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# Risk assessment of zinc oxide, a cosmetic ingredient used as a UV filter of sunscreens

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**ABSTRACT**

Zinc oxide (ZnO), an inorganic compound that appears as a white powder, is used frequently as an ingredient in sunscreens. The aim of this review was to examine the toxicology and risk assessment of ZnO based upon available published data. Recent studies on acute, sub-acute, and chronic toxicities of ZnO indicated that this compound is virtually non-toxic in animal models. However, it was reported that ZnO nanoparticles (NP) (particle size, 40 nm) induced significant changes in anemia-related hematologic parameters and mild to moderate pancreatitis in male and female Sprague-Dawley rats at 536.8 mg/kg/day in a 13-week oral toxicity study. ZnO displayed no carcinogenic potential, and skin penetration is low. No-observed-adverse-effect level (NOAEL) ZnO was determined to be 268.4 mg/kg/day in a 13-week oral toxicity study, and a maximum systemic exposure dose (SED) of ZnO was estimated to be 0.6 mg/kg/day based on topical application of sunscreen containing ZnO. Subsequently, the lowest margin of safety (MOS) was estimated to be 448.2, which indicates that the use of ZnO in sunscreen is safe. A risk assessment was undertaken considering other routes of exposure (inhalation or oral) and major product types (cream, lotion, spray, and propellant). Human data revealed that MOS values (7.37 for skin exposure from cream and lotion type; 8.64 for skin exposure of spray type; 12.87 for inhalation exposure of propellant type; 3.32 for oral exposure of sunscreen) are all within the safe range (MOS > 1). Risk assessment of ZnO indicates that this compound may be used safely in cosmetic products within the current regulatory limits of 25% in Korea.

## Introduction

Zinc oxide (ZnO) is an inorganic compound that usually appears as a white powder (Meyer et al. 2011). ZnO powder, including fine nanoparticles (NP), is used in a variety of applications, generally as an additive in products such as plastics, glass, ceramics, cement, rubber, lubricants, paints, adhesives, ointments, sealants, pigments, foods, batteries, fire retardants, ferrites, pharmaceuticals, and cosmetics (Demir, Creus, and Marcos 2014; Djurisic and Leung 2006; Fan and Lu 2005). The earth's crust is a major source of zincite, a mineral form of ZnO; however, a majority of the commercially available ZnO is produced synthetically (WHO 2001). ZnO exhibits reliable functional properties, similar to titanium dioxide ($TiO_2$), as an active sunscreen ingredient (Nohynek and Dufour 2012). Biological and physical effects of ZnO are related to particle size, shape, retention, conjugation, and dose (Silva et al. 2013; Singh et al. 2014). Accordingly, most sunscreens contain well-known ultraviolet (UV) filters such as ZnO and $TiO_2$ that protect the skin from UV damage (Leiter and Garbe 2008). ZnO effectively absorbs UV rays, primarily in the UVA [UVA1 (340 ~ 400 nm) + UVA2 (320 ~ 340 nm)] and UVB (290 ~ 320 nm) regions, depending upon particle size (Mitchnick, Fairhurst, and Pinnell 1999; Pinnell et al. 2000; Popov et al. 2005a). Therefore, this compound has been used as an active ingredient in sunscreens with broad-spectrum purpose. As an inorganic

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physical UV absorber, ZnO is chemically stable under circumstances such as the high temperature of UV rays (Becheri et al. 2008). Large surface area-to-volume ratios of the NP improve its effectiveness in blocking UV rays compared to bulk-sized compounds (Yadav et al. 2006).

Zinc oxide NP exhibit an antibacterial activity, similar to silver or other types of NP, as their particle size decreases (Blum et al. 2015; Carneiro and Barbosa 2016; Nair et al. 2009; Roberts et al. 2013; Shi et al. 2014). In spite of the widespread use of ZnO, its safety in humans is unclear. Several investigators reviewed the safety of ZnO nanomaterials (Annangi et al. 2016; Kermanizadeh et al. 2016; Kwon, Koedrith, and Seo 2014; Liu et al. 2016; Newman, Stotland, and Ellis 2009; Osmond and McCall 2010; Saptarshi, Duschl, and Lopata 2015; Singh and Nalwa 2007; Smijs and Pavel 2011; Stern and McNeil 2008), and their dermal (Hackenberg and Kleinsasser 2012) and mammalian toxicity (Sruthi and Mohanan 2016; Vandebriel and De Jong 2012). However, there has not been any apparent report on risk assessment of ZnO as a cosmetic ingredient. Several *in vitro* or *in vivo* skin penetration studies noted that ZnO NP in sunscreens did not penetrate the organism (Cross et al. 2007; Dussert, Gooris, and Hemmerle 1997; Gamer, Leibold, and Van Ravenzwaay 2006; Lin et al. 2011; Zvyagin et al. 2008). In contrast, Kuo et al. (2009) demonstrated that chemical enhancers increase skin penetration ability of ZnO NP in mice without adverse consequences. Recently, detailed toxicity information of ZnO NP was reviewed based upon ion-shedding properties (Liu et al. 2016). Several investigators indicated that ZnO NP are more cytotoxic against cancer cells than normal cells, suggesting potential penetration of ZnO NP for use as a cancer treatment in nanomedicine (Hanley et al. 2008; Nair et al. 2009). Thus, the safety of ZnO including NP in cosmetics needs to be determined to clarify potential risks to consumers using these cosmetics.

In this study, a comprehensive toxicological evaluation and risk assessment of ZnO were carried out considering routes of exposure (skin, oral, and inhalation) and various product types (cream, lotion, spray, and propellant). Most of the source literature presented originated from peer-reviewed articles searched through PubMed using search terms of ZnO, zinc oxide, toxicity, risk, absorption, exposure, distribution, and safety. In addition, grey literature, such as guidelines of cosmetics and scientific opinion documents for cosmetics, was searched through Google and open websites of regulatory agencies of South Korea, European Commission, European Chemicals Bureau, and US Food and Drug Administration. A total of 6,525 papers were searched by PubMed available, and mammalian studies providing pertinent information for risk assessment were included. The guidelines of Organization for Economic Cooperation and Development (OECD) and good laboratory practice (GLP) were considered to select papers.

## Physicochemical properties and photocatalytic behavior of ZnO

ZnO powder is a white- or gray-colored odorless compound (WHO 2001). The color of ZnO powder varies depending on its average particle size (Vandebriel and De Jong 2012). When the average particle sizes of ZnO range from 200 to 400 nm, it reflects and scatters sunlight and therefore appears white. However, as the average particle size of ZnO decreases to 40 ~ 100 nm, it absorbs visible light (still scatters UV rays), making it transparent. Generally, nanosized metal oxide particles offer greater UV protection compared to micron-sized particles (MPs), at least in the UVB region, which can be compared to nanotubes (Chatterjee et al. 2014; Popov et al. 2005b). ZnO can absorb carbon dioxide ($CO_2$) from the atmosphere. It is soluble in acids and alkalis and insoluble in water and alcohol (WHO 2001). The physical and chemical properties of ZnO are summarized in Table 1. There is no apparent current information on specific requirement of quality control and purity for cosmetics grade ZnO NP. ISO/TR13014 states that the physicochemical characterization of nanoscale materials is crucial for the identification of test material prior to toxicological assessment. The physicochemical properties include particle size/particle size distribution, aggregation/agglomeration state, shape, surface area, composition, surface chemistry, surface charge, and solubility/dispersibility (ISO/TR13014 2012). X-ray diffraction and high-resolution transmission electron microscopy are generally employed to determine particle size/particle distribution, shape, and aggregation/agglomeration of NP in commercial sunscreen sprays (Lu et al. 2015).

**Table 1.** Physical and chemical properties of ZnO.

| Properties | Value | Ref. |
|---|---|---|
| CAS number | 1314–13-2 | Gamer, Leibold, and Van Ravenzwaay (2006) |
| EINECS number | 215–222-5 | SCCNFP (2003) |
| RTECS number | ZH4810000 | CDC (2010) |
| IUPAC name | Zinc oxide | SCCNFP (2003) |
| INCI name | Zinc oxide | |
| Molecular formula | ZnO | CDC (2010) |
| Synonyms | Chinese white, zinc white, flowers of zinc, philosopher's wool | WHO (2001) |
| Molar mass | 81.4 | CDC (2010) |
| Melting point | 1975°C | |
| Boiling point | na | |
| Specific gravity | 5.61 | |
| Density | 5.61 g/cm$^3$ | Cross et al. (2007) |
| Flash point | na | CDC (2010) |
| Vapor pressure (20°C) | 0 mmHg (approx.) | |
| Water solubility (25°C)* | 1.6 µg/ml | Gamer, Leibold, and Van Ravenzwaay (2006) |

CAS, Chemical Abstracts Service; CDC, Centers for Disease Control and Prevention; EINECS, European Inventory of Existing Commercial Chemical Substances; INCI, International Nomenclature of Cosmetic Ingredients; IUPAC, International Union of Pure and Applied Chemistry; RTECS, Registry of Toxic Effects of Chemical Substances.

*At low pH simulating stomach environment at pH 2.7, the solubility of ZnO nanoparticles ranged from 89.6% (particles >3 mm) to 98.5% (particles <1 mm) (Scott et al., 1991).

ZnO is a semiconductor photocatalyst with wide band gap (3.37 eV) (Wang et al. 2012). A photon containing energy larger than 3.37 eV (wavelength less than 368 nm) possesses the potential to photoactivate ZnO and induces photocatalytic degradation of dyes (Hynek et al. 2013; Siddiquey et al. 2012; Zeng et al. 2014). Photocatalysis occurs through UV light absorption of ZnO to excite electrons from the valence band to the conduction band and produces photogenerated electro-hole pairs, thus triggering subsequent photoredox reactions (Ansari et al. 2013; Wang et al. 2012; Zheng et al. 2007). Leite-Silva et al. (2013) showed that the particle sizes of ZnO are reduced and nanoscaled to (1) decrease reflection of the visible light and (2) provide sunscreens a better transparent appearance and skin feeling. However, nanoscaled ZnO impairs the chance of recombination of photogenerated electro-hole pairs and photocatalytic activity increases. Enhanced photocatalytic activity induces degradation of organic ingredients in sunscreens. To minimize photocatalytic activity of ZnO, especially nanosized, coating materials such as silica were applied (Siddiquey et al. 2012). There have been many research efforts to improve the photocatalytic activity of ZnO by coupling with various carbons (Han et al. 2014). Mitchnick, Fairhurst, and Pinnell (1999) reported that microfine coated or non-coated ZnO (Z-Cote®, particle size of less than 200 nm) was photostable, representing a low photoreactive sunscreen formulation. Silica-coated ZnO NP (particle size, anout 40 nm) reduced the photocatalytic activity of non-coated ZnO NP maintaining UV shielding effect and transparency in the visible light spectrum (Siddiquey et al. 2012).

