Dermal absorption and short-term biological impact in hairless mice from sunscreens containing zinc oxide nano- or larger particles

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Abstract

Previous studies have shown no, or very limited, skin penetration of metal oxide nanoparticles following topical application of sunscreens, yet concerns remain about their safety compared to larger particles. Here, we assessed the comparative dermal absorption of a traceable form of Zn (⁶⁸Zn) from ⁶⁸ZnO nano-sized and larger particles in sunscreens. Sunscreens were applied to the backs of virgin or pregnant hairless mice over four days. Control groups received topical applications of the sunscreen formulation containing no ZnO particles, or no treatment. Major organs were assessed for changes in ⁶⁸Zn/⁶⁴Zn ratios, ⁶⁸Zn tracer and total Zn concentrations. Short-term biological impact was assessed by measuring levels of serum amyloid A in blood, and by performing whole-genome transcriptional profiling on livers from each group. Increased concentrations of ⁶⁸Zn tracer were detected in internal organs of mice receiving topical applications of ⁶⁸ZnO (nano-sized and larger particles), as well as in fetal livers from treated dams, compared with controls. Furthermore, concentrations of ⁶⁸Zn in organs of virgin mice treated with sunscreen containing ⁶⁸ZnO nanoparticles were found to be significantly higher than in mice treated with sunscreen containing larger ⁶⁸ZnO particles. However, no ZnO-mediated change in total Zn concentration in any of the major organs was observed. Thus, despite ⁶⁸Zn absorption, which may have been in the form of soluble ⁶⁸Zn species or ⁶⁸ZnO nanoparticles (not known), Zn homeostasis was largely maintained, and the presence of ZnO particles in sunscreen did not elicit an adverse biological response in the mice following short-term topical applications.

Introduction

Many modern sunscreens contain nanoparticles of zinc oxide (ZnO) and/or titanium dioxide (TiO₂). ZnO nanoparticles, in particular, absorb a broad spectrum of ultraviolet (UV) wavelengths and thus give excellent protection on their own against particular, absorb a broad spectrum of ultraviolet (UV) wave-lengths and thus give excellent protection on their own against sunburn (Mitchnick et al., 1999). In contrast, TiO₂ absorbs over a narrower range and so is often used in conjunction with ZnO or molecular UV absorbers to provide broad-spectrum protection (Moyal, 2012). Due to their transparency on the skin, metal oxide nanoparticles in sunscreens are aesthetically superior to their larger-sized counterparts, and their improved dispersion in formulations also provides a lighter feel on the skin (Wang & Tooley, 2011). Despite their clear advantages, however, the novel physico-chemical properties that emerge with decreasing particle size have caused some concerns regarding the use of nanoparticles in sunscreen, such as whether their small size might enable penetration through human skin and, if so, whether the toxicity observed for metal oxide nanoparticles in vitro (reviewed in Iavicoli et al., 2011, Vandebriel & Jong, 2012) could translate to cellular and/or organ damage in vivo (Hackenberg & Kleinsasser, 2012).

For the most part, ex vivo and in vivo studies have suggested that metal oxide nanoparticles do not penetrate into viable epidermis following topical application (reviewed in Osmond & McCall, 2010; TGA, 2009). However, many studies that have assessed the effects of normal sunscreen use were limited by low numbers of human subjects, and/or relatively short treatment protocols of 24–48 h in the absence of UV radiation. UV radiation disrupts normal skin barrier function (Gelis et al., 2002; Haratake et al., 2007) and has been shown to enhance the penetration of topically applied quantum dots (Mortensen et al., 2008, 2012). In addition, nanoparticles can accumulate in skin furrows and hair follicles, where they can remain for up to 10 days, although they appear largely to be removed via natural means such as sebum flow (Lademann et al., 2007). To overcome some of these limitations, a recent study by Gulsön et al. (2010) used the highly sensitive method of stable-isotope tracing to assess dermal absorption of ⁶⁸Zn from enriched ⁶⁸ZnO particles in sunscreens applied twice daily over 5 days to humans in a “real life” beach...
scenario. The study showed that very small amounts of $^{68}\text{Zn}$ tracer were found in the blood of 20 human volunteers receiving topical applications of sunscreens containing $^{68}\text{ZnO}$ nanoparticles or larger particles, with higher levels excreted in urine. The highest $^{68}\text{Zn}$ concentrations were found in females exposed to the $^{68}\text{ZnO}$ nanoparticles (Gulson et al., 2010). A major finding from this study, of importance to previous studies that were single-application or shorter in duration, was that not only did it take at least four applications over 2 days for $^{68}\text{Zn}$ tracer concentrations in blood of subjects to rise to detectable levels, but that, following the completion of the study, the $^{68}\text{Zn}$ tracer concentrations in blood continued to rise, reaching a maximum 14 days after the final sunscreen application (Gulson et al., 2012). This suggested there was either continued absorption from $^{68}\text{ZnO}$ particles lodged in hair follicles or skin folds, or the slow release of $^{68}\text{Zn}$ tracer from an internal reservoir. Due to the necessity of preparing samples by acid digestion, however, the isotopic tracing method could not determine whether the $^{68}\text{Zn}$ tracer was present in blood and urine as ZnO particles or soluble Zn species. Furthermore, the use of human subjects excluded a more comprehensive assessment of organ distribution of the $^{68}\text{Zn}$ tracer, or biological response to short-term sunscreen use.

The aim of this study was to provide an assessment of skin absorption and organ distribution of $^{68}\text{Zn}$ tracer in virgin and pregnant immune-competent hairless mice following repeated topical applications over 4 days of the same “nano” and “bulk” $^{68}\text{ZnO}$ sunscreens used previously on humans (Gulson et al., 2010). A mouse model was chosen on the basis that mouse skin is more likely than healthy human skin to permit penetration of intact ZnO particles and/or soluble Zn species released from the dissolution of ZnO particles, and thus allows an assessment of comparative absorption and biodistribution of either, depending on ZnO particle size. Additionally, the likelihood of Zn and/or ZnO absorption in this model allowed us to assess short-term biological responses to the treatments, to an extent not possible in the human study. Fetal livers were also assessed to determine transplacental transfer of $^{68}\text{Zn}$ from dams topically treated with sunscreens containing $^{68}\text{ZnO}$ particles.

