A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens

An on-line search was undertaken on the following databases: Medline, Embase, Biosis, Cabi and a Dialog search on a large number of medical and pharmaceutical databases as well as Google. The search strategy used was:

(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)

Reference 1


Summary and comment

In this study, the dermal penetration of coated microparticles of TiO2 was studied. This study aimed at explaining the findings of Tan et al (18), who found TiO2 in the epidermis and dermis of TiO2 treated human skin samples taken during surgery. It was stated that it was of great importance to determine whether TiO2 was present within dermal tissue or whether it might be localised in orifices of the hair follicles that reach down into the dermis.

The distribution of the microparticles in the horny layer was analysed using tape stripping to remove layers of skin and spectroscopic measurements of particle positioning. Biopsies were conducted to further investigate the distribution of the microparticles into deeper layers. Human volunteers had 2 mg/m² of the test substance applied to than area of 160 cm² of the left forearm; the right forearm (control) had vehicle only (-TiO2) applied to it in a similar manner. The application rate was 5 times/day on days 1, 2 and 3, and once on day 4.

Results showed that the deeper layers of non-follicular stratum corneum were free of TiO2. TiO2 concentration decreased rapidly with increasing depth of the stratum corneum. The corneocytes were free of microparticles in the lower half of the horny layer. The only structures containing microparticles of TiO2 were the pilosebaceous orifices. Potential penetration to deeper sites (studied using biopsy) was assessed, with TiO2 only found in the open part of the follicle. The detected TiO2 concentration inside these follicles was two orders of magnitude smaller than in the upper part of the horny layer. It was also noted that only some follicles contained TiO2 particles.
Penetration of microparticles of TiO2 into viable skin tissue could not be detected. The biopsy failed to detect TiO2 in the epidermal tissue outside of the follicles.

The potential for TiO2 to move out of the follicles and into the viable tissue was not discussed. However, the follicle is lined by a cellular inner and outer root sheath of epidermal origin and is invested with a fibrous sheath derived from the dermis. Penetration of TiO2 through this sheath would probably be unlikely, since no TiO2 was found in either the epidermal or dermal tissue surrounding the follicle.

Reference 2


Summary and comment

This is a review article examining the use of microemulsions (vehicle) for cutaneous drug delivery (through the skin), with focus on the influence of composition and structure of vehicles on drug delivery. Microemulsions aggregates were described as typically <150 nm, which is above the dimension (<100 nm) used to describe/define nanoparticles. Microemulsions can be formed with numerous different aqueous, surfactant and oil constituents. The article does not mention TiO2 or ZnO, which are actives (UV filters) formulated in sunscreens to stay on the skin surface to limit UV exposure of the skin.

Reference 3


Summary and comment

This study investigated the possibility that the skin was a route of exposure and sensitisation in chronic beryllium disease. Exposure to beryllium causes an incurable occupational lung disease, chronic beryllium disease (CBD), in approx. 3-5% exposed workers. CBD is a progressive granulomatous disease characterised by an MHC lymphocytosis. Industrial hygiene studies demonstrated that disease prevalence correlates with beryllium ultrafine particle counts, not with beryllium mass measurements (2 articles cited). This study examined the potential sensitisation activity of beryllium (500-1000 nm) applied topically to the ear of mice in the local lymph node assay (LLNA). In a separate in vitro assay using human skin, penetration of fluoro-spheres (0.5, 1, 2 & 4 μm dia. fluorescein isothiocynate conjugated dextran beads) in the layers of the skin was assessed. The data indicated that fluoro-spheres (≤ 1μm) did pass into the stratum corneum into the epidermis (location of immune reactive structures) of human skin when flexed in vitro; no fluoro-sphere penetration was seen in skin that was not flexed. This study showed that topically applied beryllium could trigger an increased ear thickness in a murine (LLNA) model, which is consistent with the development in a cell-mediated immune response. There have been no reports of titanium or zinc
triggering a similar response. This study did not use beryllium in the skin penetration section of the study; however, it did suggest that dermal absorption could be influenced by size of particle and flexing the skin.