## Cosmetic use

ZnO has intrinsic UV-absorbing properties and has been used as an ingredient in sunscreens as an UV blocker. As an inorganic physical UV absorber, ZnO is chemically stable under circumstances such as the high temperature of UV rays (Becheri et al. 2008). Large surface area-to-volume ratios of the NP improve its effectiveness in blocking UV rays compared to bulk-sized compounds (Yadav et al. 2006).

In the United Kingdom, a total of 308 sunscreen products were commercially available during 2005 for skin application with sun protection factor (SPF) ratings between 2 and 60 (median 20) (Wahie, Lloyd, and Farr 2007). Eleven products (3.6%) contained $TiO_2$ and/or ZnO, but no chemical UV filters, whereas 169 products (54.8%) were composed of chemical UV absorbent, but no $TiO_2$ or ZnO. The remaining 128 products (41.6%) were mixed with metal oxide reflectants and chemical absorbents. According to the survey, approximately half of the commercially available sunscreens contained $TiO_2$

**Table 2.** Cosmetics containing ZnO and the concentration range of ZnO.

| Type | Number | Concentration (g/100 g) |
|---|---|---|
| Powder | 60 | 0.5–8.0 |
| Concealer | 41 | 1.5–15.0 |
| Skin | 30 | 0.05–12.0 |
| Pact | 27 | 2.0–17.0 |
| Sunscreen | 25 | 1.0–5.0 |
| Blemish balm | 17 | 1.0–5.0 |
| Pack | 11 | 3.0 |
| Eye shadow | 9 | 1.0–5.0 |
| Camouflage cream | 6 | 0.1 |
| Foundation | 6 | 2.0–5.0 |
| Lotion | 2 | 14.0 |
| Cream | 1 | 2.0 |

Data source: Cosmetic Products of Korea (KCII, 2012)

and ZnO particles as at least one of their active ingredients. Of these, ZnO was used in 15 products (4.9%) and $TiO_2$ was used in 139 products (45.1%). In Korea, ZnO was used in 235 domestic cosmetic products with concentrations of 0.05%–17% (KCII 2012). The powder type products have the highest number (60) of ZnO-containing cosmetics (Table 2).

## Hazard identification

Repeated-dose toxicity studies examining ZnO revealed that there are minimal adverse effects (Clayton and Clayton 1981–1982; Straube, Schuster, and Sinclair 1980). Several mutagenicity studies demonstrated that ZnO NP are genotoxic (Dufour et al. 2006; Gerloff et al. 2009; Osman et al. 2010; Sharma et al. 2009, 2012), while another study did not (Yoshida, Kitamura, and Maenosono 2009). Animals feeding diets containing Zn displayed adverse effects on reproduction or the development of offspring (Bleavins et al. 1983; Ketcheson, Barron, and Cox 1969; Pal and Pal 1987; Samanta and Pal 1986; Schlicker and Cox 1968). Dermal toxicity potential of ZnO in animal models produced a mild irritation (Lansdown 1991).

### *Acute toxicity studies*

Lethal dose ($LD_{50}$) was estimated to be 240 mg/kg in rats treated intraperitoneally (ip) with ZnO (Lewis 2000). However, a higher oral $LD_{50}$ was calculated to be 7,950 mg/kg or over 5 g/kg in mice and rats treated with ZnO (ECB 2004; Lewis 2000). Acute toxicity of ZnO was markedly influenced by exposure routes (Table 3). Inhaled $LD_{50}$ was estimated to be 5.7 mg/L every 4 hr in mice (ECB 2004). In mice, manifestations of adverse effects included increased blood hemoglobin concentration, altered motor activity, and reduced ceruloplasmin activity in the plasma. Llobet et al. (1988) found administration of ZnO produced severe gastroenteritis attributed to irritation and corrosion of the mucosa in the stomach following formation of zinc chloride in the stomach due to its reaction with hydrochloric acid in the gastric juice. Wang et al. (2008) studied the acute toxicological effects of submicro- and nanoscaled ZnO powder on healthy ICR mice. Target organs for 20 and 120 nm ZnO in an acute oral toxicity study were determined to be liver, heart, spleen, pancreas, and bone when results of pathological examination, Zn accumulation, and biological assays were considered. The pathological and biochemical examinations showed that the toxicological impacts between the 20 and 120 nm ZnO particles were similar, but varied little according to dose. Pathological damage in the stomach, liver, heart, and spleen was observed at 120 nm ZnO, but with 20 nm ZnO, a less severe response was noted. Wang et al. (2008) concluded that the potential toxicity of oral exposure with low doses of small-sized 20 nm ZnO particles needs to be examined further.

Single oral administration of 50 nm ZnO NP (1.25, 2.5, or 5 g/kg body weight) resulted in accumulation in the liver, spleen, lung, kidneys, and heart within 72 hr (Li et al. 2012). Oral ZnO NP administration showed transient histopathologic alterations in liver that was not observed after treatment with 1 μm ZnO particles (Table 3). Gao et al. (2013) reported that an intranasal instillation of ZnO NP (30 nm) in Sprague Dawley (S-D) rats induced damage to the olfactory epithelium at both 10 and 40 mg/ml. In an acute mouse oral toxicity study, administration of Zn NP (58 nm, 5 g/kg body weight) and microparticles (1.08 μm) to 4-week-old ICR mice, Wang et al. (2006) demonstrated that anemia and renal damage noted in animals exposed to NP were more severe compared to that in animals exposed to microscale particles. Histological examination of two mice that died in the first week displayed intestinal obstruction initiated by aggregation of Zn NP in the intestine, although serum biomarkers of inflammation

**Table 3.** Acute, subchronic, and chronic toxicity of ZnO.

| Studies | Species | Sex | Route | Duration of treatment | Particle size (average) | Dose | Results | Ref. |
|---|---|---|---|---|---|---|---|---|
| Acute | Rat (Wistar) | – | IP | Single | na | – | $LD_{50}$: 240 mg/kg | Lewis (2000) |
| | Mouse (ICR) | – | Oral | Single | na | – | $LD_{50}$: 7950 mg/kg | Lewis (2000) |
| | Rat | – | Oral | Single | na | – | $LD_{50}$: >5 g/kg | ECB 2004 |
| | Mouse (ICR) | – | Inhalation | Single | na | – | $LC_{50}$ > 5.7 mg/L/4 h | ECB (2004) |
| | Mouse (ICR) | M, F | Oral | Single | 20 nm<br>120 nm | 1,000, 2,000, 3,000, 4,000 and 5,000 mg/kg | Alterations in hematology, clinical pathology parameters, and pathological findings (stomach, liver, heart, spleen)<br>- 120 nm: dose-dependent pathological damage in gastric, liver, heart and spleen.<br>- 20 nm: in low dose, severe toxicity observed in liver, spleen, and pancreas | Wang et al. (2008) |
| | Mouse (ICR) | M, F | Oral | Single | 58 nm<br>1.08 µm | 5,000 mg/kg | Two animals died by aggregation in the intestine in NP group<br>- Hepatic damage in both NP and microparticle (MP) | Wang et al. (2006) |
| | Rat (S-D) | M | Intranasal instillation | Single | 30 nm | 10, 40 mg/mL (40 µl instillation) | Damage to the olfactory epithelium | Gao et al. (2013) |
| | Mouse (ICR) | M, F | Oral | Single | 50 nm | 1,250, 2,500, 5,000 mg/kg | -In 5,000 g/kg, induced a body weight reduction<br>-Histopathological lesions were only observed for ZnO nanoparticles in the liver. | Li et al. (2012) |
| Subacute | Sheep | | Oral | 4 weeks (3 times/week) | na | 240 mg/kg | Pancreatic lesions. | Smith and Embling (1993) |
| | Rats and mice | | Inhalation | 20 weeks (1 h/day, 5 days/week) | ~2 µm | 1.3, 12.8, 121.7 mg/m$^3$ (as zinc) | Respiratory tract was damaged- | Marrs et al. (1988) |
| | Guinea pigs | | | 3 weeks (1 h/day, 5 days/week) | ~2 µm | 1.3, 12.8, 119.3 mg/m$^3$ (as zinc) | | |
| | Rat (SD) | M, F | Oral | 13 weeks | 40 nm | 67.1, 134.2, 268.4, 536.8 (mg/kg) | Significant changes in anemia-related hematologic parameters and mild to moderate pancreatitis observed in male and female at 536.8 mg/kg<br>- NOAEL was suggested to be 268.4 mg/kg | Seok et al. (2013) |
| Chronic | Ferrets | | Oral (in diet) | 6 months | na | 0, 500, 1,500 or 3,000 ppm | Diffuse nephrosis and active hematopoiesis in the bone marrow and the extramedullary area of the spleen.<br>- NOAEL: 500 ppm | Straube, Schuster, and Sinclair (1980) |
| | Rats (SD) Dogs, and Cats | | Oral (in diet) | 53 weeks | na | 175 to 1000 mg/day | - Glycosuria (dogs)<br>Fibrous degeneration of pancreas (cats).<br>No clinical signs (rat) | Clayton and Clayton, (1981–1982) |

generally did not differ appreciably between the two groups. The serum biochemistry demonstrated significant increases in activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in the microparticle group and rise in ALT, ALP, and LDH in NP group, indicating the presence of hepatic alterations induced by both microparticles and NP.

### Subacute and subchronic toxicity studies

To investigate subacute toxicity of $Zn^{2+}$, sheep were fed 31 mg $Zn^{2+}$/kg for 14 days and 49 days in three different treatment groups (Smith and Embling 1993). The sheep received additional amounts of $Zn^{2+}$ (from ZnO) at dose levels of 261 and 731 mg $Zn^{2+}$/kg feed (14-day study) or 731 and 1,431 mg $Zn^{2+}$/kg feed (49-day study), but adverse effects were not observed after 261 mg $Zn^{2+}$/kg feed. Pancreatic lesions were detected in all other groups. Treatment with 240 mg Zn (as ZnO)/kg body weight for 4 weeks (3 times/week) in 42 castrated sheep induced an increased incidence of pancreatic lesions.

Mice and rats were exposed to 121.7 mg of zinc chloride smoke (which also consists of ZnO, hexachlorophene, and other compounds, produced by ignition of a ZnO/hexachloroethane pyrotechnic composition) for 20 weeks (1 hr/day, 5 days/week), and then examined for an additional 13 months (Marrs et al. 1988). Guinea pigs were exposed to 119.3 mg zinc chloride smoke for 3 weeks. Data demonstrated that organ-specific toxicity rose in the high-dose group of mice with significantly elevated frequency of alveologenic carcinoma. All evaluations of animal stomachs and intestines at 18 months revealed no persistent adverse effects.

