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Titanium Dioxide and Zinc Oxide Nanoparticles in Sunscreens: A Review of Toxicological Data

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Synopsis

The positive effects of sunlight have been known for many years, and the negative ones, too. Sunscreens are physical and chemical UV absorbers. Nanotechnology has developed nanoparticles of physical blockers: titanium dioxide (TiO_2) and zinc oxide (ZnO). Their smaller diameter and increased bioreactivity are the focus of many toxicological studies. The usage of sunscreens has increased around the world, so all toxicological aspects should be carefully considered. There are *in vitro* and *in vivo* studies: studies on animal and human skin; investigations of potential genotoxicity and cytotoxicity; generation of reactive oxygen species; penetration; skin irritation; acute, subchronic, and chronic toxicity; and carcinogenesis. The experimental conditions of these studies differ from study to study, but most authors agree that there is no penetration of nanoparticles into viable skin layers. Risk-benefit analysis of TiO_2 and ZnO nanoparticles (NPs) usage in sunscreens strongly indicates that potential risks are vastly outweighed over the benefits. Because of the results of some authors indicating possible penetration through damaged skin, further studies should be conducted, primarily addressed on skin penetration mechanisms.

INTRODUCTION

UV radiation can cause harmful effects on the skin. UVC, and partly UVB, can be absorbed by molecular oxygen to produce ozone. Stratospheric ozone absorbs UV rays below 290 nm, but UVB and UVA rays reach the human skin and cause metabolic and biological reactions (1).

Although many patients and physicians believe that regular use of sunscreens provides protection from skin cancer, this protective effect has been confirmed only in the cases of squamous cell carcinoma and actinic keratoses, but for basal cell carcinoma and malignant melanoma, the results are inconclusive (2–4).

In the United States, skin cancer is the most common form of cancer. Annually, more than one million cases are diagnosed in the form of squamous cell and basal cell carcinoma, both associated with UV radiation. The incidence of melanoma is rising significantly.

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More people are diagnosed with skin cancer each year in the United States than all other cancers combined (5,6).

SUNSCREENS

The negative effects of sunlight, sunburns, photoaging, and skin cancers are reduced by the use of sunscreens. Sunscreens should provide protection against the adverse effects of both UVB and UVA radiation. Compounds that have the ability to protect from UV radiation are classified into two groups: organic and inorganic blockers. Minerals such as zinc oxide (ZnO) and titanium dioxide (TiO_2) are often used as inorganic physical sun blockers.

TiO_2 and ZnO have been used as ingredients for sunscreen formulations for more than 20 years. Their mechanism of actions involves absorption, reflection, and reflecting and the scattering of UV sunlight (7).

The disadvantage of microsized ZnO and TiO_2 particles is their poor dispersive properties, resulting in a white color that is not cosmetically appealing (8).

NANOPARTICLES

The rapid development of nanotechnology has resulted in an increasing number of nanomaterial-based products. Because of their physicochemical properties, nanomaterials have found an important role in cosmetics. When particles become smaller than 100 nm (the optimal light scattering size), visible light is transmitted across the particles. This avoids the cosmetically undesired opaqueness of inorganic sunscreens and makes the application of cosmetic products based on nanoparticles (NPs) commercially attractive, without reducing UV-blocking effectiveness (9).

Possible adverse effects have been considered. Because the surface area to volume ratio of particles increases as the particle diameter decreases, NPs may be more (bio) reactive than normal bulk materials. This is the reason why safety of cosmetic products containing NPs has been frequently discussed. Many scientists and research institutes mainly focus on various kinds of toxicological and skin penetration studies. However, safety also concerns the physicochemical properties of sunscreen ingredients to be taken up by the skin in both the absence and presence of light.

With the widespread use and the potential for TiO_2 or ZnO NPs exposure, concerns have focused on their possible resorption.

COSMETIC REGULATION

International Cooperation on Cosmetic Regulation defines a nanomaterial in cosmetics as an insoluble intentionally manufactured ingredient with one or more dimensions ranging from 1 to 100 nm in the final formulation. In addition, the nanomaterial must be sufficiently stable and persistent in biological media to disable potential interactions with biosystems (10).