Reference 4


Summary and comment

This study examined the morphologic basis for a pore-pathway (localisation) in mammalian stratum corneum. Using mice, tracer agents (ferritin HRP; lanthanum salts; sucrose; FITC-dextran; no mention of size of particle) were applied to skin (in vivo) and their movement through the skin was followed. This study used exaggerated conditions treating the skin with high energy sound (sonophoresis) and absorption enhancers to assist the passage of the tracers into the skin. It was shown that tracers invariably localised to discrete lacunar (space between cellular elements of epidermis) domains as a result of alignment following sonophoresis or the use of absorption enhancers. With the use of sonophoresis or absorption enhancers it appears the alignment of lacunae creates structural continuity and enables substances to pass through; lacunar domains remained discontinuous under normal basal conditions (limited movement of substances through skin). This study did not study the movement of nanoparticles of Ti or Zn through skin. It did look at possible pathways for the movement of substances through skin under extremely exaggerated conditions.

Reference 5


Summary and comment

The primary focus of this article was assessing the adverse effects of a surface layer of radioactive material (radio-pollutants) on the skin and clothes following accidental airborne nuclear releases. It was concluded that the dose to skin from β-emitters and the whole-body dose from γ-emitters on body surfaces were found to give potentially significant contributions to dose. In addition, it was suggested that skin penetration of some contaminants could lead to significant internal doses. Ti and Zn were not mentioned in this article. It was noted that physical clearance of skin contaminants was inversely proportional to particle size (not nano-scale particles); smaller particles had longer half-lives. It was noted that shedding of the stratum corneum would influence the clearance of skin contaminants. Also, a variable between animal models and humans, which could significantly affect skin permeability, is the number of hair follicles per unit of skin area. Some degree of skin permeability has been associated with movement of substances into hair follicles and into lower layers of skin bypassing the stratum corneum. Penetration of iodine (elemental and radioactive) was the main concern following exposure of the skin to fallout contaminants.
Reference 6


**Summary and comment**

This article investigated the influence of specific follicle properties, sebum production and hair growth on the follicular penetration of topically applied substances. This article did not include Zn, and did not examine particle size in the study protocol. In the introduction the authors discussed microparticulate TiO2 and its ability to penetrate into the stratum corneum during long-term application. Small amounts of TiO2 were found in deeper parts of the stratum corneum in the follicle orifices, while the surrounding corneocyte aggregates were free of TiO2 in these parts of the horny layer. TiO2 microparticles penetrated into the acroinfundibulum of follicles without reaching the layer of viable cells. The microparticles were not found in every hair follicle, but only in 1 in 10.

Three different penetration pathways were identified; the intercellular, the transcellular and the follicular penetration routes. It did show that the penetration process of topically applied substances depends on the phase of the hair growth cycle. The follicles are active when hair growth and/or sebum production are detected. The follicles are inactive when no hair growth and no sebum production can be measured.

Reference 7


**Summary and comment**

The distribution of micronised TiO2 (sunscreens in general) on the skin was investigated. The SPF (efficacy) of sunscreens depends on the distribution of the sunscreen on the skin. Three different products containing nanoparticles (over size range of 10-100 nm) of TiO2 (application rate 4 mg/cm² for each product) were used in this study. These products were formulated differently using different coatings (trimethyl/octylsilane, Al2O3 and SiO2) that changed the properties of the material (hydrophobic, amphiphilic). It was thought the distribution of the active TiO2 on/in the skin may vary as a result of the difference in base phases. This was an *in vivo* study using human volunteers (sunscreens applied to forearm). Punch biopsies were conducted on each subject, with the distribution of TiO2 assessed in each skin sample/section.