Seok et al. (2013) administered orally ZnO NP (40 nm; 67.1 134.2, 268.4, or 536.8 mg/kg/day) to both male and female S-D rats for 13 weeks (Table 3). Significant changes in anemia-related hematologic parameters and mild-to-moderate pancreatitis were found in both male and female rats at a dose of 536.8 mg/kg/day. Therefore, the no-observed adverse effect level (NOAEL) was suggested to be 268.4 mg/kg/day, a dose just below 536.8 mg/kg/day (Table 3).

Male mice were treated with 500 mg/kg of ZnO NP (<100 nm) orally for 21 days (Shrivastava et al. 2014). Significant oxidative stress was noted in erythrocytes, liver, and brain due to elevation in reactive oxygen species (ROS) levels and decrease in antioxidative enzyme activities. In addition, oral administration of ZnO NP in mice produced hepatoxic, nephrotoxic, and pulmonary toxicity (Esmaeillou et al. 2013).

### Chronic toxicity studies

Straube, Schuster, and Sinclair (1980) studied the chronic toxicity of ZnO using ferrets (3 ~ 5 per group) fed diets containing ZnO at 0, 500, 1,500, or 3,000 μg/g for 6 months (Table 3). The three ferrets in the 3,000 μg/g group displayed significant decreased body weights and were killed 9 ~ 13 days after treatment. The ferrets treated with 1,500 μg/g of zinc were killed 7 ~ 21 days after treatment. Histological examination of killed animals showed diffuse nephrosis and active hematopoiesis in the extramedullary area of the spleen and bone marrow. However, none of the ferrets given 500 μg/g of Zn in their diets developed clinical signs. In this study, NOAEL was estimated to be 500 μg/g, and the kidney was identified as the target organ in this species.

Clayton and Clayton (1981–1982) conducted a chronic toxicity of ZnO in dogs and cats fed diets containing 175–1000 mg ZnO/day for 3–53 weeks. Histological examinations demonstrated that glycosuria occurred in dogs, fibrous degeneration of the pancreas in some cats, and no apparent injury occurred in rats following administration of 0.5–34.4 mg ZnO/day for 1 month–1 year.

### Genotoxicity and cytotoxicity

Sharma et al. (2009) described the genotoxic potential of 30 nm ZnO NP in a human epidermal cell line (A431) at a concentration of 0.8 μg/ml, as well as in primary human epidermal cells at 14 μg/ml using a comet assay (Table 4). An *in vivo* mutagenicity study reported oxidative DNA damage and apoptosis in the liver of Swiss albino mice treated for 2 weeks with 30 nm ZnO NP orally using a comet assay (Sharma et al. 2012).

**Table 4.** Genotoxicity studies of ZnO.

| Characteristics or particle size | Test systems | Results | Ref. |
|---|---|---|---|
| Uncoated ZnO (100 nm) | (Photo) Ames test with TA98, 100, 1573 and *E. coli* WP2 | Negative | Dufour et al. (2006) |
| | Chromosome aberration in CHO cells | Clastogenic in vitro | |
| 100 nm | Ames test with TA98, 100, 1573 and *E. coli* WP2 | Negative (- S9) Marginal positive (+ S9) | Pan et al. (2010) |
| Tetramethylammonium hydroxide-coated ZnO NPs (5.4 nm) | Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and E. coli strain WP2uvrA(-) | Negative | Yoshida, Kitamura, and Maenosono (2009). |
| 100 nm | HEp-2 human cervix carcinoma cells, using the Comet assay and the cytokinesis-blocked micronucleus assay. | Positive | Osman et al. (2010) |
| 30 nm | Human epidermal cell line (A431), Comet assay, 0.001–5 μg/ml | DNA damage | Sharma et al. (2009). |
| 30 nm | Swiss albino mice, 50 and 300 mg/kg, for 14 days, oral treatment, Comet assay | DNA damage | Sharma et al. (2012). |
| 10 nm | Caco-2 cells: DNA breakage and oxidative damage test | Positive | Gerloff et al. (2009) |
| 20–30 nm | Human brain tumor cell (U87), HeLa cell, HEK cell: micronuclei test | Positive | Wahab et al. (2011) |
| 50 nm 1.2 μm | Ames test using *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA1535 | Negative | Li et al. (2012) |
| 86 nm | Primary human nasal mucosa cells, 0.01–50 μg/ml: DNA breakage test (Olive tail moment) | Positive | Hackenberg et al. (2011) |

Uncoated ZnO NP (mean diameter, 100 nm, >99% pure) were formulated as a 10% emulsion for Chinese hamster ovary (CHO) cells that were cultured in McCoy's 5A medium containing 10% fetal calf serum (FCS) (Dufour et al. 2006). ZnO NP produced chromosomal aberrations in a concentration-dependent manner in the dark. UV irradiation increased the clastogenicity up to 45%, but when pre-irradiated and simultaneously irradiated cells were compared, clastogenicity at equitoxic concentrations of ZnO NP was almost identical. Micron-sized, uncoated ZnO (particle size, <200 nm) formulated as a 10% emulsion induced negative results in the Ames test (strains: TA98, TA100, TA1573, and *E. coli* WP2). ZnO NP (100 nm) were found to be negative in the Ames test up to 1000 μg/ml in the absence of S9 metabolic activation and induced only marginal mutagenesis in *Escherichia coli* WP2 trp uvrA in the presence of S9 fraction (Pan et al. 2010).

Tetramethylammonium hydroxide-coated ZnO NP (size before coating was reported to be 5.4 nm) were found to produce negative effects in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *E. coli* strain WP2 uvrA(-), with and without metabolic activation using S9 pre-incubation (Yoshida, Kitamura, and Maenosono 2009). In the Ames test, no significant increment of revertants was recorded at any concentration of ZnO NP (50 nm) or ZnO microparticles (MPs, 1.2 μm) treatment in all testing strains (TA97, TA98, TA100, TA102, or TA1535) (Li et al. 2012).

Osman et al. (2010) noted that ZnO NP (100 nm) induced genotoxicity in HEP-2 human cervix carcinoma cells, using the comet assay and the cytokinesis-blocked micronucleus assay. In Caco-2 cells, 10 nm ZnO NP produced cytotoxicity (measured by the WST assay and LDH release) through DNA strand breakage and oxidative DNA damage (Gerloff et al. 2009). Genotoxicity was also demonstrated in other human cell systems such as the skin fibroblasts, neural cells (U87) (Wahab et al. 2011), and nasal mucosa cells (Hackenberg et al. 2011). Recently, the physicochemical transformation of ZnO NP with aging was found to play an important role in the ZnO NP-induced mutagenicity in mammalian cells. Aged ZnO NP were able to induce less cytotoxicity in the presence of relatively high degree mutation compared to fresh ZnO NP (Wang et al. 2015). Table 4 shows the summary of results of various genotoxicity studies. Interestingly, ZnO NP failed to induce genotoxic or mutagenic effects in bacteria, whereas these responses were noted in mammalian cells including human. Therefore, cautions need to be paid to potential genotoxicity of ZnO NP.

Oxidative stress seemed to play a crucial role in cytotoxicity and was determined metabolically by depletion of glutathione (GSH) levels, and activities of catalase (CAT) and superoxide dismutase

(SOD). ZnO NP were found to decrease mitochondrial activity, alter cellular morphology, and disturb the cell cycle distribution in human keratinocytes at a concentration of 10 μg/ml (Kocbek et al. 2010). Hackenberg and Kleinsasser (2012) demonstrated the cytotoxicity of ZnO NP (100 nm) at 20 μg/ml, with and without UVA-1 irradiation in human primary oral mucosa cells, whereas carcinoma cell lines were more susceptible to the photocatalytic reaction. Jeng and Swanson (2006) examined the adverse effects of metal oxide NP on mammalian cells. Marked changes in cell morphology were observed after exposure to ZnO NO for 24 hr, particularly at concentrations greater than 50 μg/ml. Cells became irregular and shrank, and at concentrations of 50–100 μg/ml, ZnO NP induced 15%–50% cell death as detected by the trypan blue dye method. Triethoxycaprylylsilane-coated ZnO NP (30 ~ 200 nm) did not induce genotoxicity in lung cells from rats exposed by inhalation as evidenced by the mouse bone marrow micronucleus test (Landsiedel et al. 2010). Although ZnO NP (60 ~ 200 nm) displayed some clastogenic activity in *in vitro* mammalian cells, there was no apparent evidence for clastogenic potential or aneugenic activity *in vivo*. Hackenberg et al. (2011) reported that repetitive exposure of human nasal mucosa cells to ~86 nm ZnO NP (5 μg/ml) induced DNA damage using the comet assay which was further increased after a 24-hr regeneration period. Coating 30 nm ZnO NP with polymethylacrylic acid (PMAA) reduced cytotoxicity and ROS generation in WIL2-NS human lymphoblastoid cells. However, significant elevation in genotoxicity was noted when compared to uncoated ZnO NP using the cytokinesis-blocked micronucleus assay (Yin et al. 2010). Song et al. (2010) found that ZnO NP (10 ~ 30, 30, or 100 nm) and 1 μm ZnO MP produced cytotoxicity in Ana-1 murine macrophages. ZnO NP were found to induce overproduction of ROS and caspase-12 and reduction of bcl-2 and caspase-9 levels in rat retinal ganglion cell damage (Gao et al. 2013). Overall results suggested that ZnO NP are non-genotoxic using a bacterial revertant mutation test, but ZnO NP are genotoxic in mammalian cells and may be associated with oxidative stress (Demir, Creus, and Marcos 2014).

### Immunotoxicity

Single intratracheal instillation of rats with 50 ~ 70 nm ZnO NP, 1,000 nm ZnO MP (1 and 5 mg/kg body weight) and 10 nm ZnO NP resulted in severe but reversible inflammation as measured in the bronchoalveolar lavage fluid (BALF) by increased LDH release, cell number, and neutrophil content. These ZnO NP also induced eosinophilic/fibrotic/granulomatous inflammation and recruitment of eosinophils and neutrophils in the BALF (Cho et al. 2010; Sayes, Reed, and Warheit 2007).

