Title

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Review of zinc oxide nanoparticles: toxicokinetics, tissue distribution for various exposure routes, toxicological effects, toxicity mechanism in mammals, and an approach for toxicity reduction

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Abstract

Zinc oxide (ZnO) nanoparticles (NPs) are widely used as a sunscreen, antibacterial agent, dietary supplement, food additive, and semiconductor material. This review summarizes the biological fate following various exposure routes, toxicological effects, and toxicity mechanism of ZnO NPs in mammals. Furthermore, an approach to reduce the toxicity and biomedical applications of ZnO NPs are discussed. ZnO NPs are mainly absorbed as Zn^{2+} and partially as particles. Regardless of exposure route, elevated Zn concentration in the liver, kidney, lungs, and spleen are observed following ZnO NP exposure, and these are the target organs for ZnO NPs. The liver is the main organ responsible for ZnO NP metabolism and the NPs are mainly excreted in feces and partly in urine. ZnO NPs induce liver damage (oral, intraperitoneal, intravenous, and intratracheal exposure), kidney damage (oral, intraperitoneal, and intravenous exposure) and lung injury (airway exposure). Reactive oxygen species (ROS) generation and induction of oxidative stress may be a major toxicological mechanism for ZnO NPs. ROS are generated by both excess Zn ion release and the particulate effect resulting from the semiconductor or electronic properties of ZnO NPs. ZnO NP toxicity can be reduced by coating their surface with silica, which prevents Zn^{2+} release and ROS generation. Due to their superior characteristics, ZnO NPs are expected to be used for

biomedical applications, such as bioimaging, drug delivery, and anticancer agents, and surface coatings and modification will expand the biomedical applications of ZnO NPs further.

Keywords Zinc oxide nanoparticles · Tissue distribution · Toxicological effects · Toxicological mechanism · Reactive oxygen species · Biomedical applications

Abbreviations

ZnO	Zinc oxide
NPs	Nanoparticles
8-OHdG	8-Hydroxydeoxyguanosine
AST	Aspartate transaminase
ALT	Alanine transaminase
ALP	Alkaline phosphatase
BUN	Blood urea nitrogen
Cre	Creatinine
IL1-β	Interleukin1-β
LDH	Lactate dehydrogenase
LD_{50}	Lethal dose 50
MRT	Mean residual time
MT	Metallothionein
ROS	Reactive oxygen species
TNF-α	Tumor necrosis factor-α
IFN-γ	Interferon-γ

Introduction

Zinc oxide (ZnO) is a white inorganic compound that is insoluble in water but highly soluble in acidic or alkaline solution. It does not exist naturally in bulk quantities [1]. ZnO nanoparticles (NPs) are prepared by various methods, such as gas evaporation, thermal evaporation, hydrothermal synthesis, solvothermal synthesis, sol gel synthesis, simple thermal sublimation, self-combustion, and the vapor-liquid-solid technique [2–4]. Green synthesis of ZnO NPs using various plant and fruit extracts is of particular interest because it is easy, cheap, and ecofriendly [4].

Because Zn is essential element and ZnO is classified as generally regarded as safe (GRAS) by the US Food and Drug Administration (FDA), ZnO NPs are widely used in commercial products [5]. For example, ZnO NPs are used in sunscreen because they efficiently reflect, scatter, and absorb UV radiation, particularly in the UV-A range, from 320 to 400 nm [1, 6]. Moreover, ZnO NPs are being investigated as an antibacterial agent [3], dietary supplement, and food additive [7]. ZnO NPs are a semiconductor material with a wide band gap (3.37 eV) and ZnO thin films [8] have been used for UV sensors, UV optical devices, and display window materials for solar cells [9]. The unique properties of ZnO NPs, such as size similarity with biomolecules, abundant functionality on large surface areas, and quantum size effect, are major

advantages for semiconductor NPs in biomedical applications [10]. ZnO NPs can be used for drug delivery and bioimaging because ZnO quantum dots have good biocompatibility, low toxicity, and good stability [10]. In addition, the use of ZnO NPs as an anticancer agent has been suggested [11].

Although ZnO NPs are less toxic than other semiconductor NPs, the increasing use of ZnO NPs has raised concerns about their potential toxicity [12]. The key factors in the toxicity of metal oxide NPs are the size, surface characteristics, dissolution, and exposure route [1]. Therefore, information about the biological fate and toxicity of ZnO NPs following exposure by various routes is important. Occupational exposure to ZnO NPs mainly occurs through the inhalation and dermal routes, and oral exposure may occur because they are used in food additives and packaging [13, 14]. Additionally, intravenous exposure may increase as ZnO NPs are used in drug delivery and bioimaging. This review summarizes the biological fate (Table 1, Fig.1) and toxicological effects of ZnO NPs for various exposure routes (Table 2) and the toxicity mechanism of ZnO NPs in mammals (Fig. 2). Methods for reducing toxicity and the biomedical applications of ZnO NPs are also discussed.

ZnO NPs absorption

NPs interact with biological systems via different mechanisms from bulk materials [15]. Size-dependent cellular uptake of NPs has been reported, and NPs are more efficiently absorbed by the body compared with bulk materials [16, 17]. NPs are readily absorbed into the gastrointestinal and respiratory systems and skin compared with bulk materials due to their smaller size and physicochemical properties [18]. Absorption for 20 nm ZnO NPs was slightly higher than that for 70 nm ZnO NPs in rats orally administrated ZnO NPs (50, 300, and 2000 mg/kg) [5].

ZnO NPs are rapidly dissolved in acidic conditions (pH 5.5) [15, 19]. When ZnO NPs are administered orally, they dissolve in the stomach because the pH of the gastric fluid is acidic (pH 1.5–2.0), and Zn^{2+} is absorbed into the systemic circulation [14]. In rats, ZnO NPs are likely to be absorbed mainly in ionic form and partially in particulate form following oral administration [20–22]. Yang et al. [23] suggested that ZnO NPs are absorbed mainly as particles in the duodenum and as particles and/or Zn^{2+} in the jejunum and ileum following single oral exposure. Amara et al. [24] suggested that ZnO NPs are absorbed in the organs in ionic form rather than particulate form following intraperitoneal injection of ZnO NPs. Following oral and intraperitoneal administration, Zn^{2+} is taken up by the liver via the first-pass effect, and then redistributed from the liver [12, 25]. In contrast, Choi et al. [12] suggested that the

particles are mainly taken up by the organs because the tissue distribution of ZnO NPs is different from that of Zn ions.

