometry showed that kidney is a major affected organ at a concentration greater than MTD after 10 days along with congestion in the glomeruli and hemorrhage into renal interstitium as a function of porosity and surface charge [154]. However, subchronic 60-day and 180-day toxicity observation of SNPs confirmed no significant kidney injuries [165].

7.1.5. Neurotoxicity

Inorganic nanoparticles have been considered for brain imaging and therapy. Nanoparticle penetration through blood brain barriers can be desirable or undesirable. Inorganic nanoparticles have been used for imaging and treatment of Alzheimer's and Parkinson's disease, treatment of tumors, and stroke (for review see [231,232]). For example, superparamagnetic iron oxide nanoparticles have been investigated for non-invasive localization of inflammatory and neurodegenerative processes and brain tumors and also selective detection of brain gliomas [233,234]. One of the concerns for undesirable transport of nanoscale materials is their ability to reach the brain through the nerve endings of the olfactory bulb and cause toxic effects [235]. The direct transfer of inorganic nanoparticles such as iron oxide [236], titanium oxide [237], gold nanoparticles [238] and magnetic oxide nanoparticles [222] from the nasal cavity to the brain has been examined. Furthermore, the sinusoidal portal circulation "Hypothalamic-hypophyseal portal circulation" is one of special concern. These specialized vessels mimic that of the liver and would likely be a target of nanoparticle accumulation. Though this is one of the least studied topics in nanotoxicology. There is an effort to use nanoparticles in diagnosis and therapy of brain tumors, neurodegenerative disorders, and stroke [239]. Of course, the concern is related to the effect of nanoparticles on central nervous system health and as a side effect of other applications of these nanoparticles, but not necessarily as a goal of delivery to the brain. The *in vitro* and *in vivo* neurotoxicity of different types of nanoparticles was reported earlier [235]. There are a few types of inorganic nanoparticles that have been under investigation for their subchronic neurotoxicities. High dose (1.32×10^6 particles/cm^3, 61 μg/m^3) repeated (6 h/day, 5 days/week) inhalation of silver nanoparticles (64.9 nm) to Sprague-Dawley rats for 28 days showed a significant increase in the brain silver concentration of both female and male rats [174]. The accumulation of PVP-Ag nanoparticles (20-30 nm) and Ag nanoparticles (11 nm) in the brain of the same strain of rats was also reported 90 days following oral gavage [172,216]. This level of Ag in the brain was sufficient to induce changes in the normal metals (Cu and Zn) in the brain that might be linked to neurodegenerative disorders [172]. Notwithstanding, none of these reports found histological alterations in the brain after nanoparticle treatment [172,216]. However, the DNA damage to the neural cells of the cerebral cortex of the rat's brain after chronic intraperitoneal injection of two sizes of gold nanoparticles (10 and 30 nm) has been clearly observed [161]. This chronic neurotoxicity was not size-dependent. However, the acute treatment showed a significant difference in brain damage using 10 and 30 nm gold nanoparticles [240]. It has been shown that titanium oxide nanoparticles with an average size of 5-6 nm can cross the blood-brain barrier during 90 days and be deposited in hippocampus of female CD-1 mice post-peroral nasal administration [181]. Due to this accumulation, subchronic neurotoxic effects including decreasing brain size and damage in the hippocampus tissue and neurons were observed. Functional analysis of the effect of nanoparticles on brain activity is scarce. A recent report by Greish et al. described a moderate decrease in memory and learning abilities of BALB/C mice upon exposure to silver nanoparticles of 150 nm [241]. Also, spatial memory of mice was significantly impaired in the presence of TiO_2 nanoparticles [181]. Magnetic oxide-based nanoparticles conjugated to tumor-specific monoclonal antibody were the first nanoparticles suggested for brain tumor imaging [242]. There are some MRI contrast agents which have been approved by the FDA for brain imaging [243]. Translocation of magnetic oxide nanoparticles to the brain and subchronic neurotoxicity and inflammation have been reported [166,236]. Significant inhibition of brain AChE as well as total Na^+, K^+, Mg^{2+} and Ca^{2+}, ATPase at an oral dose of 1000 $mg.kg^{-1}$ per day for 28 days of MnO_2 nanoparticles have been observed in female and male Wistar rats that resulted in inflammation based on histopathological examination [166]. Elder and coworkers have observed 6- to 12-day inhalation exposure of male rats to solid MnO_2 resulted in significant increase of Mn concentration in several brain regions, most notably the olfactory bulb that can result in inflammatory changes [236]. Altogether, these reports indicate the possible subchronic neurotoxicity of inorganic nanoparticles upon different routes of administration that should be considered in the future study design of inorganic nanoparticles for delivery applications.

7.1.6. Dermal toxicity

Application of inorganic nanomaterials in the cosmetic industries for many years raises concern for long-term toxicity of these products, especially to the skin. In general, these particles should be unable to cross through the stratum corneum. However, the impairment of this layer can occur in minor skin abrasions. Zinc oxide and titanium dioxide nanoparticles have been used in sun care products for many years [14]. Subchronic toxicity of titanium nanoparticles with different sizes has been reported from 2 weeks up to 8 weeks post-dermal administration [244]. In this study, Adachi et al., applied 17 nm TiO_2 nanoparticles (0.4 mg/cm^2) to the dorsal skin of hairless Wistar Yagi rats once a day for 56 consecutive days [244]. They observed focal parakeratosis and spongiosis in the epidermis since the particles distributed in the uppermost layers of the stratum corneum and in the follicular infundibulum. This mild and focal inflammation of animal skin was not considered significant, and it was concluded that TiO_2 nanoparticles do not have subchronic toxicity [244]. Wu and coworkers studied the 30-day and 60-day potential toxicity of two different sizes (4 nm and 60 nm) of titanium oxide nanoparticles on pig and mice, respectively [50]. They observed prolonged topical exposure to TiO_2 nanoparticles could cause pathological changes, including excessive keratinization, thinner dermis, and epidermis with wrinkles. These tissue changes were size dependent since the smaller size nanoparticles have a higher penetration capacity and can reach deeper layers of the skin and cause more severe pathological changes [50]. Finally, the authors concluded that the inorganic TiO_2 nanoparticles applied to the skin for a prolonged period might pose a human health risk such as skin aging [50].

Accumulation and dermal damage have also been reported for other types of inorganic nanoparticles. Silver nanoparticles (100 nm) have been found in the skin of guinea pigs 90 days post-dermal exposure with slight damages [176]. A 28-day subchronic toxicity assessment by Surekha et al., showed that continuous dermal application of 75

mg.kg^{-1} (10% of the total body surface area) of negatively charged 20 nm zinc oxide nanoparticles can reduce the collagen content in the skin of female and male rats [169]. Other investigators have not observed adverse effects with almost the same size of ZnO nanoparticles (29 ± 3 nm, -44.4 ± 1.0 mV) when applied at higher doses (up to 1000 mg.kg^{-1}) and for longer periods of time (90 days) [170]. Given the inconsistencies observed for dermal toxicity of inorganic nanoparticles, the cohort studies with the same batch of nanoparticles and defined physiochemical characteristics over time is needed to determine the long-term dermal effects of nanoparticles.

7.1.7. Pancreatitis

Chronic pancreatitis can occur as a result of long-standing inflammation in the pancreas [245]. There are a few reports regarding subchronic effects of inorganic nanoparticles on pancreas such as subacute and subchronic toxicity and inflammation of zinc oxide nanoparticles on the pancreas that have been reported for different periods of oral exposure including 14 days [246] and 90 days [168]. Wang and coworkers found chronic inflammatory cells in the pancreas of mice treated with 20 nm and 120 nm ZnO nanoparticles following 14-day oral toxicity exposure [246]. High doses (500 mg.kg^{-1}) of negative and positively charged 100 nm ZnO nanoparticles resulted in acinar cell apoptosis and chronic inflammation in the pancreas in the male and female Sprague Dawley rats at 90 days post oral gavage to the stomach [158]. The authors hypothesized that these lesions in the pancreas were resolved during the recovery period. These lesions were considered to be toxicologically significant because they were severe enough to induce functional abnormalities [158]. Another 90-day study on ZnO nanoparticles also confirmed mild to moderate pancreatitis associated with focal lymphocyte infiltration and mild acinar cell apoptosis after oral administration [168]. More experiments are needed to examine the potential of pancreatitis upon long-term exposure for other inorganic nanoparticles and by various routes of administration.

7.1.8. Reproductive organs and reproduction/developmental toxicity

The database on the subchronic and chronic developmental toxicity of engineered inorganic nanomaterials is limited [247]. Similar to the kidneys, ovaries possess special entangled architecture network of its small blood vessels (vasa recta). This special architecture may contribute to the accumulation of nanoparticles in ovaries. For example, it has been reported that TiO_2 nanoparticles can be translocated to ovaries and affect their function directly following intragastric chronic exposure [248]. Based on this study, Gao et al., showed that 15-20 nm TiO_2 nanoparticles can cause a series of damages in the ovary, including oxidative stress, an imbalance of mineral element distribution and sex hormones, decreased fertility or the pregnancy rate of mice, disturbance of primary and secondary follicle development, ovarian atrophy, irregular arrangement of cells, and a shapeless follicular antrum. Also, TiO_2 nanoparticles can cause conglomerates in the cytoplasm and nuclei of ovarian cells and cause nucleus chromatin condensation, margination and irregularity of the nuclear membrane, mitochondrial swelling and cristae breakage, with alteration of gene expression [248]. The reproduction/developmental subchronic toxicity screening test of about 8 nm silver nanoparticles at different doses (62.5, 125 and 250 mg.kg^{-1}) showed no statistically significant effects on mice fertility, implantation, mating, delivery and fetus measurement upon daily oral exposure [173].

These results of the toxic effects of inorganic nanoparticles on different organs after long-term exposure emphasize that the field suffers from lack of conclusive studies on time-dependent acute, subacute, subchronic and chronic safety evaluation of nanoproducts on different organs as a function of their physicochemical properties. Because of the influence of physicochemical properties, animal sex and strain and the administration methods of inorganic nanomaterials on their biological fate, subchronic and chronic toxicity assessment for these nanoproducts need to be intensively studied on a case by case basis. It is probably more valuable to simply look at the toxicity profile of each nanoproduct for its unique application, but follow-up studies of acute toxicities should be done on the same batch of nanoparticles and same setup of experiments.

7.2. Immunotoxicity

Immunotoxicity generally recognized and tested for various types of drug products includes morphological changes, physical changes to the major immune organs (*e.g.*, bone marrow, thymus, and spleen) and functional changes, such as immunostimulation and immunosuppression [253–259]. Over the years, it has become apparent, that drug-candidate interactions with the immune system may be complex and include multiple effects. The term, "immunomodulation" was coined in this respect to describe changes in the immune system's function that cannot be explained by simple immunostimulation or suppression [260–262]. In the world of nanotechnology-based drugs, the understanding of the acute immunotoxic effects has made tremendous progress in recent years [192,263–265]. The US FDA recommendations for the evaluation of the immunotoxicity of new drug products were found to be fully applicable to the safety assessment of nanotechnology-based products [266,267]. However, less is known about subacute, subchronic, and chronic toxicities, as well as regulatory pathways for assessing them. The conventional approach undertaken by many investigators in the past decade focuses on the investigation of phagocytic cells (*e.g.*, monocytes, macrophages, and dendritic cells), that are involved in particle clearance. Investigation of nanoparticle effects on T- and B-cells was primarily done in the context of vaccine research, and less in the areas of nanoparticle safety. Even less is known about the mechanisms of immunotoxicity, especially if the toxicity involves functional changes, the manifestations of which require a long time to pass after the initial exposure.

Immunotoxicity commonly reported for inorganic nanomaterials includes inflammation and some immunosuppressive effects. Oxidative stress is the mechanism commonly named to underline nanoparticle immunotoxicity. Some studies, however, have extended to the analysis of endoplasmic reticulum stress, and autophagy. For example, iron oxide nanoparticle effects on autophagy involving the activation of the extracellular signal-regulated kinase (ERK) pathway was reported in phagocytic cells such as dendritic cells and macrophages [268,269], and whole blood [270]. Furthermore, lysosomal activation, endoplasmic reticulum stress and mitochondrial dynamic changes [271–274] were reported for iron oxide nanoparticles, including those formulations currently used in the clinic. Ferroptosis, a form of non-apoptotic cell death, was described for iron oxide nanoparticles and attributed to the release of iron ions [275]. Ferritin, the iron-storage protein, is thought to link the autophagy and ferroptosis induced by iron oxide nanoparticles [276]. Understanding of the role of ferroptosis in the chronic toxicity of iron oxide nanoparticles, however, necessitates further investigation. Mesoporous silica nanoparticles and titanium oxide nanobelts were shown to activate inflammasome, thereby contributing to the second signal required for the cells to produce mature cytokines of the interleukin-1 family [201,277–279]. Citrate-stabilized gold nanoparticles interfered with Toll-like receptor 9 (TLR9) activation by oligonucleotides by interacting with HMGB1 (high mobility group box 1) protein in a size-dependent manner [280]. Repeated administration of silver nanoparticles resulted in both gross pathological changes in the immune organs, such as reduction of the thymus weight and an increase in the spleen weight, and functional changes in lymphocytes such as the decrease in natural killer cell cytotoxicity and inability to adequately express IFNγ [281].

