Australian Government
Department of Health
Therapeutic Goods Administration

Literature Review on the safety of titanium dioxide and zinc oxide nanoparticles in sunscreens

Scientific review report

Version 1.1, August 2016

TGA Health Safety Regulation

Contents

Summary

This scientific review report is limited to the review of safety concerns surrounding zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles (NPs) present in sunscreens. The two main issues considered in this review are the evidence for the ability of these NPs to penetrate the skin to reach viable cells and the potential toxicity exerted by them.

The TGA has been continuously monitoring the emerging scientific literature in this area and working cooperatively with international regulatory agencies to ensure that appropriate regulatory action is undertaken if any unacceptable risk of harm/toxicity is identified.

A review on these issues was first published by the Therapeutic Goods Administration (TGA) in 2006 which was updated in May 2013. This review is a further update to include relevant literature that has been published between May 2013 and August 2016.

The majority of *in vitro* studies (using both animal and human skin) and *in vivo* studies have shown that both ZnO and TiO_2 NPs either do not penetrate or minimally penetrate the *stratum corneum* and underlying layers of skin. This suggests that systemic absorption, hence toxicity, is highly unlikely.

In conclusion, on current evidence, neither TiO_2 nor ZnO NPs are likely to cause harm when used as ingredients in sunscreens and when sunscreens are used as directed.

1 Introduction

The inorganic UV filters ZnO and TiO_2 have been used as ingredients in sunscreens for over three decades. However, one apparent disadvantage of ZnO and TiO_2 is that in their macroparticulate (bulk) form in sunscreens, they are visible on the skin as an opaque layer resulting in reluctance of consumers to use the products. This undesirable visual effect has been addressed by decreasing the particle size of these metal oxides to nanoparticle (NP) form (see Section 2). When used in this NP form, these oxides cannot be seen on the skin but retain or even augment their UV-sunscreening properties.

In the USA, patents on TiO_2 and ZnO NPs were filed in the 1980s (Wang & Tooley 2011), although in Australia the use of TiO_2 and ZnO NPs in sunscreens began later. These NPs are particularly useful in sunscreens because of their intrinsic ability to filter ultraviolet (UV)A as well as UVB wave length spectra, thus providing broader protection than any other sunscreening agent.

The TGA has been continuously monitoring the emerging scientific literature in this area and working cooperatively with international regulatory agencies to ensure that appropriate regulatory action is undertaken if any unacceptable risk of harm/toxicity is identified. A review on these issues was first published by the TGA in 2006, which was updated in 2009 and again in 2013.

This scientific review report is limited to the review of safety concerns surrounding ZnO and TiO_2 NPs present in sunscreens. The two main issues considered in this review are the evidence for ability of these NPs to penetrate the skin to reach viable cells and the potential toxicity induced by them.

> *(Nanoparticle OR nanoparticles OR nanoparticulate OR nanoscale OR nanosize OR nanomaterials) AND (zinc oxide OR titanium dioxide) AND (sunscreen OR sunblock OR sun block OR sun screen OR UV blockers OR physical sunscreen) AND (safety OR toxicology OR toxic OR safe)*

2. Nanoparticle characteristics

For the purpose of this report, the definition of TiO_2 and ZnO NPs includes materials within the nanosize range of 1 to 100 nm. Nanosized TiO_2 and ZnO exist in three separate states: primary particles (5-20 nm), aggregates (30-150 nm) and agglomerates (1-100 microns). Primary particles cluster together to form aggregates and are the smallest units present in a final sunscreen formulation (Butler *et al*., 2012; SCCP, 2007; Schilling *et al*., 2010; Wang & Tooley 2011). The larger agglomerates form when aggregates bind loosely during the manufacturing process (Schilling *et al*., 2010). These are not efficient UV absorbers so they need to be broken down into the more efficient aggregates, which are chemically bound. This review includes assessments of NP preparations that contain aggregates and agglomerates. The latter have been included because of their yet unclear potential to disaggregate and disagglomerate when applied on the skin in a sunscreen formulation.

Although agglomerates are not normally found in sunscreen formulations, they may form on the skin surface after application of sunscreens, suggesting fewer primary NPs would be available for skin penetration (Tran & Salmon, 2010). A preliminary study by Bennett *et al*. (2012) suggests that exposure to sunlight can lead to disaggregation of TiO_2 NPs which facilitated penetration when tested on isolated porcine skin sections. Thus, any potential hazard linked with nanosized particles is likely to be reduced if these particles aggregate and form structures above the nanoscale, which then do not dissociate into smaller NPs, thus reducing their ability to penetrate the skin.

In addition to changes in physico-chemical properties due to aggregation and agglomeration, TiO_2 NPs (but not ZnO NPs) adopt different crystal forms: rutile, anatase and amorphous. The rutile form, or a mixture of rutile and anatase, is generally used in sunscreens (Dussert & Gooris, 1997). The anatase form is substantially more photocatalytic and adheres more strongly to skin than the rutile form (Turci *et* al., 2013; Osmond-McLeod *et al*., 2016). The form and size of the NPs used in the studies summarised in this review are specified if these parameters were described in the study.

3. Dermal Exposure

3.1 Skin irritation/sensitisation

The potential effects of photoirritation and photosensitisation of ZnO were discussed in the European Commission's Scientific Committee on Consumer Safety (SCCS) report (SCCS, 2012); there was no evidence of any positive findings in two photoirritation studies and two photosensitisation studies after topical application to intact skin of human volunteers. Furthermore, in a review of photoprotection, Lautenschlager *et al*. (2007) reported that neither TiO_2 nor ZnO NPs possess notable skin irritation or sensitisation properties when used in sunscreens on humans.

A number of studies published since these safety reviews have assessed the potential of ZnO NPs or TiO_2 NPs to cause skin irritation (reversible skin damage), corrosion (irreversible necrotic damage extending into the dermis) or sensitisation in the absence of UV or non-UV light. In a mouse model of atopic dermatitis employed to determine whether ZnO NPs could exacerbate the inflammatory pathology associated with allergic skin conditions, ZnO NPs were applied to the skin of mice sensitised to ovalbumin (Ilves et al., 2014). ZnO NPs were detected in the epidermal and dermal layers of the skin of both sensitised and non-sensitised mice in regions where skin had been tape-stripped (a procedure employed to mimic skin damage). The number of immune cells (T lymphocytes, neutrophils, eosinophils and mast cells) infiltrating the skin in antigen-sensitised mice was attenuated in the presence of ZnO NPs. Serum levels of total IgE and ovalbumin-specific IgE, however, were enhanced by ZnO NPs in antigen-sensitised mice. These

data demonstrate that ZnO NPs can potentially penetrate the dermis, exert an anti-inflammatory effect on allergic skin but enhance IgE generation. Despite the potential for an allergic response, the authors conclude that the benefits of using sunscreens to combat the development of skin malignancies outweigh the possible risks of exacerbating allergic symptoms (Ilves *et al.,* 2014).

In other studies, ZnO NPs failed to cause irritation or sensitisation. ZnO NPs (20-50 nm in diameter) did not cause acute dermal toxicity (when applied topically at a dose of 2g/kg for 24 h to rats) or dermal irritation (when applied topically at a dose of 0.5g for up to 4 h) in rabbits. ZnO NPs also failed to induce skin sensitization in guinea pigs during or up to 48 h after the elicitation phase after an induction phase that involved weekly application of NPs for 3 weeks (Kim *et al*., 2016).

A dose-dependent increase in ear swelling (used as an indicator of skin irritancy) was observed in female balb/c mice after dermal administration of TiO_2 NPs (50 µl, up to 10 % (w/v)) but skin sensitisation was not observed (Auttachoat *et al.,* 2014). In balb/c mice, pre-treatment with TiO_2 NPs (dermal application; 4 mg/mL) exacerbated subsequent sensitisation induced by dinitrochlorobenzene (Smulders *et al*., 2014). Exposure of EpiDerm™, a commercially available human 3D skin model composed of human-derived epidermal keratinocytes, to TiO_2 NPs (1 mg/mL every minute for 1 h) did not induce signs of dermal irritation (Miyani *et al*., 2016). TiO_2 NPs, ZnO NPs or a mixture of the two (at a final concentration of 25% (w/v)) did not cause irritation or corrosion when applied to a 3D human skin model, KeraSkin, or to intact rabbit skin (Choi, *et al*., 2014).)

3.2 Skin penetration

Skin penetration of TiO_2 and/or ZnO NPs through the outer layer to the viable cells within the deeper skin layers was investigated in a large set of *in vitro* and *in vivo* studies assessed for this report. As outlined below, the findings of the great majority of these studies indicated an apparent inability of TiO_2 and ZnO NPs to reach viable cells in the dermis. In addition, the SCCS reported in 2012 that "*in sunscreens, [ZnO NPs] can be considered to not pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin*". Similarly, the Committee concluded that "*TiO_2 nanomaterials in a sunscreen formulation are unlikely to lead to systemic exposure to nanoparticles through human skin to reach viable cells of the epidermis, dermis, or other organs*" (Chaudhry *et al*., 2015).

3.2.1 *In vitro* studies

Numerous *in vitro* studies have investigated the properties of nanoparticulate TiO_2 and ZnO, using various cultured cell lines and different methodological approaches. This multiplicity and variability in methodology has generated uncertainty regarding the relevance of individual study findings. For example, Lanone *et al*., (2009) and Park et al., (2009) identified a clear difference in the sensitivity between cytotoxicity assays with cell type being investigated. Further, numerous issues were identified by Park *et al,*. (2009) concerning the conduct of *in vitro* studies leading to a conclusion that "*much work has yet to be done before in vitro toxicity assays can play any role of importance within the risk/hazard assessment of nanomaterials*".