ZnO NPs absorbed into the respiratory system dissolve in the acidic lung lining fluid and ZnO NPs in the respiratory tract cross the blood-air barrier and enter the bloodstream by breaking through the alveolar wall [26, 27]. Human skin has an acid mantle with a pH of 4–6 and a pH gradient across the stratum corneum ranging from pH 4.5–5.3 at the surface to pH 6.8 in the stratum corneum that can cause the dissolution of topically applied ZnO NPs and dermal absorption of Zn [28, 29]. Topically applied ZnO NPs (size 20–30 nm) stayed on the stratum corneum or fell into skin folds or hair follicle roots in human skin after 24 h of treatment [30]. Similarly, Filipe et al. [31] showed that almost all ZnO NPs (size 20–60 nm) remained in the stratum corneum of human skin, and at undetectable levels in the epidermal layers under the stratum corneum following 2 h exposure. Khabir et al. [29] evaluated the Zn levels after topical application of ^{67}ZnO NPs (size 20 nm, containing the rare, stable ^{67}Zn isotope) to human skin for 48 h using a Franz cell, and they found that ^{67}ZnO NPs were retained on the surface or within the superficial layers of the stratum corneum. Repeated topical application of ZnO NPs (size 74.0 ± 3.1 nm) for 5 days using a Franz cell did not result

in ZnO NP penetration through the stratum corneum or cause any visible morphological or redox changes [32].

Zn transporters and metallothioneins

Zn transporters and metallothioneins (MTs) are important in Zn transport and homeostasis *in vivo.* There are two Zn transporter families: the SLC30A (ZnT) family, which reduces cytosolic Zn by exporting Zn out of the cells or into various intracellular compartments, and the SLC39A (ZIP) family, which increases cytosolic Zn by importing Zn from the extracellular space or intracellular compartments [33]. MTs are metal-binding proteins that can bind up to seven Zn ions or other divalent metals and regulate Zn storage and Zn release [34]. Upregulation of Zn transporters and MTs has been reported. Following oral ingestion of ZnO NPs, upregulation of *ZnT1*, *ZnT2*, *ZnT4*, *ZnT6*, and *ZIP5* in the duodenum, *ZnT1*, *ZnT2*, and *ZnT4* in the jejunum and ileum, and *MT1* and *MT4* in the duodenum, jejunum, and ileum was observed [23]. Sub-chronic inhalation exposure to high doses of ZnO NPs increased the levels of mRNA of *ZnT1*, *ZnT5*, and *MT2*, and low doses of ZnO NPs increased the levels of mRNA of *ZnT7* in the lungs [35]. Zn^{2+} from ZnO NPs may be transported by ZnT and ZIP and bind to MTs. However, intestinal absorption of ZnO NPs relies on endocytosis [36, 37].

Protein binding of ZnO NPs

NPs rapidly bind to proteins in blood and other biological fluids. NPs agglomerate in water, and ZnO NPs agglomerate further in biological media, increasing the amount and number of different proteins bound to the NPs [38]. Proteins that bind to ZnO NPs include immunoglobulins, lipoproteins, albumin, α-1-antichymotrypsin, α-2-macroglobulin, and transferrin [38]. Shim et al. [39] suggested that the interaction between diverse proteins and ZnO NPs could alter the function, conformation, and clearance of the proteins. The interaction between ZnO NPs and albumin reduced the hydrodynamic diameters, increased the intestinal transportation, and increased cellular uptake of ZnO NPs, which resulted in higher cytotoxicity [40].

Blood concentration and toxicokinetics of ZnO NPs

After oral administration and intraperitoneal injection of ZnO NPs (size 93.35 ± 14.53 nm, dose 2.5 g/kg) in mice, serum Zn levels were rapidly elevated within 30 min and leached to the maximum level (ca. 18 μg/g). The clearance for orally administered ZnO NPs in serum began 6 h after administration, whereas serum Zn levels were maintained over 72 h following intraperitoneal injection [41]. Choi et al.

[42] investigated the toxicokinetics of ZnO NPs in rats via single intravenous or oral injection (size <35nm, dose 3, 30 mg/kg) and found that blood Zn levels were not elevated by oral administration and increased significantly only in rats treated intravenously: peal concentration (41.07 ± 7.16 μg/mL) appeared at 5 min and returned to the normal range (5.58 ± 0.39 μg/mL) by 48 h after the injection. We have reported that blood Zn levels peaked at 5 min (1.3 ± 0.05 μg/mL) and rapidly decreased after 15 min following single intravenous injection of ZnO NPs (size 58.5 nm, dose 0.2 mg/kg) in mice [43].

After a single oral administration of ZnO NPs (size 20 nm) in male rats, the times to maximum concentration (T_{max}) were 1, 6, and 24 h for the 50, 300, and 2000 mg/kg doses, respectively [20]. In the study, other toxicokinetic parameters were reported: for the 50, 300, and 2000 mg/kg doses, the maximum concentrations (C_{max}) were 34, 94, and 179 μg/mL, the areas under the concentration-time curve (AUCs) were 105, 1063, and 7981 h × g/mL, the mean residual times (MRTs) were 7.16, 11.91, and 39.19 h, and the elimination half-lives ($T_{1/2}$) were 5.06, 8.11, and 30.28 h, respectively in male rats. C_{max}, AUC, MRT, and $T_{1/2}$ were slightly higher in male rats than in female rats, and AUC and MRT were slightly greater for the 20 nm NPs than for the 70 nm NPs. Yu et al. [16] studied the toxicokinetics of ZnO NPs (size 86.3 ± 23.8 nm) in

female rats following a single oral dose of 100 mg/kg. They reported a C_{max} of 44.8 μg/mL, AUC of 212 h × g/mL, MRT of 4.6 h, and $T_{1/2}$ of 3.3 h. The peak Zn concentration was much higher in the Zn ion-treated group than in the Zn particle-treated groups, and plasma Zn concentration was higher in the ZnO NP-treated group than in the bulk ZnO-treated group and decreased to zero by 10 h after administration.