**Methods**

**Sunscreen formulations**

Zinc oxide particles and sunscreen formulations were as described previously (Gulson et al., 2010). Briefly, ZnO enriched with $>99\%\ ^{68}\text{Zn}$ (Isoflex, USA) was manufactured into either nanoparticles with an average diameter of 19 nm using a proprietary process based on high energy attrition milling (Casey et al., 2006), or larger particles with an average diameter of 107 nm using a modification of the same method. TEM images and size distributions of the two types of $^{68}\text{ZnO}$ particles are shown in Figure 1. In the nano sunscreen, 100% of the particles were under 100 nm diameter, with 91% smaller than 30 nm. In the larger ZnO particle (bulk) sunscreen, half the measured particles were greater than 100 nm in diameter, while the remainder were between 25 and 100 nm and could be therefore be classified as nanoparticulate (ISO, 2010). A two-tailed $t$-test confirmed that the average size distributions of particles in the two sunscreens were significantly different ($p < 0.0001$). The uncoated nano and bulk particles were formulated at ~20% w/w into respective sunscreens by a commercial sunscreen manufacturer. The sunscreen formulation containing no ZnO particles was used as a control for the ingredients in the formulation alone. The formulation contained preservatives (phenoxethanol, hydroxybenzoates, diazolidinylurea), emollients (isopropyl myristate, cyclomethicone, isostearyl neopentanoate), moisturizers (tocopheryl acetate (vitamin E), C12–15 alkyl benzoate), film formers (triacontanyl PVP, isohexadecane) and emulsifiers (isopropyl lanolate, PEG-30

![Figure 1](https://example.com/figure1.png)
dipolyhydroxystearate), as well as a soothing agent (alo barbadensis leaf juice), a chelating agent (disodium EDTA), a stabilizing agent (magnesium sulphate), a humectant (propylene glycol) and a diluent (purified water).

Animal housing conditions
Female, immune-competent, hairless SKH:QS mice (8 weeks old) were supplied in-house by the CSIRO Animal House from an in-bred colony that had been developed from a seed cohort originally supplied by Sydney University, Australia. Pregnant mice entered the experiment at ~12 days gestation. Animal experiments were conducted following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004), and were approved by the CSIRO Animal Ethics Committee (ACEC 07-07). For the duration of the treatment period, mice were housed individually in polycarbonate cages in an isolated temperature (~21°C) and moisture (55–65% relative humidity) controlled room with a 14-h light/10-h dark cycle, and had ad libitum access to water and Gordon’s rat and mouse pellets (Gordon’s Specialty Stock Feeds, Australia).

Sunscreen treatment
Eighty mice were divided into eight treatment groups of 10. Four groups, each comprising 10 virgin mice, received no-treatment, topical applications of the $^{68}$ZnO nanoparticle sunscreen (nano), the $^{68}$ZnO larger particle sunscreen (bulk), or the sunscreen formulation only (containing no ZnO). Four groups, each comprising 10 pregnant mice, received the same treatments as the virgin cohort. Mice in all groups were fitted with Elizabethan collars (Braintree Scientific, Braintree, MA, USA) to prevent grooming and consequent ingestion of the sunscreen formulations. Collars were fitted one day prior to treatment to allow for behavioral adjustment and to ensure that each collar fitted correctly.

Before the first sunscreen application, the surface areas of the back and sides of each mouse were measured, giving an average of approximately 50 cm$^2$. Using a gloved fingertip, a weighed amount of sunscreen (average of 0.1 g per application) was applied to these regions to give a dose of 2 mg/cm$^2$ (COLIPA, 2006). Sunscreens were applied in the morning and afternoon for the first 2 days, and in the morning only for day 3 and 4. The average total amount of sunscreen applied over the course of the 4 day period was therefore 0.6 g, containing 0.12 g $^{68}$ZnO, of which 0.096 g was $^{68}$Zn. After each application, mice were placed back into their separate cages without bedding for 1 h, and then a minimum amount was added to enable normal nesting behaviors while minimizing the possibility of sunscreens rubbing off onto the bedding. For both the virgin and pregnant cohorts, used beddings from representative nano and bulk mice were re-used in the cages of virgin or pregnant “bedding-control” mice that received no-treatment apart from exposure to the used bedding (2–4 mice were used for each control). Elevated concentrations of $^{68}$Zn tracer in these bedding-control mice would be indicative of either oral ingestion or inhalation of $^{68}$Zn from the bedding. Mice in the no-treatment groups were also fitted with Elizabethan collars and underwent the same regime with the exception that they received no sunscreen applications.

Mice were anesthetized [intraperitoneal injection of Xylase (50 mg/kg)/ketamine (50 mg/kg)] on day 5. Body weight was recorded, and then blood was taken via cardiac puncture for measurement of serum amyloid A2 (SAA2). Mice were then killed by cervical dislocation, and dissected. Brain, liver, spleen, kidneys, lung and heart were taken and weighed wet immediately after dissection. From pregnant mice, the uterine tissue and fetal livers were also taken. Half of each organ sample was cooled in ice and then stored frozen at −80°C prior to preparation for Zn isotope ratio measurements. The other half of each sample was snap frozen in ethanol/dry-ice and then stored at −80°C. Portions of liver samples treated in this way were used for analysis of gene expression; the remaining liver and other organ samples were used for total Zn measurements.

Because of the high sensitivity of the stable-isotope tracing method, extreme care was taken throughout the entire treatment period to limit non-dermal entry (e.g. ingestion) of sunscreen components to the body, and during dissections to minimize cross-contamination of internal organs with the $^{68}$Zn tracer. Gloves, matting and other consumables were changed between mice as well as throughout the dissection of each individual mouse. Different sets of dissecting instruments were used for each mouse. Two sets were used for each mouse, one set for carefully cutting skin from the body and a second set for dissecting organs from the body cavity. Thus, no instrument that had touched the exterior of the mouse touched the internal organs. For each mouse, instruments were washed in 2% nitric acid (HNO$_3$) and rinsed in saline between each organ dissection, and care was taken that organs did not come into contact with each other or the external surface of the mouse. After dissection, organs were rinsed in fresh saline and then sectioned for analysis, using a fresh scalpel and clean cutting surface for each organ.

Mice were excluded from analysis if they removed their Elizabethan collars over the course of the experiment, or if they exhibited substantially reddened, dry or flaky skin from the sunscreen treatment. Organs from three to eight virgin and pregnant mice were analysed for Zn isotope ratios and total Zn. Blood samples from ten virgin mice were analyzed per treatment group; blood was not analyzed for pregnant mice. Organs from two to four bedding-control mice were used for the nano bedding and bulk bedding groups.