The complex nature of interactions of nanomaterials (nanoparticles/quantum dots) with cell cultures was highlighted in an *in vitro* study (Ryman-Rasmussen *et al*., 2007), where difficulties with interpretation of results from this type of study were identified. A review (Stone *et al*., 2009) on the development of *in vitro* systems for nanotechnology highlighted the complexities associated with *in vitro* studies investigating safety of nanoparticles and the need to establish validated experimental approaches to determine any biologically relevant nanoparticle-induced hazard.

For example, Nohynek *et al*. (2008) provided a comparative analysis of dermal penetration between different animal species, rating them in the order: rabbit >rat >pig >monkey >man.

They noted that pig and rat skin is up to 4 and 9-11 times, respectively, more permeable than human skin.

This inter-species variability in skin penetration demands caution when extrapolating positive findings from animal studies to potential for hazard in humans. The results of Wu *et al*., (2009) can at present only be accepted as preliminary, and speculative in terms of the hazard in humans, particularly since their findings in porcine skin *in vitro* and *in vivo* clearly indicate that TiO_2 NPs penetrated into the *stratum corneum*, *stratum granulosum*, prickle cell layer and basal cell layer, but not into the dermis. Importantly, only 4 nm TiO_2 NPs reached the basal cell layer; hence the ability of TiO_2 NPs to penetrate porcine skin was shown to be size-dependent. This is an important finding considering that a great majority of TiO_2 NPs exist in sunscreen formulation as relatively large aggregates or even larger agglomerates after the skin application (Schilling *et al*., 2010).

A few studies that reported findings suggesting that TiO_2 NPs could penetrate beyond the *stratum corneum* suffered from important methodological limitations that put the validity and extrapolation of these findings to humans in doubt (Tan *et al*., 1996, effect not statistically significant; Wu *et al*., 2009, effect observed in hairless mouse only; Sadrieh *et al*., 2010, tissue contamination likely).

Evidence that other forms of nanomaterials can penetrate into the skin has been presented. Vogt *et al*. (2006) examined dermal penetration of NP fluospheres and showed that 40 nm particles can penetrate hair follicles and reach the Langerhans cells surrounding the follicles in the dermis. However, this penetration was only achieved after tape-stripping debris from the follicle opening, as well as removing a significant proportion of the protective *stratum corneum* and subjecting the human skin samples *in vitro* to a prolonged (16 h) exposure to the test material in a special humidified chamber. Such conditions are artificial and unlikely to mimic typical use of sunscreens. Zhang & Monteiro-Riviere (2008) showed that Fluosphere NPs (quantum dots of 14 and 18 nm) did not penetrate normal, flexed or even tape-stripped skin in rats, while minimal penetration was observed in abraded skin. This was confirmed by Campbell *et al.* (2012) who demonstrated that Fluosphere polymeric NPs (20-200 nm) penetrated only into the superficial layers of the *stratum corneum* of partially damaged pig skin strips, again suggesting that the NPs are unlikely to reach viable cells below the *stratum corneum*.

The effect of absorption enhancers (oleic acid and/or ethanol) on the skin penetration of ZnO NPs was studied (Kuo *et al*., 2009) *in vitro* using skin samples from hairless mice. Results indicated that ZnO NPs penetrated into the *stratum corneum*, but not into viable cells in the presence of these penetration enhancers. This finding suggested that ZnO NPs are unlikely to penetrate into viable skin cells in humans, since this could not be achieved in an animal model with higher dermal permeability compared with human skin, even in the presence of penetration enhancers.

The possibility that hair follicles may play a part in percutaneous absorption of nanomaterials has also been examined. Otberg *et al*. (2008) found that hair follicles represented very limited sites for potential absorption (up to 0.1% of the skin surface), assuming they were permeable to NPs, although ZnO or TiO_2 NPs were not investigated. In another study, Lademann *et al*. (1999) failed to detect TiO_2 in the epidermal or dermal tissue outside of the follicles. Similarly, Pflücker *et al*. (1999) found TiO_2 NPs only in trace amounts in the upper part of the hair follicle without any evidence of uptake into follicular epithelium. Although Bennat & Muller-Goymann (2000) concluded that their findings *in vitro* suggested that TiO_2 NPs seems to penetrate human skin probably *via* the sebum lipids of the hair follicle, they could not detect any TiO_2 NPs within the sebum matrix of hair follicles.

More recent studies have essentially supported these findings that suggest neither ZnO NPs nor TiO_2 NPs penetrate the dermis to any significant degree. TiO_2 NPs (minimum 90% anatase solution) applied to either healthy or abraded human skin samples in Franz cells (an apparatus that can assess transdermal permeability) at a concentration of 1 g/L was detected only in the

epidermal, and not the dermal, layer (Crosera *et al.,* 2015). Three preparations of ZnO nanoparticles were tested for their ability to penetrate human skin: ZnO NPs suspended in either capric caprylic triglycerides (commonly used in commercial sunscreen preparations), a pH 6 aqueous solution (to mimic natural skin pH) or a pH 9 aqueous solution (which minimises damage to the epidermis) (Holmes et al., 2016). When applied to human skin samples *in* vitro for 48 h, none of these ZnO NP formulations penetrated beyond the superficial layers of the *stratum corneum* into the intact viable epidermis. It was shown, however, that ZnO hydrolysis caused an increase in Zn^{2+} ions within viable epidermal layers. The authors suggest that any systemic increases in zinc detected in humans after sunscreen application in previous studies (such as in Gulson *et al*., 2010) represent Zn^{2+} ions and not ZnO NPs (Holmes *et al*., 2016). Similarly, in another study, zinc was detected only in the *stratum corneum* and not in the viable underlying skin layers when Nanosun™ 65/30 was applied to porcine skin (Detoni *et al*., 2014).

Therefore, the currently available evidence suggests that the likelihood of penetration of TiO_2 or ZnO NPs beyond the surface layers into viable cells of the dermis is extremely low.

3.2.2 *In vivo* studies

Potential systemic absorption of ZnO NPs when applied in a sunscreen formulation was assessed under real-life conditions of sunscreen use by human volunteers over a period of five days (Gulson *et al.*, 2010). Blood and urine levels of ^{68}Zn (based on changes in $^{68}Zn/^{64}Zn$ ratio) 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, compared to females exposed to larger ^{68}ZnO particles and males exposed to particles of both sizes. However, concerns relating to the methodology and conduct of the study impact on the validity of these findings. Most critically, the determination of ^{68}Zn ion concentration in the blood as evidence of systemic or dermal exposure to ZnO NPs does not necessarily prove the dermal penetration of, or systemic exposure to, ZnO NPs. The method used to detect ^{68}Zn from the sunscreens could not distinguish between ^{68}ZnO particle or $^{68}ZnO^{2+}$ ions. However, these observations may still be relevant to human risk assessment because they show that the level of exposure to zinc following short term and real-life use of ZnO NP-formulated sunscreen was negligible compared with the zinc levels normally found in the body and in a typical daily diet. Thus, such exposure is unlikely to be of any concern to human health.

Subsequently, Filipe *et al*., (2010) investigated dermal penetration of TiO_2 and ZnO NPs in human volunteers after *in vivo* application, using punch biopsy analysis. Localisation of TiO_2 and ZnO NPs in damaged skin was evaluated using skin tape stripping before sunscreen application. Interestingly, the removal of the *stratum corneum* resulted in negligible adhesion of the sunscreen. NPs were did not penetrate beyond the stratum corneum; neither titanium (Ti) nor Zn^{2+} was detectable in the dermis after 48 h exposure to the sunscreen under occlusion.

The issue of altered penetration of nanosized ingredients through damaged skin (*i.e*., diseased or sunburnt) was reviewed (Newman *et al*., 2009). It was concluded that penetration through compromised skin was likely to be similar to normal skin (findings of other studies were cited). For psoriatic skin, application resulted in the sunscreen only being found on the top layers of the *stratum corneum*. Additional *in vitro* data (Senzui *et al*., 2010) for TiO_2 NPs applied on skin (from micropig) damaged using tape stripping showed no penetration of Ti into viable skin cells.

Sadrieh et al. (2010) conducted a dermal penetration study in minipigs using three different forms of TiO_2 NPs, including one TiO_2 NP currently used in a commercial sunscreen formulation. Using detailed transmission electron microscopy analysis, they detected minimal penetration of TiO_2 NPs into sub-epidermal layers of the skin, but found no evidence of TiO_2 NP penetration *via* expected routes such as follicular lumens. In concert, these results suggest that the risk of TiO_2 NP penetration into the dermis *via* hair follicles is very low.

Two-photon microscopy (a fluorescence imaging technique that permits the imaging of living tissue) was used to visualize the distribution of ZnO NPs *in vivo* after topical application of a

commercially available sunscreen on human skin. There was no penetration of ZnO nanoparticles beyond the *stratum corneum*, including microscopic skin wrinkles, where this layer is significantly thinner (Breuinig *et al*., 2015). ZnO NPs (< 100 nm in diameter), both non-coated or coated with triethoxycaprylylsilane (to enhance hydrophobicity), did not alter the hydration or barrier function of skin when applied topically to the forearms of human volunteers for 4 h. Coated and uncoated ZnO NPs localized predominantly within the *stratum corneum* and furrows of the epidermis. There was evidence of minimal penetration into the *stratum granulosum* of the viable epidermis only for coated ZnO NPs. Neither formulation altered the redox state of the cells in the viable epidermis (Leite-Silva *et al*., 2013). Furthermore, neither coated nor uncoated ZnO NPs penetrated into the viable epidermis of occluded skin of volunteers (occlusion was achieved by placing impermeable adhesive dressings over the sites of application). Penetration of ZnO NPs into barrier-impaired (tape-stripped) skin, which models damaged/sunburnt skin, was minimal and limited to the outermost layer of viable cells in the epidermis. There were no changes in cellular morphology or evidence of apoptosis in viable epidermal cells after topical application of either NP preparation for 6 h (Leite-Silva *et al*., 2016).