Tissue distribution of ZnO NPs following various exposure routes

Oral administration

Baek et al. [20] showed that ZnO NPs accumulated in the kidneys, liver, and lungs following single oral administration of ZnO NPs (size 20 and 70 nm) in rats. After a low dose (50 and 300 mg/kg) of ZnO NPs, elevated Zn levels were observed in the liver, lungs, and kidneys at 6–24 h post-dosing. A high dose (2000 mg/kg) resulted in high accumulation in the liver and kidney within 2–3 days and the levels returned to the normal range (ca. 20 μg/g in liver and ca. 10 μg/g in kidney) at 7 days post-dosing. Li et al. [41] reported elevated Zn levels in the liver, spleen, and kidneys in mice following single oral administration of ZnO NPs (size 93 nm, dose 2.5 g/kg) over 24–72 h post-dosing. Yang et al. [23] investigated the biodistribution of ZnO NPs (size 27.5 ± 4.1 nm, dose 45 mg/kg) following single oral administration in mice and found that the heart,

liver, spleen, lungs, kidneys, and brain exhibited increased Zn levels. The tissue distribution pattern of ZnO NPs showed a higher distribution in the lungs and a lower distribution in the kidneys and liver compared with $ZnCl_2$ [21]. For repeated oral doses of ZnO NPs in rats for 13 weeks (size 40 nm, dose 134.2–536.8 mg/kg/day), ZnO NPs were distributed to the liver and kidneys, although lung distribution was not reported in the study [14]. Similarly, following 14 consecutive oral doses of ZnO NPs (size 30 nm, dose 300 mg/kg) in mice, Zn content was significantly elevated in the liver and slightly elevated in the kidneys [13]. A study of 90-day repeated oral exposure of ZnO NPs (size 25.3 ± 5.8 nm, dose 350 mg/kg) in rats showed that elevated Zn levels were observed in bone in addition to liver and kidneys [22].

Intraperitoneal administration

Li et al. [41] performed a single intraperitoneal injection of ZnO NPs (size 93 nm, dose 2.5 g/kg) in mice and showed that Zn accumulated in various organs except the brain due to the blood-brain barrier up to 72 h post-dosing. The Zn levels in the liver were the highest, followed by those in the spleen, lungs, kidneys, and heart. Lin et al. [44] reported the accumulation of ZnO NPs (size 47.8 nm, dose 10 mg/kg) in mice in the liver, lung, kidneys, spleen, and heart 6 h after a single intraperitoneal injection. Amara et al. [24] performed repeated intraperitoneal injection of ZnO NPs (size 20–30

nm, dose 25 mg/kg every other day for 10 days) in rats and showed that there was no Zn accumulation in the liver and kidneys after 24 h of exposure withdrawal.

Inhalation exposure and intratracheal instillation

Inhalation of ZnO NPs (size 20 nm, dose 2.5 mg/kg twice daily for 3 days) in male rats led to elevated Zn content in the liver at 12 h and further elevation was observed at 36 h [45]. Vysloužil et al. [35] investigated the entry of ZnO NPs (size 37 ± 4.2 nm, dose 6.46 × 104 and 1.93 × 106 particles/cm^3) from ambient air into organs in mice, and the lower dose significantly increased the Zn content of the liver, whereas the higher dose significantly increased that of the lungs. Konduru et al. [46] reported the tissue distribution of ZnO NPs (size ~4.6 ± 2.5 nm, dose 1 mg/kg) following a single intratracheal instillation in rats. ^{65}Zn was distributed to the skeletal muscle, liver, skin, kidneys, and bone on day 2, and to the skeletal muscle, bone, and skin on days 7 and 28 post administration. Wang et al. [26] reported elevated Zn^{2+} concentrations in the lungs and liver in mice following a single intratracheal instillation of ZnO NPs (size 42 ± 18 nm, dose 2.5 mg/kg).

Intravenous administration

A single intravenous injection of gamma ray-emitting radioactive ZnO NPs in mice was mainly distributed in the lungs, and in the liver, kidneys, and spleen to a lesser extent [47]. Following single intravenous exposure to neutron-activated ZnO NPs (size 10 and 70 nm, dose 120 μg/mouse) in mice, the highest concentrations of ^{65}ZnO NPs were detected in the lungs and liver (<7 h post administration) and ^{65}ZnO NPs were distributed in the blood, liver, spleen, lungs, brain, and heart at 24 h post administration [48]. We have previously performed single intravenous administration of ZnO NPs (size 58.5 nm, dose 0.2 mg/kg) in mice and the short-term tissue distribution of ZnO NPs in the lungs, liver, kidneys, and spleen was investigated up to 1 h [43]. The Zn levels in the lungs and liver peaked at 5 min, those in the kidneys and spleen peaked at 15 min, and those in tissues rapidly decreased after 15 min and returned to the same level as the control at 1 h post dosing. Furthermore, the long-term tissue accumulation of Zn was evaluated for 6 days following intravenous injection of ZnO NPs (dose 0.05 mg/kg or 0.2 mg/kg). At a dose of 0.05 mg/kg, only the kidneys showed significantly higher Zn levels than the control group after 1 day. At a dose of 0.2 mg/kg, a significantly higher Zn level was observed after 6 days in the liver and after 1 day in the spleen.

Percutaneous administration

Systemic absorption of ZnO NPs via skin absorption appears limited.

Osmond-McLeod et al. [49] assessed the skin absorption and organ distribution of sunscreen containing ^{68}ZnO NPs (size 19 nm, dose 2 mg/cm^2, total of six applications over 4 days) in hairless mice. The highest concentration of ^{68}Zn was observed in liver, the lowest concentration was in the brain, and a relatively narrow range was observed in the heart, kidneys, lungs, and spleen.

Metabolism

As mentioned above, ZnO NPs are likely to be absorbed into the systemic circulation mainly in ionic form and partially in particulate form. The liver is the main organ responsible for Zn metabolism and it maintains systemic Zn homeostasis [50]. Absorbed ionic Zn is transported via albumin to the liver and is redistributed to other tissues [51]. There are no studies on the metabolism of ZnO NPs in animals or humans. However, diesel exhaust particles are metabolized by cytochrome P450 (CYP) and can be a source of reactive oxygen species (ROS) [52], and metals modify *CYP* expression and function [53,54,55]. Therefore, ZnO NPs may be metabolized by CYP.

Excretion

The main routes of NP elimination are via urine/kidneys and bile/liver, and ZnO

NPs are excreted through the urine and feces. Baek et al. [20] reported that 0.32% to 1.47% of the total amount of ZnO NPs following oral administration was excreted via urine and most was excreted via feces (48.55% to 97.12%) in rats. They showed that smaller particles were eliminated more rapidly than larger ones; 20 nm particles were eliminated in the feces and urine over 2 and 7 days, whereas 70 nm particles were eliminated in the feces and urine over 3 and 7 days, respectively. Cho et al. [14] showed that following oral exposure to ZnO NPs in rats, the Zn concentration in the urine increased dose-dependently and was significantly elevated in the medium- and high-dose groups, and that the Zn concentration was much higher in feces than in urine (ca. 200-fold). They suggested that most NPs were not absorbed from the gastrointestinal lumen. Liang et al. [22] reported that there was no excretion in urine and that a large amount of Zn was detected in the feces within 1–2 days following a single oral dose of ZnO NPs in rats. Of the total amount of ZnO NPs, 86% to 87% was excreted rapidly within 48 h and this rate was the same for ZnO NPs and bulk ZnO and regardless of sex. Similarly, urinary excretion of ^{65}ZnO was much lower than fecal excretion (50.04% $\pm$ 0.96%) following a single intratracheal instillation of ^{65}ZnO NPs [46]. Intravenously administered ZnO NPs were excreted in feces (12.9%) and eliminated from the body within 24 h in mice [47]. Fecal excretion, which follows biliary excretion of ZnO NPs,

after intravenous injection was also reported in rats [42].