$^{68}$Zn/$^{64}$Zn isotope ratios
Samples were prepared for analysis of Zn isotope ratios ($^{68}$Zn/$^{64}$Zn) by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS), as described previously (Gulson et al., 2010). Measurements were made using a Thermo Fisher Neptune MC-ICP-MS instrument housed at CSIRO, Waite Campus, Adelaide, Australia. The use of high resolution instrumentation enables precise measurements of Zn isotopes in samples.

Briefly, ~40 mg of wet tissue or 0.1 ml of blood was digested by repeated evaporation with a mix of concentrated HNO$_3$ and 30% hydrogen peroxide. The dried residue was dissolved in 2 M hydrochloric acid (HCl), and loaded onto AG MP-1 M resin columns (0.4 ml bed). After washing with 3 x 2 ml of 2 M HCl, the Zn was eluted with 2 ml of 0.3 M HNO$_3$ and $^{68}$Zn/$^{64}$Zn isotope ratios determined using MC-ICP-MS.

Total $^{68}$Zn tracer concentrations in major organs
Zinc has five stable isotopes, $^{64}$Zn (with a natural abundance of 48.6%), $^{66}$Zn (27.9%), $^{67}$Zn (4.1%), $^{68}$Zn (18.8%) and $^{70}$Zn (0.6%) (Gulson et al., 2012). Total Zn concentrations in organs can comprise of $^{68}$Zn absorbed from the sunscreen and naturally abundant Zn that enters the body. The advantage of this isotope tracer technique is that no shift in the $^{68}$Zn/$^{64}$Zn isotope ratio will be observed if no $^{68}$Zn tracer from the sunscreen enters the mice via dermal penetration, even if naturally occurring Zn enters the body from another source (e.g. food). This is because the $^{68}$Zn/$^{64}$Zn isotope ratio of naturally occurring Zn is essentially
RNA was isolated from mouse liver samples (30 mg) using a... downstream analysis. Micro-chips from the virgin and pregnant... changes were transferred to Greiner Bio-One MiniCollect tubes... the naturally occurring $^{68}$Zn/$^{64}$Zn ratio, and consistent with these mice receiving no direct exposure to an exogenous source of enriched $^{68}$Zn. In contrast, the $^{68}$Zn/$^{64}$Zn ratios in mice treated with the bulk or nano $^{68}$ZnO sunscreens were significantly higher in blood and all organs assessed compared with the untreated or formulation only groups. Further, the $^{68}$Zn/$^{64}$Zn isotope ratios were significantly higher in the nano-treated mice compared with the bulk-treated mice for all organs, but not blood where the difference did not reach statistical significance.

Consistent with results observed for virgin mice, higher $^{68}$Zn/$^{64}$Zn ratios were found in the livers of pregnant dams treated with either of the $^{68}$ZnO sunscreens compared to no treatment and formulation only groups (Figure 2B), but statistical significance was reached only for groups treated with the bulk sunscreen relative to formulation only. There was no statistically significant difference between bulk and nano treatments. In individual pregnant mice, $^{68}$Zn/$^{64}$Zn ratios in fetal livers closely matched those of dam livers, indicating a good correlation between maternal transplacental exposures. Increased levels of $^{68}$Zn tracer in uterine tissue from $^{68}$ZnO-treated dams were detectable, but comparatively low, and did not reach statistical significance.

To assess $^{68}$Zn intake from potential licking or dust inhalation from bedding, $^{68}$Zn/$^{64}$Zn ratios were determined for virgin and pregnant mice that had nested in second-hand bedding used previously in representative cages of $^{68}$ZnO-treated mice, but who were otherwise untreated (Figure 2A and B). The $^{68}$Zn/$^{64}$Zn ratios...
overall, contributions to increased concentrations of $^{68}$Zn tracer from these indirect routes of exposure were far less than that from the topical treatment.

The concentrations of $^{68}$Zn tracer in gastrointestinal tract (GIT) tissue were further compared between mice who retained their Elizabethan collars for the entire study, and those who managed to remove their collars and consequently had greater ability to lick the sunscreen and directly ingest $^{68}$Zn tracer (these mice were otherwise excluded from the study). It was found that the average concentration of the tracer in GIT tissue of mice who retained their collars (bulk = $3.5 \pm 0.7 \mu g/g$; nano = $3.2 \pm 0.8 \mu g/g$) was less than half that in GIT of mice who had removed them (bulk = $9 \pm 1 \mu g/g$; nano = $7 \pm 1 \mu g/g$). Also of note is that the average tracer level in the GIT of mice who retained their collars was lower than in all organs other than brain. Presumably due to the disagreeable taste, mice who had removed their collars were never seen licking the sunscreen, and on close inspection were found to have groomed only minimal areas of the sunscreen application site, and so the concentration of $^{68}$Zn tracer in their GIT tissue is likely to be only from very limited grooming of this area. The lower concentration of $^{68}$Zn tracer in GIT of mice retaining their collars indicates that the Elizabethan collars further minimized direct ingestion of the $^{68}$Zn tracer.

Upon conversion to mass concentrations (Table 1), the highest concentration of $^{68}$Zn tracer from $^{68}$ZnO in sunscreens was found in liver tissues of both bulk- (12 $\pm 2 \mu g/g$) and nano-treated (18 $\pm 2 \mu g/g$) virgin mice, whereas the lowest concentration was in brain tissue (bulk: 1.6 $\pm 0.4 \mu g/g$; nano: 3.4 $\pm 0.4 \mu g/g$). A comparatively narrow spread was observed in heart, kidney, lung and spleen tissue, with concentrations ranging from 5 $\pm 1$ to 7 $\pm 1 \mu g/g$ in bulk-treated mice, and 8.6 $\pm 0.5$ to 12 $\pm 1 \mu g/g$ in nano-treated mice. In pregnant mice, the concentrations of $^{68}$Zn tracer in liver were approximately three times higher than those in uterine tissue. Concentrations of $^{68}$Zn tracer in livers of pregnant and non-pregnant mice were not significantly different when bulk or nano groups were compared.

These results indicate that absorption of $^{68}$Zn from both sunscreens containing $^{68}$ZnO particles occurred in virgin, hairless mice. Furthermore, the absorption of $^{68}$Zn was significantly greater from sunscreen containing nanoparticulate $^{68}$ZnO compared to larger $^{68}$ZnO particles. Some absorption also occurred in sunscreen-treated pregnant mice, and transplacental exposure closely matched maternal exposure.