The immunotoxicity of inorganic nanomaterials may be desirable and undesirable under certain conditions. While mitochondrial damage and oxidative stress induced by Feraheme formulation of iron oxide nanoparticles and associated suppression of the T-cell function [282] may be perceived as undesirable, mainly when used in patients with the already weakened immune system, they may be beneficial in

conditions associated with undesirable activation of T-lymphocytes. For example, recent reports demonstrate that topical application of Feraheme might benefit the prevention of psoriatic skin lesions [283] and tooth decay [284]. Likewise, the pro-inflammatory properties of mesoporous silica nanoparticles [279] suggest using these materials with caution when drug delivery is required to avoid undesirable inflammatory reactions. However, the same property appears as a benefit for vaccine and immunotherapies indications, in which inflammasome activation is a beneficial property known to improve vaccine efficacy. Likewise, the ability of colloidal gold nanoparticles to inhibit TLR9 [280] suggests a benefit for using these particles to carry therapeutic oligonucleotides that often cause undesirable cytokine induction via activation of endosomal TLR9. The same property would be counterproductive if a gold nanoparticle delivers an oligonucleotide for vaccine adjuvant application.

Infusion reactions, (acute toxicity commonly observed for a variety of therapeutic agents, including those of nanotechnology origin) create a massive hurdle in both translational studies and clinical application of pharmaceutical products [285]. Interestingly, in the case of certain products (*e.g.*, PEGylated aptamers and PEGylated liposomes), the incidence and the severity of these reactions were attributed to the anti-PEG antibodies [285–291]. There is a hot debate in the current literature regarding the source of these antibodies and their contribution to immunotoxicity [286–292]. The anti-PEG antibodies, due to the exposure to the PEG of dietary and cosmetics origins, as well as due to the repeated administration of PEGylated drugs, are currently considered to be the culprits. Since PEG is commonly used to improve water solubility and circulation time of nanomaterials, including for inorganic nanocarriers, the issue of the induction of anti-PEG antibodies and its related safety concerns, is paramount for the clinical translation of PEGylated inorganic nanomaterials. Since anti-PEG antibody formation was reported for PEGylated liposomes [293–298] but not PEGylated gold colloids [299], it is essential to understand both structural aspects of nanoparticles and mechanistic differences in their immune recognition. What makes PEG immunogenic on one nanoparticle platform and not on another? Are there any differences in the isotype and titer of the anti-PEG antibodies generated in response to PEGylated nanoparticles with different chemical structures and compositions? Answering these questions would be instrumental in designing safe nanocarriers that do not induce PEG-antibodies, the PEG-antibody mediated infusion reactions, accelerated clearance, and other undesirable changes.

7.3. Genotoxicity

Genotoxicity remains a fundamental test before FDA approval and comet assay, Ames test, and erythrocyte micronucleus assay remain regulatory requirements for approval of new nanoconstructs [300]. Despite numerous studies on acute genotoxicity of inorganic nanoparticles, there are limited investigations of the effects of their subchronic and chronic exposure on gene expression pattern and gene toxicity [148,301]. For example, Grissa and coworkers focused on the influence of anatase titanium oxide nanoparticles on subchronic genotoxicity, blood toxicity, and bone marrow after 60 days treatment [180]. They hypothesized that high doses (100, 200 $mg.kg^{-1}$) of anatase TiO_2 nanoparticles (5-10 nm) could reach the bone marrow of Wistar rats following oral gavage and cause chromosome alteration, significant DNA damage, and hematopoietic inhibition [180]. Blood coagulation and severe damage to platelets was another hypothesis on which this group had based on their CBC analysis [180]. Based on the Kim et al., studies, no genetic toxicity in male and female rat bone marrow was observed following 28-day oral and 90-day inhalation delivery of silver NPs [171,175]. Most of these reports are on gene expression changes at specific time points after exposure. It would be essential to design studies that monitor gene expression alteration influenced by inorganic nanoproducts in a time-dependent manner. Limited resources and complexity of genetic alteration over time limit studies of subchronic and chronic toxicity, inflammation, and carcinogenic potential of inorganic nanomaterials. Since genotoxic effects cover a broad area, analysis of seemingly ambiguous results of gene alteration and DNA damage are difficult to analyze for chronic exposure in a meaningful manner.

8. Challenges, gaps, recommendations, and future directions

With regard to using inorganic nanoparticles in drug delivery and biomedical applications, greater emphasis has been placed on the success of these nanomaterials for their application than on their toxicity. Inorganic nanoparticles clearly have some potential in this area. A significant effort has been made for the development of inorganic nanomaterials as drug delivery systems and imaging agents, which are in various phases of clinical application or testing. Limited knowledge about the chronic toxicity of inorganic nanoparticles, inconsistent characterization protocols, and flaws in toxicity screening assays are impediments to the advancement of these systems to commercialization and patient use. Subchronic and chronic studies are time-consuming and resource intensive. Toxicologists have been trying to introduce study designs to minimize animal use for long-term safety evaluation of materials [130,302]. However, translation of these methodologies is challenging. Based on the standard *in vivo* methodology for subchronic and chronic safety testing of materials, there are requirements for these types of studies. Most commonly used animals for long-term toxicity study of nanomaterials are mice (CD-1, BALB/c, NMRI, C57Bl/6), rats (Sprague Dawley, Crl:CD(SD), F344 and Wistar rats), and pig models (Hartley-albino guinea) (Table 2). Studies should include two different sexes of at least two homogenous strains of rodents. The number of animals used should be large enough to see the minimum effect. Depending on the intended application, different doses of administration, different time points, and also the numbers of toxicity tests should be included (as discussed in Section 5). On the other hand, using animals that recover from toxic effects to investigate the reversibility of observed adverse effects, besides the statistical consideration for these types of studies, make the studies more and more expensive. An ongoing challenge is the inadequate characterization of nanomaterials. Prior to toxicological testing, the nanoparticles need to be characterized carefully for their size, size distribution, zeta potential, purity, stability (both colloidal and chemical) and the level of endotoxin present. Verification of the particle attributes by an independent third party, when available and feasible, may further improve the quality of the nanoparticle product and help to identify gaps in both particle manufacturing and characterization as well as update the protocols used to make and analyze the given nanoparticle product. Several inorganic nanoparticle products have been withdrawn after being marketed due to safety concerns. In 2016, Siramshetty et al., made a database of withdrawn or discontinued drugs. Toxicity issues reported were identified as the main reason for the withdrawal of about half of these drugs [303]. As nanoproducts receive approval by the regulatory bodies for various indications, a similar database is needed for nanoparticle-based drugs.

There is a limited number of studies focused on assessing the impact of inorganic nanoparticles on organ toxicity, inflammation, immunotoxicity, and genotoxicity in chronic exposure settings. Future directions for subchronic and chronic studies of inorganic nanomaterials should focus on the following areas: First, characterization of inorganic nanoparticles (nanoparticles alone and nanoparticle carrying the cargo) over time is needed to assess potential changes in their size, charge, geometry, surface properties, aggregation and agglomeration during storage. Shelf-life stability, degradation profiles in different body fluids, and when possible potential changes in their physiochemical properties in the body after administration need to be assessed. Pharmacokinetic and pharmacodynamic modeling can further aid in predicting the fate of nanoparticulate systems in the body. Second, systematic investigations should be done to understand the role of physicochemical properties of inorganic nanoparticles on their subchronic and chronic toxicity and comparisons need to be made with their acute and subacute effects.

Third, long-term biodistribution and rate of clearance of inorganic nanoparticles from different organs need to be evaluated and correlations need to be made with adverse effects. Fourth, the mechanism of their long-term toxicity in the accumulated organs should be included in the study design. Fifth, evaluation of the toxic effect of inorganic nanoparticles on different organs as a function of dose, route, and frequency of administration is needed. Sixth, having a database of acute to chronic toxicity of inorganic nanoparticles classified by different parameters affecting their toxicity would be useful to calculate the relative maximum safe doses of nanoparticles that need to be co-delivered with biological agents including drugs and imaging agents for specific delivery applications. Seventh, development of predictive *in vitro*, in silico and animal models which are more relevant to humans, is needed for long-term toxicology studies of nanomaterials. Finally, follow up studies are needed to investigate the effects of carcinogenesis for each nanoproduct.

Such studies can guide the design of safer nanoparticles for specific applications. Also knowledge of the biodistribution and elimination of nanoparticles over time aids in designing systems for effective delivery over the desired duration and with limited adverse effects due to the nanoparticle. *In vivo* subchronic and chronic tissue toxicity, immunotoxicity and genotoxicity of inorganic nanoparticles and understanding the underlying mechanisms can accelerate clinical translation. Phase IV post-marketing review after clinical applications can further help in the safe and effective use of inorganic nanoparticles.

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Declaration of Competing Interest

The authors declare that there are no commercial affiliations that might pose a potential, perceived or real conflict of interest with this study.