Toxicological effects of ZnO NPs *in vivo*

Lethal dose 50

Lethal dose 50 (LD_{50}) is a measure of acute toxicity of substance (short-term poisoning potential), which causes the death of 50% (one half) of a group of test animals. The LD_{50} of ZnO NPs by oral gavage in rats has been reported to be 10 g/kg in Kunming mice [22] and 3500 mg/kg in CD-ICR mice [56]. Intraperitoneal injection of ZnO NPs in mice had an LD_{50} of 299.9 mg/kg in male Swiss mice [57]. We estimated the LD_{50} for intravenous exposure to ZnO NPs as 0.3 mg/kg in female ICR mice [58].

Change in body and organ weight

Decreased body weight following repeated (13 weeks) oral exposure (size 89.3 ± 44.7 nm, dose 536.8 mg/kg/day) in rats [14], repeated intraperitoneal administration in mice (size~20 ± 5 nm, dose 1, 10, 100 mg/kg for 14 days) [7], and in rats (size 12–90 nm, dose 30.3 mg/kg for 28 days) [59], single intratracheal instillation in mice (size 59–68 nm, dose: 6, 18 µg/animal) [60], repeated intratracheal instillation in mice (dose 400 and 800 µg/kg for 7 days) [26], and repeated intravenous exposure (size 12–90 nm, dose

30.3 mg/kg for 28 days) [59] has been reported in rats or mice. A slight loss of body weight was observed following single oral administration at a high dose (size 20 and 70nm, 2000 mg/kg) in rats [20]. Decreases in organ weight of the heart, liver, spleen, lungs, kidneys, and brain were reported at 14 days post exposure following a single intragastric dose (size~20 ± 5 nm, 100 mg/kg) in mice [7] and repeated (28 days) intraperitoneal and intravenous exposure in rats (size 12–90 nm, dose 30.3 mg/kg for 14 and 28 days) [59].

Pro-inflammatory effect

Several studies have shown the pro-inflammatory effect of ZnO NPs *in vivo* and inflammatory cytokines are an indicator of inflammation. A significant increase in interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ in serum collected 4 weeks post exposure were observed in rats administered ZnO NPs orally (size 26.6 ± 9.71 nm, dose 350 mg/kg) [61]. IL-1β levels increased in bronchoalveolar lavage fluid of mice treated with ZnO NPs (size 42 ± 18 nm, dose 2.5 mg/kg) via single intratracheal instillation [26]. Dose-dependent increases in serum IL-8 and TNF-α levels were observed in rats following intratracheal instillation of ZnO NPs (size 30 nm, dose 0–5 mg/kg, once a week for a total of 12 weeks) [27]. Huang et al. [62] reported

increased expression of IL-5 and IL-13 (after 1 day), and increased production of TNF-α and IFN-γ after 1 and 3 days in bronchoalveolar lavage fluid of mice following exposure to ZnO NPs by inhalation five consecutive times (size <50 nm; dose 2.5 mg/m^3). Pro-inflammatory cytokines were upregulated in the lung tissue of mice exposed to ZnO NPs (size 30 nm, dose 5 mg/kg) by single intranasal instillation [63]. We also revealed the pro-inflammatory effects of ZnO NPs following intravenous injection in mice. The mRNA transcription levels of inflammation-related genes (TNF-α and IL1-β) in spleen cells treated with ZnO NPs (dose 0.2 mg/kg) were significantly elevated at 12 h post dosing, and the levels of TNF-α and IL1-β in supernatants of spleen cell cultures were significantly elevated at 24 h post dosing [64].

Liver damage

ZnO NPs are preferentially accumulated in the liver regardless of the exposure route and it is a main target organ for ZnO NPs. Because the liver is the largest detoxification organ, it is vulnerable to xenobiotic-mediated damage, and hepatic damage following ZnO exposure has been reported. Increased aspartate transaminase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH) were observed following a single oral dose of ZnO NPs [41, 65] in rats and mice. Studies of a single

intraperitoneal dose of ZnO NPs reported elevated ALT, alkaline phosphatase [7, 59], and AST [59] in rats. Elevated ALT and AST were observed for 7 days post dosing in mice after intratracheal instillation of ZnO NPs [26]. We have previously reported increased AST, ALT, and LDH following a single intravenous dose of ZnO NPs in mice [64]. Histopathological changes in the liver (i.e., severe hepatic swelling, vacuolization, cellular necrosis, congestion, and glycogen accumulation) following a single oral dose of ZnO NPs in mice have been reported [41, 65]. The Zn concentration in the liver was about 30 μg/g after a single oral dose (2.5 g/kg) [41]. A single intraperitoneal dose of ZnO NPs caused early apoptotic changes in the liver at low doses and focal inflammation in the liver at high doses in rats [7]. We reported that the hepatic sinusoid was partly dilated 1 day after a single intravenous dose (0.2 mg/kg) of ZnO NPs in mice, and the Zn concentration in the liver was 10.1 ± 8.6 μg/g [43].

Kidney damage

ZnO NPs tend to accumulate in the kidneys, which are susceptible to xenobiotics due to their high blood supply and ability to concentrate toxins [66]. Oral ZnO exposure (dose 600 mg or 1 g/kg body weight/day for 5 consecutive days) significantly decreased total renal glutathione levels, indicating functional damage to

kidney tissue [66]. Lin et al. [44] reported elevated blood urea nitrogen (BUN) and creatinine (Cre), which are biochemical markers of kidney damage, in the mice 6 h following a single intraperitoneal injection of ZnO NPs (dose 10 mg/kg), and the Zn concentration in the kidneys was about 70 μg/g. In contrast, we reported no elevation in BUN or Cre and no pathological changes in the kidneys following a single intravenous dose (0.2 mg/kg) of ZnO NPs in mice, and the Zn concentration in the kidneys was 8.6 ±1.0 μg/g [43, 64]. Histopathological examination showed necrosis, swelling, and hydropic degeneration in epithelial cells in the kidneys of mice following a single oral dose (size 20–30 nm, dose 333.33 mg/kg) [65]. Dilation of the tubules in the kidneys of mice was observed following repeated oral doses of ZnO NPs (size 30 nm, dose 300 mg/kg) over 14 days, and the Zn concentration in the kidneys was reported to be about 40 μg/g [13]. Inflammation and focal interstitial edema in the kidneys of mice following a single intraperitoneal dose of ZnO NPs (size 20 nm, dose 100 μg/mL daily for 14 days) were observed in a pathological investigation [7].

