Science of the Total Environment 420 (2012) 313–318

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Comparison of dermal absorption of zinc from different sunscreen formulations and differing UV exposure based on stable isotope tracing

Brian Gulson [a,b,*], Herbert Wong [a], Michael Korsch [b], Laura Gomez [a], Philip Casey [c], Maxine McCall [d], Malcolm McCulloch [e], Julie Trotter [e], Jenny Stauber [f], Gavin Greenoak [g]

[a] *Graduate School of the Environment, Macquarie University, North Ryde NSW 2109, Australia*
[b] *CSIRO Earth Science and Resource Engineering, North Ryde NSW 2113, Australia*
[c] *CSIRO Materials Science and Engineering, Clayton VIC 3168, Australia*
[d] *CSIRO Food and Nutritional Sciences, North Ryde NSW 2113, Australia*
[e] *Research School of Earth Sciences, Australian National University, Canberra ACT 2601, Australia*
[f] *CSIRO Land and Water, Lucas Heights, Menai, NSW, 2232, Australia*
[g] *Australian Photobiology Testing Facility, Sydney University, NSW 2006, Australia*

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 9 December 2011
Accepted 20 December 2011
Available online 7 February 2012

Keywords:
Human
in vivo
Nanoparticles
Sunscreen
Zinc isotopes
Zinc oxide

ABSTRACT

In a pilot study to determine if zinc (Zn) from zinc oxide nanoparticles in sunscreen can penetrate human skin *in vivo*, nanoparticles (~30 nm) of a stable isotope (52% ^{68}Zn enrichment) were incorporated into an essentially phytochemical-based formulation and applied to the backs of 3 human subjects twice daily for 5 days during the Southern Hemisphere winter. Blood and urine were collected prior to application and at regular intervals and up to 50 days. As observed in a larger outdoor trial following this pilot study but with a different formulation and with UV exposure: values of ^{68}Zn in blood continued to increase beyond the 5 day application phase with the highest measurement at 14 days after the first application; variable amounts of the ^{68}Zn tracer were observed in urine; and the amounts of extra Zn added to blood were small and indicate very low levels of absorption (minimal estimate <0.01% of the applied dose) through the skin. Reasons for differences in absorption detected in the stable isotope trials and previous investigations include: the sensitivity of the stable isotope method; the duration of the investigations; the number of applications of sunscreen formulation; *in vitro* methods with excised skin; lack of measurement of blood and urine; no skin flexing; and lack of UV exposure.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Stable isotope tracing offers a powerful tool in nanotechnology to address many of the public health concerns related to the production and use of nanoparticles, including occupational and end-user exposure, pharmacokinetics, and fate and transport in the environment. In this approach a rare stable isotope of the element of interest is incorporated into the product allowing a distinction between the absorbed/migrated components versus background levels in the body or environment. The stable isotope approach differs from tracing using radiolabelled metals such as ^{65}Zn which generally have a short half-life, although, in the case of ^{65}Zn, the half-life of 245 days allows for longer term investigations (Wastney et al., 1986).

Since the idea of stable isotope tracing for nanotoxicology studies was first suggested in 2006 by Gulson and Wong, the approach has been implemented in nanostudies of dermal absorption of Zn from sunscreens in human volunteers (Gulson et al., 2010) and in mice (Osmond and McCall, 2010), in the environment (Croteau et al., 2011), and reviewed in PROSPECT (2010).

The use of sunscreens is advocated to reduce the risk of skin cancer by filtering ultraviolet (UV) radiation when people are outdoors, either for recreational or occupational activities. The UV filters employ either organic chemicals or metal oxides such as zinc oxide (ZnO) or titanium dioxide (TiO_2) as active ingredients; the metal oxides may be used in combination with organic chemicals. The metal oxides are usually, but not necessarily, in the nanoparticle size range, coat the skin as a transparent film, and in the case of ZnO, filter out the full range of UVA and UVB radiation.

Several reviews (EWG, Environmental Working Group, 2009; Gonzalez, 2010; Monteiro-Riviere and Baroli, 2010; Nohynek et al., 2007, 2010; Schilling et al., 2010; TGA, 2009) and recent investigations (Cross et al., 2007; Inman et al., 2010; Monteiro-Riviere et al., 2011; Roberts et al., 2008; Sadrieh et al., 2010; Zvyagin et al., 2008) have concluded that metal oxide nanoparticles localise to, but are not absorbed from, the stratum corneum although they can lodge in hair follicles (Lademann et al., 1999; Nanoderm, 2007), sweat glands

* Corresponding author at: Graduate School of the Environment, Building E8B Room 322, Department of Environment and Geography, Faculty of Science, Macquarie University, Sydney, New South Wales 2109, Australia. Tel.: +61 2 9850 7983; fax: +61 2 9850 7972.
E-mail address: brian.gulson@mq.edu.au (B. Gulson).