The sunscreen products were found to provide protection against sunburn. It was stated that micronised TiO2 was solely deposited on the outermost surface of the stratum corneum and could not be detected in deeper skin layers (epidermis and dermis). The surface characteristics, particle size and shape of the micronised TiO2 did not affect its absorption through the stratum corneum. The coatings characteristics did not affect the absorption of TiO2 through the skin.
The authors concluded that sunscreens using TiO2 nanoparticles were both effective and safe for to provide topical protection against the sun in humans. However, these conclusions were based on limited exposure and subject numbers.

Reference 8

Nanoderm Project: Quality of skin as a barrier to ultra-fine particles. LIFE QUALITY January 2003.

Summary and comment

Unavailable for assessment.

Reference 9


Summary and comment

This article (review) described deleterious effects of micronised TiO2 on DNA. Details of study methodology were not included so an analysis of the studies (in vitro) could not be carried out. The authors indicated that they had tested for the formation of the hydroxyl (●OH) radicals produced on irradiation of TiO2 extracted from sunscreens. They verified TiO2 as an initiator of harmful reactions inducing DNA damage (strand breaks) through the generation of free radicals by photo-catalytic reactions. The indicated that the results were generated in both in vitro and in vivo studies using human cells.

The authors then described how they have focused on producing the most photo-catalytically inactive TiO2 specimens for possible use in sunscreens, while retaining the spectroscopic features of TiO2 that make it an excellent UVA/UVB blocker. They did not describe the process to alter TiO2 reactivity (appears to relate to surface activity properties of TiO2), but they stated that they had generated some promising TiO2 species that retained the photo-protection properties, but had greatly reduced photo-catalytic activity. They emphasised the need for more investigation into TiO2 prior to acknowledging that TiO2 was definitely safe for use in sunscreens.

Reference 10

RCC-CCR project, Czich A: In vitro test on induction of chromosome aberrations in V79 cells with HR 99/104702 (a), 00/T00017 (b), 00/106407 (c), after simultaneous irradiation with artificial.
Summary and comment

Unpublished, currently unable to assess.

The SCCNFP evaluated this study and their conclusions are as follows; “based on the structural chromosome aberrations observed in the absence and in the presence of UV light in both experiments, the test agent HR00/106407 (ZnO) dissolved in culture medium at different concentrations, displayed positive effects in cultured Chinese Hamster V79 cells under the conditions of the study. It should be noted that the observed effects were induced with low doses. This micronised material has clastogenic activity on mammalian cells cultured in vitro; it is also photoclastogenic in the same V79 cell system”.

Reference 11


Summary and comment

Unpublished, currently unable to assess. The SCCNFP evaluated this study and their conclusions are as follows; “based on the structural and/or numerical chromosome aberrations observed in the absence and in the presence of UV light, the test agent HR96/104702 (ZnO) dissolved in 10% emulsion in 3% Tego Care 450 at different concentrations showed positive effects in cultured Chinese Hamster Ovary (CHO) cells under the conditions of the study. Micronised material has clastogenic activity on mammalian cells cultured in vitro; it has been also shown, that the test agent displays aneugenic activity; it is also photoclastogenic and possibly photoaneugenic in the same cell system”.

Reference 12


Summary and comment

Unpublished, currently unable to assess. The SCCNFP evaluated this study and their conclusions are as follows; “based on the mean tail length (DNA damage), the test agent HR00/106407 dissolved in deionised water is considered photogenotoxic in cultured Chinese Hamster V79 cells under the conditions of the photocomet assay in vitro. It is worth noting that HaCaT cells (human keratinocytes – living cell of epidermis) were also tested in this assay, with no evidence of any DNA damage in the presence or absence of UV irradiation.

