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**Risk Assessment Branch I, Health Effects Division (7509P)** **Date:** 3/18/2011
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**Risk Assessment Branch, Antimicrobials Division (7510P)** **Date:** 3/15/2011
**Template version 02/06**

**TXR#:** 0055726

# DATA EVALUATION RECORD

**STUDY TYPE:** Dermal penetration-human, *Non-guideline*
**PC CODE:** 888502 **DP BARCODE:** D386970

**TEST MATERIAL (PURITY):** $^{68}$Zinc oxide nanoparticles (>99%), 19 ± 8 nm and 110 ± 46 nm

**SYNONYMS:** Nano zinc

**CITATION:** Gulson, B., McCall, M., Korsch, M. *et al.* Small Amounts of Zinc from Zinc Oxide Particles in Sunscreens Applied Outdoors are Absorbed Through Human Skin. Australian Photobiology Testing Facility, Sydney University, Sydney, New South Wales 2006, Australia. August 5, 2010. MRID 48387301. *Toxicological Sciences*, **118(1),** 140-149 (2010).

**SPONSOR:** CSIRO Flagship Collaboration Scheme and Macquarie University.

**EXECUTIVE SUMMARY:**

There is debate in the scientific community as to whether or not nanoparticles are likely to penetrate the skin barrier upon dermal contact. The results of *in vitro* studies using animal and human skin in the literature indicate that small amounts of different kinds of nanoparticles may penetrate skin in some cases. However, *in vitro* dermal penetration data alone is of limited utility to the Agency in the absence of evidence that the *in vitro* studies are predictive of *in vivo* results. This study provides *in vivo* evidence of penetration of nanoparticles and/or ions from nanoparticles in humans.

In a dermal penetration study conducted in human subjects (MRID 48387301) sunscreens containing 20% wt/wt $^{68}$ZnO particles enriched to 99% $^{68}$Zn were applied twice daily for 5 consecutive days to the backs of adult males and females at an average dose of 4.6 mg/cm$^2$ (males) and 3.7 mg/cm$^2$ (females). The oil-water sunscreen formulations contained 19 ± 8 nm $^{68}$ZnO particles (5 males, 6 females) or 110 ± 46 nm $^{68}$ZnO particles (5 males, 4 females). Applied sunscreens were allowed to equilibrate with skin for 30 minutes, after which time subjects were free to engage in beach-related activities at an aquatic center and beach in Australia. The mean UV exposures measured over the 5 day experimental period were 26.7 ± 10.1 W/m$^2$ (UVA) and 1.2 ± 0.6 W/m$^2$ (UVB).

Blood and urine samples were collected from the participants 8 days prior to the start of $^{68}$ZnO sunscreen exposures, before the first application of $^{68}$ZnO sunscreens and after removal of the second application of $^{68}$ZnO sunscreens during the 5 days of the trial, and 6 days after the end of the trial. Some subjects also provided urine samples during the trial and following the trial. Isotope ratios of $^{68}$Zn/$^{64}$Zn were measured by Multi-Collector Inductively Coupled Mass Spectrometry (MC-ICP-MS). Total Zn concentrations in all samples analyzed for isotope ratios were determined by ICP-MS. The percentage change (Δ) of $^{68}$Zn ($\Delta^{68}$Zn) in blood and urine samples was determined. (This value is >0 if $^{68}$Zn is absorbed). The absolute amounts of $^{68}$Zn absorbed in

blood were also calculated by adjusting $\Delta^{68}$Zn for fat-free body mass or by use of estimated blood volumes.

One subject had an adverse reaction to the sunscreen and application was discontinued on Day 4. The nature of this adverse response was not described. However, she continued to participate in the study by providing blood and urine samples.

The study indicated significant increases in blood $\Delta^{68}$Zn% after dermal exposure to both kinds of particles for both males and females ($p<0.001$). This was not detectable until Day 2, indicating a lag between dermal application and observable blood levels. Blood levels were also increased 6 days after the last sunscreen application for both particle types, indicating continued bioavailability of skin-bound residues of $^{68}$Zn.

Statistical analysis on Day 5 revealed significant increases in blood levels of $^{68}$Zn from 19 ± 8 nm nanoparticles in females compared to other $^{68}$ZnO treatment groups (110 ± 46 nm females, 110 ± 46 nm males, and 19 ± 8 nm males).

The largest increase in blood $\Delta^{68}$Zn% was observed for Subject 7, which had an adverse skin reaction to the sunscreen. The nature of the adverse reaction was not described, but her relatively high blood $\Delta^{68}$Zn% levels suggest that skin barrier function may have been compromised.

There was a concern that urine samples may have been contaminated with $^{68}$ZnO sunscreens during sample collection, particularly in females and particularly during the 5 days of sunscreen exposure. The authors conducted an analysis based on reasonable assumptions that seemed to effectively differentiate between samples that may have been appreciably and minimally contaminated. Analysis of samples considered likely of minimal contamination showed that $\Delta^{68}$Zn% was significantly greater for 19 ± 8 nm $^{68}$ZnO-treated females than other treatment groups, which was consistent with what was observed in the blood samples.

Since this study measured $^{68}$Zn isotope ratios in blood and urine samples and did not analyze for nanoparticles, it is not possible to determine if the $^{68}$Zn was associated with intact nanoparticles or dissolved $^{68}$Zn ions. Therefore, it is not possible to determine if it was intact particles and/or $^{68}$Zn ions that penetrated. The amount of $^{68}$Zn that penetrated the skin was estimated to be low, about 0.001% of the applied dose. This estimation does not include any $^{68}$Zn that may have partitioned into other organs or biological fluids other than blood and urine.