0048-9697/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2011.12.046

or skin folds (Zvyagin et al., 2008). Nevertheless, non-governmental organisations such as Friends of the Earth express concern over the use of nanoparticles in personal care products, especially sunscreens, and have questioned their use, and offered alternative products in Australia (FOE, Friends of the Earth, 2010).

We undertook a pilot study as a prelude to a larger and more expensive study of dermal penetration of Zn from ZnO particles in sunscreen in human volunteers in an outdoor setting (Gulson et al., 2010). In the pilot study of 3 volunteers under conditions of limited UV exposure, we used a Zn tracer whose enrichment was only half that of the tracer for the outdoor trial as calculations showed that even with this enrichment it would be feasible to detect absorption of <0.0 l% of the applied sunscreen dose; limited funds available for the pilot trial necessitated the use of the lower enriched tracer.

In this paper we compare the results from the pilot study with those of the outdoor trial which employed a different formulation and with UV exposure. The findings of this pilot study are also presented to allay concerns that results of the outdoor trial are at odds with all other studies.

2. Materials and methods

2.1. Sunscreen

A generic oil–water based sunscreen formulation containing ZnO nanoparticles enriched in the stable isotope ^{68}Zn tracer was the basis of these trials. Zinc has 5 stable isotopes, the abundances of which are shown in Fig. 1. ^{68}Zn has a natural abundance of 18.8% while that in the tracer is 52%.

The starting material for nanoparticle production was 30 g of the ^{68}ZnO tracer with a particle size of about 1–2 μm (Fig. 2). Nanoparticles with a final particle diameter of about 30 nm were made using a proprietary method based on high energy attrition milling (Casey et al., 2006). Protocols for the preparation of the ZnO nanoparticles and their characterisation are the same as described in Gulson et al. (2010) and are not repeated here. The uncoated nanoparticles were incorporated into a generic oil–water, essentially phytochemical-based formulation, similar to a commercial sunscreen product; the composition is shown in Table 1. The sunscreen contained ~18% w/w ZnO nanoparticles enriched with the stable isotopes of Zn as shown in Fig. 1. Scanning electron microscopy (SEM) analysis showed a relatively even distribution of the nanoparticles on the skin (Fig. 3). When applied to the back, the formulation appeared transparent on the skin with repeated applications (Fig. 4).

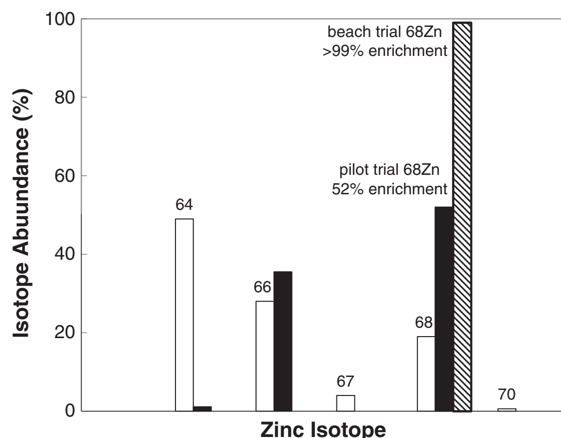

Fig. 1. Isotope abundances for Zn comparing the natural abundance (clear) with those present in the ZnO incorporated into sunscreens and used in studies described in this pilot study (52% enrichment in ^{68}Zn, black fill) and for the larger outdoor trial (>99% enrichment in ^{68}Zn, diagonal hatching).

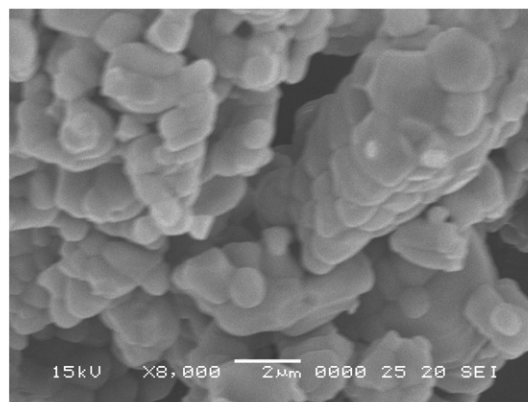

Fig. 2. SEM image showing the size of tracer ZnO particles prior to generation of nanoparticles used in the sunscreen.

2.2. Subjects and protocols

The trial was undertaken over 5 days in July 2008, winter in the southern hemisphere. It attempted to simulate a "week at the beach" in preparation for the outdoor trial described by Gulson et al. (2010). Volunteers were two males, one from an Asian background aged 28 and a Caucasian aged 66, plus a South American female aged 48 years. The older male also participated in the outdoor trial.