Expression of IL-1β and chemokine (C-X-C motif) ligand 9 (CXCL9), one of the subtypes of chemokine CXC motif, was induced by ZnO NP (20 nm) in murine bone marrow-derived dendritic cells and RAW264.7 murine macrophages. In RAW264.7 cells, ZnO NP (20 nm) induced intracellular $Ca^{2+}$ flux, lowered mitochondrial membrane potential (MMP), and loss of membrane integrity (George et al. 2010). Yazdi et al. (2010) demonstrated that ZnO NP (15 nm) failed to activate inflammasomes in THP-1 human macrophages, but ZnO NP significantly affected macrophages, monocytes, and dendritic cells. Muller et al. (2010) noted that ZnO NP exposure in macrophages resulted in LDH release, oxidative stress, intracellular $Ca^{2+}$ flux, lower MMP, and production of IL-1β and CXCL. ZnO NP (about 100 nm) initiated more severe cytotoxicity and inflammation in human monocytes than micro (about 5 μm) sized ZnO (Sahu, Kannan, and Vijayaraghavan 2014). Recently, adsorption affinity of nanoparticles to interleukin-8 was detected in A549 cells (Lee et al. 2015)

### Reproductive and developmental toxicity

To evaluate the reproductive toxicity of Zn, 18 male Charles-Foster rats were treated with a Zn-supplemented diet (4,000 μg/g zinc as $ZnSO_4$) for 30–32 days (Samanta and Pal 1986). Male rats were mated with females, and animals then killed

for measurements of Zn levels in reproductive organs and sperm. The conception incidence was significantly different between control (15/15) and Zn-supplemented females (11/18). Further, live births also significantly decreased in Zn-supplemented females. In Zn-supplemented males, Zn concentration in testes and sperm increased 25 and 18%, respectively, and motility of the sperm collected from the epididymis fell. However, there was no marked change in viability of sperm. Pal and Pal (1987) administered Zn-supplemented diet (4,000 μg/g zinc as $ZnSO_4$) to female Charles-Foster rats either from day 1 until day 18 post-coitum or from day 21 to 26 prior to mating until day 18 post-coitum. When a Zn-supplemented diet was ingested from day 1, conception incidence decreased concomitant with a fall in numbers of implantation sites in pregnant females. However, when Zn was given prior to coitus, there was no marked change in incidence of conception and implantation sites of mated females. In both experiments, there was no marked difference in stillbirths, malformed fetuses, and resorption between Zn-supplemented and control rats.

To examine the effects of high levels of Zn exposure on fetal development, adult female S-D rats were fed beginning at either 21 days prior to mating until 16 days of gestation or 0 day age of the fetus to 20 days of gestation (Schlicker and Cox 1968). Excess Zn (0.4%) administration from 0- to 15-, 16-, 18-, or 20-day-old fetuses resulted in growth reduction as evidenced by liver weight and variable degrees of fetal resorption (4%–29%), whereas no external malformations were detected. When dietary feeding of 0.4% Zn was extended to 21 days prior to mating, 100% resorption was observed. However, there were no significant effects on growth, resorption, and malformation when 0.2% Zn treatment for 21 days prior to mating until 15- and 16-day-old fetuses. The results of the reproductive toxicity studies for Zn are summarized in Table 5.

A similar study was conducted to investigate the relationship between maternal dietary Zn exposure during gestation and lactation to development and metal levels in newborns (Ketcheson, Barron, and Cox 1969). Female S-D rats were fed a Zn contained diet (0.2% and 0.5%) gestation day 0 to lactation day 14. Control animals were fed a basal diet containing 9 μg/g Zn. There were no marked changes in maternal weight and number of live fetus. No external malformations were detected in any experimental group. However, in the 0.5% Zn group, two females had all stillborn litters characterized by edema. Four stillborn animals were born to mother fed 0.2% Zn, and these animals did not show edema. There was a dose-dependent elevation in Zn content and decrease in iron levels.

To investigate excessive dietary Zn supplementation on intrauterine and postnatal development, 11 females and 3 males of natural dark ranch minks received a Zn-supplemented (1,000 μg/g) diet (Bleavins et al. 1983). Control animals were fed a basal diet containing 20.2 μg/g Zn and 3.1 μg/g copper (Cu). All 11 mated females in the control groups delivered offspring, but only eight females in the Zn-supplemented diet group produced offspring. At 12 weeks of age, body weight of male kits (newborn mink) of the Zn-supplemented group was significantly decreased compared to controls. In addition, female kits of 3–4 weeks of age fed a Zn-supplemented diet showed several clinical signs such as gray fur around ears, eyes, jaws, and genitals concomitant with hair loss and dermatosis in these areas.

Developmental toxicity was noted in the frog embryo teratogenesis study assay xenopus (FETAX). ZnO NP (40 ~ 100 nm and 10 ~ 25 $m^2$/g) were given to *Xenopus laevis*. In an acute experiment, ZnO NP were administered to embryos at concentrations of 0.1, 0.316, 1, 3.16, 10, or 31.6 mg/L (Nations et al. 2011a). The 96 hr $EC_{50}$ for malformations was 10.3 mg/L and % malformations for all experimental groups ranged from 0% to 81%. Of the malformation type, 89% of abnormalities were gut malformations. There was no marked difference in snout vent length (SVL), whereas total body length (TBL) was significantly different in the 10 and 31.6 mg/L groups. Nations et al. (2011b) administered ZnO (40 ~ 100 nm and 10 ~ 25 $m^2$/g) to *Xenopus laevis* at concentrations of 0.067, 0.159, 0.305, 0.513, or 0.799 mg/L as actual Zn beginning *in ovo* and proceeding through metamorphosis. Three doses (0.067, 0.159 or 0.305 mg/L) induced less than 10% mortality and 0.513 mg/L exposure resulted in 11% mortality. However, at 0.799 mg/L, the treated group showed a significant rise of 40% mortality compared to all treatments. In the case of groups exposed to less than 0.159 mg/L, 100% completion of metamorphosis

**Table 5.** Reproductive and developmental toxicity studies of zinc.

| Animals | Administration | Dose | Results | Ref. |
|---|---|---|---|---|
| Charles-Foster male rat | Diet | 4000 ppm $ZnSO_4$ for 30 to 32 days before mating (about 200 mg $Zn^{2+}$/kg/day) | <Male><br>- Increased zinc content in the testis (25%) and sperm (18%)<br>- Decreased sperm motility (no changes in viability)<br><Female><br>- Conception incidence of control was 15/15 whereas that of zinc treated group was 11/18<br>- Significantly decrease in number of live birth | Samanta and Pal (1986) |
| Charles-Foster female rat | Diet | 4000 ppm $ZnSO_4$ for 18 days (about 200 mg $Zn^{2+}$/kg/day) | - Conception incidence of control was 12/12, whereas that of zinc treated group was 5/12<br>- Decrease in number of implantation sites/pregnant females and /mated female<br>- No changes in incidence of conception and implantation site/mated female when zinc administration beginning at the 21 to 26 days prior to coitus and continued throughout gestation for 18 days | Pal and Pal (1987) |
| Animals | Administration | Dose | Results | Ref. |
| S-D rat | Diet for 21 days | 0.2% and 0.4% (100 and 200 mg $Zn^{2+}$/kg/day) | - –0.4% of zinc exposure from 0 day to fetal developmental period (15 ~ 20 days): fetal resorption varies from 4% to 29% whereas no external malformation<br>- 0.4% of zinc exposure before 21 days before mating to 15 days of gestation: 100% resorption<br>- 0.2% zinc exposure before 21 days before mating to 15 days of gestation: no resorption and external malformation | Schlicker and Cox (1968) |
| S-D rat | Diet from day 0 of gestation to day 14 of lactation | 0.2% and 0.5% (120 and 300 mg $Zn^{2+}$/kg/day) | - No changes the number of viable young/litter and external malformation in all experimental groups<br>- Higher still birth rate in 0.5% than 0.2%: Two females at 0.5% fed had all stillborn litters, whereas 4 stillborn pups observed in 0.2% fed group<br>- The newborns from 0.5% fed mothers showed higher levels of zinc | Ketcheson, Barron, and Cox (1969) |
| Mink (natural dark from ranch) | Diet | Beginning at 500 ppm, increased to 1000 ppm | - –8/11 females produced offspring<br>- The body weight of kits was significantly lower at 12 weeks of old<br>- Female kits of 3 to 4 weeks of old showed gray fur around eyes, ears, jaws, and genitals together with hair loss and dermatosis | Bleavins et al. (1983) |

was observed a minimum of 5 days before controls reached 90% completion. However, tadpoles treated with 0.513 mg/L demonstrated only 58% completion of metamorphosis, and no metamorphosis was detected in the 0.799 mg/L treatment group (Nations et al. 2011b). The results of the developmental toxicity studies of Zn or ZnO are summarized in Table 5.

## *Carcinogenicity*

Until now, there was no apparent adequate long-term carcinogenicity study on ZnO in animals. Further, no conclusive epidemiological evidence that Zn was carcinogenic to human exists. The US EPA classified Zn as class D indicating that it is not classifiable as to human carcinogenicity based on inadequate evidence in humans and animals (U.S. EPA 2005b).

In 1965, Walters and Roe (1965) reported no marked differences in tumor incidence between $ZnSO_4$ treated and control mice. In this study, Chester-Beatty mice were administered with 1,000 or 5,000 μg/g of $ZnSO_4$ $7H_2O$ in drinking water for 45–53 weeks. The dose of Zn was calculated as 200 or 1,000 mg $Zn^{2+}$/kg. At the end of the treatment, the tumor incidence and types of tumors were investigated. Although several types of tumors, including hepatoma, malignant lymphoma, lung adenoma, and hyperplasia in the fore-stomach epithelium, were observed in Zn treated groups, there was no significant difference between exposed and control mice.

In an epidemiological study, the association between supplementary intake of Zn and prostate cancer was examined among 46,974 US men participating in the Health Professionals Follow-Up Study (Leitzmann et al. 2003). After 14 years of follow-up study, out of ascertained prostate cancer, approximately 25% of the population consumed Zn supplements. Although high supplemental zinc intake (>100 mg/day) showed significant relative risk (RR) (2.29, 95% confidence interval = 1.06–4.95; $P_{trend}$ = 0.003) of advanced prostate cancer, less than 100 mg zn/day intake demonstrated no marked association with prostate cancer risk. More than 10 years of supplemental Zn users displayed RR (2.37, 95% confidence interval = 1.42–3.95; $P_{trend}$ < 0.001) of advanced prostate cancer, whereas no marked correlation between duration of metal use and cancer risk was found in less than 10 years user. Data indicated that strong evidence to support a specific mechanism for the association is lacking, and further study needs to be conducted to clarify the role of chronic excess Zn intake in prostate carcinogenesis.

## Neurotoxicity

Few *in vitro* and *in vivo* studies investigated ZnO NP-induced neurotoxicity. Elevated neurosecretion and increased activity in the neurohypophysis were noted in rats intragastrically exposed to ZnO (100 mg/day) for 10 days (Kozik, Gramza, and Pietrzak 1981). The neurotoxicity of ZnO depending upon concentration and particle size was determined using mouse neural stem cells (NSC) (Deng et al. 2009). For the cell viability assay, different types of ZnO NP (10, 30, 60 or 200 nm) were utilized for 24 hr with final concentration of 0, 3, 6, 12, 18, and 24 μg/g. A cytotoxic effect was found at 12 μg/g and almost all cells died at a concentration of 24 μg/g. However, there was no apparent size-dependent toxicity. The mechanism of neural cell toxicity may be attributed to be dissolved $Zn^{2+}$ within cells or in the culture medium.