References

[1] NNI, The national nanotechnology initiative supplement to the president's 2019 budget, Subcommittee on Nanoscale Science, Engineering, and Technology Committee on Technology of National Science and Technology Council, 2018.
[2] K. Greish, A. Mathur, M. Bakhiet, S. Taurin, Nanomedicine: is it lost in translation? Ther. Deliv. 9 (2018) 269–285.
[3] D.W. Hobson, Commercialization of nanotechnology, Wiley Interdiscip. Rev. Nanome.d Nanobiotechnol. 1 (2009) 189–202.
[4] D. Bobo, K.J. Robinson, J. Islam, K.J. Thurecht, S.R. Corrie, Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date, Pharm. Res. 33 (2016) 2373–2387.
[5] Y.H. Choi, H.K. Han, Nanomedicines: current status and future perspectives in aspect of drug delivery and pharmacokinetics, J. Pharm. Investig. 48 (2018) 43–60.
[6] H. Nehoff, N.N. Parayath, S. Taurin, K. Greish, *In vivo* evaluation of acute and chronic nanotoxicity, Handbook of Nanotoxicology, Nanomed. Stem Cell Use Toxicol. (2014) 65–86.
[7] G.Y. Tonga, D.F. Moyano, C.S. Kim, V.M. Rotello, Inorganic nanoparticles for therapeutic delivery: trials, tribulations and promise, Curr. Opin. Colloid Interface Sci. 19 (2014) 49–55.
[8] M. Auffan, J. Rose, J.-Y. Bottero, G.V. Lowry, J.-P. Jolivet, M.R. Wiesner, Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective, Nat. Nanotechnol. 4 (2009) 634.
[9] W. Jiang, B.Y. Kim, J.T. Rutka, W.C. Chan, Nanoparticle-mediated cellular response is size-dependent, Nat. Nanotechnol. 3 (2008) 145.
[10] J. Rejman, V. Oberle, I.S. Zuhorn, D. Hoekstra, Size-dependent internalization of particles via the pathways of clathrin-and caveolae-mediated endocytosis, Biochem. J. 377 (2004) 159–169.
[11] E.C. Cho, Q. Zhang, Y. Xia, The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles, Nat. Nanotechnol. 6 (2011) 385.
[12] E.C. Dreaden, S. Neretina, W. Qian, M.A. El-Sayed, R.A. Hughes, J.S. Preston, P. Mascher, Plasmonic enhancement of nonradiative charge carrier relaxation and proposed effects from enhanced radiative electronic processes in semiconductor −gold core−shell nanorod arrays, J. Phys. Chem. C 115 (2011) 5578–5583.
[13] C.-W. Yen, S.C. Hayden, E.C. Dreaden, P. Szymanski, M.A. El-Sayed, Tailoring plasmonic and electrostatic field effects to maximize solar energy conversion by bacteriorhodopsin, the other natural photosynthetic system, Nano Lett. 11 (2011) 3821–3826.
[14] C.S. Santos, B. Gabriel, M. Blanchy, O. Menes, D. García, M. Blanco, N. Arconada, V. Neto, Industrial applications of nanoparticles–a prospective overview, Materials Today: Proceedings, vol. 2, 2015, pp. 456–465.
[15] H. Sharma, P.K. Mishra, S. Talegaonkar, B. Vaidya, Metal nanoparticles: a theranostic nanotool against cancer, Drug Discov. Today 20 (2015) 1143–1151.
[16] A.C. Anselmo, S. Mitragotri, A review of clinical translation of inorganic nanoparticles, Am. Assoc. Pharm. Sci. J. 17 (2015) 1041–1054.
[17] S.A.M. Ealia, M. Saravanakumar, A review on the classification, characterisation, synthesis of nanoparticles and their application, IOP Conference Series: Materials Science and Engineering, IOP Publishing 2017, p. 032019.
[18] T. Persons, J.S. Droitcour, E.M. Larson, M.W. Ames, L.R. Ettaro, P.G. Farah, K.L. Howard, D.R. Johnson, S. Martinez, T.S. Ryen, S.E. Offutt, C.D. Petersen, A.O. Stevens, W.K. Vance, K.J. Wallestad, Nanomanufacturing: emergence and implications for US competitiveness, the environment, and human health, U.S.G.A. Office Ed., (Ed.), 2014.
[19] NSET/NEHI, Environmental Health, and Safety Research Strategy, 2011.
[20] C.R. Patra, R. Bhattacharya, D. Mukhopadhyay, P. Mukherjee, Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer, Adv. Drug Deliv. Rev. 62 (2010) 346–361.
[21] J. Hainfeld, D. Slatkin, T. Focella, H. Smilowitz, Gold nanoparticles: a new X-ray contrast agent, Br. J. Radiol. 79 (2006) 248–253.
[22] R. Shukla, V. Bansai, M. Chaudhary, A. Basu, R.R. Bhonde, M. Sastry, Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: a microscopic overview, Langmuir 21 (2005) 10644–10654.
[23] J.H. Fan, W.I. Hung, W.T. Li, J.M. Yeh, Biocompatibility study of gold nanoparticles to human cells, IFMBE Proc. 23 (2009) 870–873.
[24] W.-S. Cho, M. Cho, J. Jeong, M. Choi, H.-Y. Cho, B.S. Han, S.H. Kim, H.O. Kim, Y.T. Lim, N.H. Chung, J. Heong, Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles, Toxicol. Appl. Pharmacol. 236 (2009) 16–24.
[25] C.J. Murphy, A.M. Gole, J.W. Stone, P.N. Sisco, A.M. Alkilany, E.C. Goldsmith, S.C. Baxter, Gold nanoparticles in biology: beyond toxicity to cellular imaging, Acc. Chem. Res. 41 (2008) 1721–1730.
[26] P. Jiang, Y. Wang, L. Zhao, C. Ji, D. Chen, L. Nie, Applications of gold nanoparticles in non-optical biosensors, Nanomaterials 8 (2018) E977.
[27] H. Daraee, A. Eatemadi, E. Abbasi, S. Fekri Aval, M. Kouhi, A. Akbarzadeh, Application of gold nanoparticles in biomedical and drug delivery, Artif. Cells Nanomed. Biotechnol. 44 (2016) 410–422.
[28] X. Zhang, Gold nanoparticles: recent advances in the biomedical applications, Cell Biochem. Biophys. 72 (2015) 771–775.
[29] C.R. Patra, R. Bhattacharya, E. Wang, A. Katarya, J.S. Llau, S. Dutta, M. Muders, S. Wang, S.A. Buhrow, S.L. Safgren, M. Yaszemski, J.M. Reid, M.M. Ames, P. Mukherjee, D. Mukhopadhyay, Targeted delivery of gemcitabine to pancreatic adenocarcinoma using cetuximab as a targeting agent, Cancer Res. 68 (2008) 1970–1978.
[30] R. Robinson, W. Gerlach, H. Ghandehari, Comparative effect of gold nanorods and nanocages for prostate tumor hyperthermia, J. Control. Release 220 (2015) 245–252.
[31] ClinicalTrials.gov, National Institutes of Health, National Library of Medicine, 2019.
[32] H. Arami, A. Khandhar, D. Liggitt, K.M. Krishnan, *In vivo* delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles, Chem. Soc. Rev. 44 (2015) 8576–8607.
[33] N.V.S. Vallabani, S. Singh, Recent advances and future prospects of iron oxide nanoparticles in biomedicine and diagnostics, 3 Biotech. 8 (2018) 279.
[34] K.M. Krishnan, Biomedical nanomagnetics: a spin through possibilities in imaging, diagnostics, and therapy, IEEE Trans. Magnet. 46 (2010) 2523–2558.
[35] J.E. Rosen, L. Chan, D.B. Shieh, F.X. Gu, Iron oxide nanoparticles for targeted cancer imaging and diagnostics, Nanomedicine 8 (2012) 275–290.
[36] M.A. McAteer, A.M. Akhtar, C.V.Z. Muhlen, R.P. Choudhury, An approach to molecular imaging of atherosclerosis, thrombosis, and vascular inflammation using microparticles of iron oxide, Atheroscle 209 (2010) 18–27.
[37] H. Arami, Z. Stephen, O. Veiseh, M. Zhang, Chitosan-coated iron oxide nanoparticles for molecular imaging and drug delivery, in: R. Jayakumar, M. Prabaharan, R.A.A. Muzzarelli (Eds.), Chitosan for Biomaterials I, Springer Berlin Heidelberg, Berlin, Heidelberg 2011, pp. 163–184.
[38] S.M.C. Berman, P. Walczak, J.W.M. Bulte, Tracking stem cells using magnetic nanoparticles, Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology, John Wiley & Sons 2011, pp. 343–355.
[39] H. Xu, Z.P. Aguilar, I. Yang, M. Kuang, H. Duan, Y. Xiong, H. Wei, A. Wang, Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood, Biomaterials 32 (2011) 9758–9765.
[40] M. Lu, M.H. Cohen, D. Rieves, R. Pazdur, FDA report: ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease, Am. J. Hematol. 85 (2010) 315–319.
[41] C. Dos Santos, M. Seckler, A. Ingle, I. Gupta, M. Galdinero, A. Gade, M. Rai, Silver nanoparticles: therapeutical uses, toxicity, and safety issues, J. Pharm. Sci. 103 (2014) 1931–1944.
[42] L. Wei, J. Lu, H. Xu, A. Patel, Z.-S. Chen, G. Chen, Silver nanoparticles: synthesis, properties, and therapeutic applications, Drug Discov. Today 20 (2015) 595–601.

[43] S. Boca-farcau, M. Potara, T. Simon, A. Juhem, P. Baldeck, S. Astilean, Folic acid-conjugated, SERS-labeled silver nanotriangles for multimodal detection and targeted photothermal treatment on human ovarian cancer cells, Mol. Pharm. 11 (2014) 391–399.
[44] D. Zhao, X. Sun, J. Tong, J. Ma, X. Bu, R. Xu, R. Fan, A novel multifunctional nanocomposite C225-conjugated Fe_3O_4/Ag enhances the sensitivity of nasopharyngeal carcinoma cells to radiotherapy, Acta Biochim. Biophys. Sin. 44 (2012) 678–684.
[45] L.-C. Cheng, H.M. Chen, T.-C. Lai, Y.-C. Chan, R.-S. Liu, J.C. Sung, M. Hsiao, C.-H. Chen, L.-J. Her, D.P. Tsai, Targeting polymeric fluorescent nanodiamond-gold/silver multi-functional nanoparticles as a light-transforming hyperthermia reagent for cancer cells, Nanoscale 5 (2013) 3931–3940.
[46] C. Tse, M.J. Zohdy, J.Y. Ye, M. O'donnell, W. Lesniak, L. Balogh, Enhanced optical breakdown in KB cells labeled with folate-targeted silver-dendrimer composite nanodevices, Nanomedicine 7 (2011) 97–106.
[47] R. Wang, C. Chen, W. Yang, S. Shi, C. Wang, J. Chen, Enhancement effect of cytotoxicity response of silver nanoparticles combined with thermotherapy on C6 rat glioma cells, J. Nanosci. Nanotechnol. 13 (2013) 3851–3854.
[48] D. Guo, I. Zhu, Z. Huang, H. Zhou, Y.M. Ge, Wenjuan, J. Wu, X. Zhang, X. Zhou, Y. Zhang, Y. Zhao, N. Gu, Anti-leukemia activity of PVP-coated silver nanoparticles via generation of reactive oxygen species and release of silver ions, Biomaterials 34 (2013) 7884–7894.
[49] I. Sondi, B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria, J. Colloid Interface Sci. 275 (2004) 177–182.
[50] D. Wei, W. Sun, W. Qian, Y. Ye, X. Ma, The synthesis of chitosan-based silver nanoparticles and their antibacterial activity, Carbohydr. Res. 344 (2009) 2375–2382.
[51] M. Gajbhiye, J. Kesharwani, A. Ingle, A. Gade, M. Rai, Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole, Nanomedicine 5 (2009) 382–386.
[52] M. Rajarathinam, P.T. Kalaichelvan, Biogenic nanosilver as a potential antibacterial and antifungal additive to commercially available dish wash and hand wash for an enhanced antibacterial and antifungal activity against selected pathogenic strains, Intern. Res. J. Pharm. 4 (2013) 68–75.
[53] D.R. Monteiro, L.F. Gorup, S. Silfa, M. Negri, E.R.D. Camargo, R. Oliveira, D.B. Barbosa, M. Henriques, Silver colloidal nanoparticles: antifungal effect against adhered cells and biofilms of Candida Albicans and Candida Glabrata, J. Bioadhesion Biofilm Res. 27 (2011) 711–719.
[54] N.G. Kandile, H.T. Zaky, M.I. Mohamed, H.M. Mohamed, Silver nanoparticles effect on antimicrobial and antifungal activity of new heterocycles, Bull. Kor. Chem. Soc. 31 (2010) 3530–3538.
[55] S. Galdiero, A. Falanga, M. Vitiello, M. Cantisani, V. Marra, M. Galdiero, Silver nanoparticles as potential antiviral agents, Molecules 16 (2011) 8894–8918.
[56] S. Galdiero, A. Falanga, R. Tarallo, L. Russo, E. Galiero, M. Cantisani, G. Morelli, M. Galdiero, Peptide inhibitors against herpes simplex virus infections, J. Pept. Sci. 19 (2013) 148–158.
[57] C.-X. Xiang, Q. Chen, L. Pang, C.-l. Zheng, Inhibitory effects of silver nanoparticles on H1N1 influenza a virus *in vitro*, J. Virol. Methods 178 (2011) 137–142.
[58] P. Mehrbod, N. Motamed, M. Tabatabaian, E.R. Soleimani, E. Amini, M. Shahidi, M.T. Kheiri, *In vitro* antiviral effect of" Nanosilver" on influenza virus, DARU, 17, 2009 88–93.
[59] S. Gaikwad, A. Ingle, A. Gade, M. Rai, A. Falanga, N. Incoronato, I. Russo, S. Galdiero, M. Galdiero, Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3, Int. J. Med. 8 (2013) 84303–84314.
[60] R.W.-Y. Sun, R. Chen, N.P.-Y. Chung, C.-M. Ho, C.-L.S. Lin, C.-M. Che, Silver nanoparticles fabricated in HEPES buffer exhibit cytoprotective activities toward HIV-1 infected cells, Chem. Commun. (2005) 5059–5061.
[61] L. Lu, R.W.-Y. Sun, R. Chen, C.-K. Hui, C.-M. Ho, J.M. Luk, G.K.K. Lau, C.-M. Che silver nanoparticles inhibit hepatitis B virus replication, Antivir. Ther. 13 (2008) 253–262.
[62] A.M. Allahverdiyev, E.S. Abamor, M. Bagirova, C.B. Ustundag, C. Kaya, F. Kaya, M. Rafailovich, Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light, Int. J. Nanomed. 6 (2011) 2705–2714.
[63] C. Panneerselvam, S. Ponaarulselvam, D. Murugan, Potential anti-plasmodial activity of synthesized silver nanoparticle using andrographis paniculata nees (Acanthaceae), Arch. Appl. Sci. Res. 3 (2011) 208–217.
[64] R.A. Bapat, T.V. Chaubal, C.P. Joshi, P.R. Bapat, H. Choudhury, M. Pandey, B. Gorain, P. Kesharwani, An overview of application of silver nanoparticles for biomaterials in dentistry, Mater. Sci. Eng. 91 (2018) 881–898.
[65] M. Rai, A. Yadav, A. Gade, Silver nanoparticles as a new generation of antimicrobials, Biotechnol. Adv. 27 (2009) 76–83.
[66] Y. Zhang, T.P.S. Dasari, H. Deng, H. Yu, Antimicrobial activity of gold nanoparticles and ionic gold, J. Environ. Sci. Health C 33 (2015) 286–327.
[67] A. El-Nour, M.M. Kholoud, A.A. Eftaiha, A. Al-Warthan, R.A. Ammar, Synthesis and applications of silver nanoparticles, Arab. J. Chem. 3 (2010) 135–140.
[68] Y. Zhang, T.R. Nayak, H. Hong, W. Cai, Biomedical applications of zinc oxide nanomaterials, Curr. Mol. Med. 13 (2013) 1633–1645.
[69] M. Martinez-Carmona, Y. Gun'ko, M. Vallet-Regi, ZnO nanostructures for drug delivery and theranostic applications, Nanomaterials 8 (2018) 268.
[70] L.E. Shi, Z.H. Li, W. Zheng, Y.F. Zhao, Y.F. Jin, Z.X. Tang, Synthesis, antibacterial activity, antibacterial mechanism and food applications of ZnO nanoparticles: a review, Food Addit. Contam. 31 (2014) 173–186.
[71] Y. Jiang, L. Zhang, D. Wen, Y. Ding, Role of physical and chemical interactions in the antibacterial behavior of ZnO nanoparticles against *E.Coli*, Mater. Sci. Eng. 69 (2016) 1361–1366.
[72] R.K. Dutta, B.P. Nenavathu, M.K. Gangishetty, A.V. Reddy, Antibacterial effect of chronic exposure of low concentration ZnO nanoparticles on *E. Coli*, J. Environ. Sci. Health 48 (2013) 871–878.
[73] S. Soren, S. Kumar, S. Mishra, P.K. Jena, S.K. Verma, P. Parhi, Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method, Microb. Pathog. 119 (2018) 145–151.
[74] R. Kitture, K. Chordiya, S. Gaware, S. Ghosh, P.W. More, P. Kulkarni, B.A. Chopade, S.N. Kale, ZnO nanoparticles-red sandalwood conjugate: a promising anti-diabetic agent, J. Nanosci. Nanotechnol. 15 (2015) 4046–4051.
[75] P.C. Nagajyothi, S.J. Cha, I.J. Yang, T.V.M. Sreekanth, K.J. Kim, H.M. Shin, Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using Polygala tenuifolia root extract, J. Photochem. Photobiol. B Biol. 146 (2015) 10–17.
[76] J. Rasmussen, W.E. Marinez, P. Lauka, D.G. Wingett, Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications, Expert Opinion on Drug Delivery7, 2010 1063–1077.
[77] H.-M. Xiong, ZnO nanoparticles applied to bioimaging and drug delivery, Adv. Mater. 25 (2013) 5329–5335.
[78] H.-M. Xiong, Y. Xu, Q.G. Ren, Y.-Y. Xia, Stable aqueous ZnO@polymer core-shell nanoparticles with tunable photoluminescence and their application in cell imaging, J. Am. Chem. Soc. 130 (2008) 7522–7523.
[79] H. Mirzaei, M. Darroudi, Zinc oxide nanoparticles: biological synthesis and biomedical applications, Ceram. Int. 43 (2017) 907–914.
[80] T.G. Smijs, S. Pavel, Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness, Nanotechnol. Sci. Appl. 4 (2011) 95–112.
[81] J. Ruszkiewicz, A. Pinkas, B. Ferrer, T.V. Peres, A. Tsatsakis, M. Aschner, Neurotoxic effect of active ingredients in sunscreen products, a contemporary review, Toxicology Reports, vol. 4, 2017, pp. 245–259.
[82] A. Kolodzijczak-Radzimska, T. Jesionowski, Zinc oxide–from synthesis to application: a review, Materials 7 (2014) 2833–2881.
[83] S. Sahoo, M. Maiti, A. Ganguly, J.J. George, A.K. Bhowmick, Effect of zinc oxide nanoparticles as cure activator on the properties of natural rubber and nitrile rubber, J. Appl. Polym. Sci. 105 (2007) 2407–2415.
[84] M.S. Newman, M. Stotland, J.I. Ellis, The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens, J. Am. Acad. Dermatol. 61 (2009) 685–692.
[85] F.-X. Xiao, S.-F. Hung, H.B. Tao, J. Miao, H.B. Yang, B. Liu, Spatially branched hierarchical ZnO nanorod-TiO_2 nanotube array heterostructures for versatile photocatalytic and photoelectrocatalytic applications: towards intimate integration of 1D-1D hybrid nanostructures, Nanoscale 6 (2014) 14950–14961.
[86] A.E. Nel, H. Meng, X. Liu, Mesoporous Silica Nanoparticles With Lipid Bilayer Coating for Cargo Delivery, Google Patents, 2018.
[87] A.E. Nel, J.I. Zink, H. Meng, Lipid Bilayer Coated Mesoporous Silica Nanoparticles With a High Loading Capacity for One or More Anticancer Agents, Google Patents, 2016.
[88] I.I. Slowing, B.G. Trewyn, V.S.-Y. Lin, Mesoporous silica nanoparticles for intracellular delivery of membrane-impermeable proteins, J. Am. Chem. Soc. 129 (2007) 8845–8849.
[89] M. Liong, J. Lu, F. Tamanoi, J.I. Zink, A. Nel, Mesoporous Silica Nanoparticles for Biomedical Applications, Google Patents, 2018.
[90] B.G. Trewyn, I.I. Slowing, S. Giri, H.-T. Chen, V.S.-Y. Lin, Synthesis and functionalization of a mesoporous silica nanoparticle based on the sol–gel process and applications in controlled release, Acc. Chem. Res. 40 (2007) 846–853.
[91] H. Meng, M. Xue, T. Xia, Z. Ji, D.Y. Tarn, J.I. Zink, A.E. Nel, Use of size and a copolymer design feature to improve the biodistribution and the enhanced permeability and retention effect of doxorubicin-loaded mesoporous silica nanoparticles in a murine xenograft tumor model, Am. Chem. Soc. Nano 5 (2011) 4131–4144.
[92] Y. Zhou, G. Quan, Q. Wu, X. Zhang, B. Niu, B. Wu, Y. Huang, X. Pan, C. Wu, Mesoporous silica nanoparticles for drug and gene delivery, Acta Pharm. Sinica B 8 (2018) 165–177.
[93] C. Caltagirone, A. Bettoschi, A. Garau, R. Montis, Silica-based nanoparticles: a versatile tool for the development of efficient imaging agents, Chem. Soc. Rev. 44 (2015) 4645–4671.
[94] M.-X. Zhao, E.-Z. Zeng, B.-J. Zhu, The biological applications of inorganic nanoparticle drug carriers, Chem. Nanomater. 1 (2015) 82–91.
[95] X. Fang, X. Zhao, W. Fang, C. Chen, N. Zheng, Self-templating synthesis of hollow mesoporous silica and their applications in catalysis and drug delivery, Nanoscale 5 (2013) 2205–2218.
[96] Z. Tao, Mesoporous silica-based nanodevices for biological applications, R. Soc. Chem. Adv. 4 (2014) 18961–18980.
[97] D. Tarn, C.E. Ashley, M. Zue, E.C. Carnes, J.I. Zink, C.J. Brinker, Mesoporous silica nanoparticle nanocarriers: biofunctionality and biocompatibility, Acc. Chem. Res. 46 (2013) 792–8901.
[98] J.L. Vivero-Escoto, I.I. Slowing, C.-W. Wu, V.S.-Y. Lin, Photoinduced intracellular controlled release drug delivery in human cells by gold-capped mesoporous silica nanosphere, J. Am. Chem. Soc. 131 (2009) 3462–3463.
[99] L. Pan, Q. He, L. Jianan, Y. Chen, M. Ma, L. Zhang, J. Shi, Nuclear-targeted drug delivery of TAT peptide-conjugated monodisperse mesoporous silica nanoparticles, J. Am. Chem. Soc. 134 (2012) 5711–5725.
[100] D. Carmona, F. Balas, J. Santamaria, Pore ordering and surface properties of FDU-12 and SBA-15 mesoporous materials and their relation to drug loading and release in aqueous environments, Mater. Res. Bull. 59 (2014) 311–322.