Reference 13

Summary and comment

There were 3 parts to this study. Firstly, this study examined chemical oxidation of TiO2 extracted from OTC sunscreen products using an organic substrate (phenol) under conditions of irradiation (310-400nm). Secondly, TiO2 (in water at 0.025% w/v) was mixed with an equal volume of plasmid DNA in buffer and illuminated. Direct strand breaks were assayed from the conversion of supercoiled plasmid to the relaxed form. Thirdly, in an assay described as in vivo (but actually cultured human fibroblast cells; so in vitro) illumination of DNA, which examined potential for DNA damage following exposure to TiO2 (0.0125% w/v). Included in parts of the analysis were anatase and rutile forms of TiO2 and ZnO.

TiO2 (both anatase and rutile) and ZnO caused strand breaks in plasmid DNA. Furthermore, the extracted TiO2 caused DNA damage in cultured human fibroblasts. There was some evidence that hydroxyl radicals may have played a part in the observed DNA effects, since damage was suppressed by DMSO and mannitol (free radical quenchers). Oxidation of phenol was accelerated by the presence of both forms TiO2 (to varying degrees) and ZnO.

It was concluded that there appears to be possible health hazards with TiO2 if it can enter viable/living cells after penetrating the stratum corneum. The authors of this study did indicate that absorption of TiO2 has yet to be adequately demonstrated.

The authors described the systems studied as in vitro and in vivo, but none are actually conducted in vivo since they used measurements of chemical reactivity (oxidation, phenol disappearance), suspensions of plasmids and cultured human fibroblast cells. Also, whether this study dealt with nanoparticles is unsure, because the TiO2 used in this study was extracted from marketed sunscreen products with no reference to size of particle extracted.

Reference 14


Summary and comment

This article primarily described work examining the toxicology of ultra-fine (UF) particles following exposure to airborne material.

The introduction described how UF particles (<100nm) can be encountered in ambient air and at the work place. In general, background levels in urban atmosphere are 1-4x10^4 cm^-3 (mass concentration < 2 μg.m^-3). Workplace concentrations can be appreciably higher coming from metal and polymer fumes, which can lead to acute inflammatory responses in the lung following inhalation. It was suggested studying the UF material generated at the workplace, although not representative of the composition of atmospheric air, can aid in the understanding of the toxicity of UF particles.

Inhalation studies in rats using UF polytetrafluoroethylene (PTFE) showed high levels of pulmonary toxicity, toxicity decreased following agglomeration to larger particles, repeated pre-exposure for very short periods had a protective effect (reduced toxicity) and PTFE moved into epithelial, interstitial and endothelial sites.
Further studies were carried out using UF carbonaceous material (likely to be found in urban atmosphere) in rats at an inhalation exposure of 100 μg.m⁻³. Varying conditions were included in the study protocols. It was shown that UF carbon could induce slight inflammatory responses; endotoxin priming and concurrent exposure to ozone heightened the inflammatory responsiveness to UF carbon; older lungs (22 months vs 10 weeks old rats) were more susceptible to UF particle induced oxidative stress.

Data for intra-tracheally instilled TiO₂ was described, where a comparison between UF (av. size 20nm) and fine (av. size 250nm) TiO₂ found the same mass dose of UF particles had a significantly greater (36 fold) inflammatory potential than fine particles. The value of 36 fold was derived from a 3.6 times greater deposition efficiency into the alveolar and a 10 fold larger particle surface area per given mass. Inflammation was assessed using measurements of cellular and biochemical markers in lung lavage. It was suggested that the increased surface area of UF particles was an important factor in the heightened responsiveness to UF particles.

The author described, “TiO₂ particles as rather benign in nature and have been used in the past in a number of studies a control particles of low toxic potency against which effects of other particles types have been compared”. The inflammatory response seen with UF TiO₂ was suggested as likely to be similar to the oxidative stress mechanism seen with the greater pulmonary intoxicant PTFE.

Overall, this study did not examine dermal application of UF TiO₂ and has little relevance to the use of UF TiO₂ in sunscreens. It did indicate that UF TiO₂ was likely to cause a greater pulmonary inflammatory response when compared to fine TiO₂. This finding would be considered to have relevance in the industrial workplace situation.

