Dermal absorption of ZnO particles from sunscreens applied to humans at the beach

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I. INTRODUCTION

The incidence of skin cancer is increasing globally. The use of sunscreens is advocated to reduce the risk of skin cancer by filtering ultraviolet radiation when people are outdoors, either for recreational or occupational activities. Advances in formulation using nanotechnology have seen incorporation of ultraviolet (UV) absorbers titanium dioxide ($TiO_{2)}$ and zinc oxide (ZnO) into commercial sunscreens at amounts ranging from 4% to 30% w/w. These micronised sunscreens coat the skin as a transparent film and work primarily by reflecting and scattering light. The Australian Therapeutic Goods Administration stated that there are almost 400 sunscreen products, with many containing nanoparticulate TiO_2 and/or ZnO, commercially available in Australia.

Dermal penetration of metal oxide nanoparticles from personal care products remains controversial and there is ongoing media attention. Most investigations were *in vitro* using diffusion cells or animal models with very few *in vivo* human studies. Several reviews and some more investigations in recent years have concluded that nanoparticles do not penetrate the stratum corneum although they can lodge in hair follicles and sweat glands. The most comprehensive review to date of the use of nanoparticles in personal care products is by the Environmental Working Group [1], a US-based NGO, and they concluded after peer-review of more than 400 documents that: "zinc and titanium-based formulations are among the safest, most effective sunscreens on the market based on available evidence" and of 16 studies on skin absorption, "nearly all showing no absorption of small-scale zinc and titanium sunscreen ingredients through healthy skin".

II. METHODS

Concerns about dermal absorption and penetration of nanoparticles can be addressed with the approach of isotopic tracing [2], whereby an enriched stable isotope of the element of interest is incorporated into the product allowing any transfer to be detected using high resolution inductively coupled plasma mass spectrometry (ICP-MS), multi-collector ICP-MS or thermal ionization mass spectrometry.

This paper describes the use of stable isotopes of Zn for tracing potential absorption of Zn from ZnO nanoparticles in sunscreen applied to human skin under conditions of normal use. Zinc has 5 stable isotopes. One stable isotope ^{68}Zn has a natural abundance of 18.8%. The ZnO particles used in this study were enriched with ^{68}Zn to either 51% or >99%. Therefore increases in levels of ^{68}Zn in blood and urine samples would indicate dermal absorption of Zn from sunscreens. The sensitivity of the stable isotope method allows for detection of <0.1% absorption of Zn.

Three trials have been undertaken to evaluate the dermal absorption of ZnO particles from sunscreens in human volunteers. The first trial involved 2 male subjects with two applications to their backs of a formulation containing ZnO nanoparticles (with diameters of ~30nm) enriched to 51% with ^{68}Zn. Several blood and urine samples were collected throughout the day and for a number of days post trial. The second trial involved the same two males, and a female. The same formulation was applied twice daily for 5 days. Subjects experienced limited UV exposure as this trial was carried out in winter. Blood was sampled 3 times daily and urine at least 3 times daily and post trial for up to 126 days. These trials formed the basis and protocol refinement for the main beach trial in which particles of ZnO enriched to >99% with ^{68}Zn were incorporated into a different formulation. Two groups, each consisting of 10 people of various ages, skin classifications, and race, participated in the study at a Sydney beach in March 2009 (Figure 1). One group of 10 volunteers was tested with a sunscreen containing nanoparticles of ^{68}ZnO (~20nm) – the "nanoparticle" group (NP) -and the other group was tested with particles of ^{68}ZnO >100nm – the "bulk" group (Figure 2). Sunscreen was applied to the backs of the volunteers twice daily for a period of 5 days and the subjects experienced a minimum of 1 hour UV exposure in two episodes following sunscreen application. Blood was sampled twice daily and urine three times daily. Blood and urine samples were also supplied before the 5-day beach exposure and in a follow-up period.

Zinc was purified from blood and urine samples by ion exchange procedures. Changes in the isotopic abundance of ^{68}Zn of the purified samples, measured by multi-collector

inductively-coupled plasma mass spectrometry (MC-ICP-MS), were used to evaluate the dermal absorption of Zn from the sunscreens.

III. RESULTS AND DISCUSSION

Changes in the isotopic abundances are commonly expressed as ratios, in this case the enriched tracer ^{68}Zn divided by the naturally occurring ^{64}Zn, i.e. ^{68}Zn/^{64}Zn. Alternatively they can be presented as Wt% 68 tracer in the sample. In this instance we present the results as ratios.

Pilot Trials -

Results from the first two trials showed changes in the isotope ratios of <0.1% and it is estimated that this limits the dermal absorption to be <0.1%.

Beach Trial -

Changes in the ^{68}Zn/^{64}Zn ratio in blood samples for the NP group range from 0.1 to 0.8% at the end of the beach trial. Surprisingly all subjects showed significant increases in the abundance of ^{68}Zn 6 days after the completion of the trial (post-trial).

Changes in blood samples for the bulk group are similar to those for the NP group and also show the same trend of increased abundance of ^{68}Zn 6 days after the completion of the trial.

Excluding the data for 2 outliers, there is no statistically significant difference in dermal absorption for the volunteers in the NP and the bulk groups; the mean increase is about 0.4%.

Urine samples show larger increases in abundance of ^{68}Zn over the same time intervals but there is no simple relationship with changes in blood for the same volunteers.

Figure 1. Thanks to the volunteers!

Figure 2. Appearance on the skin of the formulation containing nanoparticles on the left and the bulk material on the right.

IV. CONCLUSIONS

These results provide the first conclusive evidence that Zn from ZnO particles in sunscreen penetrates healthy skin and is observed in blood and urine. Whether the Zn is present as particles or soluble Zn ions is unknown at this stage

These studies have been approved by human ethics committees at Macquarie University and CSIRO.

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