[101] X. Ma, G. Devi, Q. Qu, D.-F.K. Toh, G. Chen, Y. Zhao, Intracellular delivery of antisense peptide nucleic acid by fluorescent mesoporous silica nanoparticles, Bioconjug. Chem. 25 (2014) 1412–1420.
[102] S.B. Hartono, W. Gu, F. Kleitz, J. Liu, L. He, A.P.J. Middelberg, C. Yu, G.Q.M. Lu, S.Z. Qiao, Poly-l-lysine functionalized large pore cubic mesostructured silica nanoparticles as biocompatible carriers for gene delivery, Am. Cancer Soc. Nano 6 (2012) 2104–2117.
[103] A. Valizadeh, H. Mikaeili, M. Samiei, S.M. Farkhani, N. Zarghami, A. Akbarzadeh, S. Davaran, Quantum dots: synthesis, bioapplications, and toxicity, Nanoscale Res. Lett. 7 (2012) 480.
[104] Y. Jiao, X. Gao, J. Lu, Y. Chen, J. Zhou, X. Li, A novel method for PbS quantum dot synthesis, Mater. Lett. 72 (2012) 116–118.
[105] D. Djikanović, A. Kalauzi, M. Jeremić, J. Xu, M. Mićić, J.D. Whyte, R.M. Leblanc, R. K, Interaction of the CdSe quantum dots with plant cell walls, Colloids Surf. B 91 (2012) 41–47.
[106] J. Wu, Z.M. Wang, V.G. Dorogan, Y.I. Mazur, S. Li, G.J. Salamo, Insight into optical properties of strain-free quantum dot pairs, J. Nanopart. Res. 13 (2011) 947–952.
[107] H. Yang, W. Luan, S.-T. Tu, Z.M. Wang, Synthesis of nanocrystals via microreaction with temperature gradient: towards separation of nucleation and growth, Lab Chip 8 (2008) 451–455.
[108] Z. Zhang, A. Oehler, B. Resan, S. Kurmulis, K. Zhou, Q. Wang, M. Mangold, T. Süedmeyer, U. Keller, K. Weingarten, 1.55 μm InAs/GaAs quantum dots and high repetition rate quantum dot SESAM mode-locked laser, Sci. Rep. 2 (2012) 477.
[109] Z.M. Wang, B. Liang, K.A. Sablon, J. Lee, Y.I. Mazur, N.W. Strom, G.J. Salamo, Self-organization of InAs quantum-dot clusters directed by droplet homoepitaxy, Small 3 (2007) 235–238.
[110] K. Lee, J. Galloway, J. Park, C. Dvorack, M. Dallas, K. Konstantopoulos, A. Maitra, P. Searson, Quantitative molecular profiling of biomarkers for pancreatic cancer with functionalized quantum dots, Sci. Direct. 8 (2012) 1043–1051.
[111] M.I. Din, R. Rehan, Synthesis, characterization, and applications of copper nanoparticles, Anal. Lett. 50 (2017) 50–62.
[112] J. Bogdan, J. Pławińska-Czarnak, J. Zarzyńska, Nanoparticles of titanium and zinc oxides as novel agents in tumor treatment: a review, Nanoscale Res. Lett. 12 (2017) 225.
[113] M.R. Kalaie, A.A. Youzbashi, M.A. Meshkot, F. Hosseini-Nasab, Preparation and characterization of superparamagnetic nickel oxide particles by chemical route, Appl. Nanosci. 6 (2016) 789–795.
[114] M.A. Siddiqui, M. Ahamed, J. Ahmad, M.A. Majeed Khan, J. Musarrat, A.A. Al-Khedhairy, S.A. Alrokayan, Nickel oxide nanoparticles induce cytotoxicity, oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin, Food Chem. Toxicol. 50 (2012) 641–647.
[115] E. FarrokhTakin, G. Ciofani, G.L. Puleo, G. de Vito, C. Filippeschi, B. Mazzolai, V. Piazza, V. Mattoli, Barium titanate core-gold shell nanoparticles for hyperthermia treatments, Int. J. Nanomed. 8 (2013) 2319–2331.
[116] J.P. Ruparelia, A.K. Chatterjee, S.P. Duttagupta, S. Mukherji, Strain specificity in antimicrobial activity of silver and copper nanoparticles, Acta Biomater. 4 (2008) 707–716.
[117] S. Mandal, S. De, Catalytic and fluorescence studies with copper nanoparticles synthesized in polysorbates of varying hydrophobicity, Colloids Surf. A Physicochem. Eng. Asp. 467 (2015) 233–250.
[118] M. Vincent, P. Hartemann, M. Engels-Deutsch, Antimicrobial applications of copper, Int. J. Hyg. Environ. Health 219 (2016) 585–591.
[119] G. Ou, Z. Li, D. Li, L. Cheng, Z. Liu, H. Wu, Photothermal therapy by using titanium oxide nanoparticles, Nano Res. 9 (2016) 1236–1243.
[120] M.A. Behnam, F. Emami, Z. Sobhani, A.R. Dehghanian, The application of titanium dioxide (TiO_2) nanoparticles in the photo-thermal therapy of melanoma cancer model, Iranian J. Basic Med. Sci. 21 (2018) 1133–1139.
[121] F. Yurt, K. Ocakoglu, M. Ince, S.G. Colak, O. Er, H.M. Soylu, C. Gunduz, C. Biray Avci, C. Caliskan Kurt, Photodynamic therapy and nuclear imaging activities of zinc phthalocyanine-integrated TiO_2 nanoparticles in breast and cervical tumors, Chem. Biol. Drug Des. 91 (2018) 789–796.
[122] A.A. Lopera, A.M.A. Velasquez, L.C. Clementino, S. Robledo, A. Montoya, L.M. de Freitas, V.D.N. Bezzon, C.R. Fontana, C. Garcia, M.A.S. Graminha, Solution-combustion synthesis of doped TiO_2 compounds and its potential antileishmanial activity mediated by photodynamic therapy, J. Photochem. Photobiol. B Biol. 183 (2018) 64–74.
[123] L. Visai, L. De Nardo, C. Punta, L. Melone, A. Cigada, M. Imbriani, C.R. Arciola, Titanium oxide antibacterial surfaces in biomedical devices, Int. J. Artif. Organs 34 (2011) 929–946.
[124] A.M. Allahverdiyev, E.S. Abamor, M. Bagirova, M. Rafailovich, Antimicrobial effects of TiO(2) and Ag(2)O nanoparticles against drug-resistant bacteria and leishmania parasites, Future Microbiol 6 (2011) 933–940.
[125] J.R. Villalobos-Hernandez, C.C. Muller-Goymann, Sun protection enhancement of titanium dioxide crystals by the use of carnauba wax nanoparticles: the synergistic interaction between organic and inorganic sunscreens at nanoscale, Int. J. Pharm. 322 (2006) 161–170.
[126] A. Elsaesser, C.V. Howard, Toxicology of nanoparticles, Adv. Drug Deliv. Rev. 64 (2012) 129–137.
[127] OECD, Publicatiomns in the Series on the Safety of Manufactured Nanomaterials, www.oecd.org 2019.
[128] N.T. Program, Nanomaterials National Institute of Environmental Health Sciences (NIEHS), www.niehs.nih.gov 2019.
[129] NTP, in: H.A.H. Services (Ed.), 14th Report On Carcinogens: Cobalt And Cobalt Compounds That Release Cobalt Ions In Vivo, 2016.
[130] S.S. Sparrow, S. Robinson, S. Bolam, C. Bruce, A. Danks, D. Everett, S. Fulcher, R.E. Hill, H. Palmer, E.W. Scott, K.L. Chapman, Opportunities to minimise animal use in pharmaceutical regulatory general toxicology: a cross-company review, Regul. Toxicol. Pharmacol. 61 (2011) 222–229.
[131] S.C. Gad, Drug Safety Evaluation, John Wiley and Sons, New York, 2002.
[132] USFDA, M3(R2) nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals, 2010.
[133] C.D. Klaassen, Casarett and Doull's Toxicology, the Basic Science of Poisons, McGraw-Hill Companies Inc, 1996.
[134] E.O. Erhirhie, C.P. Ihekwereme, E.E. Ilodigwe, Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance, Interdiscip. Oxicol. 11 (2018) 5–12.
[135] S. Parasuraman, Toxicological screening, J. Pharmacol. Pharmacother. 2 (2011) 74–79.
[136] K. Greish, G. Thiagarajan, H. Ghandehari, *In vivo* methods of nanotoxicology, Methods Mol. Biol. 926 (2012) 235–253.
[137] H. Nehoff, S. Taurin, K. Greish, Toxicological assessment of nanomedicine, 2013.
[138] G. Oberdorster, Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles, Inhal. Toxicol. 8 (Suppl) (1996) 73–89.
[139] P.L. Apopa, Y. Qian, R. Shao, N.L. Guo, D. Schwegler-Berry, M. Pacurari, D. Porter, X. Shi, V. Vallyathan, V. Castranova, D.C. Flynn, Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling, Particle Fibre Toxicol. 6 (2009) 1.
[140] E.J. Park, E. Bae, J. Yi, Y. Kim, K. Choi, S.H. Lee, J. Yoon, B.C. Lee, K. Park, Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles, Environ. Toxicol. Pharmacol. 30 (2010) 162–168.
[141] E.J. Park, H. Kim, Y. Kim, J. Yi, K. Choi, K. Park, Inflammatory responses may be induced by a single intratracheal instillation of iron nanoparticles in mice, Toxicology 275 (2010) 65–71.
[142] E.J. Park, J. Yoon, K. Choi, J. Yi, K. Park, Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation, Toxicology 260 (2009) 37–46.
[143] M. van der Zande, R.J. Vandebriel, M.J. Groot, E. Kramer, Z.E. Herrera Rivera, K. Rasmussen, J.S. Ossenkoppele, P. Tromp, E.R. Gremmer, R.J. Peters, P.J. Hendriksen, H.J. Marvin, R.L. Hoogenboom, A.A. Peijnenburg, H. Bouwmeester, Sub-chronic toxicity study in rats orally exposed to nanostructured silica, Part Fibre Toxicol. 11 (2014) 8.
[144] C.M. Sayes, R. Wahi, P.A. Kurian, Y. Liu, J.L. West, K.D. Ausman, D.B. Warheit, V.L. Colvin, Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells, Toxicol. Sci. 92 (2006) 174–185.
[145] S.D. Mo, W.Y. Ching, Electronic and optical properties of three phases of titanium dioxide: Rutile, anatase, and brookite, Phys. Rev. B Condens. Matter 51 (1995) 13023–13032.
[146] M.R. Miller, J.B. Raftis, J.P. Langrish, S.G. McLean, P. Samutrtai, S.P. Connell, S. Wilson, A.T. Vesey, P.H.B. Fokkens, A.J.F. Boere, P. Krystek, C.J. Campbell, P.W.F. Hadoke, K. Donaldson, F.R. Cassee, D.E. Newby, R. Duffin, N.L. Mills, Inhaled nanoparticles accumulate at sites of vascular disease, ACS Nano 11 (2017) 4542–4552.
[147] J.L. Weaver, G.A. Tobin, T. Ingle, S. Bancos, D. Stevens, R. Rouse, K.E. Howard, D. Goodwin, A. Knapton, X. Li, K. Shea, S. Stewart, L. Xu, P.L. Goering, Q. Zhang, P.C. Howard, J. Collins, S. Khan, K. Sung, K.M. Tyner, Evaluating the potential of gold, silver, and silica nanoparticles to saturate mononuclear phagocytic system tissues under repeat dosing conditions, Part Fibre Toxicol. 14 (2017) 25.
[148] M. Yazdimamaghani, P.J. Moos, M.A. Dobrovolskaia, H. Ghandehari, Genotoxicity of amorphous silica nanoparticles: Status and prospects, Nanomedicine 16 (2019) 106–125.
[149] K.M. Tyner, N. Zheng, S. Choi, X. Xu, P. Zou, W. Jiang, C. Guo, C.N. Cruz, How has CDER prepared for the nano revolution? Rev. Risk Assess. Regul. Res. Guid. Act. AAPS J. 19 (2017) 1071–1083.
[150] J.B. Hall, M.A. Dobrovolskaia, S. Choi, A.K. Patri, S.E. McNeil, Characterization of nanoparticles for therapeutics, Nanomedicine 2 (2007) 789–803.
[151] W. Wohlleben, L. Ma-Hock, V. Boyko, G. Cox, H. Egenolf, H. Freiberger, B. Hinrichsen, S. Hirth, R. Landsiedel, Nanospecific guidance in REACH: a comparative physical-chemical characterization of 15 materials with methodical correlations, J. Ceramic Sci. Technol. 4 (2013) 93–104.
[152] A.K. Patri, J.D. Clogston, J.H. Grossman, M.A. Dobrovolskaia, P.P. Adiseshaiah, R.M. Crist, S.T. Stern, S.E. McNeil, Common pitfalls in nanotechnology: lessons learned from NCI's Nanotechnology Characterization Laboratory, Integr. Biol. 5 (2012) 66–73.
[153] S. Arora, J.M. Rajwade, K.M. Paknikar, Nanotoxicology and *in vitro* studies: the need of the hour, Toxicol. Appl. Pharmacol. 258 (2012) 151–165.
[154] T. Yu, K. Greish, L.D. McGill, A. Ray, H. Ghandehari, Influence of geometry, porosity, and surface characteristics of silica nanoparticles on acute toxicity: their vasculature effect and tolerance threshold, ACS Nano 6 (2012) 2289–2301.
[155] A. Sukhanova, S. Bozrova, P. Sokolov, M. Berestovoy, A. Karaulov, I. Nabiev, Dependence of nanoparticle toxicity on their physical and chemical properties, Nanoscale Res. Lett. 13 (2018) 44.
[156] K.L. Aillon, Y. Xie, N. El-Gendy, C.J. Berkland, M.L. Forrest, Effects of nanomaterial physicochemical properties on *in vivo* toxicity, Adv. Drug Deliv. Rev. 61 (2009) 457–466.
[157] M.A. Gatoo, S. Naseem, M.Y. Arfat, A. Mahmood Dar, K. Qasim, S. Zubair, Physicochemical properties of nanomaterials: implication in associated toxic manifestations, BioMed. Res. Int. vol. 2014 (2014).