Approximately 2–3 g (weighed) of enriched sunscreen from individual petri dishes was applied to the backs of the subjects to a specific area (up to 35 cm × 26 cm for the males and less for the female), as shown in Fig. 4, by a 63-year old female; this female also applied the sunscreen in the outdoor trial. Sunscreen was applied twice daily, over 5 days, in the morning and after 3 h. Blood was sampled before the first and second sunscreen applications, and 3 h after the second sunscreen application. Urine was collected in the morning and then whenever available (at least 3 times each treatment day). Blood and urine samples were collected for up to 50 days post-trial. Collection of blood and urine samples prior to any application of these sunscreens meant that each subject acted as his or her own control. This obviated the necessity of having a control group to which we would have applied equivalent sunscreen except with naturally occurring zinc.

Blood samples (~3 mL) were taken by venipuncture; approximately 1 mL was transferred to a preweighed and precleaned PFA Teflon Savillex beaker and the other 2 mL transferred to a Vacutainer tube. Urine was collected when available on days 1 to 5 and at the same time as blood sampling on the post-trial days. During the day, MilliQ^{TM} water was consumed when required and a mid-day vegetarian meal was supplied. Blood and urine samples were collected from the sunscreen applicator before the first application and urine on day 5 and also thereafter when the other subjects were sampled.

Table 1
Sunscreen formulation.

Water phase (~50 wt.%)	Oil phase
Glycerine	Polyglyceryl-3 diisostearate
Guar gum	Glyceryl diisostearate
Water	Ethylhexylpalmitate
Benzoic acid	Olive oil
Hyaluronic acid	Grapeseed oil
	Di-C12-15 alkyl fumarate
	Macadamia oil
	Zinc oxide (~18 wt.% of 52% enriched ^{68}Zn)
	Dicapryl maleate
	Chloroacetamide
Approximate density = 1.2 g/mL	

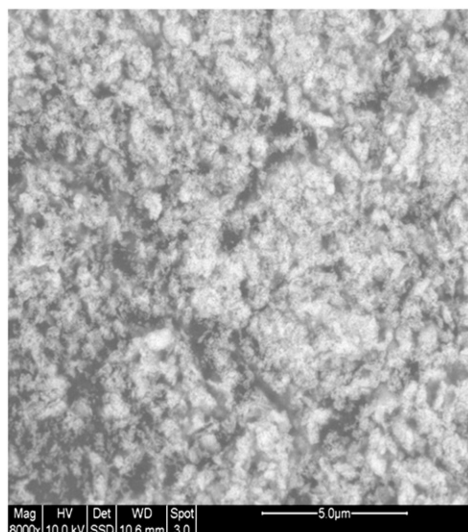

Fig. 3. Appearance of the sunscreen formulation on skin. A fixed amount of sunscreen (~ 12 mg) of the near commercial sunscreen containing ~18 wt.% stable isotope ZnO (particle diameters ~30 nm determined by XRD Rietveld refinement and confirmed by SAX) was applied to the a marked area of skin on the volar forearm with an area ~6 cm^2 (1.5×4 cm) providing equivalent to the recommended dose of 2 mg/cm^2 of sunscreen. The sunscreen was gently spread across the area by a gloved forefinger (disposable nitrile glove) to effect even coverage and mimic in situ application. Backscattered images of the contact side of the tape show relatively well dispersed coverage of nano ZnO sunscreen on skin. Back-scattered images were elementally analysed by EDAX to confirm that the white areas were ZnO.

As it was winter, the subjects experienced limited UV exposure by standing with their backs to glass windows for 30–90 min after each sunscreen application; glass windows are known to filter out the shorter wavelengths but allow partial transmission of UVA. The temperature at the surface of the backs reached 38 °C.

2.3. Laboratory procedures

Details of the laboratory procedures to process samples and determine levels of Zn isotopes are provided in Appendix 1.

2.4. Data evaluation

To determine whether Zn from sunscreens was absorbed through the skin, we evaluated the change of the ratio of $^{68}Zn/^{64}Zn$ over time. The data can also be presented in delta notation (denoted $\Delta^{68}Zn\%$) which is the percentage difference in ^{68}Zn with sunscreen application, defined as:

$$\Delta^{68}Zn\% = \left[\left({}^{68}Zn/{}^{64}Zn_{exposure} - {}^{68}Zn/{}^{64}Zn_{beforeexposure}\right)/{}^{68}Zn/{}^{64}Zn_{beforeexposure}\right] \times 100 \quad (1)$$

where the $^{68}Zn/^{64}Zn_{exposure}$ refers to the measurement for samples taken over the 5 days or post-exposure. The $\Delta^{68}Zn\%$ will be zero if no ^{68}Zn from the sunscreens enters the blood or urine via dermal absorption, even if naturally-occurring Zn is introduced to the body from another source such as ingestion. This is because the $^{68}Zn/^{64}Zn$ ratio of naturally-occurring Zn is essentially constant (e.g., Cloquet et al., 2008; Gulson et al., 2010) and therefore additional amounts of it that may enter the body during the trial period will not change the ratio measured prior to the trial period. In contrast, any increase in $^{68}Zn/^{64}Zn$ ratio or $\Delta^{68}Zn\%$ provides an indication of ^{68}Zn from ^{68}ZnO in the sunscreens entering the body.