Win-Shwe and Fujimaki (2011) proposed potential pathways for NP-induced neurotoxicity. According to their theory, NP enter the brain via either olfactory bulb or systemic circulation. Finally, NP may induce inflammation, oxidative stress and apoptosis by releasing toxic or anti-toxic mediators from microglia and astrocyte which result in neurodegeneration or neuroregeneration (Wang et al. 2014a, 2014b; Win-Shwe and Fujimaki 2011). However, Shim et al. (2014) found that the blood brain barrier (BBB) was intact after repeated oral administrations of ZnO NP for 28 days, suggesting no significant damage in the brain and no neuronal death occurred after intravenous administration of ZnO NP 4 times for 28 days.

In general, the molecular pathway of apoptosis or cell death induced by ZnO NP is mediated by cellular endocytosis through endosome and lysosome release of $Zn^{2+}$. There are four proposed pathways: firstly, increase $Ca^{2+}$ influx and disruption of cellular homeostasis leading to pro-inflammation; secondly, enhanced Bax expression and Bax/Bcl-2 ratio, decrease MMP, elevated cytochrome C release, and activated apoptotic cascade; thirdly, damage to mitochondria to initiate oxidative stress by generating reactive oxygen species (ROS) leading to apoptosis via elevated c-Jun N-terminal kinase (JNK) expression or increased cleaved poly(ADP-ribose) polymerase-1 (PARP); and finally, energy deficiency through diminished carbohydrate influx to finally produce cell death (Chang et al. 2012; Huang et al. 2010; Nel et al. 2009; Sheline, Behrens, and Choi 2000; Wang et al. 2014a; Xia et al. 2008) (Figure 1). In contrast, Goncalves and Girard (2014) reported that ZnO NP, as activators of several human neutrophil functions, inhibited apoptosis by a *de novo* protein synthesis-dependent and ROS-independent mechanism.

## Dermal toxicity

Lansdown and Taylor (1997) studied irritative potential of Zn compounds. Severe irritation was noted in rabbits, mice, and guinea pigs, inducing epidermal hyperplasia and ulceration, after daily application of Zn chloride as a 1% aqueous solution in an open patch test for 5 days. Aqueous Zn acetate (20%) was slightly less irritant. In open patch tests, ZnO (20% suspension in dilute Tween 80), zinc pyrithione (20% suspension), and zinc sulfate (1% aqueous solution) were mild irritants, produced a marginal epidermal hyperplasia, and induced hair growth. In contrast, zinc undecylenate (20% suspension) was not irritating.

Epidermal irritancy was noted when Zn interacted with epidermal keratin (Lansdown 1991). Although data for dermal irritation by ZnO have been limited, a ZnO and petrolatum formulation used in disposable diapers were associated with a significant reduction in diaper rash and skin erythema compared to a control product (Baldwin et al. 2001).

### Phototoxicity

Dufour et al. (2006) studied the photoclastogenicity of ZnO. UV irradiation induced a numerical rise in genotoxic potency of compounds that are clastogenic in the dark. Data suggested that minor induction in clastogenic potency under conditions of photogenotoxicity testing does not necessarily represent a photogenotoxic effect but might be initiated due to an enhanced sensitivity of the test system subsequent to UV irradiation (Dufour et al. 2006). Sharma et al. (2009) reported that ZnO NP might induce cellular damage even in the absence of UV irradiation. One possible mechanism is the catalysis of lysosomal-released $H_2O_2$ to -OH·during phagocytosis and degradation of NP (Sharma et al. 2009). Ma et al. (2014) also found in a lab study that toxicity of ZnO NP to *Daphnia magna* was enhanced under simulated solar UV radiation in parallel with photocatalytic ROS generation and enhanced particle dissolution. Therefore, UV co-exposure also needs to be considered in risk assessment of ZnO NP for humans.

### Inhalation toxicity

Friberg et al. (1986) reported that rabbits and cats exposed to ZnO fumes of 110 ~ 600 mg/m$^3$ for 3.5 hr showed a transient fall in body temperature followed by marked leukocytosis. Histopathological examination of heavily exposed animals displayed signs of bronchopneumonia. Guinea pigs were exposed 3-hr/day to the mean concentration of 7 mg/m$^3$ of freshly generated ZnO for 5 days (Bingham, Cohrssen, and Powell 2001). Pulmonary function of some of these animals was measured immediately after exposure on each of the 5 days. Another group of animals was exposed to a lower concentration of 2.7 mg ZnO/m$^3$ for same duration of 3hr/day for 5 days. The concentration of 7 mg ZnO/m$^3$ produced a gradual decrement in total vital capacity and lung capacity over the course of exposure. The carbon monoxide diffusing capacity (DLCO) was not markedly influenced until the fourth day when it fell to 30% below control levels. Exposures to 2.7 mg ZnO/m$^3$ did not markedly alter any parameters measured.

Male Hartley guinea pigs were exposed (nose-only exposures) to 0, 2.3, 5.9, or 12.1 mg/m$^3$ of ZnO (as ultrafine particles with an average diameter of 0.05 μm) 3 hr a day for 1, 2, or 3 consecutive days (Conner et al. 1988). Three animals were euthanized and lung tissue examined microscopically, and bronchoalveolar lavage fluid (BALF) parameters examined. Exposure to 12.1 mg ZnO/m$^3$ elevated number of nucleated cells in lavage fluid. Treatment with 5.9 and 12.1 mg ZnO/m$^3$ was associated with dose-dependent increase in protein, neutrophils, and activities of beta glucuronidase, ALP, acid phosphatase, LDH, and angiotensin-converting enzyme. Centriacinar inflammation in the lung was detected at 5.9 and 12.1 mg/m$^3$ demonstrating significant morphologic damage. The lowest dose level of 2.3 mg/m$^3$ induced minimal changes in neutrophils and activities of ALP and LDH in BALF after 3-day exposure, but no morphologic changes were observed at this level. Based on these results, 2.3 mg ZnO/m$^3$ was considered as a marginal LOAEL in the study (Conner et al. 1988).

Guinea pigs exposed to 1,000 ~ 2,600 mg ZnO/m$^3$ for an hr resulted in initial reduction in body temperature by 0.5°C–2°C, followed 6–18 hr later by a rise of 0.5°C–1°C above normal. Animals exposed up to 2,500 mg ZnO/m$^3$ for 3–4 hr died during treatment or immediately after (ACGIH 2005). Dinslage-Schlunz and Rosmanith (19,760 reported on a 12-week inhalation toxicity study in rats. Two hundred forty female Wistar rats (80/group) were exposed to 15 mg ZnO for 12 weeks via inhalation. Wistar rats killed after 14, 28, 56, or 84 days and metal content of lungs determined. Data demonstrated that independent of duration of the experiment, the greatest daily exposure time, resulted in the highest dry lung weights, while Zn levels remained almost constant. Eighty-four days of treatment significantly elevated Zn levels compared to 14 days independent of duration of daily exposure.

Sayes, Reed, and Warheit (2007) demonstrated that single intratracheal instillation of rats with 50 ~ 70 nm ZnO NP and, 1,000 nm ZnO microparticles (1 or 5 mg/kg body weight) resulted in

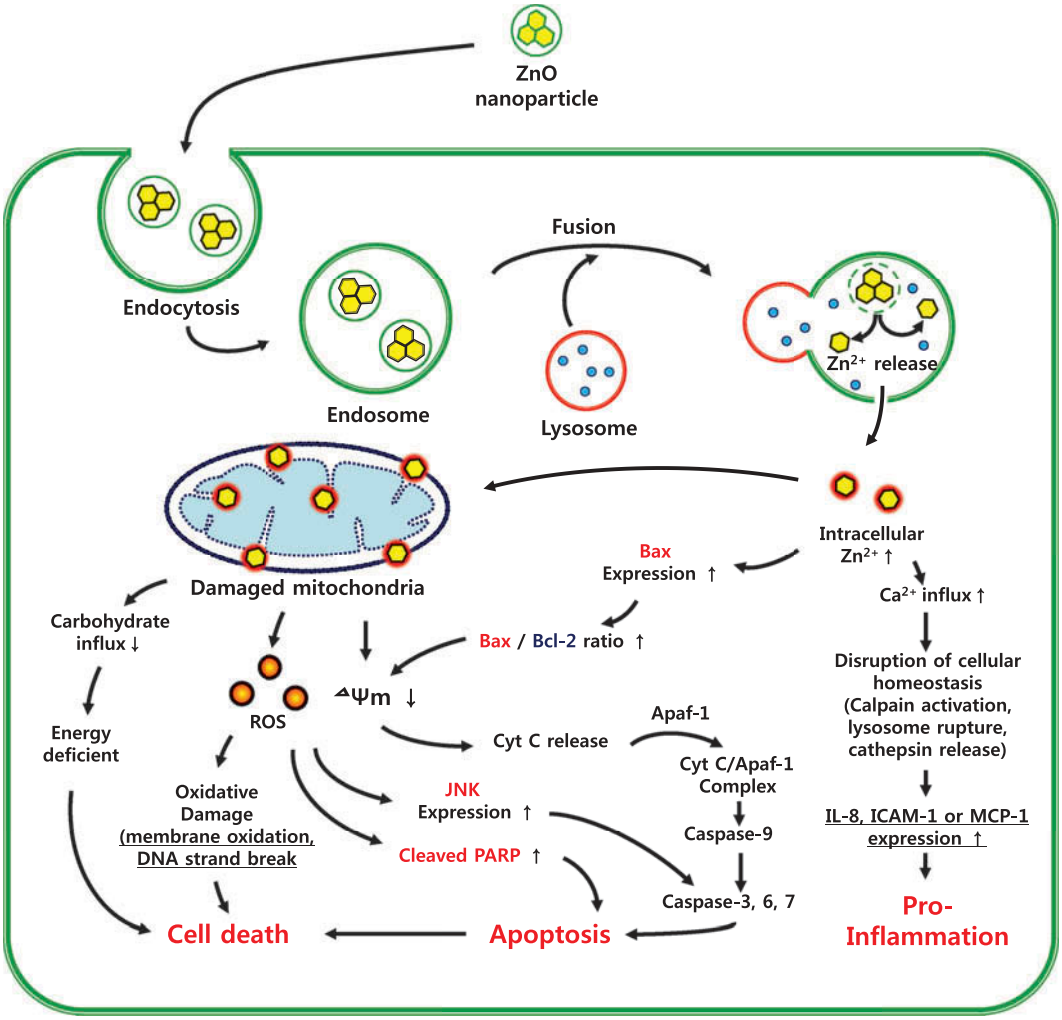


**Figure 1.** Toxicological mechanism of ZnO nanoparticle (Chang et al. 2012; Huang et al. 2010; Nel et al. 2009; Sheline, Behrens, and Choi 2000; Wang et al. 2014a; Xia et al. 2008).