Reference 15


Summary and comment

This study was described as a pilot study to examine whether ultrafine (UF) elemental carbon particles translocate to the liver and other extrapulmonary organs following inhalation as singlet particles (20-29nm) in rats. UF ¹³C particles were presented as an aerosol, which was introduced into a whole-body inhalation chamber for an exposure period of 6 hours at concentrations of 80 and 180μg.m⁻³.

Normalised to exposure concentration, the added ¹³C particles/gram of lung in the post-exposure period was approximately 9 ng/g organ/μg/m⁻³. Significant amounts of ¹³C had accumulated in the liver by 30 minutes post-inhalation, but only at the higher concentration of UF labelled particles. At 18 and 24 hours post-exposure, the ¹³C in livers of all exposed rats was approximately 5 times greater than the ¹³C content of the lung. No significant increase in ¹³C was detected in other organs examined at the end of exposure. It was concluded that UF carbon particles could translocate from the lung to the liver by 1 day after
inhalation exposure. Translocation appeared to involve absorption from the lung and possibly the GIT (licking of fur to remove aerosol). Furthermore, it was suggested that translocation to blood and extrapulmonary tissues may well be different for UF carbon compared with metallic (eg. Ti and Zn) UF particles.

Overall, this study did not examine dermal application of UF TiO2 and has little relevance to the use of UF TiO2 in sunscreens.

Reference 16


Summary and comment

Dermal uptake properties of micronised TiO2 were studied *in vitro* using a Franz-type diffusion cell on excised porcine skin for 24 hours (limited exposure since single application). Localisation of TiO2 in the skin was determined using transmission electron microscopy (TEM). Tape stripping of the stratum corneum (SC) was also used to confirm the distribution.

TiO2 was found exclusively on the outermost SC layer. Surfaces deposits (using TEM) featured clearly distinguishable agglomerates, as well as single particles with a characteristic cubic shape and a primary particle size of about 20-50nm. Micrographs initially showed an even distribution of TiO2 on the skin surface, however, skin stripping showed TiO2 was localised in the furrows and not on the partially removed ridges of the skin surface. Tape stripping initially removed TiO2 and SC layers only from the ridges and not from the deeper furrows. TiO2 was found only in trace amounts in the upper part of the follicle without any evidence of uptake into follicular epithelium. It was suggested that this shows the follicles are not a relevant route of penetration for TiO2.

Reference 17


Summary and comment

This study examined the potential cytotoxicity (in presence and absence of photo-irradiation) of ultrafine (UF) TiO2 powder in HeLa cells, the mechanism for the cytotoxicity and the distribution of the TiO2 powder. The potential use of TiO2 in photokilling of malignant cells (photodynamic therapy) was discussed.

Cultured HeLa cells were exposed (incubation) to UF TiO2 (average size 30nm) in the presence and absence of electromagnetic radiation (varying wavelengths). Appropriate controls (minus irradiation/TiO2) were included in the study. Distribution of TiO2 through treated cells was determined using TEM.
During 10 minute exposure periods to UV radiation in the presence of TiO$_2$ (100 $\mu$g/mL) HeLa cells were completely killed. HeLa cells exposed to TiO$_2$ (up to 360 $\mu$g/mL), but not irradiated, showed slight cytotoxicity. It was suggested that cytotoxicity was caused by possibly two mechanisms, the formation of reactive radicals or direct oxidation by photo-generated holes in TiO$_2$.

Distribution analysis of TiO$_2$ in the cultured cells showed incorporation into cell membrane and some particles also in the cytoplasm; there was no evidence of penetration into the nucleus.

The data presented provided evidence of cytotoxicity (by oxidative stress) of rapidly dividing tumour cells in culture with TiO$_2$ following irradiation. This study used cultured viable HeLa tumour cells, which were in intimate contact with TiO$_2$ in the culture. There was no protective barrier (stratum corneum/epidermis) that is present when TiO$_2$ containing products are applied to the skin.