Two subjects with the same value for $\Delta^{68}Zn\%$ may have different amounts of absorbed ^{68}Zn from sunscreens if the amount of naturally-occurring Zn in their blood is different. To minimise the impact from differences in the natural Zn blood reservoir between subjects, the measured total blood Zn concentration in the before-exposure blood sample was multiplied by an estimate of the individual's blood volume obtained using the formula of Nadler et al. (1962). These estimations provide amounts in micrograms (μg) of the ^{68}Zn tracer in blood for individuals and allow comparisons with the known amounts of naturally-occurring Zn in other tissues such as blood or liver.

3. Results

Because of constraints on access time to the multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) and limited funds, Zn isotopic compositions from the 3 subjects were only measured for critical blood samples (before-exposure, the end of day 5, and post-exposure days). Urine samples were analysed for days 1 to 5 (Fig. 6). The mean before-exposure (background) $^{68}Zn/^{64}Zn$ values for the 3 subjects and applicator of the sunscreen is 0.41587± 0.00030 (n = 8 measurements on different aliquots of the same samples), the same as the mean value for 21 subjects in the outdoor trial (Gulson et al., 2010). The results are presented as changes in $^{68}Zn/^{64}Zn$ ratios over time in blood and urine in Figs. 5 and 6 respectively. There was a steady increase in $^{68}Zn/^{64}Zn$ ratio in blood for the 3 subjects with the highest value observed for samples collected at 14 days from the first blood sampling (Fig. 5); that is, the amount of ^{68}Zn in blood continued to increase for 9 days even after cessation of

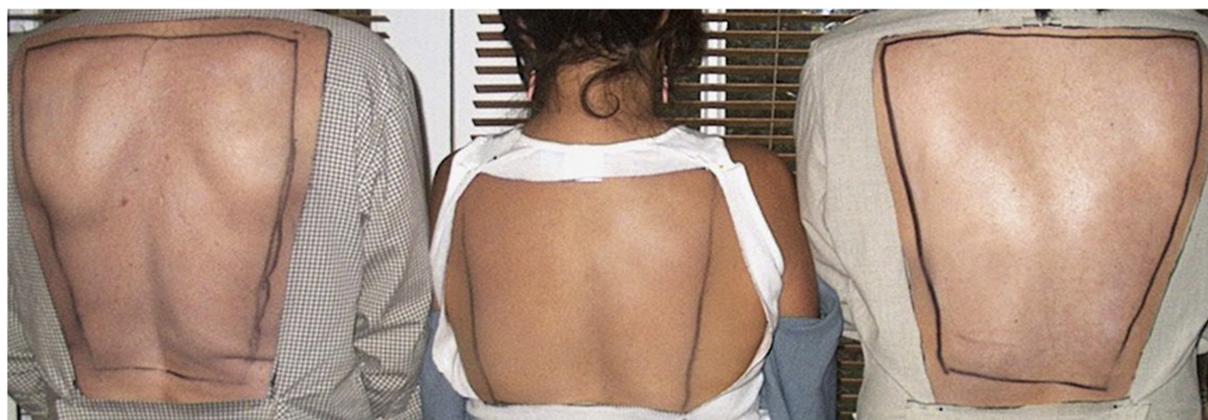

Fig. 4. Appearance of the sunscreen formulation on the backs of volunteers after 6 applications.

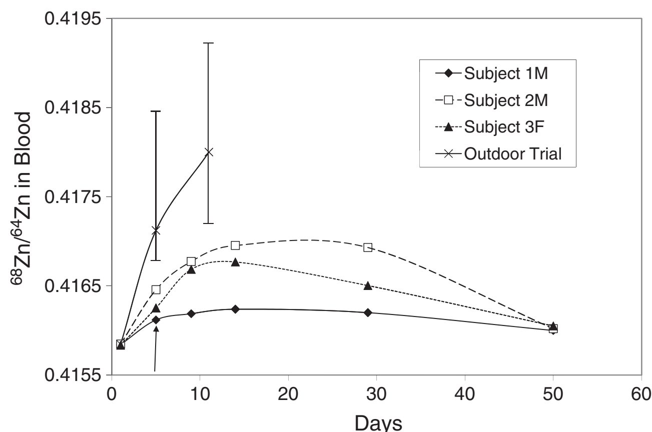

Fig. 5. Blood data expressed as $^{68}Zn/^{64}Zn$ ratio over time for the 3 volunteers on whom the nanoparticle sunscreen was applied. 1M is a Caucasian male aged 66 years; 2M is an Asian male aged 28 and 3F is a South American female aged 48. The arrow denotes the final sunscreen application.

sunscreen application, as observed in the outdoor trial. The $^{68}Zn/^{64}Zn$ ratios decreased towards the before-exposure values by day 50 although the exact ratios for day 50 are constrained by changes in machine conditions mentioned in Appendix 1. The smallest increase in $^{68}Zn/^{64}Zn$ ratio observed was for subject 1 (male, aged 66) and this was also the case for his results from the outdoor trial. The amount of ^{68}Zn tracer present in blood ranged from 12 to 35 µg compared with the total amount of natural Zn in blood ranging from 17 to 33 mg.