**Total Zn concentrations in major organs**

The concentration of total Zn in livers of untreated mice was higher for the pregnant group (40 $\pm 5 \mu g/g$) than the virgin group (27 $\pm 1 \mu g/g$; Figure 3). There was a higher concentration of total Zn in livers of both virgin and pregnant mice treated with any of the formulations, irrespective of the presence of ZnO, although this did not always reach statistical significance compared with no treatment group (Figure 3). There was no statistically significant difference in total Zn in other organs apart from the spleens of nano-treated virgin mice, although this was much smaller than in livers. Concentrations of total Zn were otherwise relatively constant across the major organs, as has been reported (as dry weights) elsewhere for several different mouse strains (Siegers et al., 1977; Wastney & House, 2008). The accumulation of hepatic Zn in groups receiving topical applications of any of the sunscreen formulations is consistent with an inflammatory reaction in response to the formulation itself rather than accumulation of Zn arising from the presence of ZnO in the sunscreens, and is discussed in more detail as follows.
The percent of sunscreen-derived $^{68}$Zn tracer compared to the total Zn concentration in organs can be found in Table 1. These were consistently higher in organs of nano-treated mice compared with bulk-treated. The lowest percentage accumulation of $^{68}$Zn tracer was in the brains of mice treated with the bulk sunscreen, with 8% of total Zn consisting of sunscreen-derived $^{68}$Zn. The largest accumulation was in the liver of mice treated with the nano sunscreen, where 50% of total Zn was $^{68}$Zn accumulated from the sunscreen Table 1. This implies a large exchange of sunscreen-derived $^{68}$Zn with endogenous total Zn in some organs, and especially so for the nano-treated mice. However, it is important to bear in mind that total Zn concentrations did not significantly change in any organ, except for increases in the liver (and spleen) which may be associated with an inflammatory response to the formulation only (see below). This indicates efficient homeostatic mechanisms in the hairless mouse model. Taken as a whole, these data suggest that although $^{68}$Zn from $^{68}$ZnO particles in the sunscreen formulations was dermally absorbed, the mice were able to adequately regulate their concentrations of total Zn in blood and organs.

It was not possible to ascertain the total absorption concentration of $^{68}$Zn from the cumulative sunscreen doses as we did not measure $^{68}$Zn in whole mouse bodies or excreted $^{68}$Zn. However, we could calculate the percent of the total $^{68}$Zn dose accumulated in selected organs. The concentration of $^{68}$Zn tracer accumulated in these organs from total sunscreen dose was found to be small. No more than 0.006% of the total $^{68}$Zn dose applied to the skin was found in each of the brain, heart, kidney, lung and spleen of mice receiving either the nano or the bulk sunscreen treatments (Table 2). In liver, the $^{68}$Zn accumulated from sunscreens was slightly higher, with 0.02% of the applied $^{68}$Zn in the bulk group, and 0.04% in the nano group (Table 2).

Levels of serum amyloid A2

Serum amyloid A2 (SAA2) was used as a biomarker for acute-phase inflammation, although it should be noted that as SAA1 and SAA2 share 91% amino acid sequence homology (Yamamoto & Migita, 1985), there is likely to be cross-hybridization from SAA1 on the SAA2 protein assay, as well as at the transcript level. Virgin mice treated with formulation only had a statistically significant 50-fold increase in SAA2 compared to untreated mice, suggestive of a formulation-mediated inflammatory response (Figure 4). In contrast, SAA2 levels were only approximately 10-fold higher in virgin mice treated with the nano and bulk

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**Table 1. Concentrations of $^{68}$Zn tracer, total Zn and percent $^{68}$Zn tracer of total Zn in major organs of virgin or pregnant mice treated with sunscreen formulations containing $^{68}$ZnO bulk or $^{68}$ZnO nanoparticles.**

|                | Bulk $^{68}$Zn (µg/g) Mean ± SEM | Nano $^{68}$Zn (µg/g) Mean ± SEM | Bulk Total Zn (µg/g) Mean ± SEM | Nano Total Zn (µg/g) Mean ± SEM | Bulk % $^{68}$Zn of Total Zn | Nano % $^{68}$Zn of Total Zn |
|----------------|----------------------------------|----------------------------------|-------------------------------|---------------------------------|-----------------------------|-------------------------------|
| **Virgin mice** |                                  |                                  |                               |                                 |                             |                               |
| Brain          | 1.6 ± 0.4<sup>a</sup>            | 3.4 ± 0.4<sup>b</sup>            | 19 ± 1                        | 19 ± 1                          | 8                           | 18                           |
| Heart          | 5 ± 1<sup>a</sup>               | 8.7 ± 0.6<sup>b</sup>            | 21 ± 1                        | 20.0 ± 0.5                      | 25                          | 44                           |
| Kidney         | 7 ± 1<sup>a</sup>               | 12 ± 1<sup>b</sup>              | 24 ± 3                        | 25 ± 2                          | 29                          | 48                           |
| Liver          | 12 ± 2<sup>a</sup>              | 18 ± 2<sup>a</sup>              | 43 ± 6                        | 36 ± 3                          | 28                          | 50                           |
| Lung           | 5.7 ± 0.7<sup>a</sup>           | 8.6 ± 0.5<sup>b</sup>           | 25 ± 5                        | 20 ± 1                          | 24                          | 43                           |
| Spleen         | 7 ± 1<sup>a</sup>               | 12 ± 1<sup>b</sup>              | 23 ± 1                        | 25 ± 2                          | 30                          | 48                           |
| **Pregnant mice** |                                    |                                  |                               |                                 |                             |                               |
| Liver          | 11 ± 2<sup>a</sup>              | 12 ± 3<sup>a</sup>              | 55 ± 3                        | 58 ± 3                          | 20                          | 21                           |
| Uterine Lining | 3.7 ± 0.5                        | 4 ± 2                           | 31 ± 3                        | 25 ± 1                          | 12                          | 16                           |

All data for $^{68}$Zn are for $^{68}$Zn sourced from $^{68}$ZnO in the sunscreen. $^{68}$Zn and total Zn concentrations are in µg/g of organ (wet) weight. Values for untreated and formulation only mice were 0 µg/g $^{68}$Zn sourced from sunscreen.

<sup>a</sup>Significantly different from no-treatment and formulation only.

<sup>b</sup>Nano significantly different from bulk. SEM standard error of the mean.

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**Effects of ZnO-containing sunscreens applied to mice**
68ZnO sunscreens, which were significantly lower than in formulations containing bulk or nanoparticles of 68ZnO, administered over 4 days. The last of the six topical applications of formulation only or sunscreens treatments, in blood of virgin and pregnant mice, 1 day after receiving the sunscreen.