A study was conducted in mice to determine whether ZnO NPs could be detected in organs after topical application of sunscreen (Osmond-Mcleod *et al*., 2014). Female, immune-competent, hairless SKH:QS mice were topically administered a total of 0.6 g of sunscreen containing ZnO NPs over a period of four days. Zn^{2+} ions derived from ZnO NPs was detected in the liver, kidneys, brain, heart, lung, blood and in the spleen and in the fetal liver when applied topically to dams. The method of zinc detection could not distinguish between free Zn^{2+} originating from ZnO NPs and zinc present in ZnO NPs, therefore it could not be determined whether ZnO NPs penetrated the skin. Irrespective of the source of the Zn^{2+} ions, there was no increase in the total zinc levels in organs suggesting that endogenous zinc homeostatic mechanisms were not altered by sunscreen application. Serum amyloid A1 and A2 levels (markers for acute phase inflammation) were reduced in ZnO NP-treated mice compared to controls and were equivalent to controls in pregnant mice, indicating that the sunscreens did not induce or exacerbate inflammation. The authors subsequently extended these investigations with longer-term studies (36 weeks) that included groups of mice treated with sunscreens that contained TiO_2 NPs and included groups that were subject to UV irradiation in combination with the sunscreens (Osmond-Mcleod *et al*., 2016). These studies revealed that sunscreen use was associated with significant protection from skin malignancies (which were observed in the UV irradiated mice) and that tissue zinc levels and hepatic titanium levels were not elevated in sunscreen-treated mice. The authors concluded that repeated, long-term application of sunscreen to mice did not result in significant dermal penetration, accumulation in organs and adverse biological outcomes. It is important to consider that these studies were conducted in hairless mice, which have skin that is significantly more permeable to NPs than intact human skin.

The potential for TiO_2 NPs to penetrate intact skin was assessed in fair-skinned individuals (Coelho *et al*., 2016). Sunscreen containing TiO_2 NPs was applied once daily for 3 days to 2 participants and once daily for 8 days to 4 participants (2 mg/cm^2 over a 5cm^2 area). One day after the final sunscreen application, biopsy specimens (approximately 4 mm in diameter) were acquired from each participant. In total, fewer than 30 confirmed TiO_2 nanoparticles or their aggregates were detected in all of the skin specimen samples and their abundance did not correlate with skin depth. The majority of NPs were detected mainly in the dermis surrounding hair follicles. Follicular accumulation of NPs has not been associated with penetration into viable skin cells (Filipe *et al.,* 2009; Lademann *et al*., 1999). In another study conducted in two human volunteers, a commercial sunscreen containing TiO_2 NPs was applied (2 mg/cm^2 over a total skin area of approximately 600 cm^2) 6 times a day for seven consecutive days to both intact and UVB-sunburned skin (Næss *et al*., 2015). TiO_2 NPs were detected in viable cells of the epidermal layer in both intact and damaged skin; 1 to 10 TiO_2 NPs were detected in a total of 3–4 skin sections each measuring approximately 200 µm × 60 µm. This study did not ascertain whether the trace levels of NPs detected in viable epidermal cells were translocated into the systemic circulation.

Therefore, based on these *in vitro* studies using both animal and human skin, and *in vivo* studies that included studies with human subjects, it can be concluded that ZnO or TiO_2 NPs minimally penetrate the underlying layers of skin, with penetration largely limited to the *stratum corneum*. This suggests that the likelihood of NPs causing cytotoxicity or pathology in internal organs or tissues is very low since systemic absorption is highly unlikely.

4. Cytotoxicity & Genotoxicity

4.1 Photocatalysis and *in vitro* cytotoxicity

Both ZnO and TiO_2 NPs can generate, via UV-induced photocatalysis, reactive oxygen species (ROS) such as superoxide anions or hydroxyl radicals (Li *et al*., 2012). ROS can damage cellular components and macromolecules (such as lipids, proteins and nucleic acids) and ultimately cause cell death if produced in excess or if they are not neutralised by innate antioxidant defences (Manke, et al., 2013; Redza-Dutordoir *et al*., 2016). ROS derived from the photocatalysis of NPs are cytotoxic to a variety of cell types (Cai *et al*., 1991; Dunford *et al*., 1997; Wamer *et al*., 1997; Afaq *et al*., 1998; Serpone *et al*., 2001; Uchino *et al*., 2002; Long *et al*., 2006; Kang *et al*., 2008; Braydich-Stolle *et al*., 2009; Liu *et al*., 2010; Yin *et al*., 2010 and Xue *et al*., 2010).

TiO_2 or ZnO NP-generated ROS have been documented to induce DNA damage and cytotoxicity in variety of cells types. These include Chinese hamster ovary (CHO) cells (Uchino *et al*., 2002), HepG2 cells (hepatocellular carcinoma cells; Shi *et al*., 2015), Caco-2 cells (human colon carcinoma cells; De Angelis *et al*., 2013), mouse bone marrow mesenchymal stem cells (Syama *et al*., 2014), normal mouse or human fibroblasts (Şeker *et al*., 2014), HUVECs (human umbilical vein endothelial cells; Chen *et al*., 2014), SW480, DlD-1 and NCM460 cells (human epithelial cells; Setyawati *et al*., 2015), mouse podocytes (Xiao *et al*., 2016) A549 cells (human non-small cell lung cancers; Wang *et al*., 2014; Ivask *et al*., 2015), 16HBE14o- cells (human bronchial epithelial cells; Yu *et al* .,2015), RAW264.7 cells (mouse macrophages) and human glial (D384) and neuronal (SH-SY5Y) cell lines (Coccini *et al*., 2015). In addition, ZnO NPs affected metabolic parameters, such as glycogenolysis and gluconeogenesis, in hepatocyte cell lines (Filippi *et al*., 2014) and interfered with the cell cycle in human intestinal epithelial cells (Setyawati *et al*., 2015). TiO_2 NPs could also induce a pre-malignant phenotype in AGS cells (human gastric epithelial cells) characterised by enhanced DNA damage and proliferation, apoptosis resistance and increased invasiveness (Botehlo *et al*., 2014). Treatment with ROS scavengers or antioxidants such as N-mercaptopropionyl-glycine (Xiao *et al*., 2016) or N-acetylcysteine (Setyawati *et al*., 2015) could prevent NP-induced cytotoxicity, highlighting the central role oxidative stress plays in NP-induced cell death induction pathways.

Manufacturers of NP-containing sunscreens attempt to block the potential production of ROS by coating NPs or adding anti-oxidant compounds to the sunscreen formulation (Tran & Salmon, 2010). Coating materials include aluminium hydroxide ($Al(OH)_3$), polymers and inert oxides of silica while anti-oxidant compounds include vitamins (A, E, C). For example, Fisichella et al. (2012) demonstrated that $Al(OH)_3$-coated TiO_2 NPs that were surface-treated by polydimethylsiloxane polymer (which have been included as an ingredient in some sunscreens) significantly attenuated ROS production compared to unmodified TiO_2 NPs and were not toxic to Caco-2 cells. It has been shown, however, that the integrity of the $Al(OH)_3$ surface layer can be disrupted, principally by Ca^{2+} and OCl^- ions (which are present in swimming pool water, for example.). This exposes the TiO_2 NPs to photocatalysis and the potential for the generation of free radicals. Thus, NPs may be stripped of their surface modifications under certain circumstances. A study into the cytotoxic properties of ZnO NPs coated with a TiO_2 NP shell revealed that the inherent cytotoxic properties of the former were markedly reduced by curtailing the release of Zn^{2+} ions and decreasing the contact area of the ZnO NP by the TiO_2 NP shell (Hsiao & Huang, 2011b). Thus, if cytotoxicity is largely related to the release of Zn^{2+} ions

from the particle surface, it is likely that these potentially harmful effects of ZnO NPs can be effectively controlled by particle coating.

Other approaches to curtailing ROS formation have been investigated. It was demonstrated that coatings based on silicon dioxide were highly effective in ameliorating UV-induced TiO_2 NP-derived ROS (Carlotti *et al*., 2009; Tsuji *et al*., 2007). ROS formation can also be attenuated by the addition of additives to the matrix the NPs are suspended in; for example, Nanosun™ 65/30, a commercial product consisting of 30 nm ZnO particles dispersed in medium-chain triglycerides at a concentration of 65% (w/v), significantly attenuated UV-induced ROS generation when applied to porcine skin *in vitro* (Detoni *et al*., 2014).

The biochemical and cellular signalling events that have been associated with the induction of ROS, DNA damage and cell death mediated by ZnO or TiO_2 NPs include the activation of NF-κB, a master transcriptional regulator of proinflammatory responses (Setyawati *et al*., 2015), proinflammatory cytokine secretion (De Angelis *et al*., 2013), disruption of calcium homeostasis (Yu *et al*., 2015), activation of executioner caspases (Syama *et al*., 2014; Wang *et al*., 2014), induction of endoplasmic reticulum stress and caspase 12 activation (Chen *et al*., 2014; Yu *et al*., 2015), and the disruption of mitochondrial membrane potential (Filippi *et al*., 2014).

Of greater relevance to the potential adverse effects of NPs in sunscreens, the cytotoxicity of TiO_2 and ZnO NPs has been investigated in various human skin models, principally HaCaT cells (transformed keratinocytes derived from histologically normal skin), human or animal-derived skin samples exposed to NPs *ex vivo*, and in human volunteers. When applied to HaCaT cells, TiO_2 NPs reduced cell viability and induced membrane damage at concentrations at or greater than 0.7μg/cm^2 regardless of the exposure time (24 h, 48 h or 7 days) (Crosera *et al*., 2015). A significant reduction in cell viability and induction of ROS, which were both enhanced by UVB irradiation, were also observed when HaCaT cells were incubated with 10-500 μg/mL TiO_2 NPs for 2 h (Rancan *et al*., 2014). It was also demonstrated that ZnO NPs (100 nm in diameter) were internalised and induced dose- and time-dependent cytotoxicity in HaCaT cells; a 50% reduction in cell viability was observed when cells were incubated with 200 μg/mL ZnO NPs for 24 h or 50 μg/mL for 48 h or 72 h. Annexin V and propidium iodide staining confirmed that ZnO NPs induced cell death by apoptosis. Preceding apoptosis, ZnO NPs induced the expression of apoptosis-related genes (such as *BAX*, *PUMA* and *NOXA*), ROS, DNA damage and cell cycle arrest at the G2/M checkpoint (Gao *et al.,* 2016).