Urine $^{68}Zn/^{64}Zn$ ratios (Fig. 6) show variations across individuals with the maximum value at day 5 as observed in the outdoor trial. As in the outdoor trial, the increase in the female urine $^{68}Zn/^{64}Zn$ ratios was up to 6 times greater than for the males.

Isotopic data for blood and urine before, and urine at the end of, the 5 day trial were also obtained for the female who applied the sunscreen twice daily to the test subjects. Her isotopic results are similar to those for the test subjects and indicate some absorption through the hands, as also found in the outdoor trial.

4. Discussion

The results of this pilot study demonstrate that small amounts of Zn from Zn oxide particles in sunscreens are absorbed through healthy human skin and are detectable in blood and urine, as observed in the outdoor trial for 21 subjects.

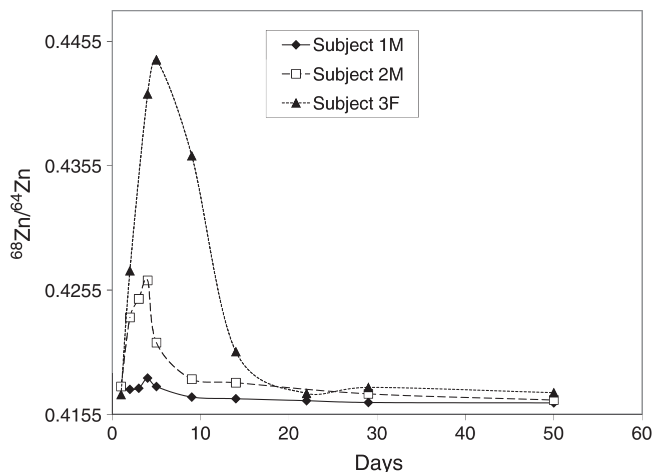

Fig. 6. Urine data expressed as $^{68}Zn/^{64}Zn$ ratio over time. Subjects 1M, 2M and 3F are as described in Fig. 5.

The peak value of $^{68}Zn/^{64}Zn$ ratio at 14 days rather than at the end of the 5-day exposure was unexpected and was taken into account when planning for the outdoor trial. Although longer monitoring for the outdoor trial would have been advantageous, it was not logistically feasible. The unexpected increase in ^{68}Zn after ceasing sunscreen application may be attributed to: substantivity (the binding and retention of residues in the lower epidermis or dermis potentially acting as a long-term chemical reservoir (Ngo and Maibach, 2010); the accumulation of the formulation in hair follicles, skin folds or sweat glands; or sequestration into, and later release from, bodily compartments such as the liver or muscle.

The profiles in $^{68}Zn/^{64}Zn$ ratio with time (Fig. 5) are similar to those observed for the 11 volunteers in the outdoor trial on whom nanoparticle sunscreen was applied. The extra amounts of ^{68}Zn tracer observed in the blood from the 3 volunteers in the pilot trial ranged from 12 to 35 µg and overlap the range from 6 to 31 µg ^{68}Zn (mean 15 µg, median 14 µg) measured in 11 volunteers from the outdoor trial to whom nanoparticles sunscreen was applied. The amounts of Zn absorbed from the sunscreen and detected in blood were small when compared to the amounts of natural Zn normally present in the human body. In this pilot trial the extra amounts of ^{68}Zn in blood are miniscule compared with the minimal blood Zn reservoir of 17 mg. The amount of ^{68}Zn tracer detected in blood post-exposure and after elimination of some of the ^{68}Zn through urine thus represents less than 0.01% of the applied dose. There are, however, major reservoirs of Zn in the body other than blood, such as muscle and liver, that we have not measured, and these could continue to exchange with, and add to, blood after cessation of sunscreen application. The tracer contribution from muscle and bone may be limited because the exchange of Zn between these compartments and plasma/blood is slow (Wastney et al., 1986; Wastney et al., 1991; Miller et al., 2000). The ^{68}Zn contribution from the liver and other easily exchangeable compartments may be significant as the liver/red blood cell ratio is of the order of 5 (Wastney et al., 1986; Wastney et al., 1991; Miller et al., 2000). However, even if ^{68}Zn from the liver and easily exchangeable compartments is 5 times that found in blood, it is still minimal after 5 days of sunscreen application compared with the amount of natural Zn in blood.