Reference 18


Summary and comment

This study was described as a pilot investigation into the potential percutaneous absorption of microfine TiO$_2$ from sunscreens. A group of patients ($n=13$; samples taken $n=16$) having surgery for skin lesions had agreed to have surplus skin removed following its treatment with a sunscreen containing 8% microfine TiO$_2$. The subjects had a mean age of 71 years (4 female, 9 males). Sunscreen was applied twice daily (morning/midday) to the skin surrounding the lesion to be excised for a period of 2-6 weeks. Treatment with the sunscreen was ceased 2 days prior to surgery. The skin was cleaned prior to surgery. After surgery, excess skin (treated with sunscreen) around the lesion was removed and used as a sample for analysis. A control sample group (n=9) came from cadaver skin collected from the hip region. Tape stripping of the skin was conducted on samples in preparation for analysis. Removal of the stratum corneum was followed by punch biopsies of the remaining tissue, which were digested (chemical breakdown) and the TiO$_2$ content measured.

It was noted that subjects applying the sunscreen (+TiO$_2$) did not sustain any trauma or rash at or around the application site. The data showed that there was no statistically significant difference in TiO$_2$ content (1.7 vs 1.2 $\mu$g/g tissue) between the treated skin (dermis) and the cadaver skin (untreated control), although the treated skin had a higher TiO$_2$ content. The investigators stated that the reason for the lack of significance was one outlining result in the control group. On this basis the investigators concluded that, “microfine TiO$_2$ may have greater potential to be percutaneously absorbed compared with commercial grade TiO$_2$”.

There must be some degree of doubt regarding the value of this study based on the age and disease state of the tissue used. Skin gets thinner and more fragile with age (mean 71 years in this study), and the lesions in the skin being removed may have caused changes the vascular...
permeability and tissue reactivity of the local and surrounding tissue to varying degrees. There was no indication as to whether the cadaver controls were age matched or had lesions near the site where skin samples were collected.

Also, distribution analysis was not conducted so the TiO2 found in the treated tissue may not be associated with release from viable cells; it could have localised in follicles in the epidermis and dermis and release on digestion of the skin prior to analysis.

The authors indicated there were inadequacies in the study based on limited sample size, TiO2 found near limit of detection and a lack of statistically significance difference between the test group and controls. Further limitations of this study may include the population of subjects sampled (old and diseased) and the lack of match controls (cadaver skin).

Reference 19


Summary and comment

This study does not examine the potential dermal absorption of TiO2, but looks at the UVA generated OH radical in the presence of different crystal forms (anatase and rutile) and sizes of TiO2 on free radical production.

UVA irradiation of the anatase form of TiO2 generated OH radicals in a dose/exposure related manner, while the rutile form (90 nm) was significantly less effective at generating OH radicals. It was noted that the crystal size had a significant influence on generation of OH radicals, but the optimum size for generation of OH radical was different for the different types of TiO2. Size and crystal form of TiO2 affected the UVA absorption characteristics, but there was no apparent relationship between UVA absorption and OH radical formation. Cytotoxicity of the OH radical was tested against cultured (in vitro) Chinese hamster ovary cells (CHO), with the CHO cells found to be sensitive to the amount of OH produced (dose-related).

Testing with UVA radiation was carried out up to 370 nm (based on filter used max. irradiation 370 nm), which would effectively omit half (370-400 nm) of the available UVA spectrum. This issue is unclear since later in the report they mention exposure at a wavelength up to 500 nm (into visible range).