Addition of three preparations of TiO_2 NPs (1nM, 100% rutile; 21nm, anatase:rutile 4:1; and 12nm, 100% anatase) to HaCaT cells for 24 h induced oxidative stress (superoxide and hydrogen peroxide induction), activated pro-apoptotic proteins (caspase 8/9), down-regulated anti-apoptotic proteins (bcl-2) and induced apoptosis (which was not enhanced by UV-C irradiation). These effects were comparable in magnitude between the different NP preparations. In addition, all NP formulations did not alter the expression of β-catenin and E-cadherin (which regulate the epithelial to mesenchymal transition where cells undergo complex biochemical and morphological changes, increasing their potential for malignant transformation) and inhibited proliferation of human lung cell lines (Wright *et al*., 2016). These data suggest that TiO_2 NPs could induce apoptosis but could not induce tumourigenic changes in HaCaT cells.

In contrast, some studies have not demonstrated NP-mediated cytotoxicity in skin or skin-derived cells. Although HaCaT cells simultaneously exposed to anatase TiO_2 NPs and to UVA irradiation exhibited impaired mitochondrial activity, oxidative stress and membrane damage (Miyani *et al*., 2016), this formulation did not generate phototoxic effects in the EpiDerm™ commercial human 3D skin model, which is composed of human-derived epidermal keratinocytes (Miyani *et al*., 2016). In another study, NPs were internalised in HaCaT cells treated with TiO_2 NPs (5-100 μg/mL for 24 h) and were distributed throughout the cytoplasm and phagosomes without causing cell death or perturbing cell cycle progression. However, mitochondrial dysfunction was observed that caused ROS formation (Tucci *et al*., 2013). Finally, there was no significant increase in oxidative stress in skin samples obtained from human

volunteers after a 2 h exposure to sunscreen containing ZnO NPs regardless of whether the treated skin was intact or tape-stripped (Hang, *et al*., 2015).

4.2 *In vivo* cytotoxicity

The toxicity of ZnO and TiO_2 NPs has been assessed in a number of animal models through a variety of administration routes.

In inhalation studies conducted in rodents, ZnO NPs caused mild acute pulmonary and systemic inflammation, pulmonary cell damage and a reduction in the antioxidant capacity of the lungs (Gosens *et al*., 2015; Liu *et al*., 2015). A robust pulmonary inflammatory response was also observed in rats administered TiO_2 intratracheally that was characterised by extensive leukocyte infiltration and ROS formation (Hurbánková *et al*., 2013). It has also been shown that inhaled ZnO NPs could potentially cause eosinophilic airway inflammation (Huang *et al*., 2015). TiO_2 NPs caused pulmonary hyperplasia and inflammation in the lungs in a 28 day study conducted in mice (Yu *et al*., 2015) and have also been implicated in causing kidney pathology in mice, where intratracheal instillation elevated serum blood urea nitrogen levels and caused a significant increase in renal oxidative stress and renal fibrosis markers (Huang *et al*., 2014). Other studies have shown that ZnO NPs cause only minor changes in the lungs; in a chronic inhalational study conducted in mice, administration of ZnO NPs (daily exposure for 13 weeks) resulted in only minimal pulmonary inflammation and toxicity (Adamcakova-Dodd *et al*., 2014).

TiO_2 and ZnO NPs can also cause pathological changes *in vivo* when administered via oral, intravenous or intraperitoneal routes. Evidence of cardiovascular pathology was observed in rats after daily oral administration of TiO_2 NPs at doses of up to 30 mg/kg for 90 days (reference?). Mice that were orally administered TiO2 daily for 6 months exhibited lower body weight gain and significant kidney pathology attributed to NP-induced oxidative stress (Gui *et al*., 2013). TiO_2 NPs caused liver damage and induced oxidative stress, DNA damage and clastogenic changes in hepatocytes when administered intraperitoneally to mice for 14 days at doses of up to 100 mg/kg (Shukla *et al*., 2014). Rats orally administered ZnO NPs (at up to 500 mg/kg per day; 90 days) had mild perturbations in some haematological and biochemical parameters and mild pathological features in the stomach and pancreas at the highest dose (Kim *et al*., 2014). Similar findings were reported in a study with a similar protocol, where in addition to these pathological features, retinal atrophy was also observed in the high dose group (Park *et al*., 2014). Orally or intravenously administered ZnO NPs failed to cross the blood brain barrier in a 28 day repeat dose study conducted in rats (Shim *et al*., 2014) but both ZnO and TiO_2 NPs were detected within neurons in a mouse study where NPs were administered orally for 21 days at a dose of 500 mg/kg (Shrivastava *et al*., 2014). In the latter study, evidence of oxidative stress was evident in the liver and brain and the levels of some neurotransmitters (norepinephrine, dopamine and 5-hydroxytryptamine) within the cerebral cortex were significantly increased in both ZnO NP- and TiO_2 NP-treated mice. Finally, in a study conducted in human volunteers TiO_2 NPs (as a single oral 5 mg/kg dose; 15 nm or 100 nm in size) were not absorbed systemically because of particle agglomeration in the gastrointestinal tract (Jones *et al*., 2015).

4.3 Genotoxicity

The genotoxicity and/or mutagenicity of ZnO and TiO_2 NPs have been addressed by several studies and reviews. Three unpublished studies investigating photomutagenicity effects (possibly linked to ROS formation) of micronized (≤200 nm) ZnO had been reviewed by the Scientific Committee Cosmetic Non-Food Products (SCCNFP 2003) to the European Commission, which concluded that "[*M]icronised material (ZnO) has been found to be clastogenic, possibly aneugenic and inducing DNA damage in cultured mammalian cells in vitro, under the influence of UV light*". In the same review, the SCCNFP (2003) noted that micronised ZnO was non-photomutagenic in an Ames test. However, the general validity of Ames assay findings in

nanoparticulate genotoxicity testing has been questioned by Landsiedel *et al*., (2009) and Warheit & Donner (2010) because NPs may not penetrate the bacterial cell wall. Nevertheless, the OECD guidance manual on nanoparticle genotoxicity testing includes the Ames assay in the list of *in vitro* methods that should be included in application dossiers submitted in the EU (Warheit & Donner, 2010).

A review by Nohynek *et al*., (2008) suggests that possible photogenotoxicity associated with *in vitro* exposure to ZnO NPs may be influenced by UV radiation-induced increases in background sensitivity of experimental cell cultures. This could partly explain why levels of photogenotoxicity determined by Dufour *et al*. (2006) using the micronucleus assay (following exposure to ZnO) were relatively small (2 to 4-fold increase) compared with a potent effect of a known photo-clastogenic agent. Consequently, Nohynek *et al*. (2008) questioned the validity of evidence from *in vitro* studies presented by Dufour *et al*. (2006) implicating ZnO NPs as a photogenotoxic agent, and the same review documented data suggesting that the TiO_2 NPs were not genotoxic. Furthermore, TiO_2 NPs were found to be of low hazard based on a series of toxicity studies conducted by Warheit *et al*. (2007), and Nohynek *et al*. (2008) concluded that the hazard associated with the use of TiO_2 and ZnO NPs appears to be low. This view was recently confirmed by the review conducted by industry representatives (Schilling *et al*., 2010), which stated that "*the in vitro genotoxic and photogenotoxic profiles of these nano-structured metal oxides are of no consequence to human health*".

A variety of transformed cell lines, as well as primary cell cultures, have been used to investigate the potential genotoxic effects of ZnO and TiO_2 NPs. The findings of Gopalan *et al*., (2009), who investigated the photogenotoxic effects of TiO_2 and ZnO NPs in human lymphocytes and in sperm cells, were inconsistent. Whereas lymphocytes showed significant levels of photogenotoxicity in a concentration-dependent manner for both oxides, the sperm showed greater photogenotoxicity at the lowest concentrations of both TiO_2 and ZnO. The relevance of these findings to dermal exposure remains unclear. As discussed by McCall *et al*., (2013), a better interpretation of the results could be made if it had been accompanied by physico-chemical characterisation of the NPs used in this biological study.

TiO_2 NPs (uncoated anatase and coated rutile forms, average crystal size 20 nm for both) were not cytotoxic or genotoxic *in vitro* (using rat liver epithelial cells in micronucleus assay) in the presence or absence of UV light (366 nm) (Linnainmaa *et al*., 1997). The authors concluded that TiO_2 NPs do not cause direct chromosomal damage and suggested that tumour responses to TiO_2 NP dust observed in experimental animals were most likely a consequence of inflammatory effects rather than genotoxicity. In a more recent study, TiO_2 NPs induced clastogenic changes in human lymphocytes at concentrations of up to 300 µg/ml, however, a dose-dependent effect was not observed (Tavares *et al*., 2014). Both ZnO and TiO_2 caused dose-dependent increases in DNA damage (assessed by comet assays) in human lymphocytes at concentrations up to 500 ppm (Khan *et al*., 2015).