Lung injury

Inhalation is the main route of occupational exposure to ZnO NPs. Pulmonary toxicity and injury caused by ZnO NPs have mainly been investigated by inhalation

exposure, intratracheal instillation, and intranasal exposure. Airway exposure to ZnO NPs is the most hazardous exposure route according to *in vivo* studies [67]. In general, metal oxide NPs are trapped in the lung mucus, although ZnO NPs <50 nm in size penetrated the airway mucus [68]. Because the lung lining fluid is an acidic environment, ZnO NPs dissolve and release Zn^{2+}, inducing inflammatory responses and necrosis caused by ROS generation [69].

Single intratracheal instillation of ZnO NPs (size 21 nm, dose 70 μg/mL) in rats increased lipid peroxide, heme oxygenase-1 (HO-1), and α-tocopherol levels in the lungs, and the ZnO NPs stayed in the lungs and continually released Zn^{2+}, inducing high oxidative stress [70]. Jacobsen et al. [60] reported that a single intratracheal instillation of ZnO NPs (size 12 ± 3 nm, low dose ≥0.3 mg/kg) was associated with large acute pulmonary inflammation, excessive desquamation of epithelial cells of the alveolar barrier, and histological changes (e.g., increased proliferation and hypertrophy of bronchiole epithelial cells, lymphoid cell infiltration, and edema) in mice. Lung injury was observed, as indicated by increased lung weight/body weight ratio and histopathological changes including pulmonary fibrosis and inflammation, following intratracheal instillation of ZnO NPs for 7 days in mice [26]. Long-term intratracheal instillation of a ZnO NP suspension (size 30 nm, dose 0, 1.25, 2.5, and 5 mg/kg once a

week for 12 weeks) in rats induced both lung and systemic inflammation, dyslipidemia, increased levels of serum HO-1, and pathological aortic damage [27]. Huang et al. [62] investigated the effect of inhalation of ZnO NPs (dose 2.5 mg/m^3, 5 h/day over 5 days) in mice and suggested that ZnO NP inhalation might play a role in the development of allergic airway inflammation. Saptarshi et al. [63] revealed pulmonary inflammation, which was confirmed by upregulation of eotaxin mRNA in the lung tissue and release of pro-inflammatory cytokines in the sera, in mice 24 h after acute single intranasal exposure to ZnO NPs (size 30 nm, dose 5 mg/kg). Histopathological changes in mouse lungs following ZnO NP exposure were observed after a single oral exposure (serous inflammation, severe hyperemia in the alveoli, and edema) [65], a single intraperitoneal dose (moderate interstitial inflammation) [7], and a single intravenous dose (pulmonary emphysema) [43].

Blood coagulation

In our preliminary study, high mortality in mice was observed within 5 min of the intravenous injection of ZnO NPs at high doses (10 and 30 mg/kg), with unusual blood coagulation observed in the mice postmortem that may have been related to the cause of death [58]. Divalent ions activate platelet aggregation [71] and transition

elements (Ni, Zn, and Mn) cause faster platelet aggregation [72]. A recent study *in vitro* showed that ZnO NPs cause erythrocyte aggregation and activation of human platelets [73]. Thus, intravenous administration of Zn^{2+} could induce platelet aggregation.

Skin irritation

The skin accounts for 10% of body mass and the dermal exposure route for ZnO NPs is important. Generally, ZnO NP-based sunscreens are considered safe because the ZnO NPs do not penetrate through the stratum corneum [28,29,30,31]. The toxicological effect of NPs in skin comprises NP-induced skin irritation. There are no reports of skin irritation by ZnO NPs *in vivo* following short-term exposure [48, 74]. For ZnO NPs up to a dose of 1000 mg/kg, 90 day repeated dermal exposure to rats resulted in dose-dependent irritation at the site of ZnO NP exposure, although there were no adverse effects, as determined based on clinical observations, organ weight, ophthalmologic examinations and urinalysis, hematology and biochemistry, and pathological examinations [75].

Blood-brain barrier penetration

The blood-brain barrier is important for controlling homeostasis and for

stopping the transfer of foreign substances into the central nervous system. Studies have indicated that there was little transfer of ZnO NPs across the blood-brain barrier. Shim et al. [76] examined the toxicological effects on the brain following oral, dermal, and intravenous exposure to ZnO NPs (size 20 nm, dose 500 mg/kg) for 28 days in rats. The blood-brain barrier was not damaged and could block penetration of ZnO NPs. No accumulation in the brain was observed following intraperitoneal administration of ZnO NPs (size 93 nm, dose 2.5 g/kg) in mice [41].

Maternal-fetal transfer

The blood-placental barrier is crucial in normal fetal development. Maternal transfer of ZnO NPs via the blood-placental barrier has been reported [77,78,79,80,81]. Chen et al. [80] showed a significant increase in Zn concentration in the uterus, placenta, and fetus following gavage of ZnO NPs (size 27.5 ± 4.1 nm, dose 180 mg/kg) in pregnant mice. In addition, the maternal transfer of ZnO NPs via lactation in rats has been reported [77].

Toxicological mechanism

The toxicological mechanisms of ZnO NPs have not been fully elucidated [82]. NPs have different physicochemical characteristics from larger particles because the surface area is proportionally greater, and this may be related to toxicity [75]. When ZnO NPs enter the body, they release Zn^{2+} as they dissolve, and some researchers have suggested that the toxicity of ZnO NPs is induced by Zn^{2+} [37, 83]. ROS generation and induction of oxidative stress may be a major toxicological mechanism for ZnO NPs [84]. ROS are generated by both the release of excess Zn ions and the particulate effect resulting from the semiconductor or electronic properties of ZnO NPs [84,85,86].