In 2012, the International Organization for Standardization underlined that the physicochemical characterization of nanomaterials was critical for the identification of test materials before toxicological assessment (11).

Important physicochemical parameters for their characterization are particle composition, size/particle size distribution, surface charge, solubility/dispersibility, aggregation/agglomeration state, shape, surface area, and surface chemistry (12).

INSTRUMENTAL METHODS FOR THE CHARACTERIZATION OF TiO_2 AND ZnO NPs

Sunscreen preparations with micronized TiO_2 and/or ZnO are complex and opaque, so NPs detection and characterization are complicated. Tyner has evaluated the ability of 20 analytical methods to detect TiO_2 and ZnO NPs in unmodified commercial sunscreens (13). Variable-pressure scanning electron microscopy, laser scanning confocal microscopy, X-ray diffraction, and atomic force microscopy were considered applicable and complimentary for NPs characterization in sunscreens. Guidelines on the safety assessment of nanomaterials in cosmetics from the Scientific Committee on Consumer Safety suggested the use of at least two methods, of which one should be electron microscopy, preferably high-resolution transmission electron microscopy, to determine the size of nanomaterial particles (14).

TOXICOLOGICAL CONCERNS

There are conclusive results that titanium can cause lung cancer after inhalation, so it is the reason for increased concerns about potential toxicity after dermal applications. Titanium is classified into group 2B of carcinogens. Organized, accurate, and detailed studies must be conducted to give information about dermal permeation, local effects, and eventually generalized effects. It is necessary because sunscreens are applied to the skin in some countries during the whole year, in large amounts, on almost the whole skin area.

DERMAL PENETRATION OF ZnO AND TiO_2

There are few parameters that must be considered during the research of dermal permeation of substance. One of them is the characteristics of the studied substance. Theoretically, only those materials with an adequate log P coefficient (octanol/water partition coefficient) and low molecular weight (<ca. 500) can penetrate the intact human skin through the stratum corneum (SC).

The second parameter is the skin itself. The SC represents the outermost layer of the skin and plays an important role in protecting the human organism against penetration by xenobiotics. It should be emphasized that although cosmetics and sunscreens containing ZnO and TiO_2 are normally used on healthy skin, injuries to the skin can occur under certain circumstances (physical force or sunburn), which can cause enhancement of resorption. This is the reason why skin penetration studies of TiO_2 and ZnO particles are usually investigated *in vivo* and *in vitro* with both intact skin and stripped skin which mimics an injured skin (15).

The ingredients of sunscreen formulation must also be known. The biopharmaceutical characteristics are not the same comparing O/W emulsions, W/O emulsions, silicone-based emulsions, or aerosol sprays. Researchers investigating dermal absorption should underline all properties and circumstances of the experiment.

ABSORPTION OF TiO_2 AND ZnO—*IN VITRO* STUDIES

Many authors reported the penetration of NPs from sunscreens onto the human skin. Human epidermal penetration of a transparent TiO_2 and ZnO sunscreen formulation was determined using Franz-type diffusion cells and electron microscopy to verify the location of NPs in exposed membranes. In most studies, no particles could be detected in the lower layers of SC or viable epidermis by electron microscopy, suggesting that minimal nanoparticle penetration occurs through the human epidermis. Thus, some researchers have concluded that NPs penetrate to 13 layers into the UVB-damaged SC, whereas only seven layers in the intact skin. The experiment conditions and effects are shown in Table I.

ABSORPTION OF TiO_2 AND ZnO—ANIMAL SKIN

Gamer et al. reported the dermal absorption of ZnO and TiO_2 particles through the skin of domestic pigs. In addition, the results show that microfine ZnO particles were not able to penetrate the porcine-dermatomed skin preparations (20).

Wu et al. (21) and Adachi et al. (22) have concluded that after prolonged application, NPs can penetrate through the SC and they can be located in the deep layer of the epidermis. Wu et al. investigated the penetration and toxicity of TiO_2 NPs after *in vivo* animal (BALB/c hairless mice) dermal application. After 60 d of dermal exposure in hairless mice, TiO_2 NPs not only penetrated the skin but also reached different tissues and induced pathological lesions in several major organs. In addition, they found TiO_2 NPs in the mouse brain without inducing any pathological changes (21).