ZnO NPs induced cytotoxicity and genotoxicity (at concentrations of up to 100 µg/mL) in a rat kidney epithelial cell line (NRK-52E) (Uzar *et al*., 2015). In human peripheral blood lymphocytes, ZnO caused cytotoxicity only at very high concentrations (>0.5 mM for 24 h) but caused DNA damage (both single- and double-strand breaks; assessed by alkaline and neural comet assays, respectively) at 10µM (Swilinska *et al*., 2015). In this study it was shown that a significant proportion of the DNA damage was attributed to the action of NP-induced ROS. ZnO NPs were not cytotoxic to human embryonic kidney cells (HEK293) or mouse embryonic fibroblast cells (NIH/3T3) but caused dose-dependent genotoxicity and clastogenicity (as assessed by comet and micronucleus assays, respectively) and induced cell-anchorage independent growth (an attribute indicative of metastatic potential) in both of these cell lines (Demir *et al*., 2014). ZnO NPs induced clastogenic changes in Chinese hamster lung fibroblasts (assessed by cytokinesis block micronucleus assay) but was found not to be mutagenic in the *Drosophila* somatic mutation and recombination test (which assesses the potential of a substance to induce loss of heterozygosity resulting from DNA damage or clastogenic changes) (Reis *et al.,* 2015). Topical

application of ZnO NP to female SKH mice caused mild skin damage, ROS generation, apoptosis, leukocyte infiltration, DNA damage and induction of COX-2 (an inflammatory marker) in the skin. All of these parameters were augmented by UV irradiation (Pal *et al*., 2016a; Pal et al., 2016b).

Other studies have not demonstrated a genotoxic effect by TiO_2 or ZnO NPs. TiO_2 NPs were internalised by immortalised human skin fibroblasts, but did not induce cytotoxicity (assessed by clonogenic survival assay) or clastogenicity (assessed by scoring chromosomal aberrations during metaphase) after a 24 h exposure at concentrations of up to 100 μg/cm^2 (Browning *et al*., 2014). ZnO NPs (20 nm or 70 nm diameter, positively or negatively charged) did not significantly increase the number of revertant *Salmonella typhymurium* and *Escherichia coli* colonies (with or without metabolic activation) in bacterial reverse mutation assays (Kwon *et al*., 2014).. In the same study, all ZnO NP types failed to induce clastogenic changes *in vitro* in Chinese hamster lung fibroblasts (at a concentrations up to 15μg/mL) and *in vivo* in rats (as assessed by bone marrow micronucleus assay) administered ZnO NPs at up to 2000 mg/kg. Similarly, in another study, injection of rats with TiO_2 NPs (approximately 20 nm in diameter, 5 mg/kg administered once intravenously) did not induce genotoxicity or clastogenicity in the bone marrow, as assayed by comet and micronucleus assays (Dobrzyńska *et al.,* 2014). In Caco-2 cells, only ZnO, and not TiO_2, induced micronuclei and DNA damage (Zijno *et al.,* 2015). Finally, there was no evidence of DNA damage in HaCaT cells after exposure to ZnO (presumably micronised form; ≤200 nm) in the presence or absence of UV irradiation (SCCNFP 2003).

The SCCS (2012) comprehensive review of ZnO NPs assessed both *in vitro* and *in vivo* studies on photo-mutagenicity/genotoxicity and concluded that there is no conclusive evidence to ascertain whether or not ZnO NPs pose a mutagenic/genotoxic, photo-toxic or photo-mutagenic/genotoxic risk to humans. A similar position was upheld in their review of TiO_2 NPs (Chaudhry *et al*., 2015). Since skin penetration is limited to the upper layers, it is very unlikely that harmful effects would occur at the systemic level in humans.

5. Conclusion

There is conclusive *in vitro* evidence that in the presence of UV light, ZnO and TiO_2 NPs can induce ROS, which have the capacity to damage cellular components. Furthermore, ZnO NP- and TiO_2 NP-mediated cytotoxicity and genotoxicity have been demonstrated in a wide range of cell types. In addition, there are numerous studies that demonstrate a diversity of potential pathological sequelae upon administration of ZnO and TiO_2 NPs to experimental animals via a range of administration routes.

However, a number of factors need to be considered in order to draw sound conclusions from this evidence with regard to potential NP toxicity from topically applied sunscreens in humans. Of paramount importance is the finding that the vast majority of studies do not demonstrate NP skin penetration; the current weight of evidence suggests that TiO_2 and ZnO NPs do not reach viable skin cells (even in compromised skin) or the general circulation, but rather remain on the skin surface and in the outer layer of the *stratum corneum*, a surface layer of non-viable, keratinized cells. It is therefore highly likely that if sunscreens are used as is intended, NPs from sunscreens applied dermally will not achieve significant concentrations in the systemic circulation.

Consequently, it is highly unlikely that NPs will induce the cytotoxic responses or pathological outcomes outlined in this review in the *in vitro* and animal studies, respectively. The data from the reviewed *in vitro* experiments should be interpreted with caution given that the findings from studies conducted in cell lines are of limited value in assessing the potential toxicity NPs pose to humans from topically applied sunscreens. Similarly, the limitations of the reviewed animal studies, where NPs were administered at relatively high concentrations through exposure routes that are not relevant in the context of sunscreen use and at high frequency,

should also be acknowledged. Given the majority of studies found no evidence of skin penetration of NPs when applied dermally, it is highly unlikely that the high systemic NP concentrations attained in these experimental animals would be achieved in people, even if accidental intake occurred via these non-dermal routes. Therefore, any deductions made regarding the safety of topically applied sunscreens in humans by extrapolating these findings in animals to humans, are of limited value.

It is also crucial to emphasize that NPs present in sunscreens are modified to reduce their potential to generate ROS, which largely mediate NP-induced cytotoxicity and genotoxicity. During the manufacturing process the surface of the NPs may be coated to reduce the formation of ROS, even after UV exposure. Sunscreens may also contain antioxidants in order to neutralise ROS generated by NPs. Additionally, endogenous protective mechanisms, such as antioxidant activity mediated by a range of intracellular enzymes and factors, will likely protect against the damaging effects of oxidative stress generated by any exposure to nanoparticles. Minimal dermal penetration of NPs is likely to be adequately counteracted by these natural cellular defences.

In conclusion, on current evidence, neither TiO_2 nor ZnO NPs are likely to cause harm when used as ingredients in sunscreens. The current state of knowledge strongly indicates that the minor risks potentially associated with NPs in sunscreens are vastly outweighed by the benefits that NP-containing sunscreens afford against skin damage and, importantly, skin cancer.

6. Bibliography

Adamcakova-Dodd A, Stebounova LV, Kim JS, Vorrink SU, Ault AP, O'Shaughnessy PT, et al (2014). Toxicity assessment of zinc oxide nanoparticles using sub-acute and sub-chronic murine inhalation models. Particle and fibre toxicology.11:15.

Afaq F, Abidi P, Matin, R. & Rahman, Q. (1998) Cytotoxicity, pro-oxidant effects and antioxidant depletion in rat lung alveolar macrophages exposed to ultrafine titanium dioxide. J Appl Toxicol. 18(5): 307-312.

Alvarez-Roman R, Naik A, Kalia Y, Guy R & Fessi H. (2004) Skin penetration and distribution of polymeric nanoparticles. J. Control Release. 99: 53-62.

Auttachoat W, McLoughlin CE, White Jr KL, Smith MJ. (2014) Route-dependent systemic and local immune effects following exposure to solutions prepared from titanium dioxide nanoparticles . Journal of immunotoxicology.11(3):273-82.

Barker PJ & Branch A. (2008) The interaction of modern sunscreen formulations with surface coatings. Prog Org Coat. 62: 313-320.

Bennat C & Muller-Goymann CC. (2000) Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. Internat J Cosmet Sci. 22(4): 271-283.

Bennet SW, Zhou D, Mielke R & Keller AA. (2012) Photoinduced disaggregation of TiO_2 nanoparticles enables transdermal penetration. PLOS ONE. 7: 1-7.

Berube DM. (2008) Rhetorical gamesmanship in the nano debate over sunscreens and nanoparticles. J Nanoparticle Res. 10: 23-37.

Botelho MC, Costa C, Silva S, Costa S, Dhawan A, Oliveira PA, et al (2014). Effects of titanium dioxide nanoparticles in human gastric epithelial cells in vitro. Biomedicine and Pharmacotherapy.68(1):59-64.

Braydich-Stolle LK, Schaeublin NM, Murdock RC, Jiang J, Biswas P, Schlager JJ, et al. (2009) Crystal structure mediates mode of cell death in TiO2 nanotoxicity. J Nanopart Res. 11: 1361-74.

Breunig HG, Weinigel M, Konig K. In Vivo Imaging of ZnO Nanoparticles from Sunscreen on Human Skin with a Mobile Multiphoton Tomograph. BioNanoScience. 2014;5(1):42-7.

Browning CL, The T, Mason MD, Wise JP, Sr. (2014) Titanium Dioxide Nanoparticles are not Cytotoxic or Clastogenic in Human Skin Cells. Journal of environmental & analytical toxicology.4(6).

Butler MK, Prow TW, Guo Y-N, Lin LL, Webb RI & Martin DJ. (2012) High-pressure freezing/freeze substitution and transmission electron microscopy for characterisation of metal oxide nanoparticles within sunscreen. Nanomedicine. 7: 541-51.

Cai R, Hashimoto K, Itoh K, Kubota Y & Fujishima A. (1991) Photo-killing of malignant cells with ultrafine TiO_2 powder. Bull Chem Soc Jpn. 64: 1268-1273.

Campbell CSJ, Contreras-Rojas LR, Delgado-Charro MB & Guy RH (2012) Objective assessment of nanoparticle disposition in mammalian skin after topical exposure. J Controlled Rel. 162: 20-207

Carlotti ME, Ugazio E, Sapino S, Fenoglio I, Greco G, Fubini B. (2009) Role of particle coating in controlling skin damage photoinduced by titania nanoparticles. Free Radical Res. 43(3): 312-322.

Chaudhry Q. Opinion of the Scientific Committee on Consumer safety (SCCS) - Revision of the opinion on the safety of the use of titanium dioxide, nano form, in cosmetic products. Regulatory toxicology and pharmacology : RTP. 2015;73(2):669-70.