The increase in ^{68}Zn tracer in whole blood for 9 days after cessation of sunscreen application and ongoing maintenance of these elevated levels needs to be considered in the context of life-time use of sunscreens especially for occupational users. This may or may not be of concern but is contingent on the species of retained Zn. If the Zn is still present as nanoparticles there may be cause for concern but if it is as soluble Zn ions there should be less concern, especially given the homeostatic control of Zn in the body. Whether the Zn is present as particles or soluble Zn ions is unknown at this stage and, especially for the low levels observed in blood, would be a challenge to characterise. For those working in the cosmetics industry, there is little doubt that some of the ZnO is soluble and may be absorbed through the skin (M. Nearn, personal communication, 2010). This is also demonstrated by studies with larger ZnO particles. For example, Ågren (1990) measured the penetration of Zn for 48 h through normal skin on the flexor side of the lower arm of 15 healthy volunteers using occlusive dressings containing Zn oxide (25% w/w). The particle size was approximately 1 µm (M. Ågren, email communication, 2009). After 48 h, suction blisters were raised. The Zn concentration of the epidermis, blister fluid and dermis was increased beneath the Zn dressing compared with control-treated skin. The estimated mean release rate of Zn to the skin was 5 µg/cm^2/h.

As mentioned earlier, concern has been expressed that the results of the outdoor trial are at odds with all other investigations involving nanoparticles. There could be several reasons for this apparent discrepancy apart from the fact that our trials are the first to use the highly sensitive stable isotope method. Other reasons could be the

short time of the investigations with many being less than 48 h; the limited number of applications of the formulation; the limited number of subjects or animals; *in vitro* methods with excised skin; lack of measurement of blood and urine; no skin flexing; and lack of UV exposure. For example, apart from the trial of Sadrieh et al. (2010) with swine over a 4 week period (but with no UV exposure), other exposure times were short, commonly for only 24 h; in the outdoor trial, Gulson et al. (2010) did not detect any of the ^{68}Zn tracer in blood until the end of the second day, after 4 applications of sunscreen.

We have identified numerous limitations and problems in this study which were taken into account when planning for the later outdoor trial, or that need to be considered by others using the stable isotope approach. Some of the limitations include: limited numbers of subjects and expense of both the tracer and the analyses, although we think this is compensated for by the sensitivity of the method; the short 5-day period of the trial, equivalent to a week “at the beach” or holiday, but longer studies using humans are very expensive, inconvenient to volunteers and the tracer is non-recyclable; the low UV exposure as the study was carried out in winter in preparation for the outdoor trial; and complexities arising from using a less enriched tracer, as described in the appendix. For the outdoor trial, the tracer was >99% enriched ^{68}Zn and, even though expensive, minimises complications that we found with the 52% enriched tracer. Where investigators are seeking small changes in isotopic composition it is recommended the highly enriched tracer is used, rather than the cheaper alternative.

5. Conclusions

It is worth repeating the conclusion of Hostynek (2003) about percutaneous absorption of Zn: “Mechanisms which regulate Zn absorption via the skin are not understood and measurements of the rate of Zn penetration have been contradictory. Apparently its absorption does not take place by simple diffusion, but it seems to be regulated by homeostasis, uptake inversely related to the body's actual load of the metal.” Furthermore, the dynamics of homeostasis are important as there is a rapid exchange between skin-applied Zn and the large pool of endogenous metal. The stable isotope approach at least offers the possibility of distinguishing between the applied Zn and the endogenous pool. The results of both trials using different sunscreen formulations and different UV exposures demonstrate that small amounts of Zn from ZnO particles in sunscreen are absorbed through the skin in healthy subjects and can be detected in blood and urine. However, the amounts of extra Zn added to blood over a 5 day period are minimal compared to the body burden of Zn.

Acknowledgements

We thank David Ellis and Associates for producing the sunscreen formulation; Mary Salter for phlebotomy; Richard Kriege and Les Kinsley for guiding the first and second authors through the intricacies of the mass spectrometer; The CSIRO Flagship Collaboration Scheme and Macquarie University for providing partial funding for these trials; Dianne Gulson as the applicator and assistance with logistics; Hong Yin and Lynne Waddington for SEM and TEM analyses and images; valuable comments on the research protocol from Leland Miller, Melaney Wastney, and Paul Mushak; and advice from Jon Woodhead on Zn isotope measurements. These studies have been approved by human research ethics committees at Macquarie University and CSIRO.