Recently, Adachi et al. (23) found signs of irritant dermatitis with focal parakeratosis in the SC and epidermal spongiosis after applying uncoated TiO_2 NPs for a long time.

The experiment conditions and results of other researchers are shown in Table II.

Table I
Absorption of TiO_2 and ZnO *in vitro*

Type of NPs	Formulation	Experimental system	Effects	Ref.
ZnO NPs	Transparent formulation	Franz-type diffusion cells	No particles could be detected in the lower SC	(16)
	w/o emulsion	Franz-type diffusion cells on the excised porcine skin	No particles could be detected in the lower SC	(17)
TiO_2 NPs	Suspensions (1.0 g/L), 24 h	Franz cells using intact and needle-abraded human skin	Not detectable in receiving solutions for both intact and damaged skin	(18)
TiO_2 and ZnO NPs	1) 10% coated TiO_2 in w/o, 2) 10% coated TiO_2 o/w, 3) 5% coated ZnO in o/w, and 4) 5% uncoated ZnO in o/w	Skin in flow-through diffusion cells	1) TiO_2 in w/o penetrated deeper in UVB-damaged SC; 2) penetrated 13 layers into UVB-damaged SC, whereas only seven layers in normal; and 3) and 4) were localized to the upper one to two SC layers	(19)

ABSORPTION OF TiO_2 AND ZnO—INTACT AND DAMAGED HUMAN SKIN

Many studies showed that there was no penetration of TiO_2 and ZnO NPs. Also, they have shown that particle shape and formulation have no significant impact on penetration through the SC in *in vivo* studies. The experiment conditions and results are shown in Table III.

Gulson et al. have conducted the study to detect possible amounts of ZnO in blood and urine. The experiment was assessed under real-life conditions. Sunscreen was applied to the skin of volunteers for 5 d. Blood and urine levels of ^{68}Zn from ^{68}ZnO particles in sunscreens increased in all subjects over the period of exposure, with significantly higher levels of ^{68}Zn in females exposed to a sunscreen containing NPs of ^{68}ZnO than in females exposed to larger ^{68}ZnO particles and males exposed to particles of both sizes (27).

Tan et al. (28) have researched if there is a difference in resorption of NPs in elderly patients. There was no significant difference in the level of TiO_2 in dermis compared with the control group.

Bennat et al. found that TiO_2 NPs are able to penetrate through hair follicles or pores, but no closer information is given on the fate of those particles. This result can show the importance of permeation through hair follicles (29).

Zvyagin et al. investigated the distribution of topically applied ZnO on excised human skin. The lack of penetration of these NPs suggests that safety concerns are not objective and evidence based (26).

Presently, only a few studies have been conducted with TiO_2 or ZnO NPs applied to the damaged skin and on what normally happens when sunscreens are reapplied to sunburned skin. UVB-damaged skin slightly enhanced TiO_2 NPs or ZnO NPs penetration in sunscreen formulations, but no transdermal absorption was detected (19).

Table II
Dermal Absorption of TiO_2 and ZnO NPs; Animal Skin

Type of NPs	Formulation	Skin type	Effect	Ref.
TiO_2 and ZnO NPs	Sunscreen formulation 25 µL	UVB sunburned skin pigs	Slightly enhanced penetration, but no detection of transdermal resorption	(19)
TiO_2, ZnO NP	o/w emulsion 24 and 48 h	Normal and UVB-sunburned skin pigs	UVB-damaged skin slightly enhanced penetration, but no transdermal absorption was detected	(19)
Ultrafine TiO_2	10% W/O emulsion	Hairless rat skin	Neither penetrate viable cell layers nor biologically cause any cellular changes	(22)
Ultrafine TiO_2	10% W/O emulsion 2, 4, 8 week	Dorsal skin of hairless Wistar Yagi rats	No evidence of penetration after the subchronic exposure	(21)
Micronized TiO_2		Human skin transplanted to immuno-deficient mice	Do not penetrate through the intact epidermal barrier	(23)