Figure 4. Relative levels of serum amyloid A2 (SAA2), compared to no treatment.Virgin mice treated with the formulation only (showing a total of 6485 mouse kidneys) and pregnant mice treated with any of the formulations showed an even stronger release of SAA2, with a 150- to 200-fold increase compared to untreated pregnant mice.

Unlike the results observed for virgin mice, however, the presence of ZnO in the sunscreen, although bulk ZnO historically has been effective in treating skin diseases (Agren, 1990), was not ameliorated by the presence of ZnO in the sunscreen. Total organ 68Zn (µg) concentrations were calculated by multiplying 68Zn (µg/g) (Table 1) by average organ weights. Percent 68Zn tracer in organs, from total sunscreen applied, was calculated using an accumulated dose of 0.096 g 68Zn from six sunscreen applications.

All data for 68Zn are for 68ZnO in the sunscreen. 68Zn concentrations were calculated by multiplying 68Zn (µg/g) (Table 1) by average organ weights. Percent 68Zn tracer in organs, from total sunscreen applied, was calculated using an accumulated dose of 0.096 g 68Zn from six sunscreen applications.

Table 2. Average organ wet weights, total accumulated 68Zn in organs, and percent organ-accumulated 68Zn of total sunscreen applied.

|                | Average total organ wet weight (g) | Bulk estimated total organ 68Zn (µg/g) | Nano estimated total organ 68Zn (µg/g) | Bulk % 68Zn of total sunscreen applied | Nano % 68Zn of total sunscreen applied |
|----------------|-----------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| Virgin mice    |                                   |                                       |                                       |                                       |                                       |
| Brain          | 0.44 ± 0.01                       | 0.7                                   | 1.5                                   | 0.0007                                | 0.001                                |
| Heart          | 0.26 ± 0.02                       | 1.3                                   | 2.3                                   | 0.001                                 | 0.002                                |
| Kidney         | 0.47 ± 0.02                       | 3.3                                   | 5.6                                   | 0.003                                 | 0.006                                |
| Liver          | 1.98 ± 0.08                       | 23.8                                  | 35.6                                  | 0.02                                  | 0.04                                 |
| Lung           | 0.19 ± 0.01                       | 1.1                                   | 1.6                                   | 0.001                                 | 0.002                                |
| Spleen         | 0.16 ± 0.01                       | 1.1                                   | 1.9                                   | 0.001                                 | 0.002                                |
| Pregnant mice  |                                   |                                       |                                       |                                       |                                       |
| Liver          | 3.0 ± 0.2                         | 33                                    | 36                                    | 0.03                                  | 0.04                                 |

Figure 4. Relative levels of serum amyloid A2 (SAA2), compared to no treatment, in blood of virgin and pregnant mice, 1 day after receiving the last of the six topical applications of formulation only or sunscreens containing bulk or nanoparticles of 68ZnO, administered over 4 days.

*Significantly different from no-treatment. #Significantly different from formulation only.

68ZnO sunscreens, which were significantly lower than in the formulation only group. These results suggest the formulation-mediated inflammatory response was somewhat ameliorated by the presence of ZnO in the sunscreen, although not to untreated levels. Pregnant mice treated with any of the formulations showed an even stronger release of SAA2, with a 150- to 200-fold increase compared to untreated pregnant mice. Unlike the results observed for virgin mice, however, the presence of ZnO in the formulation did not mitigate this stronger response in pregnant mice.

Whole-genome gene-expression profiling in liver

Mouse livers were chosen for whole-genome gene-expression profiling due to the observed inflammatory response in treated mice. Gene expression data were deposited at the Gene Expression Omnibus at NCBI (http://www.ncbi.nlm.nih.gov/geo/) under Accession Number: GSE46568 ID 200046568. Transcriptional activity was more greatly perturbed in livers of mice treated with the formulation only (showing a total of 6485 differentially transcribed genes compared to no treatment) than for mice treated with the nano or bulk sunscreens (with a total of 862 and 118 perturbed genes, respectively, compared to no treatment). Figure 5(A) shows treatment interactions at the transcriptional level separated into genes perturbed by one treatment only (unique to a treatment) or those similarly perturbed across two or more treatments (shared across different treatments). The treatment interactions again indicate that the majority of unique transcriptional activity was confined to the formulation only group (5880 genes). In contrast, only a small proportion of transcripts were perturbed across all treatments (79 genes shared by all treatments). The associated representation of data as a map in Figure 5(B), where differential expression levels of individual genes are color-coded, highlights the types of gene functions that were perturbed, with the majority being categorized as enzymatic or "other" by IPA analysis. For the formulation only group, the majority of genes encoding enzymes, phosphatases, translation regulators and transporters were slightly down-regulated (black or red bars). In contrast, genes encoding proteins related to cytokines, growth factors and transcription regulators were more often positively regulated (green bars). This pattern suggests these mice may be exhibiting deregulated transcription and translation. Additionally, the up-regulation of genes for G-coupled protein receptors, ion channels and trans-membrane receptors indicates increased trans-membrane activity for the formulation only group relative to others.

The majority of perturbed transcripts in livers of mice treated with the nano sunscreen were shared with the formulation only group (526 genes), far fewer than the proportion shared with the bulk sunscreen group (31 genes). In marked contrast, the bulk and formulation only groups had no perturbed transcripts in common. Of the 31 genes shared between the two ZnO-treated groups, all showed the same direction of perturbation (i.e. a gene would be up-regulated by both treatments, or down-regulated by both treatments). However, when this reduced, uniquely shared dataset was probed more deeply, there was no indication of a specific ZnO-mediated biological outcome in response to exposure to ZnO bulk or nanoparticles, possibly due to the small number of genes. At the phenotypic level, genes shared between the nano and formulation only groups were associated with dermatological diseases and conditions, whereas this association was not one of the main biological functions perturbed by treatment with the bulk sunscreen. The significance of this is not yet known, although bulk ZnO historically has been effective in treating skin diseases (Agren, 1990).