Chen R, Huo L, Shi X, Bai R, Zhang Z, Zhao Y, et al. Endoplasmic reticulum stress induced by zinc oxide nanoparticles is an earlier biomarker for nanotoxicological evaluation. ACS nano. 2014;8(3):2562-74.

Choi J, Kim H, Choi J, Oh SM, Park J, Park K. Skin corrosion and irritation test of sunscreen nanoparticles using reconstructed 3D human skin model. Environmental health and toxicology. 2014;29:e2014004.

Coccini T, Grandi S, Lonati D, Locatelli C, De Simone U. Comparative cellular toxicity of titanium dioxide nanoparticles on human astrocyte and neuronal cells after acute and prolonged exposure. Neurotoxicology. 2015;48:77-89.

Coelho SG, Patri AK, Wokovich AM, McNeil SE, Howard PC, Miller SA. Repetitive application of sunscreen containing titanium dioxide nanoparticles on human skin. JAMA dermatology. 2016;152(4):470-2.

Crosera M, Prodi A, Mauro M, Pelin M, Florio C, Bellomo F, et al. Titanium dioxide nanoparticle penetration into the skin and effects on HaCaT cells. International journal of environmental research and public health. 2015;12(8):9282-97.

Cross SE, Innes B, Roberts MS, Tsuzuki T, Robertson TA & McCormick P. (2007) Human skin penetration of sunscreen nanoparticles: In vitro assessment of a novel micronized zinc oxide formulation. Skin Pharmacol Physiol. 20: 148-154.

Deng XY, Luan QX, Chen WT, Wang YL & Jiao Z. (2009) Nanosized zinc oxide particles induce neural stem cell apoptosis. Nanotechnology. 20: 115101.

Doak SH, Griffiths SM, Manshian B, Singh N, Williams PM, Brown AP & Jenkins GJS. (2009) Confounding experimental considerations in nanogenotoxicology. Mutagenesis. 24: 285-293.

De Angelis I, Barone F, Zijno A, Bizzarri L, Russo MT, Pozzi R, et al. (2013) Comparative study of ZnO and TiO2 nanoparticles: Physicochemical characterisation and toxicological effects on human colon carcinoma cells. Nanotoxicology.7(8):1361-72.

Demir E, Akca H, Kaya B, Burgucu D, Tokgun O, Turna F, et al.(2014) Zinc oxide nanoparticles: genotoxicity, interactions with UV-light and cell-transforming potential. Journal of hazardous materials.;264:420-9.

Detoni CB, Coradini K, Back P, Oliveira CM, Andrade DF, Beck RC, et al. (2014) Penetration, photo-reactivity and photoprotective properties of nanosized ZnO. Photochemical & photobiological sciences. 13(9):1253-60.

Dobrzynska MM, Gajowik A, Radzikowska J, Lankoff A, Dusinska M, Kruszewski M. (2014) Genotoxicity of silver and titanium dioxide nanoparticles in bone marrow cells of rats in vivo. Toxicology. 315:86-91.

Dufour E, Kumaravel T, Nohynek G, Kirkland D & Toutain H. (2006) Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells. Mutat Res. 607(2): 215-224.

Dunford R, Salinaro A, Cai L, Serpone N, Horikoshi, Hidaka H & Knowland J. (1997) Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS Lett. 418: 87-90.

Dusinska M, NanoTEST consortium. (2009) Testing strategies for the safety of nanoparticles used in medical applications. Nanomedicine. 4: 605-607.

Dussert AS & Gooris E. (1997) Characterisation of the mineral content of a physical sunscreen emulsion and its distribution onto human stratum corneum. Int J Cosmet Sci. 19: 119-129.

Environmental Working Group (EWG) 2009 Sunscreen Investigation. Section 4: Nanotechnology and Sunscreens. http://www.ewg.org/cosmetics/report/sunscreen09/ investigation/Nanotechnology-Sunscreens. Date accessed 16/09/10.

Fisichella M, Berenguer F, Steinmetz G, Auffan M, Rose J & Prat O. (2012) Intestinal toxicity evaluation of TiO_2 degraded surface-treated nanoparticles: a combined physico-chemical and toxicogenomics approach in Caco-2 cells. Particle & Fibre Toxicol. 9: 18-30.

Filipe P, Silva JN, Silva R, Cirne de Castro JL, Marques-Gomes M, Alves LC, Santus R & Pinheiro T. (2010) Stratum corneum is an effective barrier to TiO2 and ZnO nanoparticle percutaneous absorption. Skin Pharmacol Physiol. 22: 266-275.

Filippi C, Pryde A, Cowan P, Lee T, Hayes P, Donaldson K, et al. (2015) Toxicology of ZnO and TiO2 nanoparticles on hepatocytes: impact on metabolism and bioenergetics. Nanotoxicology.9(1):126-34.

Gamer A, Leibold E & Van Ravenzwaay B. (2006) The in vitro absorption of microfine zinc oxide and titanium through porcine skin. Toxicol In Vitro. 20: 301-307.

Gao F, Ma N, Zhou H, Wang Q, Zhang H, Wang P, et al.(2016) Zinc oxide nanoparticles-induced epigenetic change and G2/M arrest are associated with apoptosis in human epidermal keratinocytes. International journal of nanomedicine.11:3859-74.

Gojova A, Guo B, Kota RS, Rutledge JC, Kennedy IM & Barakat AI. (2007) Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environ Health Perspect. 115: 403-9.

Gopalan RC, Osman IF, Amani A, DeMatas M & Anderson D. (2009) The effect of zinc oxide and titanium dioxide nanoparticles in the Comet assay with UVA photoactivation of human sperm and lymphocytes. Nanotoxicology. 3: 33-39.

Gosens I, Kermanizadeh A, Jacobsen NR, Lenz AG, Bokkers B, de Jong WH, et al. (2015) Comparative hazard identification by a single dose lung exposure of zinc oxide and silver nanomaterials in mice. PLos One 2015/05/13:[e0126934].

Gui S, Li B, Zhao X, Sheng L, Hong J, Yu X, et al. (2013) Renal injury and Nrf2 modulation in mouse kidney following chronic exposure to TiO(2) nanoparticles. Journal of agricultural and food chemistry.61(37):8959-68.

Gulson B, McCall M, Korsch M, Gomez L, Casey P, Oytam Y, Taylor A, Kinsley L & Greenoak G. (2010) Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci. 118: 140-149.

Hang LYT, Lin LL, Yamada M, Soyer HP, Raphael AP, Prow TW. (2015) Microbiopsy skin sampling in volunteers reveals no oxidative stress detected after topically applying sunscreen with zinc oxide nanoparticles. Journal of the American Academy of Dermatology. 1:AB210.

Holmes AM, Song Z, Moghimi HR, Roberts MS. (2016) Relative Penetration of Zinc Oxide and Zinc Ions into Human Skin after Application of Different Zinc Oxide Formulations. ACS nano.10(2):1810-9.

Hsiao IL & Huang YJ. (2011a) Effects of various physicochemical characteristics on the toxicities of ZnO and TiO_2 nanoparticles toward human lung epithelial cells. Sci Total Environ. 409(7): 1219-28.

Hsiao IL & Huang YJ. (2011b) Titanium oxide shell coatings decrease the cytotoxicity of ZnO nanoparticles. Chem Res Toxicol. 24: 303-13.

Huang KL, Lee YH, Chen HI, Liao HS, Chiang BL, Cheng TJ. (2015) Zinc oxide nanoparticles induce eosinophilic airway inflammation in mice. Journal of hazardous materials. 297:304-12.

Huang KT, Wu CT, Huang KH, Lin WC, Chen CM, Guan SS, et al. (2015) Titanium nanoparticle inhalation induces renal fibrosis in mice via an oxidative stress upregulated transforming growth factor-beta pathway. Chemical research in toxicology. 28(3):354-64.

Hurbankova M, Cerna S, Kovacikova Z, Wimmerova S, Hraskova D, Marcisiakova J, et al. (2013) Effect of TiO2 nanofibres on selected bronchoalveolar parameters in acute and subacute phase--experimental study. Central European journal of public health.21(3):165-70.

Ivask A, Titma T, Visnapuu M, Vija H, Kakinen A, Sihtmae M, et al. (2015) Toxicity of 11 Metal Oxide Nanoparticles to Three Mammalian Cell Types In Vitro. Current topics in medicinal chemistry.15(18):1914-29.

Jonaitis TS, Card JW & Magnuson B. (2010) Concerns regarding nano-sized titanium dioxide dermal penetration and toxicity study. Toxicol Lett. 192: 268-9. (comments on Wu et al., 2009)

Jones K, Morton J, Smith I, Jurkschat K, Harding AH, Evans G. (2015) Human in vivo and in vitro studies on gastrointestinal absorption of titanium dioxide nanoparticles. Toxicology letters.233(2):95-101.

Kang JL, Moon C, Lee HS, Lee HW, Park EM, Kim HS & Castranova V. (2008) Comparison of the biological activity between ultrafine and fine titanium dioxide particles in RAW264.7 cells associated with oxidative stress. J Toxicol Environ Health Part A; 71: 478-485.

Kim YR, Park JI, Lee EJ, Park SH, Seong NW, Kim JH, et al. (2014b) Toxicity of 100 nm zinc oxide nanoparticles: a report of 90-day repeated oral administration in Sprague Dawley rats. International journal of nanomedicine. 9 Suppl 2:109-26.

Kim SH, Heo Y, Choi SJ, Kim YJ, Kim MS, Kim H, et al. (2016) Safety evaluation of zinc oxide nanoparticles in terms of acute dermal toxicity, dermal irritation and corrosion, and skin sensitization. Molecular and Cellular Toxicology.12(1):93-9.

Kuo TR, Wu CL, Hsu CT, lo W, Chiang SJ, Lin SJ, Dong CY & Chen CC. (2009) Chemical enhancer induced changes in the mechanisms of transdermal delivery of zinc oxide nanoparticles. Biomaterials. 30: 3002-3008.