[158] Y.R. Kim, J.I. Park, E.J. Lee, S.H. Park, N.W. Seong, J.H. Kim, G.Y. Kim, E.H. Meang, J.S. Hong, S.H. Kim, S.B. Koh, M.S. Kim, C.S. Kim, S.K. Kim, S.W. Son, Y.R. Seo, B.H. Kang, B.S. Han, S.S. An, H.I. Yun, M.K. Kim, Toxicity of 100 nm zinc oxide nanoparticles: a report of 90-day repeated oral administration in Sprague Dawley rats, Int. J. Nanomed. 9 (Suppl. 2) (2014) 109–126.
[159] W.H. De Jong, L.T.M. Van Der Ven, A. Sleijffers, M.V.D.Z. Park, E.H.J.M. Jansen, H. Van Loveren, R.J. Vandebriel, Systemic and immunotoxicity of silver nanoparticles in an intravenous 28 days repeated dose toxicity study in rats, Biomaterials 34 (2013) 8333–8343.
[160] J. Chen, H. Wang, W. Long, X. Shen, D. Wu, S.S. Song, Y.M. Sun, P.X. Liu, S.J. Fan, F.Y. Fan, X.D. Zhang, Sex differences in the toxicity of polyethylene glycol-coated gold nanoparticles in mice, Int. J. Nanomed. 8 (2013) 2409–2419.
[161] E. Cardoso, G.T. Rezin, E.T. Zanoni, F. de Souza Notoya, D.D. Leffa, A.P. Damiani, F. Daumann, J.C. Rodriguez, R. Benavides, L. da Silva, V.M. Andrade, M.M. da Silva Paula, Acute and chronic administration of gold nanoparticles cause DNA damage in the cerebral cortex of adult rats, Mutat. Res. 766-767 (2014) 25–30.
[162] B.A. Katsnelson, T.D. Degtyareva, I.I. Minigalieva, L.I. Privalova, S.V. Kuzmin, O.S. Yeremenko, E.P. Kireyeva, M.P. Sutunkova, I.I. Valamina, M.Y. Khodos, A.N. Kozitsina, V.Y. Shur, V.A. Vazhenin, A.P. Potapov, M.V. Morozova, Subchronic systemic toxicity and bioaccumulation of Fe_3O_4 nano- and microparticles following repeated intraperitoneal administration to rats, Int. J. Toxicol. 30 (2011) 59–68.
[163] Y.S. Kim, M.Y. Song, J.D. Park, K.S. Song, H.R. Ryu, Y.H. Chung, H.K. Chang, J.H. Lee, K.H. Oh, B.J. Kelman, I.K. Hwang, I.J. Yu, Subchronic oral toxicity of silver nanoparticles, Particle Fibre Toxicol. 7 (2010).
[164] J.H. Sung, J.H. Ji, J.D. Park, J.U. Yoon, D.S. Kim, K.S. Jeon, M.Y. Song, J. Jeong, B.S. Han, J.H. Han, Y.H. Chung, H.K. Chang, J.H. Lee, M.H. Cho, B.J. Kelman, I.J. Yu, Subchronic inhalation toxicity of silver nanoparticles, Toxicol. Sci. 108 (2009) 452–461.
[165] R. Mohammadpour, M. Yazdimamaghani, D.L. Cheney, J. Jedrzkiewicz, H. Ghandehari, Subchronic toxicity of silica nanoparticles as a function of size and porosity, J. Control. Release 304 (2019) 216–232.
[166] S.P. Singh, M. Kumari, S.I. Kumari, M.F. Rahman, M. Mahboob, P. Grover, Toxicity assessment of manganese oxide micro and nanoparticles in Wistar rats after 28 days of repeated oral exposure, J. Appl. Toxicol. 33 (2013) 1165–1179.
[167] Y. Zhao, Y. Wang, F. Ran, Y. Cui, C. Liu, Q. Zhao, Y. Gao, D. Wang, S. Wang, A comparison between sphere and rod nanoparticles regarding their *in vivo* biological behavior and pharmacokinetics, Sci. Rep. 7 (2017) 4131.
[168] S.H. Seok, W.S. Cho, J.S. Park, Y. Na, A. Jang, H. Kim, Y. Cho, T. Kim, J.R. You, S. Ko, B.C. Kang, J.K. Lee, J. Jeong, J.H. Che, Rat pancreatitis produced by 13-week administration of zinc oxide nanoparticles: biopersistence of nanoparticles and possible solutions, J. Appl. Toxicol. 33 (2013) 1089–1096.
[169] P. Surekha, A.S. Kishore, A. Srinivas, G. Selvam, A. Goparaju, P.N. Reddy, P.B. Murthy, Repeated dose dermal toxicity study of nano zinc oxide with Sprague-Dawley rats, Cutan. Ocul. Toxicol. 31 (2012) 26–32.
[170] H.J. Ryu, M.Y. Seo, S.K. Jung, E.H. Maeng, S.Y. Lee, D.H. Jang, T.J. Lee, K.Y. Jo, Y.R. Kim, K.B. Cho, M.K. Kim, B.J. Lee, S.W. Son, Zinc oxide nanoparticles: a 90-day repeated-dose dermal toxicity study in rats, Int. J. Nanomedicine 9 (Suppl. 2) (2014) 137–144.
[171] Y.S. Kim, J.S. Kim, H.S. Cho, D.S. Rha, J.M. Kim, J.D. Park, B.S. Choi, R. Lim, H.K. Chang, Y.H. Chung, I.H. Kwon, J. Jeong, B.S. Han, I.J. Yu, Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats, Inhal. Toxicol. 20 (2008) 575–583.
[172] T. Garcia, D. Lafuente, J. Blanco, D.J. Sanchez, J.J. Sirvent, J.L. Domingo, M. Gomez, Oral subchronic exposure to silver nanoparticles in rats, Food Chem. Toxicol. 92 (2016) 177–187.
[173] J.S. Hong, S. Kim, S.H. Lee, E. Jo, B. Lee, J. Yoon, I.C. Eom, H.M. Kim, P. Kim, K. Choi, M.Y. Lee, Y.R. Seo, Y. Kim, Y. Lee, J. Choi, K. Park, Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test, Nanotoxicology 8 (2014) 349–362.
[174] J.H. Ji, J.H. Jung, S.S. Kim, J.U. Yoon, J.D. Park, B.S. Choi, Y.H. Chung, I.H. Kwon, J. Jeong, B.S. Han, J.H. Shin, J.H. Sung, K.S. Song, I.J. Yu, Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats, Inhal. Toxicol. 19 (2007) 857–871.
[175] J.S. Kim, J.H. Sung, J.H. Ji, K.S. Song, J.H. Lee, C.S. Kang, I.J. Yu, *In vivo* genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure, Saf. Health Work 2 (2011) 34–38.
[176] M. Korani, S.M. Rezayat, S. Arbabi Bidgoli, Sub-chronic dermal toxicity of silver nanoparticles in Guinea pig: Special emphasis to heart, bone and kidney toxicities, Iran J. Pharm. Res. 12 (2013) 511–519.
[177] C. Rambanapasi, J.R. Zeevaart, H. Buntting, C. Bester, D. Kotze, R. Hayeshi, A. Grobler, Bioaccumulation and subchronic toxicity of 14 nm gold nanoparticles in rats, Molecules 21 (2016).
[178] X.D. Zhang, H.Y. Wu, D. Wu, Y.Y. Wang, J.H. Chang, Z.B. Zhai, A.M. Meng, P.X. Liu, L.A. Zhang, F.Y. Fan, Toxicologic effects of gold nanoparticles *in vivo* by different administration routes, Int. J. Nanomed. 5 (2010) 771–781.
[179] A. Jafari, Y. Rasmi, M. Hajaghazadeh, M. Karimipour, Hepatoprotective effect of thymol against subchronic toxicity of titanium dioxide nanoparticles: Biochemical and histological evidences, Environ. Toxicol. Pharmacol. 58 (2018) 29–36.
[180] I. Grissa, J. Elghoul, L. Ezzi, S. Chakroun, E. Kerkeni, M. Hassine, L. El Mir, M. Mehdi, H. Ben Cheikh, Z. Haouas, Anemia and genotoxicity induced by sub-chronic intragastric treatment of rats with titanium dioxide nanoparticles, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 794 (2015) 25–31.
[181] Y. Ze, L. Sheng, X. Zhao, X. Ze, X. Wang, Q. Zhou, J. Liu, Y. Yuan, S. Gui, X. Sang, Q. Sun, J. Hong, X. Yu, L. Wang, B. Li, F. Hong, Neurotoxic characteristics of spatial recognition damage of the hippocampus in mice following subchronic peroral exposure to TiO2 nanoparticles, J. Hazard. Mater. 264 (2014) 219–229.
[182] D.B. Warheit, S.C. Brown, E.M. Donner, Acute and subchronic oral toxicity studies in rats with nanoscale and pigment grade titanium dioxide particles, Food Chem. Toxicol. 84 (2015) 208–224.
[183] I.C. Lee, J.W. Ko, S.H. Park, N.R. Shin, I.S. Shin, C. Moon, J.H. Kim, H.C. Kim, J.C. Kim, Comparative toxicity and biodistribution assessments in rats following subchronic oral exposure to copper nanoparticles and microparticles, Part Fibre. Toxicol. 13 (2016) 56.
[184] J. Ai, E. Biazar, M. Jafarpour, M. Montazeri, A. Majdi, S. Aminifard, M. Zafari, H.R. Akbari, H.G. Rad, Nanotoxicology and nanoparticle safety in biomedical designs, Int. J. Nanomed. 6 (2011) 1117–1127.
[185] M. Gochfeld, Sex differences in human and animal toxicology, Toxicol. Pathol. 45 (2017) 172–189.
[186] R.L. Lipnick, J.A. Cotruvo, R.N. Hill, R.D. Bruce, K.A. Stitzel, A.P. Walker, I. Chu, M. Goddard, L. Segal, J.A. Springer, R.C. Myers, Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures, Food Chem. Toxicol. 33 (1995) 223–231.
[187] S.L. Klein, K.L. Flanagan, Sex differences in immune responses, Nat. Rev. Immunol. 16 (2016) 626.
[188] D.L. Smith, X. Dong, S. Du, M. Oh, R.R. Singh, R.R. Voskuhl, A female preponderance for chemically induced lupus in SJL/J mice, Clin. Immunol. 122 (2007) 101–107.
[189] S.L. Klein, I. Marriott, E.N. Fish, Sex-based differences in immune function and responses to vaccination, Trans. R. Soc. Trop. Med. Hyg. 109 (2015) 9–15.
[190] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, M.H. Cho, Antimicrobial effects of silver nanoparticles, Nanomedicine 3 (2007) 95–101.
[191] K.H. Chen, D.J. Lundy, E.K. Toh, C.H. Chen, C. Shih, P. Chen, H.C. Chang, J.J. Lai, P.S. Stayton, A.S. Hoffman, P.C. Hsieh, Nanoparticle distribution during systemic inflammation is size-dependent and organ-specific, Nanoscale 7 (2015) 15863–15872.
[192] M.A. Dobrovolskaia, Pre-clinical immunotoxicity studies of nanotechnology-formulated drugs: Challenges, considerations and strategy, J. Control. Release 220 (2015) 571–583.
[193] M.L. Donnell, A.J. Lyon, M.R. Mormile, S. Barua, Endotoxin hitchhiking on polymer nanoparticles, Nanotechnology 27 (2016) 285601.
[194] A.N. Ilinskaya, S. Man, A.K. Patri, J.D. Clogston, R.M. Crist, R.E. Cachau, S.E. McNeil, M.A. Dobrovolskaia, Inhibition of phosphoinositol 3 kinase contributes to nanoparticle-mediated exaggeration of endotoxin-induced leukocyte procoagulant activity, Nanomedicine 9 (2014) 1311–1326.
[195] Y. Li, Z. Shi, I. Radauer-Preiml, A. Andosch, E. Casals, U. Luetz-Meindl, M. Cobaleda, Z. Lin, M. Jaberi-Douraki, P. Italiani, J. Horejs-Hoeck, M. Himly, N.A. Monteiro-Riviere, A. Duschl, V.F. Puntes, D. Boraschi, Bacterial endotoxin (lipopolysaccharide) binds to the surface of gold nanoparticles, interferes with biocorona formation and induces human monocyte inflammatory activation, Nanotoxicology 11 (2017) 1157–1175.
[196] L. Talamini, M.B. Violatto, Q. Cai, M.P. Monopoli, K. Kantner, Z. Krpetic, A. Perez-Potti, J. Cookman, D. Garry, P.S. C, L. Boselli, B. Pelaz, T. Serchi, S. Cambier, A.C. Gutleb, N. Feliu, Y. Yan, M. Salmona, W.J. Parak, K.A. Dawson, P. Bigini, Influence of size and shape on the anatomical distribution of endotoxin-free gold nanoparticles, ACS Nano 11 (2017) 5519–5529.
[197] K. Inoue, Promoting effects of nanoparticles/materials on sensitive lung inflammatory diseases, Environ. Health Prev. Med. 16 (2011) 139–143.
[198] K. Inoue, H. Takano, Aggravating impact of nanoparticles on immune-mediated pulmonary inflammation, Sci. World J. 11 (2011) 382–390.
[199] K. Inoue, H. Takano, R. Yanagisawa, S. Hirano, T. Kobayashi, Y. Fujitani, A. Shimada, T. Yoshikawa, Effects of inhaled nanoparticles on acute lung injury induced by lipopolysaccharide in mice, Toxicology 238 (2007) 99–110.
[200] K. Inoue, H. Takano, R. Yanagisawa, S. Hirano, M. Sakurai, A. Shimada, T. Yoshikawa, Effects of airway exposure to nanoparticles on lung inflammation induced by bacterial endotoxin in mice, Environ. Health Perspect. 114 (2006) 1325–1330.
[201] R.F. Hamilton, N. Wu, D. Porter, M. Buford, M. Wolfarth, A. Holian, Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity, Part Fibre Toxicol. 6 (2009) 35.
[202] C. U.S. FDA, CBER, CMV, CDRH, ORA, Guidance for industry, Pyrogen Endotoxins Test. (2012).
[203] USP 30 NF 25., <85>, Bacterial Endotoxins Test, 2007.
[204] R. Engelhardt, A. Mackensen, C. Galanos, Phase I trial of intravenously administered endotoxin (Salmonella abortus equi) in cancer patients, Cancer Res. 51 (1991) 2524–2530.
[205] R. Engelhardt, F. Otto, A. Mackensen, R. Mertelsmann, C. Galanos, Endotoxin (Salmonella abortus equi) in cancer patients. Clinical and immunological findings, Prog. Clin. Biol. Res. 392 (1995) 253–261.
[206] A. Mackensen, C. Galanos, R. Engelhardt, Treatment of cancer patients with endotoxin induces release of endogenous cytokines, Pathobiology 59 (1991) 264–267.
[207] F. Otto, P. Schmid, A. Mackensen, U. Wehr, A. Seiz, M. Braun, C. Galanos, R. Mertelsmann, R. Engelhardt, Phase II trial of Intravenous endotoxin in patients with colorectal and non-small cell lung cancer, Eur. J. Cancer 32a (1996) 1712–1718.
[208] S.K. Biswas, E. Lopez-Collazo, Endotoxin tolerance: new mechanisms, molecules and clinical significance, Trends Immunol. 30 (2009) 475–487.
[209] J.M. Cavaillon, M. Adib-Conquy, Bench-to-bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis, Crit. Care 10 (2006) 233.
[210] E. Lopez-Collazo, C. del Fresno, Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences, Crit. Care 17 (2013) 242.
[211] A.E. Medvedev, K.M. Kopydlowski, S.N. Vogel, Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression, J. Immunol. 164 (2000) 5564–5574.