The top 10 up-regulated genes across all treated groups were strikingly similar, featuring genes involved in inflammation and...
cellular stress responses (Table 3). The top gene, SAA1, is closely homologous to SAA2, and both are expressed in the liver; the SAA2 gene was also highly up-regulated (but not amongst the top 10 genes), consistent with elevated levels of the SAA2 protein measured in blood samples. Orm1, encoding the inflammation-induced, acute-phase plasma protein, Alpha-1-acid glycoprotein 1, was the second-most strongly up-regulated across all treatments. Two metallothionein subtypes (MT1H and MT1E), proteins that can bind Zn, were up-regulated across all treatments, but only MT1H was in the top 10 for formulation only and bulk-treated mice. Slco1a1 and Mup1, encoding proteins involved in ion transport and glucose metabolism, respectively, were two of the top three down-regulated genes for all treatments. One potentially interesting exception to these kinds of functions was the shared down-regulation of the neuronal PAS domain protein 2 (NPAS2) and aryl hydrocarbon receptor nuclear translocator-like (ARNTL) genes, associated with the circadian clock in mammals, which were in the top 10 down-regulated genes in livers of mice treated with bulk and nano sunscreens, but which were not differentially expressed at all in the formulation only group.

Overall, results from the gene-expression study suggest that exposure to the 68ZnO particles in either nano or bulk form did not elicit a strong ZnO-dependent response at the transcriptional level in livers of virgin mice, and may have had a beneficial effect on the inflammatory response induced by the formulation only, particularly the larger-sized particles.

In contrast to the virgin cohort, livers of pregnant mice receiving any treatment had far fewer differentially transcribed genes compared to pregnant mice receiving no treatment (Figure 6A). Treatment with formulation only elicited the smallest transcriptional perturbation in pregnant mice, with only 11 differentially transcribed genes, none of which were unique to the formulation only group. Unlike the virgin cohort, the bulk of differential transcriptional activity occurred within the groups treated with the bulk or nano sunscreens, with the bulk group showing the largest transcriptional perturbation (49 differentially transcribed genes, 30 being unique to bulk). Once more, the majority of perturbed transcripts were classified as "enzymatic" or "other" by IPA (Figure 6B). However, when these datasets were analyzed using IPA, no canonical transcriptional pathways were meaningfully flagged as being differentially activated, likely due to the small number of genes in each dataset. Consistent with other data presented here, genes encoding proteins associated with an inflammatory response were prominent in the top ten up-regulated genes across all treatments, whereas genes in the top 10 down-regulated tended more towards cellular metabolism (Table 4).

Discussion

A major concern arising from the use of metal oxide nanoparticles in modern sunscreens has been whether those nanoparticles might penetrate the skin, translocate throughout the body and, potentially, elicit the toxicity in vivo as demonstrated in vitro. Existing studies have largely addressed the first aspect, showing that, for the most part, metal oxide nanoparticles do not penetrate intact, healthy human skin, although questions remain over damaged or otherwise compromised skin (Osmond & McCall, 2010). Additionally, the relevance of very short-term (i.e. 24–48 h) or single-application studies to the effects of multiple use over a longer period has been questioned (Gulson et al., 2010). Here, we serially applied sunscreens containing nano or larger-sized ZnO particles enriched with the stable isotope 68Zn, or formulation only containing no ZnO, to hairless mice as a biological model for highly permeable skin (Godin & Touitou, 2007). The aims were to
determine organ distribution and short-term biological impact of systemically absorbed Zn species. We reasoned that if ZnO particles or soluble Zn species from sunscreen are ultimately found to penetrate through mouse skin under some circumstances, then the transport of, and biological response to, those ZnO particles, or exposure to increased exogenous concentrations of Zn, in vivo would be of interest to industry and consumers.

$^{68}$Zn/$^{64}$Zn ratios were found to be significantly higher in the blood and major organs of virgin mice that had received topical applications of sunscreen containing $^{68}$ZnO nanoparticles compared to mice receiving no treatment or applications of the formulation only. The concentrations of $^{68}$Zn tracer were always higher in mice receiving no treatment or applications of the formulation /C0 than pregnant mice exposed to the bulk sunscreen had higher concentrations of$^{68}$Zn tracer in total blood Zn ($^{68}$Zn tracer in organs of the much thinner-skinned hairless mice, ranging from 8% of total brain Zn in bulk-treated mice to 50% of total liver Zn in nano-treated mice. Furthermore, the estimated retention in selected organs of $^{68}$Zn from total sunscreen dose was small, the highest being 0.04% in liver, and at least an order of magnitude lower in the brain, heart, kidney, lung and spleen. However, the key finding with respect to this influx of exogenous $^{68}$Zn was that total Zn concentrations did not increase in major organs of mice, excluding the likely inflammation-mediated increases found in liver and, to a much lesser extent, spleen. This implies that Zn homeostatic mechanisms were not statistically significant in blood. These observations are consistent with a human study, which observed the highest levels of $^{68}$Zn in blood and urine of females who received applications of the $^{68}$ZnO nano sunscreen compared to bulk (Gulson et al., 2010). $^{68}$Zn/$^{64}$Zn ratios were slightly higher in dam and pup livers of pregnant mice treated with $^{68}$ZnO-containing sunscreens compared with formulation only and untreated controls, but only marginally higher in uterine tissue. In contrast to the results for the virgin mice, pregnant mice exposed to the bulk sunscreen had higher concentrations of $^{68}$Zn tracer in dam (and fetal) liver than pregnant mice exposed to the nano sunscreen. We are unable to account for the difference in findings between pregnant and virgin mice. Also of note from the pregnant cohort is that the concentrations of $^{68}$Zn tracer detected in fetal liver closely matched those in dam livers, indicating a good correlation between maternal and placental exposures arising from topically applied sunscreens. Previous work has reported silica and TiO$_2$ nanoparticles in mouse fetal tissues following maternal intravenous injection, although only at the highest concentrations measured (0.8 mg/mouse; Yamashita et al., 2011). To the best of our knowledge, this is the first report of Zn from topically applied ZnO detected in fetal tissue.