Kwon JY, Lee SY, Koedrith P, Lee JY, Kim KM, Oh JM, et al. (2014) Lack of genotoxic potential of ZnO nanoparticles in in vitro and in vivo tests. Mutation research Genetic toxicology and environmental mutagenesis.761:1-9.

Lademann J, Knorr F, Richter H, Blume-Peytavi U, Vogt A, Antoniou C, Sterry W & Patzelt A. (2008) Hair follicles – an efficient storage and penetration pathway for topically applied substances. Skin Pharmacol Physiol. 21: 150-155.

Lademann J, Otberg N, Richter H, Weigmann HJ, Lindemann U, Schaefer H & Sterry W. (2001) Investigation of follicular penetration of topically applied substances. Skin Pharmacol Appl Skin Physiol. 14: 17-22.

Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G & Sterry W. (1999) Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol. 12: 247-256.

Landsiedel R, Kapp MD, Schulz M, Wiench K & Oesch F. (2009) Genotoxicity investigations on nanomaterials: Methods, preparation and characterization of test material, potential artifacts and limitations-Many questions, some answers. Mutat Res. 681(2-3): 241-58.

Lanone S, Rogerieux F, Geys J, Dupont A, Maillot-Marechal E, Boczkowski J, Lacroix G, Hoet P. (2009) Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Particle Fibre Toxicol. 6(14): 1-12.

Lautenschlager S, Wulf HC, Pittelkow MR. (2007) Photoprotection. The Lancet. 370: 528-537.

Leite-Silva VR, Le Lamer M, Sanchez WY, Liu DC, Sanchez WH, Morrow I, et al. (2013) The effect of formulation on the penetration of coated and uncoated zinc oxide nanoparticles into the viable epidermis of human skin in vivo. European Journal of Pharmaceutics & Biopharmaceutics.84(2):297-308.

Leite-Silva VR, Liu DC, Sanchez WY, Studier H, Mohammed YH, Holmes A, et al. (2016) Effect of flexing and massage on in vivo human skin penetration and toxicity of zinc oxide nanoparticles. Nanomedicine : nanotechnology, biology, and medicine.;11(10):1193-205.

Li Y, Zhang W, Niu J, Chen Y. (2012) Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. ACS nano. 6(6):5164-73.

Linnainmaa K, Kivipensas P & Vainio H. (1997) Toxicity and cytogenetic studies of ultrafine titanium dioxide in cultured rat liver epithelial cells. Toxicol in Vitro. 11: 329-335.

Liu HL, Yang HL, Lin BC, Zhang W, Tian L, Zhang HS, et al. (2015) Toxic effect comparison of three typical sterilization nanoparticles on oxidative stress and immune inflammation response in rats. Toxicology Research.4(2):486-93.

Liu S, Xu L, Zhang T, Ren G & Yang Z. (2010) Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. Toxicology. 267(1-3): 172-7.

Long T, Saleh N, Tilton R, Lowry G & Veronesi B. (2006) Titanium dioxide (P25) produces reactive oxygen species in immortalised brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol. 40(14): 4346-4352.

Mavon A, Miquel C, Lejeune O, Payre B & Moretto P. (2007) In vitro percutaneous absorption and in vivo stratum corneum distribution of an organic and a mineral sunscreen. Skin Pharmacol Physiol. 20: 10-20.

McCall MJ, Coleman VA, Hermann J, Kirby JK, Gardner IR, Brent PJ & Johnson CM. (2013) A tiered approach. Nature Nanotechnol. 8: 307-308.

Miyani VA, Hughes MF. (2016) Assessment of the in vitro dermal irritation potential of cerium, silver and titanium nanoparticles in a human skin equivalent model. Cutaneous & Ocular Toxicology.1-27.

Monteiro-Riviere NA, Wiench K, Landsiedel R, Schulte S, Inman AO & Riviere JE. (2011) Safety Evaluation of Sunscreen Formulations Containing Titanium Dioxide and Zinc Oxide Nanoparticles in UVB Sunburned Skin: An In Vitro and In Vivo Study. Toxicol Sci. 123(1): 264-280.

Naess EM, Hofgaard A, Skaug V, Gulbrandsen M, Danielsen TE, Grahnstedt S, et al. (2016) Titanium dioxide nanoparticles in sunscreen penetrate the skin into viable layers of the epidermis: A clinical approach. Photodermatology Photoimmunology and Photomedicine.32(1):48-51.

NANODERM. (2007) Quality of skin as a barrier to ultra-fine particles (Project ID: QLK4-CT-2002-02678).

Nash JF. (2006) Human Safety and Efficacy of Ultraviolet Filters and Sunscreen Products. Dermatol Clin. 24: 35-51.

Newman MD, Stotland M, Ellis JI. (2009) The safety of nanosized particles in titanium dioxide and zinc oxide based sunscreens. J Am Acad Dermatol. 614: 685-692.

Nohynek GJ, Antignac E, Re T & Toutain H. (2010) Safety assessment of personal care products/cosmetic and their ingredients. Toxicol Appl Pharmacol. 243: 239-259.

Nohynek GJ, Dudour EK & Roberts MS. (2008) Nanotechnology, cosmetics and the skin; is there a health risk? Skin Pharmacol Physiol. 21: 136-149.

Oberdoerster G. (2000) Toxicology of ultrafine particles: in vivo studies. Phil Trans R Soc Lond. 358: 2719-2740.

Osmond-Mcleod MJ, Oytam Y, Kirby JK, Gomez-Fernandez L, Baxter B, McCall MJ. Dermal absorption and short-term biological impact in hairless mice from sunscreens containing zinc oxide nano- or larger particles. Nanotoxicology. 2014;8(SUPPL. 1):72-84.

Osmond-McLeod MJ, Oytam Y, Rowe A, Sobhanmanesh F, Greenoak G, Kirby J, et al. (2016) Long-term exposure to commercially available sunscreens containing nanoparticles of TiO2 and ZnO revealed no biological impact in a hairless mouse model. Particle and fibre toxicology.;13(1):44.

Otberg N, Patzelt A, Rasulev U, Hagemeister T, Linscheid M, Sinkgraven R, Sterry W & Lademann J. (2008) The role of hair follicles in the percutaneous absorption of caffeine. Brit J Clin Pharmacol. 65(4): 488-492.

Pal A, Alam S, Chauhan LKS, Saxena PN, Kumar M, Ansari GN, et al. (2016a) UVB exposure enhanced the dermal penetration of zinc oxide nanoparticles and induced inflammatory responses through oxidative stress mediated by MAPKs and NF-kappaB signaling in SKH-1 hairless mouse skin. Toxicology Research.;5(4):1066-77.

Pal A, Alam S, Mittal S, Arjaria N, Shankar J, Kumar M, et al. (2016b)UVB irradiation-enhanced zinc oxide nanoparticles-induced DNA damage and cell death in mouse skin. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 807:15-24.

Park HS, Shin SS, Meang EH, Hong JS, Park JI, Kim SH, et al. (2014) A 90-day study of subchronic oral toxicity of 20 nm, negatively charged zinc oxide nanoparticles in Sprague Dawley rats. International journal of nanomedicine.;9 Suppl 2:79-92.

Park MVDZ, Lankveld DPK, van Loveren H & de Jong WH. (2009) The status of in vitro toxicity studies in the risk assessment of nanomaterials. Nanomedicine. 4: 669-685.

Pflücker F, Hohenberg H, Hoelzle E, Will T, Pfeiffer S, Wepf R, Diembeck W & Gers-Barlag H. (1999) The outermost stratum corneum layer is an effective barrier against dermal uptake of topically applied micronized titanium dioxide. Int J Cosmet Sci; 21: 399-411.

Pflücker F, Wendel V, Hohenberg H, Gärtner, Will T, Pfeiffer S, Wepf R & Gers-Barlag H. (2001) The human stratum corneum layer: an effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide. Skin Pharmacol Appl Skin Physiol. 14(suppl 1): 92-97.

Popov A, Lademann J, Priezzhev A & Myllyla R. (2005) Effect of size of TiO2 nanoparticles embedded into stratum corneum on ultraviolet-A and ultraviolet-B sunblocking properties of the skin. J Biomed Optics. 10(6): 064037-1 - 064037-9.

Position of Titanium Dioxide Stewardship Council: http://www.cefic.org/Documents/About%20us/Industry%20sectors/Industry-responds-to-Nano-TiO2-study-published-in-American-Association-for-Cancer-Research-Journal.pdf

Rancan F, Nazemi B, Rautenberg S, Ryll M, Hadam S, Gao Q, et al. (2014) Ultraviolet radiation and nanoparticle induced intracellular free radicals generation measured in human keratinocytes by electron paramagnetic resonance spectroscopy. Skin research and technology :;20(2):182-93.

Reddy KM, Feris K, Bell J, Wingett DG, Hanley C & Punnoose A. (2007) Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett. 90: 213902-1–213902-3.

Reis Ede M, de Rezende AA, Santos DV, de Oliveria PF, Nicolella HD, Tavares DC, et al. (2015) Assessment of the genotoxic potential of two zinc oxide sources (amorphous and nanoparticles) using the in vitro micronucleus test and the in vivo wing somatic mutation and recombination test. Food and chemical toxicology.;84:55-63.

Rostan EF, DeBuys HV, Madey DL, Pinnell SR. (2002) Evidence supporting zinc as an important antioxidant for skin. Int J Dermatol; 41: 606-611.

Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA (2007) Variables influencing interactions of untargeted quantum dot nanoparticles with skin cells and identification of biochemical modulators. Nano Lett; 7(5): 1344-1348.

Sadrieh N, Wokovich AM, Gopee NV, Zheng J, Haines D, Parmiter D, Siitonen PH, Cozart CR, Patri AK, McNeil SE, Howard PC, Doub WH & Buhse LF. (2010) Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO2 particles. Toxicol Sci; 115(1): 156-66.