Table III
Absorption of TiO_2 and ZnO on Human Skin *in vivo*

Type of NPs	Formulation	Skin type	Effect	Ref.
TiO_2 (20 nm)	w/o emulsion, 5 h	Human skin	No penetration	(24)
Micronized TiO_2, hydrophobic 100 nm, amphiphilic 10–15 nm, and hydrophilic 20 nm	w/o emulsion, 6 h	Human forearms	Particle shape and formulation nonsignificant impact on penetration	(25)
Micronized TiO_2 (10–50 nm)	w/o emulsion	Older patients' skin, 59–85 years and 9–31 d	The insignificant level in dermis higher than in control	(25)
ZnO (26–30 nm)	w/o emulsion,	Human skin	Remained in the SC	(26)
^{68}ZnO (19 nm)	w/o emulsion, 5 d	Human skin	Small increases ^{68}Zn in blood and urine	(27)
TiO_2 (20 nm)	w/o emulsion and aqueous suspension	Hairy skin	May be able to penetrate through hair follicles and pores	(28)
TiO_2, ZnO	w/o emulsion	Excited human skin	Remained on the surface	(29)
TiO_2, ZnO NP	w/o emulsion	Mechanically, physically, and chemically damaged skin	Very small particles to cross to the SC increases relative to control	(29)
TiO_2 NP	w/o emulsion	Healthy and psoriatic skin	Deeply penetrated to psoriatic relative to the normal skin	(30)

Pincheiro et al. have investigated the difference in resorption of ZnO through healthy and psoriatic skin. In psoriatic skin, the fragility of the SC seemed to facilitate the penetration of the NPs, although they did not reach living cell layers. However, the desquamation of the SC hindered the adequate distribution of the cream along the skin surface (30).

SKIN IRRITATION BY TOPICAL APPLICATION OF ZNO AND TIO_2 AND STUDIES OF ACUTE TOXICITY

Effects of ZnO NPs and TiO_2 NPs, and their mixtures on skin corrosion and irritation were investigated by using *in vitro* 3D human skin models (KeraSkin™), and the results were compared with those of an *in vivo* animal test. The results provide the evidence that ZnO NPs and TiO_2 NPs and their mixture are "nonirritant" and "noncorrosive" to the human skin by a globally harmonized classification system. *In vivo* test using animals may be replaced by an alternative *in vitro* test (31).

The potential effects of photosensitization and photo irritation of ZnO on the human skin were also discussed. There was no evidence of any positive findings in two photo irritation studies and two photosensitization studies after topical application on the intact human skin. Furthermore, in a review of photoprotection, Lautenschlager et al. reported that neither TiO_2 nor ZnO NPs possess skin irritation or sensitization properties when used in sunscreens on humans (32).

Using acute dermal irritation studies in rabbits and local lymph node assay in mice (CBA/JHsd), Warheit et al. concluded that water solution of TiO_2 NPs used in different

concentrations from 0% to 100% applied for three consecutive days were not a dermal sensitizer or skin irritant (33).

In a 14-d toxicity study, TiO_2 NPs applied topically to rat skin (Wistar) induced short-term toxicity at the biochemical level (34). Enzymes for which concentrations increased are lactate dehydrogenase, lipid peroxidase, serum glutamic pyruvic transaminase, and serum glutamic oxaloacetic transaminase. Depletion in the levels of catalase and glutathione S-transferase (GST) activity was detected. They concluded that short-term exposure to TiO_2 NPs can cause hepatic and renal toxicity in rats. It should be underlined that the doses used in these studies are high (14, 28, 42, and 56 mg/kg) and humans are not exposed to those high concentrations (35).

INFLUENCE OF TiO_2 AND ZnO ON ROS GENERATION AND POTENTIAL CYTOTOXICITY

Results of the recent studies provided the information that both ZnO and TiO_2 NPs can generate reactive oxygen species (ROS): superoxide anions, hydroxyl radicals, and singlet oxygen (36,37). The mechanism of the reaction is UV-induced photocatalysis. ROS can damage cellular components and macromolecules, and ultimately cause cell death if produced in excess or if they are not neutralized by antioxidant defenses. ROS derived from the photocatalysis of NPs are cytotoxic to a variety of cell types (38).