Together, these data indicate that dermal penetration of $^{68}$ZnO particles 110 ± 46 and 19 ± 8 nm in size in oil-water formulations with a penetration enhancer and Zn chelator may occur, but that penetration is low.

This metabolism study in humans is classified **acceptable/non-guideline**.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements **were not** provided.

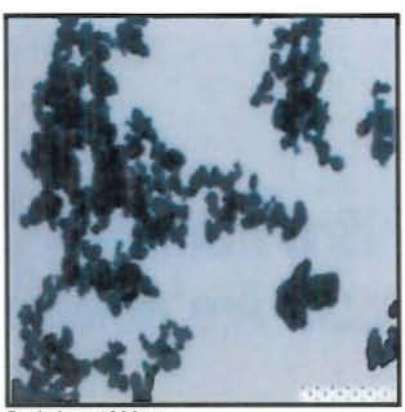
# I. MATERIALS AND METHODS

## A. MATERIALS:

1. **Test compound:** $^{68}$ZnO particles ("bulk" particles and nanoparticles) prepared from >99% $^{68}$Zn

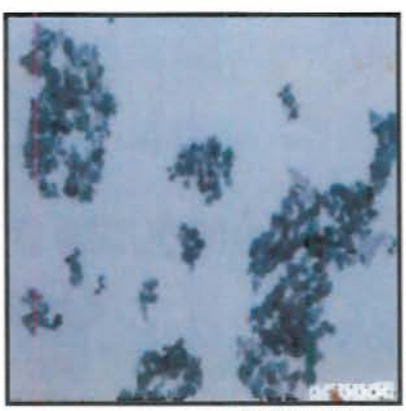
| | |
|---|---|
| **Description:** | "Bulk" particles (110 ± 46 nm, range 25-284 nm)<br>Nanoparticles (19 ± 8 nm, range 3-60 nm)<br>Note: Although the study authors consider the 110 ± 46 nm particles as "bulk" particles, the Agency considers these nanoparticles, since there is a large fraction ≤100 nm. However, for purposes of distinguishing these two sizes and for consistency with the literature study, the nomenclature of "bulk" is retained. |
| **Lot/batch #:** | Not stated |
| **Purity:** | 20% wt/wt in sunscreen preparations |
| **Contaminants:** | Not stated |
| **CAS # of TGAI:** | 1314-13-2 (zinc oxide) |

**Structures:**



Scale bar=1000 nm

**"Bulk" particles ($^{68}$ZnO=100 ± 46 nm)**



Scale bar=200 nm

**Nanoparticles ($^{68}$ZnO=19 ± 8 nm)**

2. **Vehicle:** Oil-water sunscreen formulation with isopropyl myristate (a penetration enhancer) and EDTA (a Zn chelator) prepared by commercial process for sunscreens by Baxter Pharmaceuticals (Lot/Batch #; Purity not stated).

3. **Human subjects:**

**Ethical Consideration**

All research was certified by the authors as being done in full compliance with Australian government policies and the Helsinki Declaration. The studies were approved by human ethics committees at Macquarie University and CSIRO.

**Pilot studies**

*Sunscreen formulation*

The distribution of sunscreen containing $^{68}$ZnO nanoparticles was determined by even application of sunscreen to the underside of the forearm of one male and one female subject (ages unknown, skin types unknown) at a

dose of 2 mg/cm$^2$. Sunscreen was removed by application of tape, followed by application of 200 g/cm$^2$ pressure to the area for 30 s, followed by tape removal.

*Sunscreen exposure protocol development*
The experimental protocol for the sunscreen exposure protocol in the main study was developed in a pilot study. In this pilot study, three subjects (ages, sex, and skin types not provided) were exposed to sunscreen in which the ZnO active ingredient was only 51% enriched with $^{68}$Zn (instead of 99% in the main study).

**Main study**

Ten males (aged 20-66) and 11 females (aged 19-64) with skin types I-IV participated in the study. There were two subjects (ages, sex, and skin types not provided) who withdrew from the study after the first night. Subject 2 had an unforeseen commitment on Day 5. Subject information is summarized in Table 1, along with key experimental results.

Subjects wore UV-protective upper body garments with a specific section cut out of the back to reveal uncovered skin. Sunscreen containing 20% wt/wt "bulk" $^{68}$ZnO or $^{68}$ZnO nanoparticles was applied twice daily at an average dose of 4.6 mg/cm$^2$ (males) and 3.7 mg/cm$^2$ (females) by the same investigator. After each sunscreen application, skin was allowed to equilibrate for 30 minutes with the sunscreen, followed by sun exposure of participants by lying on their stomachs in the sun for a minimum of 30 minutes. After this 30 minute period, subjects were free to pursue normal activities while keeping a diary of UV exposure, activities, garment changes, and any concerns over touching their backs or other unusual happenings. The use of commercial sunscreens of similar formulation but with chemical UV absorbers (instead of ZnO) to uncovered body areas other than the back was encouraged. $^{68}$ZnO sunscreens were removed from the subject's backs at the end of each day with alcohol/lanoline wipes.

$^{68}$ZnO sunscreen exposures were performed for 5 days, with the first day at an aquatic center and the following 4 days at a Sydney beach. The mean UV exposures measured over the 5 day experimental period were 26.7 ± 10.1 W/m$^2$ (UVA) and 1.2 ± 0.6 W/m$^2$ (UVB).

Blood and urine samples were collected from the participants 8 days prior to the start of $^{68}$ZnO sunscreen exposures, before the first application of $^{68}$ZnO sunscreens and after removal of the second application of $^{68}$ZnO sunscreens during the 5 days of the trial, and 6 days after the end of the trial. Some subjects also provided urine samples during the trial and following the trial.