Sayes CM & Warheit DB. (2009) Characterization of nanomaterials for toxicity assessment. Wiley Interdiscip Rev Nanomed Nanobiotechnol; 1(6): 660-70.

Sayes MC, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB & Colvin VL. (2006) Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci; 92: 174–85.

SCCS (Scientific Committee on Consumer Safety), Opinion on ZnO (nano form), 18 September 2012

Schilling K, Bradford B, Castelli D, Dufour E, Nash JF, Pape W, Schulte S, Tooley I, van den Bosch J & Schellauf F. (2010) Human safety review of "nano" titanium dioxide and zinc oxide. Photochem Photobiol Sci; 9(4): 495-509.

Shim KH, Jeong KH, Bae SO, Kang MO, Maeng EH, Choi CS, et al. (2014) Assessment of ZnO and SiO2 nanoparticle permeability through and toxicity to the blood-brain barrier using Evans blue and TEM. International journal of nanomedicine.;9 Suppl 2:225-33.

Shrivastava R, Raza S, Yadav A, Kushwaha P, Flora SJS. (2014) Effects of sub-acute exposure to TiO2, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. Drug and chemical toxicology.37(3):336-47.

Shukla RK, Kumar A, Vallabani NV, Pandey AK, Dhawan A. (2014) Titanium dioxide nanoparticle-induced oxidative stress triggers DNA damage and hepatic injury in mice. Nanomedicine (London, England).9(9):1423-34.

Schulz J, Hohenberg H, Pflücker F, Gärtner E, Will T, Pfeiffer S, Wepf R, Wendel V, Gers-Barlag H, Wittern KP. (2002) Distribution of sunscreens on skin. Adv Drug Deliv Rev; 54(1): 157-163.

Seker S, Elcin AE, Yumak T, Sinag A, Elcin YM. (2014) In vitro cytotoxicity of hydrothermally synthesized ZnO nanoparticles on human periodontal ligament fibroblast and mouse dermal fibroblast cells. Toxicology in vitro : an international journal published in association with BIBRA. 28(8):1349-58.

Senzui M, Tamura T, Miura K, Ikarashi Y, Watanabe Y, Fujii M. (2010) Study on penetration of titanium dioxide (TiO2) nanoparticles into intact and damaged skin in vitro. J Toxicol Sci; 35(1): 107-113.

Serpone N, Salinaro A, Emeline A. (2001) Deleterious effects of sunscreen titanium dioxide nanoparticles on DNA: efforts to limit DNA damage by particle surface modification. Proc. SPIE; 4258:86-98.

Setyawati MI, Tay CY, Leong DT. (2015) Mechanistic Investigation of the Biological Effects of SiO2, TiO2, and ZnO Nanoparticles on Intestinal Cells. Small (Weinheim an der Bergstrasse, Germany).11(28):3458-68.

Scientific Committee on Consumer Products (SCCP, 2007): Opinion on safety of nanomaterials in cosmetic products. SCCP/1147/07. European Commission.

Shi Z, Niu Y, Wang Q, Shi L, Guo H, Liu Y, et al. (2015) Reduction of DNA damage induced by titanium dioxide nanoparticles through Nrf2 in vitro and in vivo. Journal of hazardous materials. 298:310-9.

Sliwinska A, Kwiatkowski D, Czarny P, Milczarek J, Toma M, Korycinska A, et al. (2015) Genotoxicity and cytotoxicity of ZnO and Al2O3 nanoparticles. Toxicology mechanisms and methods. 25(3):176-83.

Smulders S, Golanski L, Smolders E, Vanoirbeek J, Hoet PH. (2015) Nano-TiO2 modulates the dermal sensitization potency of dinitrochlorobenzene after topical exposure. The British journal of dermatology.;172(2):392-9.

Stone V, Johnston H, Schins PF. (2009) Development of in vitro systems for nanotechnology: methodological considerations. Crit Rev Toxicol. 39(7): 613-626.

Syama S, Sreekanth PJ, Varma HK, Mohanan PV. (2014) Zinc oxide nanoparticles induced oxidative stress in mouse bone marrow mesenchymal stem cells. Toxicology mechanisms and methods. 24(9):644-53.

Tan MH, Commens CA, Burnett L, Snitch PJ. (1996) A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. Aust J Dermatol. 37: 185-187.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP, 2003): Evaluation and opinion on zinc oxide. Report no. SCCNFP/0649/03, final. COLIPA no. S76.

Tran DT & Salmon R. (2010) Potential photocarcinogenic effects of nanoparticle sunscreens. Austral. J Dermatol.

Trouiller B, Reliene R, Westbrook A, Solaimani P & Schiestl RH. (2009) Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer Res. 69(22): 8784-9.

Tsuji JS, Maynard AD, Howard PC, James JT, Lam C, Warheit DB & Santamaria AB. (2007) Research Strategies for Safety Evaluation of Nanomaterials. Part IV: Risk Assessment of Nanoparticles Toxicol. Sci. 89: 42-50.

Tucci P, Porta G, Agostini M, Dinsdale D, Iavicoli I, Cain K, et al. (2013) Metabolic effects of TiO2 nanoparticles, a common component of sunscreens and cosmetics, on human keratinocytes. Cell death & disease. 4:e549.

Turci F, Peira E, Corazzari I, Fenoglio I, Trotta M, Fubini B. (2013) Crystalline phase modulates the potency of nanometric TiO(2) to adhere to and perturb the stratum corneum of porcine skin under indoor light. Chemical research in toxicology. 26(10):1579-90.

Uchino T, Tokunaga H, Ando M, Utsumi H. (2002) Quantitative determination of OH radical generation and its cytotoxicity induced by TiO(2)-UVA treatment. Toxicol In Vitro 16(5): 629-635.

Uzar NK, Abudayyak M, Akcay N, Algun G, Ozhan G. (2015) Zinc oxide nanoparticles induced cyto- and genotoxicity in kidney epithelial cells. Toxicology mechanisms and methods. 25(4):334-9.

Virkutyte J, Souhail R.A. & Dionysiou DD. (2012) Depletion of the protective aluminium hydroxide coating in Ti-O_2-based sunscreens by swimming pool water ingredients. Chem. Engineering J. 191:95-103.

Vogt A, Combadiere B, Hadam S, Stieler K, Lademann J, Schaefer H, Autran B, Sterry W, Blume-Peytavi U. (2006) 40 nm, but not 750 or 1500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. J Invest Dermatol; 126: 1316-1322.

Wamer WG, Yin JJ, Wei RR. (1997) Oxidative damage to nucleic acids photosensitised by titanium dioxide. Free Radical Biol Med; 23(6): 851-858.

Wang SQ & Tooley IR. (2011) Photoprotection in the era of nanotechnology. Semin Cutan Med Surg. 30: 210-213.

Wang Y, Cui H, Zhou J, Li F, Wang J, Chen M, et al. (2015) Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. Environmental science and pollution research international.22(7):5519-30.

Warheit DB & Donner EM. (2010) Rationale of genotoxicity testing of nanomaterials: regulatory requirements and appropriateness of available OECD test guidelines. Nanotoxicology; 4: 409-13.

Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM. (2007) Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicol Lett; 171: 99-110.

Wright C, Iyer AK, Wang L, Wu N, Yakisich JS, Rojanasakul Y, et al. (2016) Effects of titanium dioxide nanoparticles on human keratinocytes. Drug & Chemical Toxicology. 1-11.

Wu J, Liu W, Xue C, Zhou S, Lan F, Bi L, Xu H, Yang X & Zeng FD. (2009) Toxicity and penetration of TiO2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. Toxicol Lett; 191(1): 1-8.

Xiao L, Liu C, Chen X, Yang Z.(2016) Zinc oxide nanoparticles induce renal toxicity through reactive oxygen species. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association.90:76-83.

Xue C, Wu J, Lan F, Liu W, Yang X, Zeng F & Xu H. (2010) Nano titanium dioxide induces the generation of ROS and potential damage in HaCaT cells under UVA irradiation. J Nanosci Nanotechnol; 10(12): 8500-7.

Yu KN, Chang SH, Park SJ, Lim J, Lee J, Yoon TJ, et al. Titanium Dioxide Nanoparticles Induce Endoplasmic Reticulum Stress-Mediated Autophagic Cell Death via Mitochondria-Associated Endoplasmic Reticulum Membrane Disruption in Normal Lung Cells. PloS one. 2015;10(6):e0131208.

Yu KN, Sung JH, Lee S, Kim JE, Kim S, Cho WY, et al. (2015) Inhalation of titanium dioxide induces endoplasmic reticulum stress-mediated autophagy and inflammation in mice. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association.85:106-13.

Zhang LW, Monteiro-Riviere NA. (2008) Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. Skin Pharmacol Physiol; 21: 166-180.

Zijno A, De Angelis I, De Berardis B, Andreoli C, Russo MT, Pietraforte D, et al. (2015) Different mechanisms are involved in oxidative DNA damage and genotoxicity induction by ZnO and TiO2 nanoparticles in human colon carcinoma cells. Toxicology in Vitro. 29(7):1503-12.

Zvyagin AV, Zhao X, Gierden A, Sanchez W, Ross JA, Roberts MS. (2008) Imaging of zinc oxide nanoparticle penetration in human skin in vitro and in vivo. J Biomed Optics; 13(6): 064031-1 - 064031-9.

Version history

Version	Description of change	Author	Effective date
V1.0	Original publication	Toxicology Section, Scientific Evaluation Branch	August 2013
V1.1	Update	Toxicology Section, Scientific Evaluation Branch	August 2016

Therapeutic Goods Administration

PO Box 100 Woden ACT 2606 Australia
Email: info@tga.gov.au Phone: 1800 020 653 Fax: 02 6203 1605
https://www.tga.gov.au

Reference/Publication #