This was an assay examining chemical reactions between TiO2 containing agents and UVA (limited) radiation; no biological cells were involved until the second phase, when CHO cells were exposed to the OH radicals that were generated. OH radicals were cytotoxic to CHO in vitro, depending on the conditions; it was noted that rutile did not change the viability of CHO cells significantly, while anatase did reduce the viability of CHO cells. The authors suggested that further clarification of the relationship between TiO2 type and size, and possible adverse biological activity is required.
Reference 20


Summary and comment

This study examined the potential adverse effects of introducing ultrafine TiO2 into the lungs of rats for up to 16 days. Ultrafine TiO2 (<30nm) was instilled (single dose) into the trachea at a dose of 2 mg/rat in physiological saline in a volume of 0.5 mL. A group of control rats received only saline. Animals (6/time interval) exposed to TiO2 and saline controls were sacrificed at 1, 4, 8 and 16 days following treatment. The sacrificed animals had their lungs lavaged 3 times with a collection buffer, with the collected lung lavage analysis for the presence of released biochemical markers (LDH, AP, macrophages, GSH, etc).

Significant increases in alveolar macrophages (AM) were seen at all times, but peaked at day 8. Acid phosphatase (AP) and lactate dehydrogenase (LDH) levels were increased on all analysis days, and also peaked on day 8. Peak levels were 59% and 75% above control levels for AP and LDH, respectively. Markers for lipid peroxidation were increased during the course of the study. Increased glutathione (GSH) redox reaction activity in AMs was observed throughout the study period. A significant and progressive depletion of GSH was seen in AMs in TiO2 exposed rats. It was suggested that this data exposure to TiO2 may lead to oxidative stress in the lungs of rats and potentially pathological changes.

This study would have greater relevance to occupational health issues, where exposure to powdered TiO2 during formulating products could lead to intake by the lungs. TiO2 in cream/lotion bases for sunscreens would not appear to be an issue for exposure to the lungs. Some of the parameters measured in this study have been described as having limited diagnostic value in rat models due to the variation in backgrounds levels when compared to humans; 12 fold higher in rats normally. Also, the process of lung lavage/perfusion on its own can cause large increases in the release of biochemical markers.

Reference 21


Notes

Article in German; no translation available.

Reference 22

Oberdoster E: Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. Environmental Health Perspectives, vol 112, no 10, July 2004
Summary and comment

This was a study examining the potential for a manufactured nanoparticle, fullerenes (C₆₀), to induce oxidative stress in the brain of juvenile largemouth bass (fish). Concern regarding the environmental impact of nanoparticles, in the form of adverse effects on wildlife, was the stimulus behind this study. The results of this study were interpreted as showing oxidative damage and depletion of GSH following exposure of a limited number of fish to manufactured nanoparticles.

This study did not examine dermal application/administration of UF TiO₂ in a mammalian species and has little relevance to the use of UF TiO₂ in sunscreens.

The issue of potential environmental contamination by nanoparticles was raised in the context that sunscreens containing nanoparticles may wash into the environment.

Reference 23

Zs Kertesz, Z Szikszai, A Z Kiss: Quality of skin as a barrier to ultra-fine particles. Contribution of the IBA Group to the NANODERM EU-5 PROJECT IN 2003 – 2004

Summary and comment

This report provides details of a consortium (consisting of 12 European universities and scientific institutes) that has used cutting edge techniques to investigate the possible penetration of micronised Ti, Zn and Si–oxides into the skin. Ion microscopy, electron microscopy and autoradiography are used to trace the penetration of the nanoparticles into the skin layers, while molecular and cell-biological methods are applied to assess the skin response and activation of dermal cells. Studies have been conducted using porcine and human skin samples. Resolution was described as, “quantitative elemental composition in all strata of the skin with detection limits of approximately 1μg/g and lateral resolution of 1-2μm”. Validation (consistency and reliability) across the laboratories (with varying methods) for accuracy was conducted, fairly good agreement (standard deviation 17-20%) across 6 laboratories.

Initial reported results were for 22 pig skin, 11 transplanted human skin and 13 human skin samples. The results generated using ion microscopy or electron microscopy shows that in healthy skin the nanoparticles penetrate into the deepest corneocyte layer of the skin, but never reach the vital layers. This report did not provide details of the application methods for the test substances.