**4. Preparation and characterization of sunscreens containing bulk or nano-sized $^{68}$ZnO:**

ZnO powder enriched to >99% $^{68}$Zn (a stable (non-radioactive), but uncommon isotope of Zn) was purchased and used to make nanoparticles using a proprietary method based on high-energy attrition milling and larger "bulk" particles based on a modification of this same method. The particles were characterized by determining crystal size and phase by x-ray diffraction. The crystal structure of both $^{68}$ZnO preparations was identical to those used in commercial preparations. The size of the "bulk" particle preparation was 110 ± 46 nm (range 25-284 nm) and the size of the nanoparticle preparation was 19 ± 8 nm (range 3-60 nm).

Sunscreens containing "bulk" and nanoparticle $^{68}$ZnO were prepared by incorporation into an oil-water formulation using a commercial process for preparing sunscreens by Baxter Pharmaceuticals. The final concentration of $^{68}$ZnO in both preparations was 20% wt/wt. Sunscreens were characterized by determining sun protection factor (SPF) and the distribution of $^{8}$ZnO particles in sunscreen on human skin. The latter was determined by even application of the sunscreens to the forearms of human subjects at a dose of 2 mg/cm$^2$, followed by tape stripping and imaging of the stripped tape by scanning electron microscopy (SEM) in back-scattered mode, which verified an even distribution of $^{68}$ZnO particles on skin.

## B. STUDY DESIGN AND METHODS:

### 1. Exposure and sample collection:

The design of the main study was based on a pilot study in which three subjects (ages, sex, and skin types not provided) in which the ZnO active ingredient was only 51% enriched with $^{68}$Zn instead of 99% in the main study. No further details were provided on this pilot study.

In the main study, blood and urine samples were collected from the participants 8 days prior to the start of $^{68}$ZnO sunscreen exposures and just prior to the first sunscreen application to facilitate establishment of baseline $^{68}$Zn/$^{64}$Zn levels. Blood and urine were also collected after removal of the last application on each of the 5 days of the trial (to determine $^{68}$Zn/$^{64}$Zn levels during treatment) and 6 days after the end of the trial (to determine $^{68}$Zn/$^{64}$Zn levels after treatment).

The first day of the sunscreen exposure was conducted at an aquatic center (to refine protocols) and the following 4 days were conducted at a Sydney beach. Subjects wore UV-protective upper body garments with a specific section cut out of the back to reveal uncovered skin. A single investigator applied sunscreen containing ZnO bulk or nanoparticles enriched with 99% $^{68}$Zn. Sunscreen was allowed to equilibrate with skin for 30 minutes prior to sun exposure, in which subjects laid on their stomachs for 30 minutes to expose the back. Subjects were then free to engage in desired activities. A second, similar application was then performed. It was not stated how far apart the two applications were. At the end of each day, sunscreen was removed from the subject's backs with an alcohol/lanoline wipe.

A summary of the experimental subjects and key experimental results are summarized in Table 1.

### 2. Prevention of sample contamination:

Since the isotope method is very sensitive, subjects were continuously reminded orally and with signage to minimize contamination, especially during urine collection. Subjects also recorded whether or not they changed garments or touched their backs. Clean beach towels and a paper towel covering were provided each day. Towels and the UV-protective upper body garments were washed daily by two study organizers.

**Table 1. Subject Information and Key Experimental Results for Main Study**

| Subject ID # | $^{68}$ZnO sunscreen type | Gender | Age | Skin type | Country | Relationship | Average dose (mg/cm$^2$) | $\Delta^{68}$Zn (beach) | $\Delta^{68}$Zn (post) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | NP | Male | 60 | IV | South America | | 5.1 | 0.18 | 0.26 |
| 2[a] | NP | Male | 20 | II | Australia | | 5.3 | NA[a] | 0.24 |
| 3 | NP | Female | 23 | II | Australia | Sibling of 4 | 3.7 | 0.20 | 0.37 |
| 4 | NP | Male | 20 | II/III | Australia | Sibling of 3 | 4.7 | 0.22 | 0.42 |
| 5 | Withdrew after first night. No other information available. | | | | | | | | |
| 6 | Bulk | Male | 24 | I/II | Australia | | 5.1 | 0.16 | 0.22 |
| 7[b] | NP | Female | 44 | II/III | South America | | 3.8 | 0.83 | 1.31 |
| 8 | Bulk | Female | 21 | III | Australia | | 4.0 | 0.30 | 0.43 |
| 9 | NP | Female | 60 | II/III | South America | | 3.9 | 0.27 | 0.52 |
| 10 | Bulk | Female | 34 | I | UK | | 3.7 | 0.09 | 0.15 |
| 11 | Withdrew after first night. No other information available. | | | | | | | | |
| 12 | NP | Male | 66 | I/II | Australia | | 4.6 | 0.08 | 0.24 |
| 13 | Bulk | Male | 23 | II/III | Australia | | 4.6 | 0.20 | 0.41 |
| 14 | Bulk | Male | 21 | II/III | Australia/ South American parents | Brother of 20 | 3.8 | 0.26 | 0.40 |
| 15 | Bulk | Female | 27 | I | Germany | | 3.7 | 0.25 | 0.42 |
| 16 | NP | Male | 27 | I | Australia | Son of 17 | 4.3 | 0.11 | 0.23 |
| 17 | Bulk | Male | 59 | I | Australia | Father of 16 | 5.3 | 0.06 | 0.17 |
| 18 | NP | Female | 21 | IV | South America | | 3.2 | 0.45 | 0.69 |
| 19[c] | NP | Female | 19 | III | United States | | 4.1 | NA[c] | 0.80 |
| 20 | Bulk | Male | 20 | IV | Australia/ South American parents | Brother of 14 | 5.2 | 0.18 | 0.32 |
| 21 | Bulk | Female | 24 | IV | South America | Twin of 22 | 3.3 | 0.10 | 0.22 |
| 22 | NP | Female | 24 | IV | South America | Twin of 21 | 4.0 | 0.28 | 0.58 |
| Sunscreen applicator[d] | NP and Bulk | Female | 64 | I/II | Australia | | Unknown[d] | 0.35 | 0.45 |

NP=nanoparticles, 19 ± 8 nm, Bulk=100 ± 46 nm

[a]Subject had an unforeseen commitment on Day 5.