Fukui et al. [70] performed intratracheal instillation of ZnO NPs in rats and suggested that ZnO NPs stay in the lungs and continually release Zn^{2+}, which induces high oxidative stress. The levels of 8-hydroxydeoxyguanosine (8-OHdG), a major product of ROS that is widely used as a marker for oxidative DNA damage, were elevated by intratracheal instillation and inhalation of ZnO NPs. ZnO NPs may generate large amounts of free radicals, which may induce oxidative stress [87]. Wang et al. [26] also reported that 8-OHdG was highly concentrated in the lungs and lung apoptosis was increased following intratracheal instillation of lipopolysaccharides plus ZnO NPs. Inflammation may be mediated via oxidative stress, which can lead to DNA damage and apoptosis. They also suggested that ZnO NPs can permeate the destroyed blood-air

barrier, and then enter the liver from circulating blood, thereby also causing liver injury. We previously investigated 8-OHdG levels in urine following a single intravenous administration of ZnO NPs. A significantly higher concentration was observed after 1 day, the concentration decreased gradually over 6 days, and a dose-dependent effect was observed. The oxidative stress marker of superoxide dismutase levels in serum following intravenous injection of 0.2 mg/kg ZnO NPs was significantly elevated at 24 and 48 h post dosing [43]. An *in vitro* study showed that ZnO NPs enter cells as Zn^{2+} and NPs. Zn^{2+} affects enzyme balance, transcription factors, and signaling pathways, whereas NPs cause cell inactivation, oxidative stress, mitochondrial damage, and intracellular Ca^{2+} overload [67, 88]. When the toxicity of ZnO NPs and bulk ZnO was compared in astrocytes, both destroyed mitochondrial function, and ROS production and caspase activity were higher in astrocytes exposed to ZnO NPs than in those exposed to bulk ZnO [89].

The genetic responses toward ZnO NPs and its involvement in toxicological effect of ZnO NPs have been reported. Tang et al. [90] reported that oral administration of ZnO NPs (dose 100, 300, and 600 mg/kg for 1 week) reduced *CYP1A2* mRNA expression, increased *CYP2C11* and *CYP3A2* mRNA expression, and induced pathological changes in liver and kidney tissues (vacuolization and substantial

swelling). Yu et al. [16] observed the upregulation of CYP related genes (*CYP1A1*, *CYP 3A2*, and *CYP8B1*), injury repair- or metabolism-related genes (aldo-keto reductase family 1: *AKR1B8* and glutathione S-transferase alpha 3: *GSTA3*), and a gene involved in immune response (nuclear receptor subfamily 1 group D member 1: *NR1D1*) in rat liver following 14 days oral administration of ZnO NPs (dose 100 mg/kg). They suggested that the toxicological mechanism of ZnO NPs may be related to the functions of heme, metal binding, and CYP, and that the signaling pathway involving inflammation response could be a target for further studies on the toxicological mechanism. The toxicological mechanism of ZnO NPs is summarized in Fig. 2.

Toxicity reduction by surface coating

Silica coatings improve the UV stability and stabilize the dispersion of NPs [91]. In general, coatings are effective for preventing ROS generation and a silica coating may also prevent Zn^{2+} release from ZnO NPs; therefore, a silica coating should reduce the toxicity of ZnO NPs. We have shown that death was not observed following intravenous injection of silica-coated ZnO NPs (size 81 nm, doses: 10, 30, and 130 mg/kg), whereas high mortality (100%) was observed following intravenous injection of uncoated ZnO NPs [58]. Similarly, *in vitro* studies showed that silica coatings are highly

effective in reducing the toxicity of ZnO NPs by preventing their dissolution and release of Zn^{2+} [83, 92].

Biomedical applications

Because ZnO NPs are easily absorbed by the body due to their small particle size and are classified as GRAS by the US FDA, their potential biomedical applications, including those in bioimaging and drug delivery, have been examined [93, 94]. Xiong et al. [95] synthesized stable ZnO@polymer core-shell NPs that exhibited luminescence in aqueous solution, and they applied them to cell imaging of human hepatoma cells, which were stained with ZnO-1 (green fluorescence) and ZnO-2 (yellow fluorescence). Alves et al. [96] showed that ZnO NPs obtained via green synthesis using apple extract can be used for bioimaging because they are safe and have blue emissions [95]. The potential use of ZnO NPs as an anticancer agent was suggested because ZnO NPs can destroy cancer cells by producing ROS [11]. Drug-loaded nanostructured materials can enter cells through intracellular endocytic pathways and release drugs at target sites [10]. Zhang et al. [97] developed ZnO@polymer-doxorubicin, which released 90% of the doxorubicin loaded on the ZnO surface in pH 5 buffer solution. They suggested that surface modification is crucial for protecting ZnO NPs from dissolution during drug

delivery. Wiesmann et al. [98] investigated the cellular toxicity of ZnO NPs and ZnO NPs@SiO_2 NPs. The cellular viability was the same for both types of NP, but the toxicity of ZnO NPs@SiO_2 NPs was delayed compared with ZnO NPs, suggesting that the silica coating did not hinder the anticancer effect. The delay is useful because it could allow the NPs to be transported safely to the tumor site.

Conclusions

ZnO NPs are absorbed mainly in ionic form and partly in particulate form, distributed to tissues quickly, and cleared rapidly. The NPs are not accumulated in the tissue for a long time and are largely excreted in the feces. Regardless of exposure route, elevated Zn concentrations in the liver, kidneys, lungs, and spleen were observed following ZnO NP exposure, and these are the target organs for ZnO NPs. ZnO NPs are mainly accumulated in the liver and histopathological changes and liver damage were initiated by various exposure routes. Kidney damage was observed following single oral and intraperitoneal exposure. Airway exposure *in vivo* is the most hazardous and induces pulmonary inflammation. Lung injury was observed by intratracheal instillation and inhalation exposure. However, there are no reports of skin irritation by ZnO NPs *in vivo* following short-term exposure. The toxicological mechanism of ZnO NPs is

attributed to induction of high oxidative stress due to generation of large amounts of ROS. ROS are generated by both Zn^{2+} release from ZnO NPs and the particulate effect resulting from the semiconductor or electronic properties of ZnO NPs. The pro-inflammatory effect of ZnO NPs *in vivo* and inflammation may be mediated by oxidative stress. The toxicity of ZnO NPs can be reduced by coating their surface with silica, which prevents ROS generation and Zn^{2+} release. Due to the superior characteristics of ZnO NPs, they are expected to have biomedical applications in bioimaging and drug delivery, and surface coating and modification will expand their applications.

Statements and Declaration

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Competing Interests

The authors declare that they have no known competing financial interests or

personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

JF: Writing – original draft, review and editing, visualization. NN: Writing – review and editing, and visualization.