Apaf-1, Apoptotic protease activating factor 1; Cyt C, cytochrome C; ICAM-1, intracellular cell adhesion molecule-1; IL-8, interleukin-8; MCP-1, monocyte chemotactic protein-1; JNK, c-Jun N-terminal kinase; $\Delta\psi_{m,}$ mitochondria membrane potential; PARP, poly(ADP-ribose) polymerase-1; ROS, reactive oxygen species.

reversible inflammation as evidenced by increased LDH release, cell number, and neutrophil content, which was resolved 1 month after instillation. Warheit, Sayes, and Reed (2009) reported inhalation exposure of 3 μm ZnO microparticles (25 or 50 mg/m$^3$) and instillation of 300 nm ZnO NP (1 or 5 mg/kg body weight) of rats produced transient inflammation measured in BALF as determined by elevation in LDH release and levels of protein and neutrophils. Wang et al. (2010) noted that inhalation of 20 nm ZnO NP (2.5 mg/kg body weight) in rats twice daily for 3 days increased Zn content in the liver after 12 hr and in kidneys after 36 hr. Histopathology revealed damage in liver and lung tissues (Wang et al. 2010).

C57BL/6 mice were exposed to ZnO NP (3.5 mg/m$^3$, 4 hr/day) via inhalation for 2 or 13 weeks and were killed within either 1 hr or 3 weeks post-exposure (Adamcakova-Dodd et al. 2014). The particle size of ZnO NP was 15 ± 4 nm (mean ± SD), and it was zincite crystalline. In two-week study, ZnO NP increased macrophage levels in BALF and numerical rise in IL-12 and MIP-1α of animals necropsied within 1-hr post-exposure, but these changes were not significant in animals necropsied 3-week post-exposure. In BALF, LDH activity was significantly elevated 3-week post-exposure in two-week study. In a 13-week study, ZnO NP increased number of macrophages in BALF of animals necropsied within 1-hr or 3-

week post-exposure, However, lung histopathological changes were not observed in both 2-week and 13-week studies. Therefore, data suggested that ZnO NP exerted low 13-week toxic potential by inhalation route (Adamcakova-Dodd et al. 2014).

Rats exposed to coated ZnO NP (20–200 nm) by aerosols (ranging from 0.5, 2.5 to 12.5 mg/m$^3$) for 5 days were observed 14- or 21-day post-exposure (Landsiedel et al. 2014). BALF and histopathology of respiratory tract were examined. Five-day ZnO NP exposure increased total cell counts and polymorphonuclear neutrophils (PMN), lymphocytes, monocytes, total protein content, and activities of gamma-glutamyl transpeptidase (GGT), LDH, ALP, and N-acetyl-β-(D)-glucosaminidase (NAG) in BALF. Various mediators including cytokine-induced neutrophil chemoattractant 1 (CINC-1), clusterin, cystatin C, granulocyte chemotactic protein 2 (GCP-2), monocyte chemoattractant protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF), macrophage-derived chemokine (MDC), myeloperoxidase (MPO), and osteopontin (OPN) were elevated in BALF of rats after 5-day ZnO NP treatment. Moderate multifocal necrosis of olfactory epithelia in nasal cavity and granulocytic infiltration in lung was also detected in rats after 5 days. It should be also noted that there might be significant variation in adverse outcomes attributed to ZnO NP dependent upon various characteristics. The characteristics include types of NP, physicochemical properties, dose, size, deposition, susceptibility of organisms, purity, time of exposure, duration and routes of exposure (Alaraby et al. 2016; Braakhuis et al. 2016; Kang, Lim, and Han 2013; Kermanizadeh et al. 2016; Kim et al. 2015; Lim et al. 2014).

## Toxicokinetics

Absorption, distribution, metabolism, and excretion of ZnO in animals and humans are informative to perform risk assessment. Several studies were available for toxicokinetics of ZnO.

### Absorption

Kapur et al. (1974) examined percutaneous absorption of ZnO in rabbits. Retention of $^{65}$Zn on skin ranged from 3% to 65%, 6–24 hr after administration. $^{65}$Zn was also found in the keratogenous area of the hair shaft and subcutaneous muscle layer. When sunscreens containing $^{68}$ZnO nanosized and larger particles were dermally applied to hairless mice, the probability of skin penetration of ZnO was markedly enhanced (Osmond-McLeod et al. 2014).

A human case report showed that ZnO penetrated wounded skin (Hallmans 1977). During wound treatment of burn patients, Zn adhesive tape that contained approximately 7.5 g ZnO/100 g was applied to the wounded site. After 3 ~ 18 days of treatment, the maximum serum Zn levels elevated to 28.3 μmol/l. Agren (1991) found Zn delivery from ZnO dressings in injured human forearm skin. The dressing was maintained for 48 hr. As a result, 12% of the applied dose (450 μg) of Zn entered the wounded skin site. Pirot et al. (1996) demonstrated percutaneous absorption of Zn released from ZnO. Ointment containing ZnO was applied topically to human skin *in vitro*. Percutaneous absorption of Zn from the ointment was estimated to be 0.36% (0.09%–1.19%) of the applied dose after 72 hr.

Dussert, Gooris, and Hemmerle (1997) investigated the distribution and penetration of sunscreen emulsions on human skin *in vitro*. Abdominal human skin was obtained during plastic surgery. Spectra veil mineral oil or caprylic/capric triglyceride (MOTG, Tioxide specialties, UK) was used as a test emulsion, which was a 60% dispersion of ZnO (average length, 116.8 nm ± 8.5). Data demonstrated that topical application onto the skin resulted in an almost regular distribution of ZnO on the stratum corneum. In addition, intracellular or intercellular penetration of ZnO into the skin was not detected. Lansdown and Taylor (1997) conducted an *in vivo* penetration study of ZnO in New Zealand White rabbits by topically administering 0.2 ml of 20% ZnO. There were no adverse effects and no penetration into either the dermis or epidermis. Gamer, Leibold, and Van Ravenzwaay (2006) investigated the penetration potential of ZnO *in vitro* in excised skin from a 5-month-old domestic pig. Microfine ZnO (uncoated, 80 nm) particles were applied with an exposure dose of approximately 400 μg/cm$^2$. The mean total recovery of Zn ranged from 102% to 107% of the applied dose. It was found that ZnO did not penetrate through the stratum

corneum of porcine skin under these experimental conditions. Cross et al. (2007) noted that penetration of ZnO into the skin was negligible. Three sunscreen formulations were applied to volunteer female human skin. Their formulations were as follows: dispersion with 60 wt% of siliconate-coated ZnO, sunscreen emulsion with 20 wt% ZnO and sunscreen emulsion without ZnO. Average particle size of the ZnO was approximately 15 ~ 40 nm. Treatment of the three formulations onto the human epidermal membrane over 24 hr showed that less than 0.03% of the applied ZnO was absorbed; therefore, ZnO is not expected to penetrate the human epidermal membrane. Zvyagin et al. (2008) reported the results of topical administration of ZnO onto excised and *in vivo* human skin. Data demonstrated that ZnO NP were located in the stratum corneum, skin folds, and hair follicle roots, suggesting that ZnO NP lacked dermal penetration potential. Lin et al. (2011) did not observe any skin penetration of ZnO NP in intact and tape stripped the human skin using time-correlated single-photon counting (TCSPC). Tape stripping was conducted after the application of ZnO NP onto the human subject's forearm. Among the volunteer subjects, there were eight with psoriasis or atopic dermatitis. Siliconate-coated ZnO NP were applied at doses of 2 mg/cm$^2$ (for 4 or 24 hr) to healthy volunteers and 14 mg/cm$^2$ (for 2 hr) to individuals with skin lesions. Real-time quantification of ZnO NP using the TCSPC technique demonstrated that ZnO NP did not penetrate into the human skin in any group. Table 6 summarizes dermal penetration of ZnO.

Cho et al. (2013) found that ZnO NP were readily absorbed and then distributed compared to $TiO_2$ administered orally. ZnO NP (mean ± SD, 89.2 ± 44.7 nm; hexagonal crystalline) of 134.2, 268.4, or 536.8 mg/kg/day were administered orally for 13 weeks to male and female SD rats. Systemic absorption of ZnO NP was dose dependent, but Zn levels in whole blood were low with concentrations less than 6 μg/g in blood at the highest dose of 536.8 mg/kg/day.

### Distribution

Ansari et al. (1975) investigated changes in Zn levels in rat organs, including heart, kidneys, liver, muscle, small intestine, and tibia. Male rats were fed a diet containing 600 μg/g of Zn supplement for 42 days. Results showed numerical increases in tissue metal concentration. Nonetheless, there was no pattern in alterations of Zn, and only a few changes were significant. Further, Ansari et al. (1976) examined metal concentration in tissues including heart, kidneys, liver, muscle, and tibia, after ZnO was added to the diet in concentrations up to 8,400 μg/g for 21 days in male rats. There were no significant alterations in tissue Zn levels up to 1,200 μg/g. Zinc concentrations in the heart and muscles were not markedly affected by Zn supplements at any dose. Zinc treatment with of 2,400 ~ 7,200 μg/g elevated tissue Zn levels in bone, liver, and kidneys.

Aamodt et al. (1979) reported an *in vivo* human study of Zn distribution after oral or intravenous (iv) administration. Seventeen patients were administered 50 μCi of Zn-69 m via oral or iv route. Data showed that Zn transported into the liver after ingestion was subsequently distributed to other parts of the body. Sturniolo et al. (1991) investigated factors affecting Zn absorption using a Zn tolerance test conducted with 11 healthy human volunteers and found that metal levels in plasma peaked after 3 hr oral intake. Schiffer et al. (1991) examined the effects produced by dietary exposure to Zn cations. In female SJL mice fed a diet containing supplemental Zn sulfate, high accumulations of metal occurred in bone, kidneys, liver, and pancreas. Recently, two ZnO NP (20 and 70 nm) were orally administered to male and female SD rats (Baek et al. 2012). ZnO NP were mainly deposited in organs such as liver, lung, and kidneys within 72 hr, with no marked difference between particle sizes or genders. Baek et al. (2012) suggested that liver, lung, and kidneys might serve as potential target organs for distribution and toxicity of ZnO NP regardless of NP size or gender. Cho et al. (2013) also found that oral administration of ZnO NP (mean ± SD, 89.2 ± 44.7 nm; hexagonal crystalline) of 134.2, 268.4, or 536.8 mg/kg/day revealed the highest deposition of Zn in liver (about 75 μg/g liver at highest dose of 536.8 mg/kg/day). Choi et al. (2015) recently demonstrated that a single iv injection of ZnO NP in rats was distributed mainly in liver, kidneys, lung and spleen, but not thymus, brain, and testes.