Gulson et al. (2010) reported a very low percentage of $^{68}$Zn tracer in total blood Zn (~0.1%) of female subjects exposed to the same $^{68}$ZnO-containing sunscreens used here. We found higher percentages of $^{68}$Zn tracer in organs of the much thinner-skinned hairless mice, ranging from 8% of total brain Zn in bulk-treated mice to 50% of total liver Zn in nano-treated mice. Furthermore, the estimated retention in selected organs of $^{68}$Zn from total sunscreen dose was small, the highest being 0.04% in liver, and at least an order of magnitude lower in the brain, heart, kidney, lung and spleen. However, the key finding with respect to this influx of exogenous $^{68}$Zn was that total Zn concentrations did not increase in major organs of mice, excluding the likely inflammation-mediated increases found in liver and, to a much lesser extent, spleen. This implies that Zn homeostatic mechanisms were not statistically significant in blood. These observations are consistent with a human study, which observed the highest levels of $^{68}$Zn in blood and urine of females who received applications of the $^{68}$ZnO nano sunscreen compared to bulk (Gulson et al., 2010). $^{68}$Zn/$^{64}$Zn ratios were slightly higher in dam and pup livers of pregnant mice treated with $^{68}$ZnO-containing sunscreens compared with formulation only and untreated controls, but only marginally higher in uterine tissue. In contrast to the results for the virgin mice, pregnant mice exposed to the bulk sunscreen had higher concentrations of $^{68}$Zn tracer in dam (and fetal) liver than pregnant mice exposed to the nano sunscreen. We are unable to account for the difference in findings between pregnant and virgin mice. Also of note from the pregnant cohort is that the concentrations of $^{68}$Zn tracer detected in fetal liver closely matched those in dam livers, indicating a good correlation between maternal and placental exposures arising from topically applied sunscreens. Previous work has reported silica and TiO$_2$ nanoparticles in mouse fetal tissues following maternal intravenous injection, although only at the highest concentrations measured (0.8 mg/mouse; Yamashita et al., 2011). To the best of our knowledge, this is the first report of Zn from topically applied ZnO detected in fetal tissue.

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Posibility of absorption of particles over dissolved ionic species, of this bulk sample were dermally absorbed. In support of the true nano sample), it is possible that only nano-sized components 100 nm, although most of these are larger than all particles in the where approximately half were in the “nano” range (i.e. less than 100 nm, although most of these are larger than all particles in the true nano sample), it is possible that only nano-sized components of this bulk sample were dermally absorbed. In support of the possibility of absorption of particles over dissolved ionic species, the stratum corneum has been described as a microenvironment unlikely to be conducive to ZnO dissolution due to its relatively dry nature (Yang et al., 2010), and a recent review of nano-sized particles in cosmetic formulations listed ZnO as an insoluble component in sunscreen (Nohynek & Dufour, 2012). Wu et al. (2009) reported that Ti could be detected in internal organs, but not in blood, following 60 days exposure to topically applied (insoluble) TiO₂ to hairless mice, suggesting that blood may be a low internal reservoir for intact nanoparticles; hence our observation of a lower ⁶⁸Zn/⁶⁴Zn ratio in blood compared with any of the major organs (except brain), for hairless mice treated with the nano sunscreen, could be consistent with some absorption of ZnO in nanoparticulate form.

Attributing the influx of ⁶⁸Zn to penetration of intact ⁶⁸ZnO nanoparticles from sunscreen, however, seems at odds with the weight of evidence suggesting that metal oxide nanoparticles do not rapidly penetrate the outer epidermal layers (Osmond & McCall, 2010), and may reflect the source of internally absorbed ⁶⁸Zn as being due to soluble ⁶⁸Zn species formed upon dissolution of ⁶⁸ZnO particles in sunscreens applied to the skin. The dissolution of ZnO particles in sunscreen or on skin has not yet been established, and should not be assumed from studies assessing dissolution of ZnO nanoparticles in aqueous media (Mudunkotuwa et al., 2011; Yang & Xie, 2006). However, if dissolution of (oily coated) ZnO particles on skin does occur, it is possible that the rate of dissolution might be faster for nanoparticles than for larger particles, resulting in more soluble ⁶⁸Zn created within a given period for subsequent dermal absorption. Furthermore, an earlier study comparing absorption of radioactive ⁶⁵Zn in rats treated topically with suspensions of ⁶⁵ZnO (pH8) or ⁶⁵ZnCl₂ (pH4) found no difference in the levels of ⁶⁵Zn detected in internal organs between the two treatments (Hallmans & Liden, 1979), suggestive of ZnO particle dissolution on the skin surface and a subsequent ionic absorption and distribution profile. Finally, if intact ZnO particles were accumulating in the organs, as opposed to absorbed soluble ⁶⁸Zn species undergoing homeostatic exchange with endogenous Zn, we might expect to see higher concentrations of total Zn in all organs, but this was not the case (except for liver, but see below).

Inflammation is associated with an accumulation of hepatic Zn (Aydemir et al., 2012). Higher concentrations of total Zn were seen in livers of mice exposed to formulation only as well as the two ⁶⁸ZnO-containing sunscreens, compared to no treatment. Higher levels of SAA2, an acute phase liver biomarker for inflammation, were seen in the same groups showing hepatic Zn accumulation. An up-regulation of genes encoding metallothioneins, whose functions include Zn storage and detoxification, was seen across all treatment groups, but most strongly in formulation only. Therefore, we suggest that the increases in hepatic total Zn concentrations, hepatic metallothionein transcripts, and SAA2 transcript and protein levels relate to an inflammatory response in mice following topical exposure to the actual sunscreen formulation. This is also consistent with the skin reddening we observed for some mice in these three groups.

It could therefore be argued that the measured dermal absorption of ⁶⁸Zn was due to treatments being applied to inflamed skin, similar to the UVR-enhancement of quantum dot penetration through murine skin (Mortensen et al., 2008). However, mice that were noted to have developed substantially reddened, dry or flaky skin were largely excluded from the study. A small subset of mice displaying less obvious signs of dermal inflammation was retained in the study, but their concentrations of ⁶⁸Zn tracer in organs were not significantly higher than in mice that displayed no obvious physical inflammatory response (data not shown). Therefore, the effect of compromised skin was not the
Table 4. Top 10 up- and down-regulated genes in liver tissue from pregnant mice treated with sunscreen formulations containing no ZnO particles, 68ZnO bulk or 68ZnO nanoparticles.