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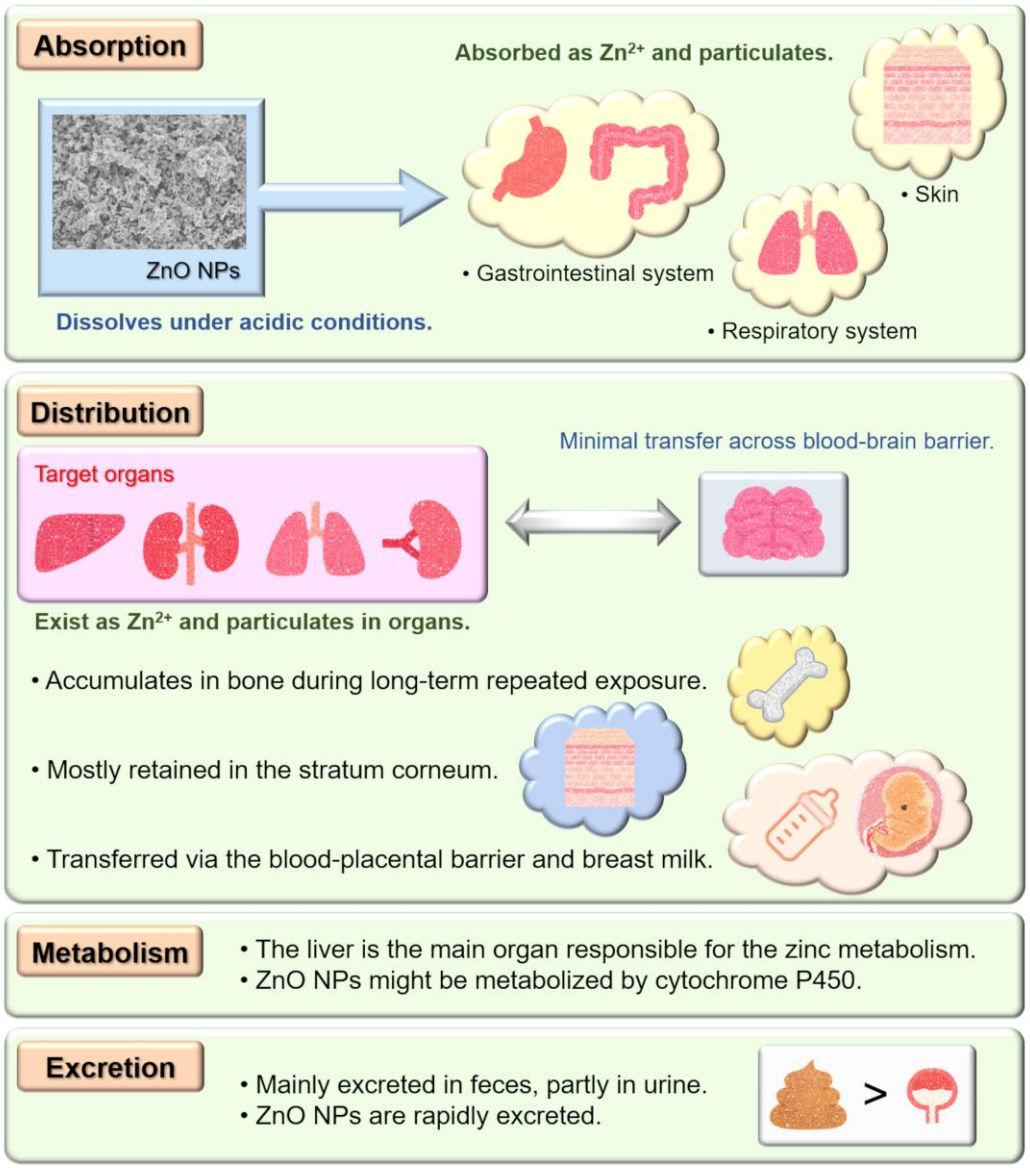

Fig. 1. Summary of the absorption, distribution, metabolism, and excretion of ZnO NPs.

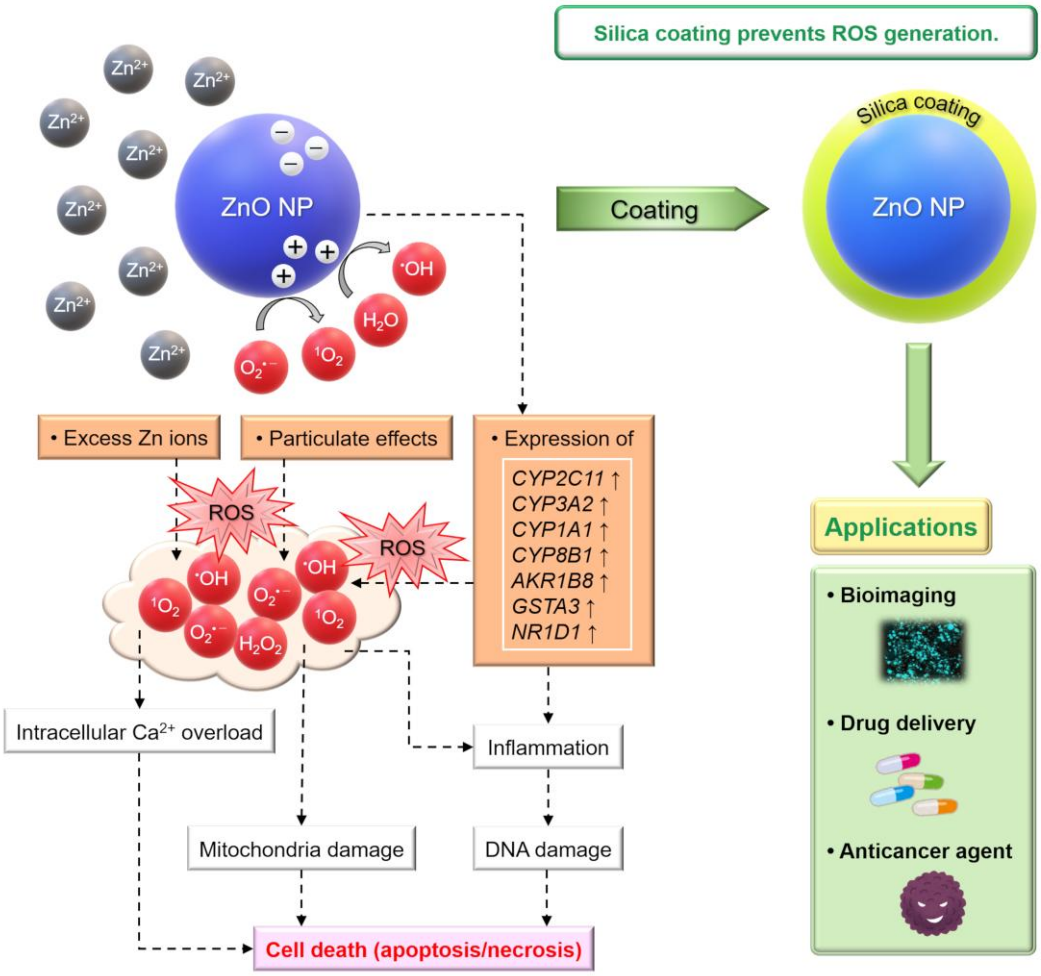

Fig. 2. Schematic of the toxicity mechanism of ZnO NPs. ROS: reactive oxygen species; CYP: cytochrome P450; AKR1: aldo-keto reductase family 1; glutathione S-transferase alpha 3: GSTA3; NR1D1: nuclear receptor subfamily 1 group D member 1.