Sayes et al. have investigated the difference between two crystal forms of TiO_2 NPs in producing ROS. They reported that anatase NPs generated more ROS than rutile after UV irradiation. It has been concluded that TiO_2 anatase has a greater toxic potential than TiO_2 rutile. Also, anatase ROS production does not occur under ambient light conditions (39).

A study by Lewicka et al. (40) reported a greater generation of ROS by ZnO NPs than TiO_2 NPs.

The cytotoxicity of TiO_2 NPs was demonstrated in keratinocytes, using different tests and exposures, with or without UV exposure, but many *in vivo* experiments on animals did not confirm this effect (41–43).

Cytotoxicity studies on HaCaT cells gave an important result that TiO_2 NPs induce cytotoxic effects only at very high concentrations after 7 d (44).

In vitro toxicity was also observed. Vinaredell et al. used the EpiSkin model, to determine the differences between ZnO and ZnO NPs. Formulations with ZnO and ZnO NPs were first applied for 15 min and for 24 h, but cytotoxic effects were not observed. The percentage of viability of the treated cells was around 100% for all ZnO materials, regardless of their size (45).

Kiss et al. investigated *in vivo* penetration and effects on cell viability of TiO_2 on human skin transplanted to immunodeficient mice. They demonstrated that with TiO_2 NPs, there was no penetration through the skin, but when exposed directly to cell culture *in vitro*, they have significant effects on cell viability (23).

Liu et al. have conducted an important study. During the PC12 cells treatment with different concentrations of TiO_2 NPs, the viability of cells was significantly decreased in the periods of 6, 12, 24, and 48 h, showing a significant dose effect and time-dependent manner. The number of apoptotic PC12 cell increased with the increasing concentration of TiO_2 NPs (35) (Table IV).

GENOTOXICITY

TiO_2 and ZnO NPs were investigated for their potential genotoxicity in *in vitro* and *in vivo* test systems. No genotoxicity was observed *in vitro* (Ames' Salmonella gene mutation test and V79 micronucleus chromosome mutation test) or *in vivo* (mouse bone marrow micronucleus test and Comet DNA damage assay in lung cells from rats exposed by inhalation) (46).

The SCCS (2012) comprehensive review of ZnO NPs revised both *in vitro* and *in vivo* studies on photo-mutagenicity/genotoxicity and concluded that there is no definite evidence to claim if ZnO NPs pose a mutagenic/genotoxic, phototoxic, or photomutagenic/genotoxic risk to humans (47).

CARCINOGENESIS

There are reports that dermal application of noncoated rutile TiO_2 does not exhibit a promoting effect on UVB-induced skin carcinogenesis in rats. Xu et al. researched c-Ha-ras proto-oncogene transgenic rats, which are sensitive to skin carcinogenesis, and their wild-type siblings were exposed to UVB radiation. Their back skin is shaved twice weekly for 10 weeks. On the shaved area, a suspension of 100 mg/ml TiO_2 NPs was applied. In the observed groups, the tumor incidence was not different (48).

Sagawa et al. have reached the same conclusion, after studying the promoting effect of silicone-coated TiO_2 NPs suspended in silicone oil and noncoated TiO_2 NPs suspended in Pentalan 408 on a two-stage skin chemical carcinogenesis model (49).

Newman et al. also suggested that TiO_2 NPs are not carcinogenic to the skin. However, the authors emphasized that further studies for the safety evaluation of the TiO_2 NPs in sunscreens must be performed to simulate real-world conditions, particularly in sunburned skin and under UV exposure (50).