| Treatment | Gene   | Log ratio | Function                  | Gene   | Log ratio | Function                  |
|-----------|--------|-----------|---------------------------|--------|-----------|---------------------------|
| Formulation | Saa3   | 4.2       | Acute phase response      | Slc10a1| −1.8      | Transporter protein        |
|           | SAA1   | 4.0       | Acute phase response      | PPP1R3C| −1.3      | Protein phosphorylation    |
|           | SAA2   | 3.7       | Acute phase response      | Mup3   | −1.4      | Lipocalin protein          |
|           | Orm1   | 3.0       | Acute phase response      | CAR3   | −1.4      | Carbonic anhydrase isomerase|
|           | SULT1E1| 3.0       | Sulphate conjugation      | SNORD116| −1.3      | Small nucleolar RNA        |
|           | LCN2   | 1.8       | Innate immune response    | Gmc11I | −1.2      | p53 response               |
|           | Sfa21I | 1.6       | Putative protease inhibitor| ARNTL  | −1.2      | Transcription factor        |
|           | A2M    | 1.3       | Proteinase inhibitor      | Gm8020 | −1.1      | not known                  |
|           | NGR4   | 1.3       | Neuregulin                | CLPX   | −1.0      | Proteinase                 |
| Bulk      | Saa3   | 5.4       | Acute phase response      | Slc10a1| −2.2      | Transporter protein        |
|           | SAA1   | 4.5       | Acute phase response      | PPP1R3C| −1.9      | Protein phosphorylation    |
|           | SAA2   | 4.0       | Acute phase response      | Mup3   | −1.4      | Lipocalin protein          |
|           | Orm2   | 3.2       | Acute phase response      | CAR3   | −1.4      | Carbonic anhydrase isomerase|
|           | SULT1E1| 3.1       | Sulphate conjugation      | SNORD116| −1.3      | Small nucleolar RNA        |
|           | LCN2   | 2.4       | Innate immune response    | Gmc11I | −1.2      | p53 response               |
|           | S100a8 | 2.2       | Calcium binding protein   | ARNTL  | −1.2      | Transcription factor        |
|           | A2M    | 2.2       | Proteinase inhibitor      | Gm8020 | −1.1      | not known                  |
|           | BC100530| 2.0       | Not known                 | Cyp2a22| −1.0      | Cellular metabolism        |
| Nano      | SAA1   | 3.6       | Acute phase response      | Slc10a1| −1.7      | Transporter protein        |
|           | SAA2   | 3.2       | Acute phase response      | Mup3   | −1.2      | Lipocalin protein          |
|           | Orm2   | 2.8       | Acute phase response      | Gmc11I | −1.1      | p53 response               |
|           | Saa3   | 2.8       | Acute phase response      | PPP1R3C| −1.0      | Protein phosphorylation    |
|           | SULT1E1| 2.7       | Sulphate conjugation      | SNORD116| −1.0      | Small nucleolar RNA        |
|           | LCN2   | 1.5       | Innate immune response    | Gm8020 | −0.9      | not known                  |
|           | S100a8 | 1.5       | Calcium binding protein   | Cyp2a22| −0.9      | Cellular metabolism        |
|           | Sfa21I | 1.4       | Putative protease inhibitor| Scl34a2| −0.9      | Transporter protein        |
|           | S100a9 | 1.4       | Calcium binding protein   | Hsd1b2 | −0.8      | Steroid hormone production|
|           | Orm3   | 1.3       | Acute phase response      | ME1    | −0.8      | Cellular metabolism        |

over-riding factor in the results reported here, although it may have contributed to some extent.

The short-term biological response of mice treated with the sunscreen formulations was additionally assessed by whole-genome transcriptional profiling of liver tissue, which showed that, consistent with a formulation-mediated inflammatory response, the strongest perturbation of transcriptional activity in virgin mice was elicited by treatment with formulation only, whereas far fewer genes were differentially expressed in mice treated with either of the ZnO sunscreens compared to untreated mice. In support of this, transcript levels for SAA2 were amongst the most strongly up-regulated for all treatments, consistent with the increased protein levels as measured by the SAA2 ELISA. Furthermore, the acute phase response signaling pathway, the NRF2-mediated oxidative stress response pathway, as well as pathways involved in repair of damaged DNA, were moderately perturbed in the formulation only group at the transcriptional level, whereas they were either more weakly or not at all perturbed in the nano and bulk groups.

An interesting point of difference at the transcriptional level was the shared down-regulation of the NPAS2 and ARNTL genes in ZnO nano- and bulk-treated mice, which was not present in the formulation only group. NPAS2 and ARNTL together form a heterodimer associated with the circadian clock in mammals. Both genes are positively regulated by period homolog 2 (PER2), which was not differentially regulated here. Single nucleotide polymorphisms in these genes have been associated with an increased risk of seasonal affective disorder in humans (Partonen et al., 2007), distinctive sleep patterns in the elderly (Evans et al., 2013) and reproductive success (Kovanen et al., 2010). Concentrations of serum Zn fluctuate over a 24 h period in humans (Kanabrocki et al., 2008; Scales et al., 1988), however the significance of the shared down-regulation of Arntl and Npas2 specifically in mice treated with sunscreens containing ZnO here is not yet known.

In contrast to the relative high level of transcriptional perturbation in the virgin cohort, far fewer genes were differentially transcribed in treated pregnant mice compared to untreated pregnant mice. We observed a greater variation in global gene-expression patterns between untreated pregnant mice compared to untreated virgin mice and it is likely that the increased variability in the control pregnant mice diminished the impact of treatment-related variability in gene expression, such that far fewer differentially regulated genes attained statistical significance. Furthermore, whereas treatment with the formulation only elicited the strongest response in the virgin mice, it induced the least amount of differential transcriptional activity in the pregnant mice, and most in the bulk-treated pregnant group. This is consistent with SAA2 protein levels for the pregnant cohort, where the addition of ZnO to the sunscreen formulation did not mitigate the inflammatory response induced by topical application, as opposed to the virgin cohort where it had a protective effect.

Conclusions
In conclusion, by using hairless mice, we assessed the absorption of 68Zn tracer from topically applied 68ZnO nano or larger particles in sunscreen formulations, as well as the short-term biological impact of those sunscreens, compared to formulation only or untreated mice. Use of the hairless mouse model markedly enhanced the probability of skin penetration due to its much thinner skin compared to humans, and therefore the concentrations of Zn absorption in the hairless mouse cannot be directly...
extrapolated to humans, which, we emphasize, was not the primary intent of this study. The presence of 68Zn tracer in blood and major internal organs was indicative of absorption of 68Zn from 68ZnO sunscreens, either as 68ZnO nanoparticles (or larger particles) or as soluble 68Zn species, but was not associated with an overall change in the accumulation of Zn in organs, suggestive of adequate Zn homeostatic mechanisms in the face of regular ZnO sunscreen use in the short term. Furthermore, no ZnO-dependent adverse biological impact was found in the short-term using a biomarker for acute-phase inflammation or gene profiling, and in fact the presence of ZnO (and particularly the larger particles) may have mitigated the response to inflammatory dermal stimuli, consistent with its role in wound healing and general skin health. However, the inflammatory response to the sunscreen formulation itself, and transplacental presence of 68Zn tracer from sunscreen formulations may warrant further, longer-term investigations.

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Declaration of interest

The authors declare no competing interests. This work was supported by the CSIRO Animal, Food and Health Sciences Division, the CSIRO Advanced Materials Transformational Capabilities Platform (Nanosafety), and the CSIRO Future Manufacturing Flagship.

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