Table 1. Tissue distribution of ZnO NPs following various exposure routes

Exposure route	Single/repeated	Animal	ZnO NP size (nm)	Dose	Tissue distribution	Reference
PO	Single	Rats	20 and 70	50, 300, and 2000 mg/kg	Low dose: liver, lungs, and kidneys (6–24 h post dosing) High dose: liver and kidneys (2–3 days post dosing)	[20]
		Mice	93	2.5 g/kg	Liver, spleen, and kidneys (24, 48, 72 h post dosing)	[41]
			27.5 ± 4.1	45 mg/kg	Heat, liver, spleen, lungs, kidneys, and brain (24 h post dosing)	[23]
	Repeated (13 weeks)	Rats	40	134.2–536.8 mg/kg/day	Liver and kidneys (after last administration)	[14]
	Repeated (14 days)	Mice	30	300 mg/kg	Liver and kidneys (after last administration)	[13]
	Repeated (90 days)	Rats	25.3 ± 5.8	350 mg/kg	Bone in addition to liver and kidneys (after last administration) Bone (after 28 days from withdrawal)	[22]
IP	Single	Mice	93	2.5 g/kg	Liver, spleen, lungs, kidneys, and heart (24, 48, and 72 h post dosing)	[41]
			47.8	10 mg/kg	Liver, lung, kidneys, spleen, and heart (6 h post dosing)	[44]
	Repeated (every other day for 10 days)	Rats	20–30	25 mg/kg	Zn levels were not elevated in liver and kidneys 24 h post dosing	[24]
IH	Repeated (twice daily for 3 days)	Rats	20	2.5 mg/kg	Liver (12 and 36 h post dosing)	[45]
	Repeated (12 weeks)	Mice	37 ± 4.2	6.46×10^4 and 1.93×10^6 particles/cm^3	Low dose: liver (after last administration) High dose: lungs (after last administration)	[35]
IT	Single	Rats	~4.6 ± 2.5	1 mg/kg	Skeletal muscle, liver, lungs, skin, and bone (2 days post dosing) Skeletal muscle, bone, and skin (7 and 28 days post dosing)	[46]
		Mice	42 ± 18	2.5 mg/kg	Lungs and liver (24 h post dosing)	[26]
IV	Single	Mice	40–100	–	Lungs, pancreas, liver, spleen, and kidneys (1 h post dosing) Pancreas, lungs, spleen, liver, and intestines (24 h post dosing)	[47]
			10 and 70	120 μg/mouse	Short-term (<7 h): lungs and liver At 24 h: liver, spleen, lungs, brain, and heart	[48]
			58.5	0.2 mg/kg 0.05 mg/kg, 0.2 mg/kg	Short-term (<1 h): lungs, liver, kidneys, and spleen Long-term (up to 6 days): kidneys, liver, and spleen	[43]
PC	Single (for 5 min)	Humans	20–30	6.0 mg/cm^2 (sunscreen)	Stratum corneum or fell into skin folds or hair follicles	[30]
	Single (for 2 h)		20–60	0.5–1.0 mg/cm^2 (sunscreen)	Stratum corneum	[31]
	Repeated (for 4 days)	Hairless mice	19	2 mg/cm^2	Liver, heart, kidneys, lung, spleen, and brain (24 h post dosing)	[49]

PO: oral administration
IP: intraperitoneal injection
IH: inhalation exposure
IT: intratracheal instillation
IV: intravenous administration
PC: percutaneous administration

Table 2. Toxicological effects on organs observed following ZnO NPs exposure in experimental animals

Organ	Toxicological effect	Reference
Liver	Abnormal serum liver enzyme levels	
	• AST, ALT, ALP, and LDH elevation by single PO, IP, IT, and IV	[7, 26, 41, 59, 64, 65]
	Histopathological changes	
	• Severe hepatic swelling, vacuolization, cellular necrosis, congestion, and glycogen accumulation by single PO, IP	[41, 65]
	• Apoptotic changes in the liver at low doses and focal inflammation by single IP	[7]
	• Partly dilatation of hepatic sinusoid by single IV	[43]
Kidneys	Abnormal levels in kidney damage marker and glutathione	
	• BUN and Cre elevation by single IP	[44]
	• Decreased total renal glutathione levels by single PO	[66]
	Histopathological changes	
	• Necrosis, swelling, and hydropic degeneration by single PO	[65]
	• Dilation of the tubules by repeated by sub-acute PO	[13]
	• Inflammation and focal interstitial edema by single IP	[7]
Lungs	Increased levels of marker for oxidative stress and observation of inflammation	
	• Increased lipid peroxide, heme oxygenase-1 (HO-1), and α-tocopherol levels in the lungs by single IT	[70]
	• Large acute pulmonary inflammation, excessive desquamation of epithelial cells of the alveolar barrier by single IT	[60]
	• Lung and systemic inflammation, dyslipidemia, increased levels of serum HO-1 by long-term IT	[27]
	Histopathological changes	
	• Increased proliferation and hypertrophy of bronchiole epithelial cells, lymphoid cell infiltration, and edema by single IT	[60]
	• Pulmonary fibrosis and inflammation by IT for 7 days	[26]
	• Aortic damage by long-term IT	[27]
	• Serous inflammation, severe hyperemia in the alveoli, and edema by single PO	[65]
	• Moderate interstitial inflammation by single IP	[7]
	• Pulmonary emphysema by single IV	[43]

AST: aspartate transaminase
ALP: alkaline phosphatase
ALT: alanine transaminase
LDH: lactate dehydrogenase
BUN: blood urea nitrogen
Cre: creatinine
PO: oral administration
IP: intraperitoneal injection
IT: intratracheal instillation
IV: intravenous administration