[212] L.A. Haile, M. Puig, L. Kelley-Baker, D. Verthelyi, Detection of innate immune response modulating impurities in therapeutic proteins, PLoS One 10 (2015), e0125078.
[213] Y.N. Zhang, W. Poon, A.J. Tavares, I.D. McGilvray, W.C.W. Chan, Nanoparticle-liver interactions: Cellular uptake and hepatobiliary elimination, J. Control. Release 240 (2016) 332–348.
[214] E. Sadauskas, G. Danscher, M. Stoltenberg, U. Vogel, A. Larsen, H. Wallin, Protracted elimination of gold nanoparticles from mouse liver, Nanomedicine 5 (2009) 162–169.
[215] M. Longmire, P.L. Choyke, H. Kobayashi, Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats, Nanomedicine 3 (2008) 703–717.
[216] J.W. Yun, S.H. Kim, J.R. You, W.H. Kim, J.J. Jang, S.K. Min, H.C. Kim, D.H. Chung, J. Jeong, B.C. Kang, J.H. Che, Comparative toxicity of silicon dioxide, silver and iron oxide nanoparticles after repeated oral administration to rats, J. Appl. Toxicol. 35 (2015) 681–693.
[217] S. Najimi, M. Shakibaie, E. Jafari, A. Ameri, N. Rahimi, H. Forootanfar, M. Yazdanpanah, H.R. Rahimi, Acute and subacute toxicities of biogenic tellurium nanorods in mice, Regul. Toxicol. Pharmacol. 90 (2017) 222–230.
[218] H. Nishimori, M. Kondoh, K. Isoda, S. Tsunoda, Y. Tsutsumi, K. Yagi, Histological analysis of 70-nm silica particles-induced chronic toxicity in mice, Eur. J. Pharm. Biopharm. 72 (2009) 626–629.
[219] Y. Yu, Y. Li, W. Wang, M. Jin, Z. Du, Y. Li, J. Duan, Y. Yu, Z. Sun, Acute toxicity of amorphous silica nanoparticles in intravenously exposed ICR mice, PLoS One 8 (2013), e61346.
[220] J.J. Li, S. Muralikrishnan, C.T. Ng, L.Y. Yung, B.H. Bay, Nanoparticle-induced pulmonary toxicity, Exp. Biol. Med. 235 (2010) 1025–1033.
[221] A. Adamcakova-Dodd, L.V. Stebounova, J.S. Kim, S.U. Vorrink, A.P. Ault, P.T. O'Shaughnessy, V.H. Grassian, P.S. Thorne, Toxicity assessment of zinc oxide nanoparticles using sub-acute and sub-chronic murine inhalation models, Part. Fibre Toxicol. 11 (2014).
[222] A. Elder, R. Gelein, V. Silva, T. Feikert, L. Opanashuk, J. Carter, R. Potter, A. Maynard, Y. Ito, J. Finkelstein, G. Oberdorster, Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, Environ. Health Perspect. 114 (2006) 1172–1178.
[223] W.S. Cho, R. Duffin, C.A. Poland, S.E. Howie, W. MacNee, M. Bradley, I.L. Megson, K. Donaldson, Metal oxide nanoparticles induce unique inflammatory footprints in the lung: important implications for nanoparticle testing, Environ. Health Perspect. 118 (2010) 1699–1706.
[224] D. Schwotzer, H. Ernst, D. Schaudien, H. Kock, G. Pohlmann, C. Dasenbrock, O. Creutzenberg, Effects from a 90-day inhalation toxicity study with cerium oxide and barium sulfate nanoparticles in rats, Part Fibre Toxicol. 14 (2017) 23.
[225] M. Cataldi, C. Vigliotti, T. Mosca, M. Cammarota, D. Capone, Emerging role of the spleen in the pharmacokinetics of monoclonal antibodies, nanoparticles and exosomes, Int. J. Mol. Sci. 18 (2017).
[226] S. Ivanov, S. Zhuravsky, G. Yukina, V. Tomson, D. Korolev, M. Galagudza, *In vivo* toxicity of intravenously administered silica and silicon nanoparticles, Materials 5 (2012) 1873–1889.
[227] M. Valko, H. Morris, M. Cronin, Metals, toxicity and oxidative stress, Curr. Med. Chem. 12 (2005) 1161–1208.
[228] S.J. Stohs, D. Bagchi, Oxidative mechanisms in the toxicity of metal ions, Free Radic. Biol. Med. 18 (1995) 321–336.
[229] I. Iavicoli, L. Fontana, G. Nordberg, The effects of nanoparticles on the renal system, Crit. Rev.Toxicol. 46 (2016) 490–560.
[230] S.M. Moghimi, A.C. Hunter, T.L. Andresen, Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective, Annu. Rev. Pharmacol. Toxicol. 52 (2012) 481–503.
[231] C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, L. Bernardino, Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurodegenerative diseases, J. Control. Release 235 (2016) 34–47.
[232] Y. Zhou, Z. Peng, E.S. Seven, R.M. Leblanc, Crossing the blood-brain barrier with nanoparticles, J. Control. Release 270 (2018) 290–303.
[233] K.G. Petry, C. Boiziau, V. Dousset, B. Brochet, Magnetic resonance imaging of human brain macrophage infiltration, Neurotherapeutics 4 (2007) 434–442.
[234] H. Xie, Y.H. Zhu, W.L. Jiang, Q. Zhou, H. Yang, N. Gu, Y. Zhang, H.B. Xu, H.B. Xu, X.L. Yang, Lactoferrin-conjugated superparamagnetic iron oxide nanoparticles as a specific MRI contrast agent for detection of brain glioma *in vivo*, Biomaterials 32 (2011) 495–502.
[235] Y.L. Hu, J.Q. Gao, Potential neurotoxicity of nanoparticles, Int. J. Pharm. 394 (2010) 115–121.
[236] B. Wang, W.Y. Feng, M. Wang, J.W. Shi, F. Zhang, H. Ouyang, Y.L. Zhao, Z.F. Chai, Y.Y. Huang, Y.N. Xie, H.F. Wang, J. Wang, Transport of intranasally instilled fine Fe_2O_3 particles into the brain: Micro-distribution, chemical states, and histopathological observation, Biol. Trace Elem. Res. 118 (2007) 233–243.
[237] J.X. Wang, Y. Liu, F. Jiao, F. Lao, W. Li, Y.Q. Gu, Y.F. Li, C.C. Ge, G.Q. Zhou, B. Li, Y.L. Zhao, Z.F. Chai, C.Y. Chen, Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles, Toxicology 254 (2008) 82–90.
[238] A.J.D. de Lorenzo, Taste and smell in vertebrates, Churchhill, London, 1970.
[239] D.M. Teleanu, C. Chircov, A.M. Grumezescu, A. Volceanov, R.I. Teleanu, Impact of nanoparticles on brain health: An up to date overview, J. Clin. Med. 7 (2018).
[240] E. Cardoso, G.T. Rezin, E.T. Zanoni, F. de Souza Notoya, D.D. Leffa, A.P. Damiani, F. Daumann, J.C.O. Rodriguez, R. Benavides, L. Da Silva, Acute and chronic administration of gold nanoparticles cause DNA damage in the cerebral cortex of adult rats, Mutat. Res. Fundam. Mol. Mech. Mutagen. 766 (2014) 25–30.
[241] K. Greish, A.A. Alqahtani, A.F. Alotaibi, A.M. Abdulla, A.T. Bukelly, F.M. Alsobyani, G.H. Alharbi, I.S. Alkiyumi, M.M. Aldawish, T.F. Alshahrani, V. Pittalà, S. Taurin, A. Kamal, The effect of silver nanoparticles on learning, memory and social interaction in BALB/C mice, Int. J. Environ. Res. Public Health 16 (2019) 148.
[242] L.G. Remsen, C.I. McCormick, S. Roman-Goldstein, G. Nilaver, R. Weissleder, A. Bogdanov, I. Hellstrom, R.A. Kroll, E.A. Neuwelt, MR of carcinoma-specific monoclonal antibody conjugated to monocrystalline iron oxide nanoparticles: the potential for noninvasive diagnosis, AJNR Am. J. Neuroradiol. 17 (1996) 411–418.
[243] J.A. Frank, B.R. Miller, A.S. Arbab, H.A. Zywicke, E.K. Jordan, B.K. Lewis, L.H. Bryant Jr., J.W. Bulte, Clinically applicable labeling of mammalian and stem cells by combining superparamagnetic iron oxides and transfection agents, Radiology 228 (2003) 480–487.
[244] K. Adachi, N. Yamada, Y. Yoshida, O. Yamamoto, Subchronic exposure of titanium dioxide nanoparticles to hairless rat skin, Exp. Dermatol. 22 (2013) 278–283.
[245] V. Patel, F. Willingham, The management of chronic pancreatitis, Med. Clin. N. Am. 103 (2019) 153–162.
[246] B. Wang, W. Feng, M. Wang, T. Wang, Y. Gu, M. Zhu, H. Ouyang, J. Shi, F. Zhang, Y. Zhao, Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice, J. Nanopart. Res. 10 (2008) 263–276.
[247] K.S. Hougaard, L. Campagnolo, P. Chavatte-Palmer, A. Tarrade, D. Rousseau-Ralliard, S. Valentino, M.V. Park, W.H. de Jong, G. Wolterink, A.H. Piersma, A perspective on the developmental toxicity of inhaled nanoparticles, Reprod. Toxicol. 56 (2015) 118–140.
[248] G. Gao, Y. Ze, B. Li, X. Zhao, T. Zhang, L. Sheng, R. Hu, S. Gui, X. Sang, Q. Sun, J. Cheng, Z. Cheng, L. Wang, M. Tang, F. Hong, Ovarian dysfunction and gene-expressed characteristics of female mice caused by long-term exposure to titanium dioxide nanoparticles, J. Hazard. Mater. 243 (2012) 19–27.
[249] L.I. Privalova, B.A. Katsnelson, N.V. Loginova, V.B. Gurvich, V.Y. Shur, I.E. Valamina, O.H. Makeyev, M.P. Sutunkova, I.A. Minigalieva, E.P. Kireyeva, V.O. Rusakov, A.E. Tyurnina, R.V. Kozin, E.Y. Meshtcheryakova, A.V. Korotkov, E.A. Shuman, A.E. Zvereva, S.V. Kostykova, Subchronic toxicity of copper oxide nanoparticles and its attenuation with the help of a combination of bioprotectors, Int. J. Mol. Sci. 15 (2014) 12379–12406.
[250] I.A. Minigalieva, B.A. Katsnelson, V.G. Panov, L.I. Privalova, A.N. Varaksin, V.B. Gurvich, M.P. Sutunkova, V.Y. Shur, E.V. Shishkina, I.E. Valamina, I.V. Zubarev, O.H. Makeyev, E.Y. Meshtcheryakova, S.V. Klinova, *In vivo* toxicity of copper oxide, lead oxide and zinc oxide nanoparticles acting in different combinations and its attenuation with a complex of innocuous bio-protectors, Toxicology 380 (2017) 72–93.
[251] B.A. Katsnelson, I.A. Minigaliyeva, V.G. Panov, L.I. Privalova, A.N. Varaksin, V.B. Gurvich, M.P. Sutunkova, V.Y. Shur, E.V. Shishkina, I.E. Valamina, Some patterns of metallic nanoparticles' combined subchronic toxicity as exemplified by a combination of nickel and manganese oxide nanoparticles, Food Chem. Toxicol. 86 (2015) 351–364.
[252] S. Lee, M.S. Kim, D. Lee, T.K. Kwon, D. Khang, H.S. Yun, S.H. Kim, The comparative immunotoxicity of mesoporous silica nanoparticles and colloidal silica nanoparticles in mice, Int. J. Nanomedicine 8 (2013) 147–158.
[253] N. Bhogal, Immunotoxicity and immunogenicity of biopharmaceuticals: design concepts and safety assessment, Curr. Drug Saf. 5 (2010) 293–307.
[254] W.H. De Jong, H. Van Loveren, Screening of xenobiotics for direct immunotoxicity in an animal study, Methods 41 (2007) 3–8.
[255] J. Descotes, Importance of immunotoxicity in safety assessment: a medical toxicologist's perspective, Toxicol. Lett. 149 (2004) 103–108.
[256] J. Descotes, Immunotoxicology: role in the safety assessment of drugs, Drug Saf. 28 (2005) 127–136.
[257] J. Descotes, Methods of evaluating immunotoxicity, Expert Opin. Drug Metab. Toxicol. 2 (2006) 249–259.
[258] L. Peyton Myers, Clinical Immunotoxicology, Methods Mol. Biol. 1803 (2018) 15–26.
[259] B.S. Zolnik, A. Gonzalez-Fernandez, N. Sadrieh, M.A. Dobrovolskaia, Nanoparticles and the immune system, Endocrinology 151 (2010) 458–465.
[260] S.M. Anderton, S. Fillatreau, Editorial overview: Immunomodulation: New insights for future treatments, Curr. Opin. Pharmacol. 23 (2015) vii–ix.
[261] S.E. Bates, Disruptive Immunology, Clin. Cancer Res. 22 (2016) 1844.
[262] Q. Jiao, L. Li, Q. Mu, Q. Zhang, Immunomodulation of nanoparticles in nanomedicine applications, Biomed. Res. Int. 2014 (2014) 426028.
[263] M.A. Dobrovolskaia, S.E. McNeil, Immunological properties of engineered nanomaterials, Nat. Nanotechnol. 2 (2007) 469–478.
[264] M.A. Dobrovolskaia, M. Shurin, A.A. Shvedova, Current understanding of interactions between nanoparticles and the immune system, Toxicol. Appl. Pharmacol. 299 (2016) 78–89.
[265] M.A. Dobrovolskaia, D.R. Germolec, J.L. Weaver, Evaluation of nanoparticle immunotoxicity, Nat. Nanotechnol. 4 (2009) 411–414.
[266] S. Bancos, K.M. Tyner, J.L. Weaver, Immunotoxicity testing for drug-nanoparticle conjugates: Regulatory considerations, in: M.L. Yarmush, D. Shi (Eds.), Frontiers in Nanobiomedical Research, World Scientific Publishing, Singapoor, 2012.
[267] S. Bancos, K.M. Tyner, J.L. Weaver, Immunotoxicity testing for drug-nanoparticle conjugates: regulatory considerations, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of immunological properties of engineered nanomaterials, World Scientific Publishing Co, Singapore 2013, pp. 671–685.
[268] Y. Mou, Y. Xing, H. Ren, Z. Cui, Y. Zhang, G. Yu, W.J. Urba, Q. Hu, H. Hu, The effect of superparamagnetic iron oxide nanoparticle surface charge on antigen cross-presentation, Nanoscale Res. Lett. 12 (2017) 52.
[269] E.J. Park, D.H. Choi, Y. Kim, E.W. Lee, J. Song, M.H. Cho, J.H. Kim, S.W. Kim, Magnetic iron oxide nanoparticles induce autophagy preceding apoptosis through