[b]Subject had an adverse reaction to the sunscreen (nature of reaction not specified) and application was discontinued on Day 5, however she continued to provide blood and urine samples.

[c]Subject was unable to provide blood samples at the beach provided a blood sample before exposure and post-exposure and urine samples throughout.

[d]This was the individual that applied sunscreen to the subjects. Her exposure level was unknown.

## 2. Isolation of Zn and Measurement of $^{68}Zn/^{64}Zn$ in blood and urine:

Zn was purified from blood (0.2 ml) and urine (2-6 ml) samples by ion exchange through macroporous resin following digestion with ultraclean nitric acid and hydrogen peroxide. Total Zn levels in blank controls were routinely less than 3 ng, which is much less than the amounts naturally occurring in blood and urine.

Isotope ratios of $^{68}Zn/^{64}Zn$ measured by Multi-Collector Inductively Coupled Mass Spectrometry (MC-ICP-MS). Due to limited access to instrumentation, only critical samples were measured for all subjects (before exposure, end of Day 5 at the beach, post-exposure, while more complete data were observed for four subjects. In order to allow comparisons between laboratories, the isotope ratios of $^{66}Zn/^{64}Zn$ were normalized to a value of 0.596. Total Zn concentrations in all samples analyzed for isotope ratios were determined by ICP-MS.

## 3. Calculations

Zn is an essential element that naturally occurs in bodily tissues.

The levels of Zn naturally occurring in the body tissues reflect the sum of the 5 stable isotopes [$^{64}$Zn (48.89%) + $^{66}$Zn (27.81%) +$^{67}$Zn (4.11%) +$^{68}$Zn (18.57) + $^{70}$Zn (0.62%)].

The "bulk" and NP sunscreens used in this study were made with 99% $^{68}$Zn, and so were enriched from naturally occurring levels of $^{68}$Zn (18.57).

Determining the absolute amount of $^{68}$Zn (in mass) absorbed from the $^{68}$ZnO in sunscreen was achieved in two steps:

1) The percentage change ($\Delta$) of $^{68}$Zn in blood and urine samples was calculated after measuring isotope ratios of $^{68}Zn/^{64}Zn$ by MC-ICP-MS on Day 5 of sunscreen exposure and 6 days after exposure:

$$\Delta^{68}Zn\% = [(^{68}Zn/^{64}Zn_{\text{during exposure}} - {}^{68}Zn/^{64}Zn_{\text{after exposure}}) \div {}^{68}Zn/^{64}Zn_{\text{before exposure}}] \times 100\%$$

Where $\Delta^{68}Zn > 0$ only if $^{68}$ZnO from sunscreens are absorbed, since the $^{68}Zn/^{64}Zn$ ratio is otherwise constant.

2) In order to estimate the absolute amounts of $^{68}$Zn absorbed from sunscreen, two approaches were used.
   a) Determine fat-free body mass based on body mass index (BMI, Deurenberg et al. 1991, Appendix 1). Use this value and measured blood Zn levels (via ICP-MS) to adjust the $\Delta^{68}Zn$ value for each individual. Since the BMI calculations in this paper accounts for gender and age, these potential biases are controlled.

Body Fat %= 1.20 X BMI +( 0.23 x age )-(10.8 x sex)-5.4
Where BMI = (body weight in kg) /(height in m)$^2$ (Quetelet 1869)
Age= age in years
Sex: males = 1, females =0

b) Estimate an individual's blood volume based on the method of Nadler *et al.* (1962, Appendix II). Multiply this value by the measured total blood Zn concentration before exposure.

## 4. Statistics:

Subject "X", the technician who applied both sunscreen formulations, was not included in any of the statistical analyses.

The number of subjects per treatment group was: NP (5 males, 6 females), "bulk" (5 males, 4 females). The dependent variable ($\Delta^{68}$Zn%) was positively skewed (skewness index 2.1) and so data were transformed to the $\log_{10}$ prior to analysis.

A Wilcoxan test was used for within-group comparisons of $\Delta^{68}$Zn%between exposure and post exposure groups. A 2x2 independent ANOVA was used to determine effects of sex and time on Deurenberg fat-free mass adjusted (and $\log_{10}$ transformed) $\Delta^{68}$Zn% (approach 2a above). Differences in the amount of blood $^{68}$Zn (in µg) were also evaluated by a 2x2 independent ANOVA. Effect sizes in both cases were calculated using partial eta-squared ($\eta_p^2$).

## II. RESULTS:

### 1. Adverse responses:

Subject 7 had an adverse reaction to the sunscreen and application was discontinued on Day 4. The nature of this adverse response was not described. However, she continued to participate in the study by providing blood and urine samples.

### 2. Blood measurements:

The average $^{68}$Zn/$^{64}$Zn $_{\text{before exposure}}$ ratio in blood was 0.41584±0.00002 for the "bulk" and NP subjects, with a variation of ±0.006% (N=21). $^{68}$Zn/$^{64}$Zn ratios in blood increased during sunscreen exposure and after sunscreen exposure was discontinued in both the "bulk" and NP groups (Appendix, Figure 1). Although the isotopic ratios of $^{68}$Zn/$^{64}$Zn increased with exposure to $^{68}$ZnO, the total Zn content in the blood remained unchanged in both sexes before and after exposure to sunscreens containing "bulk" or NP . $^{68}$ZnO, However, males had significantly higher mean natural levels of Zn. This was observed both before exposure (males 3.83 mg/l, females 3.05 mg/ml, p=0.01) and after exposure (males 3.63, females 3.21, p=0.04).