### Metabolism

Based on a physiological perspective, Zn is not metabolized in humans (US EPA 2005a), but is bound to proteins or located in organelles as a divalent cation (Frazzini et al. 2006). Zinc may interact electrostatically with various anions or proteins with negatively charged moieties.

### Excretion

Changes in Zn levels were determined in tissues of rats fed a diet containing 600 μg/g metal for up to 42 days (Ansari et al. 1975) and 1,200–8,400 μg/g of Zn for 21 days (Ansari et al. 1976). In these studies, Zn excretion increased linearly as dietary intake rose. ZnO NP may be excreted in feces and

**Table 6.** Dermal penetration potential of ZnO.

| Test system | Coating | Size (average or range) | Application conditions | Results | Ref. |
|---|---|---|---|---|---|
| *in vitro* | | | | | |
| Human skin | na | Microfine | Ointment, for 72 h | - −0.36% absorption of zinc applied dose | Pirot et al. (1996) |
| Human skin | na | 116.8 nm | Commercial sunscreen formulation (w/o emulsion) | - No intracellular penetration | Dussert, Gooris, and Hemmerle (1997) |
| Porcine skin | Uncoated | 80 nm | W/o emulsion with 10.3% ZnO, nominal dose of test formulation, 4 mg/cm$^2$ for 24 h | - No significant penetration | Gamer, Leibold, and Van Ravenzwaay (2006) |
| Human skin | Polymethylsilsesquioxane | 15–40 nm | 2 Sunscreen formulations, 10 mg/cm$^2$ for 24 h: 60% ZnO dispersion in caprylic/capric triglyceride; typical o/w emulsion of 20% ZnO | - Limited penetration of stratum corneum | Cross et al. (2007) |
| Human skin | na | 26–30 nm | Commercial sunscreen formulation with 19% ZnO, 6 mg/cm$^2$ for 24 h | - No evidence of penetration, accumulation into skin folds and/or hair follicle roots | Zvyagin et al. (2008) |
| Human skin | Coated | 100–200 nm | W/o emulsion with 1% ZnO, 2 mg/cm$^2$ for 24 h | No evidence of penetration, accumulation into skin folds and/or hair follicles | Durand et al. (2009) |
| Nude mouse skin | Uncoated | 10 nm | Penetration-enhancing vehicle with 10% ZnO | Penetration into stratum corneum | Kuo et al. (2009) |
| *in vivo* | | | | | |
| Rabbit | na | < 2–20 μm | Suspension with 20% ZnO for 4 h (1 day) or 2 h daily (3 days) | - No significant penetration | Lansdown and Taylor (1997) |
| Human skin | na | 26–30 nm | Commercial sunscreen formulation with 19% ZnO, 6 mg/cm$^2$ for 24 h | - No evidence of penetration, accumulation into skin folds and/or hair follicle roots | Zvyagin et al. (2008) |
| Human intact skin; psoriasis/atopic disease | Siliconate | 35 nm | Sunscreen formulation, 2 and 14 mg/cm$^2$ for 4 and 24 h: 60% ZnO dispersion in caprylic capric triglyceride | - No significant penetration | Lin et al. (2011) |
| Human intact skin; tape strips (over 15 times) and occlusion; psoriasis | na | 20–60 nm | Commercial sunscreen formulation, for 2 h | No evidence of skin penetration | Filipe et al., (2009) |
| Human intact skin | Uncoated | 19 nm; >100 nm | Commercial sunscreen formulation with o/w: ~20% $^{68}$ZnO, 2 mg/cm$^2$, repeated application twice a day for 5 days | - Minimal penetration observed in both 19 and >100 nm | Gulson et al. (2010) |

biliary system, but a small fraction of NP might be cleared via urine (Baek et al. 2012; Paek et al. 2013). The biokinetics of ZnO NP were reviewed in detail by Choi and Choy (2014).

## Dose-response assessment

To calculate a reference dose (RfD) for humans, data from the 13-week oral repeated toxicity animal studies were analyzed to obtain NOAEL (Seok et al. 2013). The oral NOAEL is considered to be 268.4 mg/kg/day for rats.

$$\mathrm{RfD} = \frac{\mathrm{NOAEL}}{\mathrm{UF_A} \times \mathrm{UF_H}} = \frac{268.4\mathrm{mg/kg/day}}{10 \times 10} = 2.68\mathrm{mg/kg/day}$$

An uncertainty factor of 10 for intraspecies differences in humans ($UF_H$) and 10 for interspecies differences between animals and humans ($UF_A$) is used. Subsequently, using the NOAEL, one can calculate oral RfD for ingestion of ZnO by humans as 2.68 mg/kg/day.

## Exposure assessment

In Korea, ZnO was used in 235 domestic cosmetic products with concentrations of 0.05%–17% (KCII (The Foundation of Korea Cosmetic Industry Institute) 2012). The powder type products possess the highest number (60) of ZnO-containing cosmetics (Table 2). There was no apparent information on particle size of ZnO. Nanosized ZnO raw materials are currently commercially available (Z-Cote®, BASF SE; Nanox, Elementis; Nano TEC® 50 and Nano® 60, Grillo Zinkoxid GmbH; Finex-50, Sakai Chemical; MZ 30, Tayca; Zinc Oxide Neutral, Symrise GmbH; Zano® 10, Umicore; Z-Cote® HP1, ZnO coated with triethoxycaprylylsilane, BASF SE; Z-Cote® MAX, ZnO coated with dimethoxydiphenylsilanetriethoxycaprylylsilane cross-polymer, BASF SE; Zinc Oxide NDM, ZnO coated with dimethicone, Symrise GmbH; Zano® 10 Plus, ZnO coated with octyltriethoxysilane, Umicore) (SCCS 2012b). In addition, final cosmetic products with nanosized ZnO raw materials are also available (W/O emulsion, 8.4% uncoated ZnO, Unilever; W/O emulsion, 20% coated ZnO, Umicore; O/W emulsion, 9% coated ZnO, Umicore; O/W emulsion, 2.2% coated ZnO, Unilever; O/W emulsion, coated ZnO, Proctor & Gamble) (SCCS 2012b). Table 2 indicates that skin is the major exposure route of ZnO-based cosmetic product types. Recently, sunscreen sprays were developed and marketed (Lu et al. 2015). Humans may be exposed to ZnO NP via inhalation using sunscreen sprays. Therefore, one needs to consider inhalation route for human exposure of ZnO NP. For lipstick application, oral intake of ZnO NP cannot be ignored, although there was no cosmetic product of lipstick containing ZnO NP in Korea (KCII 2012). Thus, oral route for human exposure to ZnO NP was also considered. The mean level of cosmetics exposure to adults was obtained from the Cosmetic, Toiletry, and Fragrance Association (CTFA 2005). The maximum concentration (17%) of ZnO in cosmetics was selected as the highest value of the respective cosmetics type. In addition, the highest result (1.19%) is used for the value of absorption rate reported by Pirot et al. (1996). Our finding demonstrated that dermal SED of ZnO ranged from 0.0035 to 0.5988 mg/kg/day (Table 7). The dermal SED is calculated as following equation:

| | |
|---|---|
| SED = | A (g/day) × 1,000 mg/g × C (%)/100 × DAp (%)/100<br>60 kg |
| **SED** (mg/kg bw/day) | : Systemic exposure dosage |
| **A** (g/day) | : Amount of cosmetics daily used |
| **C** (%) | : Maximum allowed concentration of cosmetic ingredient |
| **DAp** (%) | : Dermal absorption rate of cosmetic ingredient |
| **60 kg** | : Average body weight |

Furthermore, the exposure assessment of ZnO NP used in sunscreen by routes (skin, oral, and inhalation) and types of products (cream/lotion, propellant spray type, and pump spray type) is described in Table 9(A, B, C).

## Risk characterization

The systemic exposure dose (SED) and margin of safety (MOS) for ZnO through use of cosmetic products in Koreans were estimated using NOAEL from animal studies (Table 8). Calculated MOS ranged from 448.2 to 76,685.7 (Table 8). Therefore, the estimated exposure to ZnO is considered to be safe because MOS exceeds 100. Risk assessments of ZnO in sunscreens considering product types (cream, lotion, spray, and propellant) and all routes (skin, oral, and inhalation) were also performed using

**Table 7.** Calculation of systemic exposure dose (SED) of ZnO by cosmetic products in Koreans.

| Type | N | [a] Average amount of cosmetics applied (g) | [b] Adult weight (kg) | [c] Maximum concentration (%) | [d] Dermal absorption rate (%) | SED (mg/kg/day) |
|---|---|---|---|---|---|---|
| Powder | 60 | 17.76 | 60 | 8.0 | 1.19 | 0.2818 |
| Concealer | 41 | | | 15.0 | | 0.5284 |
| Skin | 30 | | | 12.0 | | 0.4227 |
| Pact | 27 | | | 17.0 | | 0.5988 |
| Sunscreen | 25 | | | 5.0 | | 0.1761 |
| Blemish balm | 17 | | | 5.0 | | 0.1761 |
| Pack | 11 | | | 3.0 | | 0.1057 |
| Eye shadow | 9 | | | 5.0 | | 0.1761 |
| Camouflage cream | 6 | | | 0.1 | | 0.0035 |
| Foundation | 6 | | | 5.0 | | 0.1761 |
| Lotion | 2 | | | 14.0 | | 0.4931 |
| Cream | 1 | | | 2.0 | | 0.0704 |

[a]Average amount of cosmetics applied to adult in the USA (CTFA, 2005); [b]typical body weight of adult; [c]maximum concentration of ZnO in their respective cosmetic product type (KCII 2012); [d]Absorption rate (Pirot et al. 1996).

human NOAEL of 0.166 mg/kg/day (internal dose), derived from sensitive human subpopulation (ECB 2004; SCCS 2012b) (Table 9). In this assessment, the safety limit of MOS 1 may be applied because of the use of human NOAEL. MOS values of dermal exposure were estimated to be 7.37 for cream/lotion type and 8.64 for spray types, respectively.

**Table 8.** Estimation of the margin of safety (MOS) of ZnO through the use of cosmetic products in Koreans.

| Type | SED (mg/kg/day) | a NOAEL (mg/kg) | MOS |
|---|---|---|---|
| Powder | 0.2818 | 268.4 | 952.4 |
| Concealer | 0.5284 | | 507.9 |
| Skin | 0.4227 | | 635.0 |
| Pact | 0.5988 | | 448.2 |
| Sunscreen | 0.1761 | | 1,524.1 |
| Blemish balm | 0.1761 | | 1,524.1 |
| Pack | 0.1057 | | 2,539.3 |
| Eye shadow | 0.1761 | | 1,524.1 |
| Camouflage cream | 0.0035 | | 76,685.7 |
| Foundation | 0.1761 | | 1,524.1 |
| Lotion | 0.4931 | | 544.3 |
| Cream | 0.0704 | | 3,812.5 |

[a]NOAEL from Seok et al. (2013)
NOAEL, no observed adverse effect level; SED, systemic exposure dose.