mitochondrial damage and ER stress in RAW264.7 cells, Toxicol. in Vitro 28 (2014) 1402–1412.
[270] M. Shi, L. Cheng, Z. Zhang, Z. Liu, X. Mao, Ferroferric oxide nanoparticles induce prosurvival autophagy in human blood cells by modulating the Beclin 1/Bcl-2/VPS34 complex, Int. J. Nanomedicine 10 (2015) 207–216.
[271] X. Zhang, H. Zhang, X. Liang, J. Zhang, W. Tao, X. Zhu, D. Chang, X. Zeng, G. Liu, L. Mei, Iron oxide nanoparticles induce autophagosome accumulation through multiple mechanisms: Lysosome impairment, mitochondrial damage, and ER stress, Mol. Pharm. 13 (2016) 2578–2587.
[272] B. Halamoda Kenzaoui, C. Chapuis Bernasconi, S. Guney-Ayra, L. Juillerat-Jeanneret, Induction of oxidative stress, lysosome activation and autophagy by nanoparticles in human brain-derived endothelial cells, Biochem. J. 441 (2012) 813–821.
[273] M.I. Khan, A. Mohammad, G. Patil, S.A. Naqvi, L.K. Chauhan, I. Ahmad, Induction of ROS, mitochondrial damage and autophagy in lung epithelial cancer cells by iron oxide nanoparticles, Biomaterials 33 (2012) 1477–1488.
[274] T. Yokoyama, J. Tam, S. Kuroda, A.W. Scott, J. Aaron, T. Larson, M. Shanker, A.M. Correa, S. Kondo, J.A. Roth, K. Sokolov, R. Ramesh, EGFR-targeted hybrid plasmonic magnetic nanoparticles synergistically induce autophagy and apoptosis in non-small cell lung cancer cells, PLoS One 6 (2011), e25507.
[275] S.J. Dixon, K.M. Lemberg, M.R. Lamprecht, R. Skouta, E.M. Zaitsev, C.E. Gleason, D.N. Patel, A.J. Bauer, A.M. Cantley, W.S. Yang, B. Morrison III, B.R. Stockwell, Ferroptosis: an iron-dependent form of nonapoptotic cell death, Cell 149 (2012) 1060–1072.
[276] W. Hou, Y. Xie, X. Song, X. Sun, M.T. Lotze, H.J. Zeh 3rd, R. Kang, D. Tang, Autophagy promotes ferroptosis by degradation of ferritin, Autophagy 12 (2016) 1425–1428.
[277] A. Garcia-Fernandez, G. Garcia-Lainez, M.L. Ferrandiz, E. Aznar, F. Sancenon, M.J. Alcaraz, J.R. Murguia, M.D. Marcos, R. Martinez-Manez, A.M. Costero, M. Orzaez, Targeting inflammasome by the inhibition of caspase-1 activity using capped mesoporous silica nanoparticles, J. Control. Release 248 (2017) 60–70.
[278] R.F. Hamilton, N. Wu, C. Xiang, M. Li, F. Yang, M. Wolfarth, D.W. Porter, A. Holian, Synthesis, characterization, and bioactivity of carboxylic acid-functionalized titanium dioxide nanobelts, Part Fibre. Toxicol. (2014) 11–43.
[279] X. Zhang, J. Luan, W. Chen, J. Fan, Y. Nan, Y. Wang, Y. Liang, G. Meng, D. Ju, Mesoporous silica nanoparticles induced hepatotoxicity via NLRP3 inflammasome activation and caspase-1-dependent pyroptosis, Nanoscale 10 (2018) 9141–9152.
[280] C.Y. Tsai, S.L. Lu, C.W. Hu, C.S. Yeh, G.B. Lee, H.Y. Lei, Size-dependent attenuation of TLR9 signaling by gold nanoparticles in macrophages, J.Immunol. 188 (2012) 68–76.
[281] R.J. Vandebriel, E.C. Tonk, L.J. de la Fonteyne-Blankestijn, E.R. Gremmer, H.W. Verharen, L.T. van der Ven, H. van Loveren, W.H. de Jong, Immunotoxicity of silver nanoparticles in an intravenous 28-day repeated-dose toxicity study in rats, Part Fibre. Toxicol. 11 (2014) 21.
[282] A. Shah, C.I. Mankus, A.M. Vermilya, F. Soheilian, J.D. Clogston, M.A. Dobrovolskaia, Feraheme(R) suppresses immune function of human T lymphocytes through mitochondrial damage and mitoROS production, Toxicol. Appl. Pharmacol. 350 (2018) 52–63.
[283] A. Shah, E. Cedrone, C.L. Sanders, D. Butcher, A.M. DeFrancesco, C.L. DeGrange, S. Difilippantonio, E.F. Edmondson, M.A. Dobrovolskaia, The potential utility of iron oxide nanoparticles for the treatment of skin inflammation in a mouse model of psoriasis, Prec. Nanomed. 2 (2019) 249–255.
[284] Y. Liu, P.C. Naha, G. Hwang, D. Kim, Y. Huang, A. Simon-Soro, H.I. Jung, Z. Ren, Y. Li, S. Gubara, F. Alawi, D. Zero, A.T. Hara, D.P. Cormode, H. Koo, Topical ferumoxytol nanoparticles disrupt biofilms and prevent tooth decay *in vivo* via intrinsic catalytic activity, Nat. Commun. 9 (2018) 2920.
[285] J. Szebeni, D. Simberg, A. Gonzalez-Fernandez, Y. Barenholz, M.A. Dobrovolskaia, Roadmap and strategy for overcoming infusion reactions to nanomedicines, Nat. Nanotechnol. 13 (2018) 1100–1108.
[286] Y. Le, W.M. Toyofuku, M.D. Scott, Immunogenicity of murine mPEG-red blood cells and the risk of anti-PEG antibodies in human blood donors, Exp. Hematol. 47 (2017) 36–47.
[287] B. Li, Z. Yuan, H.C. Hung, J. Ma, P. Jain, C. Tsao, J. Xie, P. Zhang, X. Lin, K. Wu, S. Jiang, Revealing the immunogenic risk of polymers, Angew. Chem. Int. Ed. Eng. 57 (2018) 13873–13876.
[288] A. Moreno, G.A. Pitoc, N.J. Ganson, J.M. Layzer, M.S. Hershfield, A.F. Tarantal, B.A. Sullenger, Anti-PEG antibodies inhibit the anticoagulant activity of pegylated aptamers, Cell Chem. Biol. 26 (2019) 634–644.
[289] S.M. Poppenborg, J. Wittmann, W. Walther, G. Brandenburg, R. Krahmer, J. Baumgart, F. Leenders, Impact of anti-PEG IgM antibodies on the pharmacokinetics of pegylated asparaginase preparations in mice, Eur. J. Pharm. Sci. 91 (2016) 122–130.
[290] J.T. White, S.D. Newsome, B.C. Kieseier, R.A. Bermel, Y. Cui, A. Seddighzadeh, S. Hung, M. Crossman, M. Subramanyam, Incidence, characterization, and clinical impact analysis of peginterferon beta1a immunogenicity in patients with multiple sclerosis in the ADVANCE trial, Ther. Adv. Neurol. Disord. 9 (2016) 239–249.
[291] P. Zhang, F. Sun, S. Liu, S. Jiang, Anti-PEG antibodies in the clinic: Current issues and beyond PEGylation, J. Control. Release 244 (2016) 184–193.
[292] B.W. Neun, Y. Barenholz, J. Szebeni, M.A. Dobrovolskaia, Understanding the role of Anti-PEG antibodies in the complement activation by Doxil *in vitro*, Molecules 23 (2018) 1700.
[293] H. Ando, A.S. Abu Lila, M. Kawanishi, T. Shimizu, K. Okuhira, Y. Ishima, T. Ishida, Reactivity of IgM antibodies elicited by PEGylated liposomes or PEGylated lipoplexes against auto and foreign antigens, J. Control. Release 270 (2018) 114–119.
[294] Y. Hashimoto, T. Shimizu, A.S. Abu Lila, T. Ishida, H. Kiwada, Relationship between the concentration of anti-polyethylene glycol (PEG) immunoglobulin M (IgM) and the intensity of the accelerated blood clearance (ABC) phenomenon against PEGylated liposomes in mice, Biol. Pharm. Bull. 38 (2015) 417–424.
[295] Y.C. Hsieh, H.E. Wang, W.W. Lin, S.R. Roffler, T.C. Cheng, Y.C. Su, J.J. Li, C.C. Chen, C.H. Huang, B.M. Chen, J.Y. Wang, T.L. Cheng, F.M. Chen, Pre-existing anti-polyethylene glycol antibody reduces the therapeutic efficacy and pharmacokinetics of PEGylated liposomes, Theranostics 8 (2018) 3164–3175.
[296] Y. Mima, A.S. Abu Lila, T. Shimizu, M. Ukawa, H. Ando, Y. Kurata, T. Ishida, Ganglioside inserted into PEGylated liposome attenuates anti-PEG immunity, J. Control. Release 250 (2017) 20–26.
[297] T. Shimizu, A.S. Abu Lila, R. Fujita, M. Awata, M. Kawanishi, Y. Hashimoto, K. Okuhira, Y. Ishima, T. Ishida, A hydroxyl PEG version of PEGylated liposomes and its impact on anti-PEG IgM induction and on the accelerated clearance of PEGylated liposomes, Eur. J. Pharm. Biopharm. 127 (2018) 142–149.
[298] T. Shimizu, Y. Mima, Y. Hashimoto, M. Ukawa, H. Ando, H. Kiwada, T. Ishida, Anti-PEG IgM and complement system are required for the association of second doses of PEGylated liposomes with splenic marginal zone B cells, Immunobiology 220 (2015) 1151–1160.
[299] S.K. Libutti, G.F. Paciotti, A.A. Byrnes, H.R. Alexander Jr., W.E. Gannon, M. Walker, G.D. Seidel, N. Yuldasheva, L. Tamarkin, Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine, Clin. Cancer Res. 16 (2010) 6139–6149.
[300] V. Patravale, P. Dandekar, R. Jain, Nanotoxicology: evaluating toxicity potential of drug-nanoparticles, in: V. Patravale, P. Dandekar, R. Jain (Eds.), Nanoparticulate Drug Delivery, Woodhead Publishing 2012, pp. 123–155.
[301] T. Chen, J. Yan, Y. Li, Genotoxicity of titanium dioxide nanoparticles, J. Food Drug Anal. 22 (2014) 95–104.
[302] K.L. Chapman, H. Holzgrefe, L.E. Black, M. Brown, G. Chellman, C. Copeman, J. Couch, S. Creton, S. Gehen, A. Hoberman, L.B. Kinter, S. Madden, C. Mattis, H.A. Stemple, S. Wilson, Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation, Regul. Toxicol. Pharmacol. 66 (2013) 88–103.
[303] V.B. Siramshetty, J. Nickel, C. Omieczynski, B.O. Gohlke, M.N. Drwal, R. Preissner, WITHDRAWN: A resource for withdrawn and discontinued drugs, Nucleic Acids Res. 44 (2016) D1080–D1086.
[304] S. Sharifi, S. Behzadi, S. Laurent, M.L. Forrest, P. Stroeve, M. Mahmoudi, Toxicity of nanomaterials, Chem. Soc. Rev. 41 (2012) 2323–2343.
[305] J.L. Frandsen, H. Ghandehari, Recombinant protein-based polymers for advanced drug delivery, Chem. Soc. Rev. 41 (2012) 2696–2706.
[306] M.A. Dobrovolskaia, S.E. McNeil, Understanding the correlation between *in vitro* and *in vivo* immunotoxicity tests for nanomedicines, J. Control. Release 172 (2013) 456–466.
[307] N.R.J. Committee, Nature Journal, 1869 www.nature.com/subjects (Accessed july 2019).
[308] A current view on inflammation, Nat. Immunol. 18 (2017) 825.