Notes

Quoted in: Referenced in ATOMKI annual report

Reference 24

Summary and comment

This study characterised the ZnO (2.5%) and TiO2 (11.5%) content (type & size) of a commercially available sunscreen formulated without organic filters, as well as assessing the distribution of the sunscreen agents through the skin. Assessment of distribution of the sunscreen (1 mg/cm²) through the skin was conducted using abdominal human skin (from plastic surgery) in vitro. Physical characteristics of Zn and Ti were determined using X-ray diffraction and electron microscopy.

Analysis revealed TiO2 was present as a mixture of rutile and anatase. Microfine ZnO particles had a length of 116.8 nm and a length to width ratio of 2.03:1. The ZnO particles were described as being larger than the ultrafine TiO2 particles (no dimensions given), which indicates the TiO2 particles should be in the nanoparticle range.

Scanning of the skin after topical application of the sunscreen emulsion displayed an almost regular mineral coating of the stratum corneum. Mineral crystals appear to surround the desquamating corneocytes. However, both intercellular and intracellular penetration of mineral crystallites was not evident in transmission electron microscopy.

OVERVIEW

The studies summarised in the preceding report represent available published articles on issues relating to the use of nanoparticulate TiO2 and ZnO as UV filters in sunscreens; some related studies have been identified but were not in the public domain and not able to be accessed (or summarised). A number of the studies presented did not directly address the main issues identified below; type of toxicity of TiO2 or ZnO and ability of these inorganic materials to penetrate the skin to reach viable cells. Study numbers in the brackets below identify studies that provide relevant information on these issues.

Toxicity (pathological changes) that could occur following exposure to inorganic titanium dioxide and zinc oxide (UV filters) has been linked to free radical generation (9, 13, 17, 19 and 20). In these limited number of studies, there is evidence that TiO2 and ZnO can induce the production of free radicals (likely hydroxyl radicals through oxidation) and cause adverse effects in isolated cell experiments.

There were three unpublished studies (10, 11 and 12) investigating photo-mutagenicity (possibly linked to free radical formation) that had been discussed by the SCCNFP in their review of micronised ZnO. The SCCNFP conclusions for these studies have been recorded under “Notes” in the preceding document, since we don’t have access to these articles. The overall conclusion by the SCCNFP was that, “micronised 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”. A fourth article looking at photo-mutagenicity of micronised ZnO in cultured bacteria (Ames assay) was negative for mutagenic activity. Photo-toxicity was assessed by the SCCNFP on intact skin of human volunteers, with no evidence of any reactions in 2 photo-irritation studies (n=40) and 2 photosensitisation studies (n=55). Toxicokinetic assessment (in vivo) using human volunteers with healthy and diseased skin (psoriasis) found no evidence of an increase in systemic Zn levels after dermal
application of ZnO. An *in vitro* assay using human skin (with stratum corneum stripped away) indicated 0.34% absorption of an applied ZnO dose based on recovery from receptor fluid.

Dermal penetration (studies 1, 6, 7, 16, 18, 23, 24) of TiO₂ and ZnO through the outer layer of the skin was investigated in 8 studies, with 7 of the 8 indicating an inability of TiO₂ and ZnO to reach viable cells. The only study to suggest TiO₂ (not shown statistically) penetrated beyond the stratum corneum (18) had several limitations that put the validity of this study in doubt (ZnO not examined in this study).

It is possible that TiO₂ and ZnO in the presence of sunlight could catalyse the generation of free radicals, however the potential toxicity associated with this event would be nullified if it did not take place in viable cells. The available evidence suggests that the likelihood of penetration beyond the stratum corneum into viable cells is very low.

**Conclusion:**

There is evidence from isolated cell experiments that ZnO and TiO₂ can induce free radical formation in the presence of light and that this may damage these cells (photo-mutagenicity with ZnO). However, this would only be of concern in people using sunscreens if the ZnO and TiO₂ penetrated into viable skin cells. The weight of current evidence is that they remain on the surface of the skin and in the outer dead layer (stratum corneum) of the skin.

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