The differences in $\Delta^{68}$Zn% between exposure and post-exposure results was highly significant via a Wilcoxan test (p<0.001), with a mean increase in $\Delta^{68}$Zn% on the last day of exposure of

0.23 compared to a mean increase of 0.42 post-exposure. Analysis of a subset of individuals, one male and one female in each of the two particle treatment groups, showed increases in $\Delta^{68}$Zn% during exposure and 6 days following exposure (Appendix, Figure 2).

Multi-level ANOVAs and post hoc tests were pursued to determine the interaction of gender and sunscreen particle type that contributed to this significant difference. For fat-free adjusted $\Delta^{68}$Zn%, no significant effects were observed at alpha=0.05. However, an interaction of gender and particle type approached significance (p=0.053). Further analysis revealed that this difference was not significant for males (p=0.83), but that it was for females (p=0.016). Similar results were obtained by comparing the amounts of absorbed Zn from sunscreens compared to estimated blood volumes, with an interaction between particle and gender identified that approached significance (p=0.051) with females identified as the source of the difference (p=0.012). Results from subjects obtained 6 days post-exposure are depicted graphically in the Appendix in Figure 3.

Since a significant effect of particle type was identified in females, particular attention was paid to whether or not Subject 7, who had absorbed the highest levels of $^{68}$Zn, possibly as a result of adverse reactions to the screen, had unduly influenced the results. Residuals and Cooks distance were calculated for each subject and Subject 7 was found to be well within the range of these values, with a residual of 1.21 (less than the highest value of 1.47) and a Cook's distance of 0.09 (less than the highest value of 0.20).

Further statistical comparisons with covariates of age, average dose of sunscreen, skin type (treated as a numeric variable) and country were used for a 2x2 ANCOVA. For females, the effect of particle remained with each covariate held constant: age (p=0.01), skin type (p=0.032), country (p=0.029). No significant effect was observed for males, however.

Together, these data show that males have naturally higher blood levels of Zn than females, and that exposure to sunscreens containing $^{68}$ZnO particles did not change overall Zn blood levels in males or females. For females only, nanoparticle type ("bulk" or NP) affects dermal absorption of $^{68}$Zn (with greater penetration of NP), and this effect is independent of age, skin type, or country.

**3. Urine measurements:**

The variation in the average $^{68}$Zn/$^{64}$Zn $_{\text{before exposure}}$ ratio in the urine was ±0.022% (N=21). Larger increases in $\Delta^{68}$Zn% were observed in blood than urine samples. $\Delta^{68}$Zn% peaked around the last day of sunscreen exposure and decayed onwards, with $\Delta^{68}$Zn% values detectable for all subjects 6 days after the last sunscreen application and out to 25-40 days after a application in a subset of 4 subjects (one female and three males, all of which were exposed to NP sunscreen). These results are summarized graphically in the Appendix, Figure 4.

Statistical analysis in the $\Delta^{68}$Zn% in urine samples of all subjects revealed no differences. There was a large variation in peak $\Delta^{68}$Zn% values across subjects. Most subjects at peak $\Delta^{68}$Zn% from 0-4, but six subjects had peak values ranging from 5-330. These large variations led to concerns about contamination of urine with sunscreen during sample collection.

Although contamination cannot be unequivocally ruled out, assumptions were made that contaminated samples would have 1) Larger peak $\Delta^{68}$Zn% values on Days 1-5 while samples were collected 2) Smaller ratios of $\Delta^{68}$Zn% on Day 11/Day 5 (since subjects would have applied sunscreen on Day 5 but not Day 11): this is called the "retention ratio" and 3) there would be a mathematical relationship between retention ratio and peak $\Delta^{68}$Zn% .

Scatter log plots of the retention ratio versus peak $\Delta^{68}$Zn% revealed two populations (see Appendix, Figure 5), one that displayed no mathematical relationship between retention ratio and peak $\Delta^{68}$Zn% , indicative of minimal contamination and one that displayed a linear relationship, indicative of contamination.

A 2x2 ANOVA (with gender and particle type as factors) of the subset of samples suspected of minimal contamination on Day 5 revealed that females in an NP group showed significantly higher $\Delta^{68}$Zn% values compared to NP males (p=0.0007), bulk females (p=0.009), bulk females (p=0.009), and bulk males (p=0.002). A similar analysis was performed on Day 11, from which no samples were excluded (both minimal contamination and samples suspected of contamination were included) and yielded similar results. Females in the NP group on Day 11 showed significantly higher $\Delta^{68}$Zn% values compared to NP males (p=0.002), bulk females (p=0.041), and bulk males (p=0.009).

## III. DISCUSSION AND CONCLUSIONS:

### A. INVESTIGATORS CONCLUSIONS:

The investigators noted four key findings from the study:

1) Contrary to the dominant view, this study provides unequivocal evidence that Zn from ZnO particles in sunscreens in absorbed through healthy human skin exposed to sunlight and is detectable in blood and urine.
2) The total amounts of Zn absorbed from sunscreen were small compared with the amounts of Zn normally present in the human body.
3) Particle size and gender interacted to determine the levels of absorption. This may be due to differences in skin thickness (female skin generally thinner than male) or other gender-related factors such as skin pH and surface lipid content.
4) There is a time lag between sunscreen application and the first detection of tracer $^{68}$Zn in samples. Detection is first detected in blood after the fourth sunscreen application on the second day. This implies that studies with fewer applications, a shorter observation time, or the use of less sensitive methods to detect absorption may not have been able to observe effects.