Table IV
Cytotoxicity of TiO_2 NPs *in vivo*

Type of NPs	Cells	Effect	Ref.
TiO_2 NPs < 20 nm	Human HaCaT and keratinocytes	Induction of the mitochondrial "common deletion" in HaCaT cells following exposure to TiO_2 NPs, which strongly suggests a ROS-mediated cytotoxic and genotoxic potential of NPs.	(41)
TiO_2, 25 nm dispersion in serum-free medium	Immortalized keratinocyte cells and HaCaT cells	Increase production of ROS, the toxicological effects can be simplified into six events	(43)
TiO_2 NPs	Keratinocyte cells	Alter the calcium homeostasis and induced a decrease in cell proliferation associated with early keratinocyte differentiation, without any indication of cell death.	(42)
TiO_2 NPs (anatase, rutile, and anatase–rutile) sizes (4, 10, 21, 25, and 60 nm) UVA radiation	Human keratinocyte and HaCaT cells	Induced ROS resulted in oxidative stress in these cells by reducing SOD and increasing MDA levels and damage HaCaT cells	(44)

The lack of penetration through the epidermis is considered as the main reason for the absence of skin carcinogenesis–promoting effects.

INTERACTIONS OF SUNSCREENS WITH OTHER SUBSTANCES

Sunscreens are not only dermal preparations applied on the skin. Many cosmetic preparations, dermocosmeceutic, and dermal preparations are applied every day. It is important to investigate potential interactions between sunscreens containing NPs, to find out whether sunscreens enhance or block resorption. Effects of drugs applied to the skin for many diseases can be modulated and disturbed. Peire et al. have researched interactions with amphotericin. TiO_2 NPs can modulate the transdermal permeation of the amphotericin. The main reason is the superficial chemistry of TiO_2 (51).

ROS generated by TiO_2 and ZnO NPs can increase skin permeability. Transdermal drug and other substances (dyes, pesticides, and toxins) penetration can be favored or reduced by modulating the TiO_2 surface charge (coating) and its oxidative potential (crystalline phases), so the enhancer effect of TiO_2 NPs can be adjusted and converted up or downward (52,53).

People are encouraged to use sunscreens when they are exposed to sunlight, and it is often on fields where pesticides are applied. The dermal penetration of the herbicide 2,4-dicholorophenoxyacetic acid (2,4-D) is enhanced by the formulations containing chemical UV absorbers, the absorbers themselves, and the insect repellent DEET. Brand et al. investigated whether commercially available sunscreens containing TiO_2 or ZnO enhance the transdermal absorption of pesticides. For *in vitro* studies, hairless mouse skin was used. *In vitro* permeability studies were performed with the pesticides: parathion, malathion, 2,4-D, and paraquat. The data demonstrate that there was significant penetration enhancement of malathion, parathion, and paraquat when compared with controls. The difference between ZnO and TiO_2 was noticed because ZnO can interfere with 2,4-D penetration and TiO_2 had no effect. Although, the risk-benefit analysis gives the recommendation for using sunscreens (54).

CONCLUSION

There are conclusive pieces of evidence that TiO_2 and ZnO NPs do not penetrate through intact and healthy human skin, but further studies are necessary to confirm their penetration through damaged and sensitive skin. Of paramount importance is the finding that most studies do not demonstrate NPs' skin penetration and that no significant concentrations are found in layers of viable cells. It must be emphasized that cytotoxic and pathological outcomes are presented in studies using high concentrations of NPs, which are impossible to be used for human purpose.

Results of *in vivo–in vitro* and human–animal studies should be cautiously extrapolated.

Some studies have given conclusive pieces of evidence about the potential of NPs to induce ROS *in vitro*, which largely mediate NP-induced cytotoxicity and genotoxicity, but the important real-situation information is that NPs used in sunscreens have modified the surface so it has less possibility to produce ROS, even after UV exposure. Sunscreens also contain antioxidants to neutralize generated ROS, and endogenous antioxidants can

protect against oxidative stress. These are important factors for the prevention of possible harmful effects of ROS.

Risk-benefit analysis of TiO_2 or ZnO NPs usage in sunscreens strongly indicates that potential risks are vastly outweighed by the benefits. Sunscreens afford protection against UV-induced skin damages, such as photoaging and, importantly, skin cancer.

Possibilities of artificial intelligence, especially machine learning, as one of the most powerful technique, must be applied in further studies in the prediction of the mechanism of penetration and toxicity.

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