Glossary

Nanoparticle/nanomaterial: Small particles which are less than 100 nm in at least one dimension.
Inorganic nanoparticle: A class of nanoparticles made up of metal or metal oxide materials [17].
Nanomedicine: The branch of medical science which uses nanotechnology to diagnose, prevent, or treat diseases [304].
Nanotoxicology: A branch of toxicology concerned with the toxicity of nanomaterials in living organisms and the environment [136].
Drug delivery: The science related to the delivery of therapeutics to the desired site of action in the organism [305].
Subchronic and chronic toxicity: A classification of toxicity upon long-term exposure;1-3 month for subchronic and more than 3 months up to years for chronic [136].
Immunotoxicity: Described as any undesirable reaction to the structure and function of immune system or causing the immune system dysfunction in living organisms by exposure to any substances [306,307].
Physicochemical properties: Refers to any chemical and physical characterization of materials including, but not limited to, materials' charge, size, size distribution, density, shape, geometry, and hydrophobicity.
Inflammation: A biological response of the body to any harmful foreign or self-stimuli which triggers the immunological reaction to repair the tissue [308].
Endotoxin: A toxin which is present on the outer membrane of gram-negative bacteria. Endotoxin is a large, heat-stable lipopolysaccharide molecule which is responsible for many physiochemical reactions following this type of bacterial infection. [194]