The authors also noted that $^{68}$Zn detected in blood and urine could be in the form of $^{68}$ZnO particles or ions. The amount of UV exposure did not appear to influence absorption. Some subjects may have experienced greater sweating and increased skin temperature associated with being more active and this possibility has been mentioned in other studies, but these variables were difficult to quantify in this study.

## B. EPA REVIEWER COMMENTS:

This study investigated the dermal absorption of 19 ± 8 nm $^{68}$ZnO nanoparticles and 110 ± 46 nm "bulk" $^{8}$ZnO formulated as sunscreens in human subjects. Importantly, although the publication referred to the 110 ± 46 nm as "bulk" particles, the Agency considers particles in this size range as nanoparticles, due to the presence of a fraction ≤ 100 nm in size.

Sunscreens were applied twice per day for 5 days and urine and blood isotope levels of $^{68}$Zn monitored prior to the study, during sunscreen application days, and 6 Days following the last sunscreen application.

The study indicated significant increases in blood $\Delta^{68}$Zn% after dermal exposure. This was not detectable until Day 2, indicating a lag between dermal application and observable blood levels. Blood levels were also increased 6 Days after the last sunscreen application, indicating continued bioavailability of skin-bound residue. This was observed for both the 19 ± 8 nm nanoparticle and the 110 ± 46 nm "bulk" sunscreens.

Statistical analysis on Day 5 revealed significant increases in blood levels of $^{68}\Delta$ Zn from 19 ± 8 nm particles in females compared to other treatment groups (110 ± 46 nm females, 110 ± 46 nm males, and 19 ± 8 nm males).

The largest increase in blood $\Delta^{68}$Zn% was observed for Subject 7, which had an adverse skin reaction to the sunscreen. The nature of the adverse reaction was not described, but her relatively high blood $\Delta^{68}$Zn% levels suggest that skin barrier function may have been compromised.

The urine data were equivocal. The analysis conducted by the investigators seemed to effectively differentiate between samples that may have been appreciably and minimally contaminated, however, the contamination status of each sample could not be unequivocally determined. Even so, the result that $\Delta^{68}$Zn% was significantly greater for 19 ± 8 nm $^{68}$ZnO-treated females than other treatment groups was consistent with what was observed in the blood.

Since this study measured $^{68}$Zn isotope ratios in blood and urine samples and did not analyze for nanoparticles, it is not possible to determine if the $^{68}$Zn was associated with intact nanoparticles or dissolved $^{68}$Zn ions. Therefore, it is not possible to determine if it was intact particles and/or $^{68}$Zn ions that penetrated. The amount of $^{68}$Zn that penetrated the skin was estimated to be low, about 0.001% of the applied dose. This estimation does not include any $^{68}$Zn that may have partitioned into other organs or biological fluids other than blood and urine.

## C. STUDY DEFICIENCIES:

The following study deficiencies were noted. These deficiencies were not considered severe enough to impact the regulatory utility of the study.

- No information was provided on how blood was drawn from patients and stored.
- It was not stated if the subject's height and weight were measured by the investigators or if this information was provided by the subjects. However, since BMI calculations were

used, this information must have been obtained.

- It was not stated if/how subjects were compensated for participation in the study.
- It was not stated how data skewness was determined.