Appendix 1

Laboratory procedures. Zn separations were carried out in a Class 350 laboratory. Zinc was purified from blood (0.2 ml) and urine (2–6 ml) samples by ion exchange through Bioradmacroporous resin following digestion with ultraclean nitric acid and hydrogen peroxide. Total Zn levels in blank controls processed by these procedures were routinely less than 3 ng, which is insignificant compared with the total amounts of Zn in the blood and urine samples. Changes in the isotopic abundance of ^{68}Zn of the purified samples were measured by multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at the Research School of Earth Sciences, Australian National University, and used to evaluate the dermal absorption of Zn from the sunscreens. A Zn solution of concentration 400 μg/L Zn with naturally-occurring (normal) isotopic ratios was measured several times during each analytical session to obtain an estimate of the precision of the isotopic ratios. In addition to measurements of the Zn standard solution, a composited sample of blood from the Australian Red Cross Blood Bank and urine collected from one of the volunteers prior to sunscreen application were also processed as “standards” along with the unknown samples. The relatively low enrichment of the ^{68}ZnO tracer (52%) necessitated complex deconvolution of the isotopic measurements, brief details of which are provided in the following section. The total Zn concentrations in all samples analysed for their Zn isotopic ratios were determined by ICP-MS.

Isotopic measures. Isotopic ratios were measured on a Neptune MC-ICP-MS (ThermoElectron Corporation, Bremen, Germany) in autosampling mode. The isotopes were measured simultaneously for ^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn and ^{70}Zn along with ^{62}Ni, ^{63}Cu, ^{65}Cu and ^{71}Ga. ^{62}Ni was monitored in order to correct for isobaric interferences of ^{64}Ni with ^{64}Zn. Incorporating an efficient ion-exchange separation procedure minimises isobaric interference from most metals. The measuring procedure involved a 10 second “wash” with clean 2% HNO_3, a 2 minute aspiration with “Triton X100” solution, a 2 minute wash with clean 2% HNO_3, a 2 minute “blank” measurement with clean 2% HNO_3, and 400 μg/L Zn standard solution or unknown sample. Integration time on each peak was 4 s, and for blanks, standards and samples a delay of 90 second aspiration was implemented before data collection began. Blood and urine samples were initially measured with standard bracketing, i.e., the 400 μg/L Zn standard solution was measured before and after the unknown samples along with the “washing” procedures. However, there were differences in the “raw” (as measured) isotopic ratios for the 400 μg/L Zn standard solution and the blood and urine solutions which we attribute to matrix effects and so it was not feasible to use the standard bracketing procedure to correct the isotopic ratios for mass fractionation effects in the unknown samples (e.g., Mason et al., 2004). The quality of the isotopic measurements is constrained by mass spectrometer effects. Raw isotope ratios measured by plasma-source mass spectrometry typically deviate from their true values by up to 10% per atomic mass unit (Mason et al., 2004). These deviations arise from potential interference with the Zn isotopes from other elements, and mass discrimination, which refers to fractionation or changes in the amount of isotopes during measurement whereby the heavier isotope (in this case ^{70}Zn) is transmitted more rapidly into the mass spectrometer section. On scatter plots of isotope ratios (e.g. $^{68}Zn/^{64}Zn$ versus $^{66}Zn/^{64}Zn$) the fractionated data lie along well-defined linear trends when samples and standards are measured in one session. If, however, the session is interrupted for any number of reasons, the data will often lie along a line of similar slope to the earlier analyses but show a “shift” in isotope ratios, requiring a correction to be applied to the data. Approaches to correct for mass bias and nonspectral mass discrimination are outlined in Mason et al. (2004) and include adding an ultrapure solution of copper to the separated Zn solution or to measure a Zn standard solution between each unknown sample. As described above, the latter approach was found to be unsatisfactory, and we encountered difficulties in using the copper method.

If dermal penetration occurs then a sample of blood or urine will contain a mixture of natural Zn and tracer Zn with the isotopic

compositions shown in Fig. 1. For simple two component mixing of the natural and tracer Zn, scatter plots of isotope ratios as mentioned above will also lie on well-defined linear arrays. Hence the measured data can lie on two linear arrays, one arising from isotopic fraction within the mass spectrometer and the other from mixing of natural and tracer Zn. To derive the amount of tracer Zn in a sample, the isotopic measurements are deconvoluted or unmixed using conventional methods from mass spectrometry. Normally, this would be a straightforward process but in the present study because of the low enrichment of 52% of the tracer compared with 18.8% in naturally-occurring Zn, the lines for fractionation and mixing almost coincide, complicating the unmixing calculations.

Nevertheless, it is possible to obtain satisfactory data but not to the same degree of certainty that can be obtained with a high purity tracer such as the >99% enriched ^{68}ZnO used in the subsequent outdoor trial.

References

Ågren MS. Percutaneous absorption of zinc from zinc oxide applied topically to intact skin in man. Dermatologica 1990;180:36–9.

Casey PS, Rossouw CJ, Boskovic S, Lawrence KA, Turney TW. Incorporation of dopants into the lattice of ZnO nanoparticles to control photoactivity. Superlattices Microstruct 2006;39:97-106.

Cloquet C, Carignan J, Lehmann MF, Vanhaecke F. Variation in the isotopic composition of zinc in the natural environment and the use of zinc isotopes in biogeosciences: a review. Anal Bioanal Chem 2008;390:451–63.