MOS value of inhalation exposure was estimated to be 12.87 for propellant spray type. MOS values of oral and inhalation exposure were 2.54 for propellant spray type and 2.42 for pump spray type, respectively (Table 9 A, B, C). Oral MOS value of oral exposure was also estimated to be 3.32 for lip application via sunscreens. Based on the formulation types, MOS values were estimated to be 2.29 for cream/lotion, 1.2 for pump spray, and 1.13 for propellant spray, respectively. All equations and parameters for MOS calculation are presented in detail in Table 9.

## Summary and conclusion

Zinc oxide is a versatile compound that has been utilized in many applications, including cosmetic products as an effective physical UV blocker (Djurisic and Leung 2006). ZnO NP exert the broadest UV protection of all active ingredients currently available in commercial sunscreens (Pinnell et al. 2000). Therefore, it is natural that many cosmetic products contain physical UV blockers such as ZnO or TiO2. However, little is known regarding the pharmacokinetics and tissue distribution of ZnO NP in biological systems. Currently, ZnO NP have not been comprehensively assessed with respect to potential adverse effects on human health from exposure to commercial cosmetic products. For that reason, safety evaluations of ZnO have been addressed based on data available. Generally, it is reported that chronic oral exposure of ZnO NP did not produce noticeable apparent toxic responses in animals (Clayton and Clayton 198182; Straube, Schuster, and Sinclair 1980). In addition, topically administered ZnO produced mild irritation in animal experiments (Lansdown 1991). However, there was some evidence of reproductive or developmental toxic effects of ZnO (Bleavins et al. 1983; Ketcheson, Barron, and Cox 1969; Pal &Pal 1987; Samanta and Pal 1986; Schlicker and Cox 1968). ZnO NP were also shown to be genotoxic in *in vitro* and *in vivo* systems (Dufour et al. 2006; Gerloff et al. 2009; Osman et al. 2010; Sharma et al. 2009, 2012).

**Table 9.** Calculation of the systemic exposure dose (SED) and margin of safety (MOS) for ZnO in sunscreens.

| Parameters | | | |
|---|---|---|---|
| Parameters for skin exposure calculation | | Values | Ref. |
| Amount (cream/lotion type applied) | Ac | 18,000 mg | MFDS, (2013); SCCS, (2012a) |
| Amount (spray type applied)[a] | Ass | 15,300 mg | Bremmer, Pruˇıd Homme De Lodder, and Van Engelen (2006a); Rothe et al. (2011) |
| Concentration of ZnO[b] | C | 25% | MFDS (2014) |
| Absorption through the skin | ABSs | 0.03% | SCCS (2012b) |
| Body weight | BW | 60 kg | |
| NOAEL | NOAEL (oral, human sensitive subpopulation) | 0.166 mg/kg/day (internal dose) | ECB (2004); SCCS (2012b) |
| **SED and MOS through the skin** | | | |
| Skin exposure and MOS (Cream/lotion type) | SEDsc = (Ac × C × ABSs/BW) | 0.0225 mg/kg/day | |
| | MOSsc = (NOAEL/SEDsc) | 7.37[g] | |
| Skin exposure and MOS (spray type) | SEDss = (Ass × C × ABSs/BW) | 0.0192 | |
| | MOSss (NOAEL/SEDss) | 8.64 | |
| **Parameters for exposure calculation through inhalation** | | | |
| Inhaled amount: spray type[100] | As | 18,000 mg | |
| Concentration of ZnO[b] | C | 25% | MFDS (2014) |
| Airborne fraction[500] | AF | 15% | Bremmer, Pruˇıd Homme De Lodder, and Van Engelen (2006a); Rothe et al. (2011) |
| Substance amount for relevant exposure | EA (As × C × AF) | 675 mg | |
| Distribution volume at exposure time (t1) | V1 | 2,000 L | Bremmer, Pruˇıd Homme De Lodder, and Van Engelen (2006b); Rothe et al. (2011) |
| Distribution volume at exposure time (t2) | V2 | 10,000 L | |
| Inhalation rate[e] | IR | 10.9 L/min | Jang et al. (2007) |
| Exposure time 1 | t1 | 2 min | Bremmer, Pruˇıd Homme De Lodder, and Van Engelen (2006b); Rothe et al. (2011) |
| Exposure time 2 | t2 | 18 min | |
| Potential amount that may be inhaled during the first 2 min | IA1 = {[(EA/V1)] × IR × t1} | 7.3575 | |
| Potential amount that may be inhaled during the subsequent 18 min | IA2 = [(EA/V2)] × IR × t2 | 13.2435 | |
| Substance exchange[f] | G | 0.75 | ECB, (2003) |
| Fraction reaching alveoli, fraction <10 μm | RFpps (respirable fraction) (propellant spray type) | 5% | CIR, (2012) |
| Fraction not reaching alveoli, fraction >10 μm | NRFpps (propellant spray type) | 95% | |
| Fraction not reaching alveoli, fraction >10 μm | NRFps (pump spray type) | 100% | Rothe et al. (2011) |
| Absorption through the lung | ABSl | 100% | ECB (2004) |
| Absorption through the oral | ABSo | 20% | |
| Body weight | BW | 60 kg | |
| NOAEL | NOAEL: oral, human sensitive subpopulation | 0.166 mg/kg/day (internal dose) | ECB (2004); SCCS (2012b) |
| **SED and MOS through inhalation** | | | |
| Inhalation exposure and MOS via sunscreen (propellant spray type) | SEDi = [(IA1+IA2) × G × RF × ABSl/BW] | 0.0129 mg/kg/day | |
| | MOSi = (NOAEL/SEDi) | 12.87 | |
| Oral exposure and MOS through inhalation via sunscreen (propellant spray type) | SEDopps = (IA1 + IA2) × NRFpps × ABSo/BW | 0.0653 mg/kg/day | |
| | MOSop = (NOAEL/SEDopps) | 2.54 | |

(*Continued*)

**Table 9.** (Continued).

| Parameters for skin exposure calculation | | Values | Ref. |
|---|---|---|---|
| Oral exposure and MOS through inhalation via sunscreen (pump spray type) | SEDops = [(IA1+IA2) × NRFps × ABSo/BW] | 0.0687 mg/kg/day | |
| | MOSops = (NOAEL/SEDops) | 2.42 | |
| **Parameters for oral exposure calculation through lips application** | | | |
| Amount of sunscreen applied on lips | AI | 60 mg | MFDS (2013); SCCS (2012a) |
| Maximum concentration of ZnO[b] | C | 25% | MFDS (2014) |
| Absorption through the oral | ABSo | 20% | ECB (2004) |
| Body weight | BW | 60 kg | |
| NOAEL | NOAEL (oral, human sensitive subpopulation) | 0.166 mg/kg/day (internal dose) | ECB (2004); SCCS (2012b) |
| **SED and MOS through the oral (lips application)** | | | |
| Oral exposure and MOS via sunscreen (lips application) | SEDo = (AI × C × ABSo/BW) | 0.05 mg/kg/day | |
| | MOSo = (NOAEL/SEDo) | 3.32 | |
| **Exposure via sunscreen product types** | | | |
| Cream/lotion type | SEDc = (SEDsc + SEDo) | 0.0725 mg/kg/day | |
| Pump spray type | SEDps = (SEDo + SEDss + SEDops) | 0.1379 mg/kg/day | |
| Propellant spray type | SEDpps = SEDo + SEDss + SEDopps + SEDi | 0.1474 mg/kg/day | |
| **MOS via sunscreen product types** | | | |
| Cream/lotion type | MOSc = (NOAEL/SEDc) | 2.29[h] | |
| Pump spray type | MOSps = (NOAEL/SEDps) | 1.20[h] | |
| Propellant spray type | MOSpps = (NOAEL/SEDpps) | 1.13[h] | |

[a]Sprayed amount of sunscreen spray was assumed to be 18 g/day (equal to sunscreen lotion), and amount of sunscreen applied to the skin was assumed to be 85% of sprayed amount (18 g).
[b]Cosmetic regulation on ZnO, maximum concentration in Korea.
[c]Sprayed amount of sunscreen spray was assumed to be 18 g/day (equal to sunscreen lotion).
[d]Airborne fraction was assumed to be 15% of the sprayed amount.
[e]Mean inhalation rate of Korean male.
[f]25% of the air and airborne particles was exhaled by the lung without substance retention.
[g]SCCS risk assessment on ZnO nanoparticles in sunscreen results in a MOS of 7.4, which was rounded off to one decimal place (SCCS 2012b).
[h]Minimal MOS = 1.

Further, phototoxicity, neurotoxicity, and immunotoxiciy were attributed to ZnO NP; however, there was no apparent evidence of carcinogenicity.

ZnO NP lack dermal penetration potential in human skin (Lin et al. 2011; Zvyagin et al. 2008). Since NP exhibit different properties in various conditions, it is essential to understand how size and particle nature of ZnO NP affect their pharmacokinetics *in vivo*. Dose-response assessments revealed that NOAEL, RfD, and SED for ZnO NP are estimated to be 268.4, 2.68, and a maximum of 0.5988 mg/kg/day, respectively, based upon recent reports (KCII 2012; Seok et al. 2013). The highest SED of ZnO is expected to be at most 0.5988 mg/kg/day of ZnO NP, safe exposure level, in the use of all types of cosmetic products in Koreans.

Risk characterization of ZnO demonstrated that the lowest MOS of 448.2 obtained in this study is clearly within the safe limits of MOS = 100, when oral NOAEL of 268.4 mg/kg/day was used (Table 8). It was not possible to calculate accurate MOS from dermal exposed ZnO in cosmetics. When functions of gut and skin are considered regarding absorption, it was possible to calculate an oral NOAEL for ZnO. Risk assessments with respect to other routes (oral and inhalation) and product types (cream, lotion, spray, and propellant) noted that MOS values were all greater than 1. Based upon human internal exposure dose of 0.166 mg/kg/day (Table 9) evidence indicates that the use of ZnO NP in sunscreen may not pose any significant threat to consumers, although SCCS (Scientific Committee on Consumer Safety) was concerned with lung inflammation induced by ZnO NP (ECB 2004; SCCS 2012b). However, when one takes into account inhalation of overspray sunscreen aerosols with other ingredients and potential impact on children and other susceptible subpopulations, exposure of ZnO via inhalation of cosmetic products may be of concern for the health of children and other susceptible subpopulations.

Hence, based on the risk assessment of ZnO, this agent is not considered a threat to consumer's health, and it may be safely used in cosmetic products under current regulations, although there are some toxicities. However, it should be noted that UV co-exposure to ZnO producing synergistic toxicity might be a factor to be considered for the comprehensive risk assessment of ZnO, which requires further investigation.

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