## APPENDIX

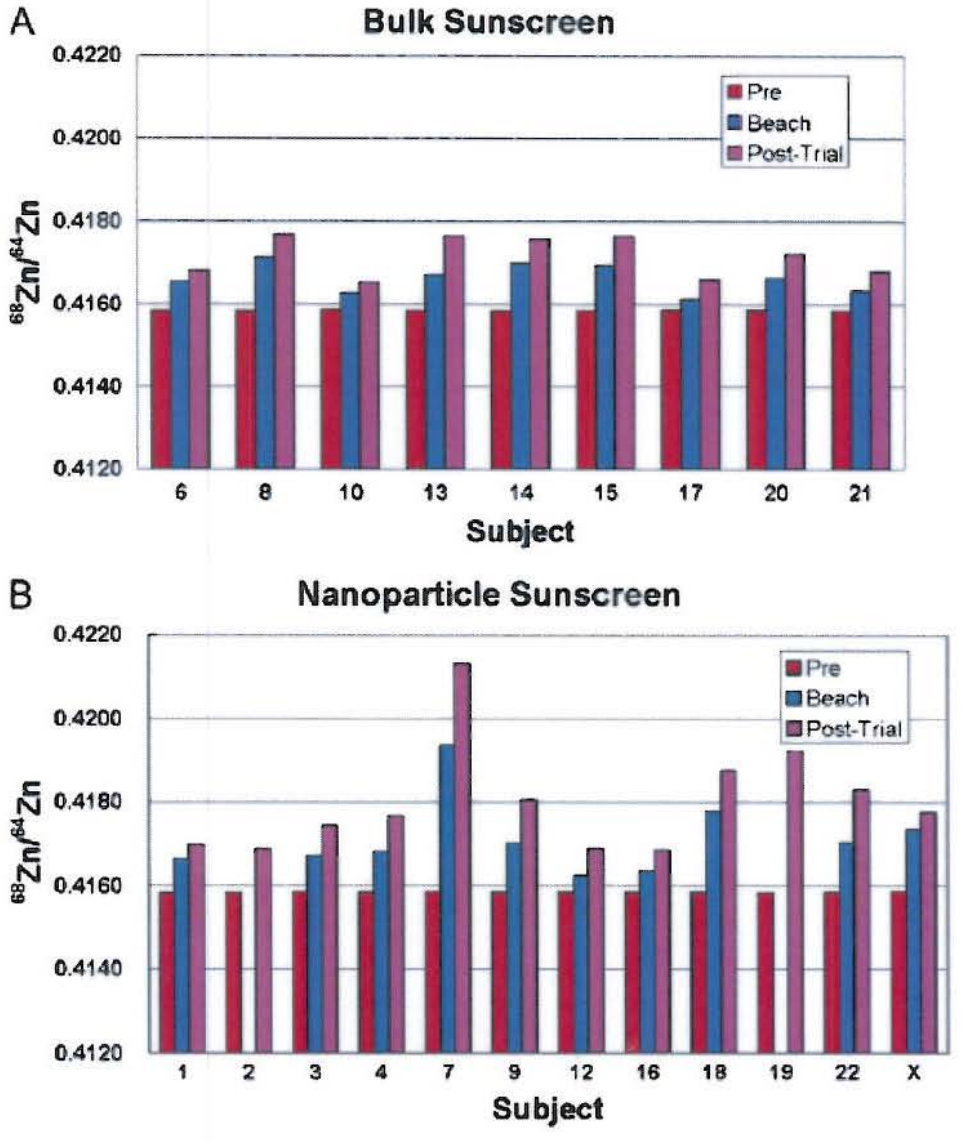



**Figure 1.** $^{68}$Zn/$^{64}$Zn ratios in blood of subjects exposed to sunscreens with 110 ± 46 nm "bulk" particles (Panel A) or 19 ± 8 nm nanoparticles (Panel B) of $^{68}$ZnO. Red bars ("Pre) represent blood samples drawn before any dermal application of any $^{68}$ZnO sunscreens. Blue bars ("Beach) represent blood samples drawn on the last day (Day 5) of dermal application of $^{68}$ZnO sunscreens. Purple bars ("Post) represent blood samples drawn 6 days after the last dermal application of $^{68}$ZnO sunscreens. Subject numbers refer to the Subjects as described in Table 1 of the main text, with subject "X" referring to the technician that applied both sunscreens.

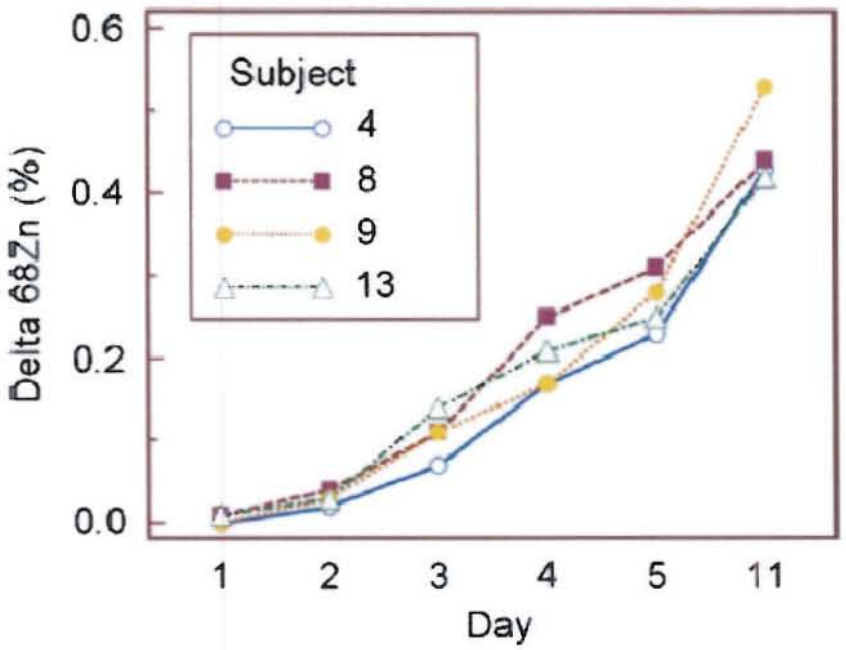



**Figure 2.** $\Delta^{68}$Zn in blood of subjects exposed to sunscreens with 110 ± 46 nm "bulk" particles (Subjects 8 (female) and 13 (male)) or 19 ± 8 nm nanoparticles (Subjects 4 (males) and 9 (female)) of $^{68}$ZnO.

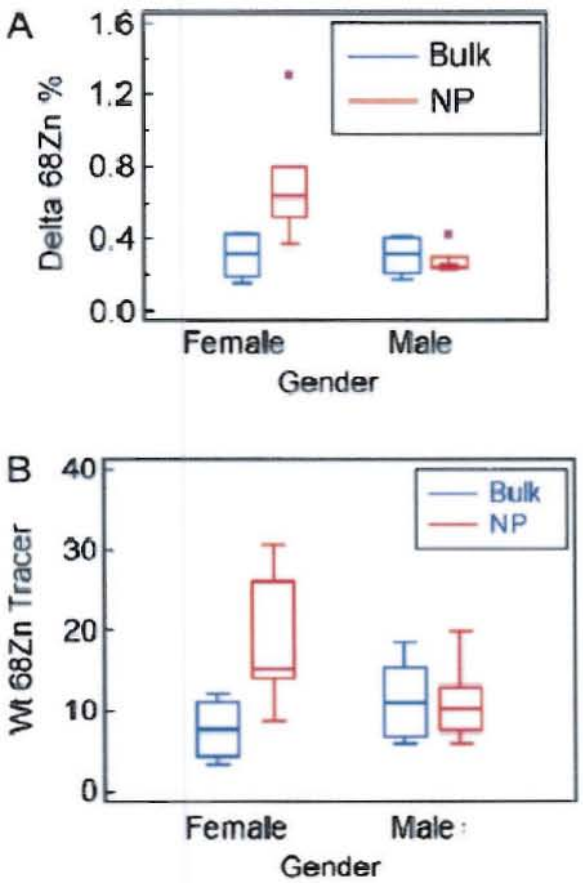



**Figure 3.** $\Delta^{68}$Zn (Panel B) and amounts of $^{68}$Zn (in µg, Panel B) in blood samples 6 days following exposure to $^{68}$ZnO sunscreens. Blue box plots represent subjects exposed to sunscreens with 110 ± 46 nm "bulk" and red box plots represent subjects exposed to 19 ± 8 nm nanoparticles.