Cross SE, Innes B, Roberts MS, Tsuzuki T, Robertson TA, McCormick P. Human skin penetration of sunscreen nanoparticles: in-vitro assessment of a novel micronized zinc oxide formulation. Skin Pharmacol Physiol 2007;20:148–54.

Croteau M-L, Dybowska AD, Luoma SN, Valsami-Jones E. A novel approach reveals that zinc oxide nanoparticles are bioavailable after dietary exposures. Nanotoxicology 2011;5:79–90.

EWG (Environmental Working Group). Sunscreen Investigation. Section 4. Nanotechnology & Sunscreens. www.ewg.org/cosmetics/report/sunscreen09/investigation/Nanotechnology-Sunscreens2009.

FOE (Friends of the Earth). Safe Sunscreen Guide summer 2010–2011. http://nano.foe.org.au/sunscreens2010.

Gonzalez H. Percutaneous absorption with emphasis on sunscreens. Photochem & Photobiol Sci 2010;9:489–94.

Gulson B, Wong H. Stable isotopic tracing—a way forward for nanotechnology. Environ Health Perspect 2006;114:1486–8.

Gulson B, McCall M, Korsch M, Gomez L, Casey P, Oytam Y, et al. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci 2010;118:140–9.

Hostynek JJ. Factors determining percutaneous metal absorption. Food Chem Toxicol 2003;41:327–45.

Inman AO, Landsiedel R, Wiech K, Riviere JE, Schulte S, Monteiro-Riviere NA. Assessment of UVB-damaged skin in vivo with sunscreen formulations containing titanium and zinc nanoparticles. Toxicologist Abstr 2010;2067:439.

Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G, et al. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol 1999;12:247–56.

Mason TFD, Weiss DJ, Horstwood M, Parrish RR, Russell SS, Mullane E, et al. High-precision Cu and Zn isotope analysis by plasma source mass spectrometry. Part 2. Correcting for mass discrimination effects. J Anal At Spectrom 2004;19:218–26.

Miller LV, Krebs NF, Hambidge KM. Development of a compartmental model of human skin metabolism: identifiability and multiple studies analyses. Am J Physiol Regul Integr Comp Physiol 2000;279:1671–84.

Monteiro-Riviere NA, Baroli B. Nanomaterial penetration. In: Monteiro-Riviere NA, editor. Toxicology of the skin. New York: Informa healthcare; 2010. p. 333–46.

Monteiro-Riviere NA, Wiech K, Landsiedel R, Schulte S, Inman AO, Riviere JE. 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 2011;123:264–80.

Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962;51:224–32.

Nanoderm. Quality of skin as a barrier to ultra-fine particles. Final report; 2007.. QLK4-CT-2002-02678.

Ngo MA, Maibach HI. Dermatotoxicology: historical perspective and advances. Toxicol Appl Pharmacol 2010;243:225–38.

Nohynek GJ, Lademan J, Ribaud C, Roberts MS. Grey Goo on the skin? Nanotechonology, Cosmetic and Sunscreen Safety. Crit Rev Toxicol 2007;37:251–77.

Nohynek GJ, Antignac E, Re T, Toutian H. Safety assessment of personal care products/cosmetics and their ingredients. Toxicol Appl Pharmacol 2010;243:239–59.

Osmond MJ, McCall MJ. Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. Nanotoxicology 2010;4:15–41.

PROSPECT. Stable isotope tracing of engineered ZnO nanoparticles. Scientific Report to the OECD WPNM; 2010.. 27 pp.

Roberts MS, Roberts MJ, Robertson TA, Sanchez W, Th rling C, Zou U, et al. In vitro and in vivo imaging of xenobiotic transport in human skin and in the rat liver. J Biophotonics 2008;1:478–93.

Sadrieh N, Wokovich AM, Gopee NV, Zheng J, Haines D, Parmiter D, et al. Lack of significant penetration of titanium dioxide from sunscreen formulations. Toxicol Sci 2010;115:156–66.

Schilling K, Bradford B, Castelli D, Dufour E, Nash JF, Pape W, et al. Human safety review of "nano" titanium dioxide and zinc oxide. Photochem & Photobiol Sci 2010;9:495–509.

TGA (Therapeutic Goods Administration), Australia. A review of the scientific literature on the safety of nanoparticulate titanium dioxide or zinc oxide in sunscreens. www.tga.gov.au/npmeds/sunscreen-zotd.htm2009.

Wastney ME, Aamodt RL, Rumble WF, Henkin RI. Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol Regul Integr Comp Physiol 1986;251:398–408.

Wastney ME, G kmen IG, Aamodt RL, Rumble WF, Gordon GE, Henkin RI. Kinetic analysis of zinc metabolism in humans after simultaneous administration of ^{65}Zn and ^{70}Zn. Am J Physiol Regul Integr Comp Physiol 1991;260:134–41.

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