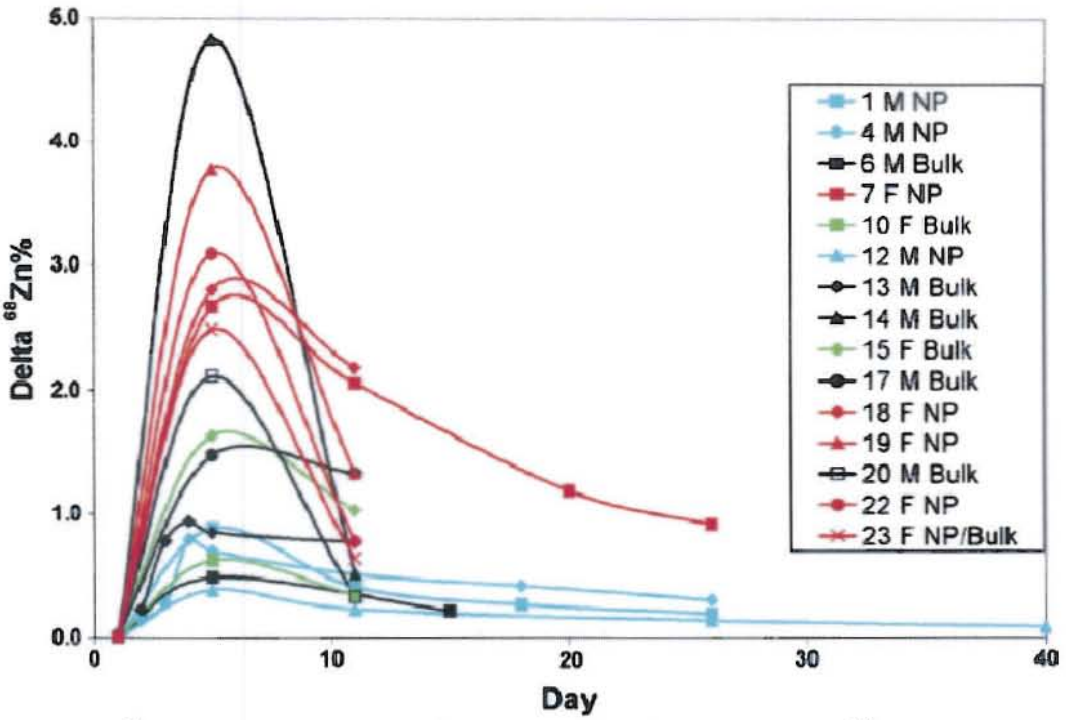



**Figure 4.** $\Delta^{68}$Zn in urine samples of Subjects following exposure to $^{68}$ZnO sunscreens. "Bulk" sunscreens contained 110 ± 46 nm $^{68}$ZnO particles and "NP" sunscreens contained 19 ± 8 nm nanoparticles. Days start at 1 day prior to exposure and terminate for most subjects at Day 11, which is 6 days after the last exposure. For a subset of subjects exposed to "NP" sunscreens (one female and three males) $\Delta^{68}$Zn was detected in urine 25-40 days following dermal exposure.

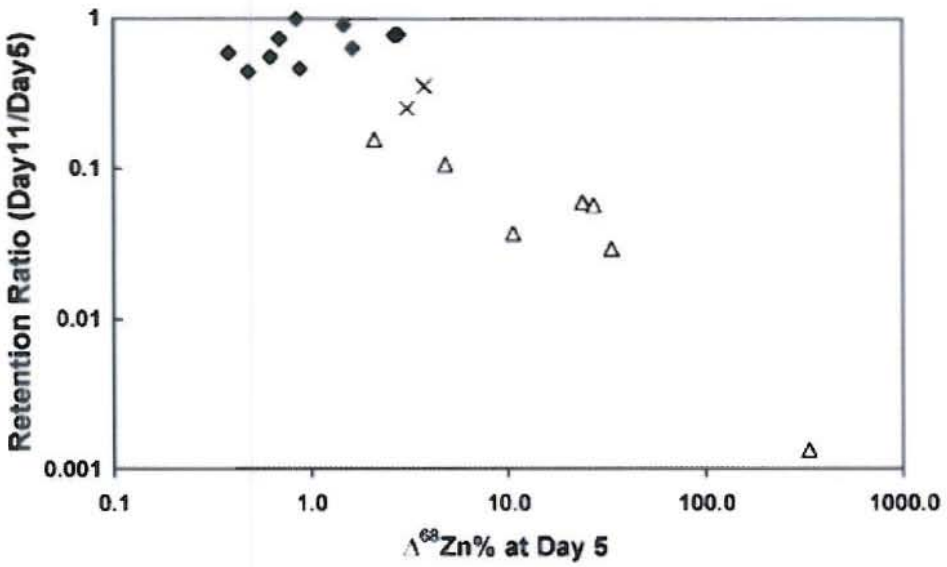



**Figure 5.** Plot of log (retention rates) verses log (peak $\Delta^{68}$Zn) to evaluate possible urine contamination with $^{68}$Zn from sunscreen. Two populations were identified: one showing no relationship between retention ratio and peak $\Delta^{68}$Zn indicating minimal contamination (♦) and one showing a linear relationship (slope ~ -1) indicating contamination (Δ). Samples marked (X) do not affect statistical outcomes.