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Submission regarding SCCS Opinion on Zinc oxide (nano form), October 2012

1. Summary

The National Toxics Network Australia is pleased to note and shares the SCCS's conclusion that Zinc Oxide in nanoform shows a potential for risk to humans , as indicated by a number of positive toxic responses in some of tests. Similarly we also share the SCCS's concerns around the issue of inhalation in regards the use of zinc oxide (ZnO) in cosmetic products. Overall we believe that the forms of nano-zinc oxide outlined in the document are potentially unsafe for use in sunscreens, due to the potential that skin penetration may occur.

NTN shares the SCCS's conclusion that:

> *"on the basis of available information, the use of ZnO nanoparticles in spray products cannot be considered safe."* (p. 96)
>
> *"clear positive toxic responses in some of these tests clearly indicate a potential for risk to humans."* (p. 96)

NTN strongly disagrees with the SCCS's conclusion that:

> *"where ZnO nanoparticles are applied on the skin in a sunscreen formulation, there is sufficient evidence to conclude that due to the very low if any systemic exposure, the risk to the consumer is negligible."* (p. 95)

NTN strongly disagrees with the assertion that "*from the available information, there is no indication for penetration of ZnO nanoparticles through the skin*" (p. 96) because of the limitations in skin penetration studies already outlined.

We are in particular concerned about the following:

1. **Nano zinc oxide has high demonstrated levels of toxicity.**
   Various aspects of nano-zinc oxide toxicity are clearly demonstrated and acknowledged in the SCCS Opinion. Moreover, nano-zinc oxide has been demonstrated to have greater potential for toxicity and skin penetration than bulk zinc oxide.

2. **The SCCS's reliance relies on unrealistic ZnO skin penetration research data provided by industry groups with a vested interest in negating such concerns.**
   The majority of the skin penetration studies evaluated by the SCCS are short term, *in vitro* and don't consider the role of flexing, skin condition and the widespread use of penetration enhancers in cosmetics, hence this makes them unrealistic.

3. **It is essential that the extent of nano zinc oxide penetration data be investigated in vivo.**

There are still no long-term human *in vivo* studies looking at the extent of penetration into the basal skin layer. In formulating its opinion we believe the SCCS failed to adequately consider evidence of skin penetration by other nanomaterials, which may give an indication of the situation present. The SCCS also appears to have failed to consider the strong affinity nanoparticles have with certain proteins and how this might impact the extent of ionisation and skin penetration.

4. **The numerous data gaps regarding certain aspects of the toxicity and fate of nano ZnO.**
   The SCCS noted numerous data gaps regarding certain aspects of the toxicity and fate of nano ZnO, including information regarding long term toxicity, solubility, phototoxicity and carcinogenicity.

5. **Methodological problems in the existing research which prevent reliable conclusions from being drawn.**
   Many of the studies cited by the SCCS have methodological flaws, such as very small sample size,which limit their value in assessing the extent of toxicity and skin penetration.

Overall NTN believes that there is insufficient evidence regarding the fate of absorbed nano zinc oxide in the body to warrant the SCCS's conclusion that nano zinc oxide in sunscreens is safe.

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2. Nano zinc oxide has high demonstrated levels of toxicity

The toxicity of nano-zinc oxide is clearly demonstrated and acknowledged in the SCCS Opinion. Moreover, nano-zinc oxide has been demonstrated to pose higher potential for toxicity and skin penetration.

Examples of the toxicity of ZnO include:

- Acute oral toxicity (p. 30)
    - alarmingly, it was found *"The incidences of microscopic lesions in the liver, pancreas, heart and stomach were higher at lower doses of nano-size zinc oxide compared to higher doses,"* and that *"The nano-sized ZnO induced toxicity at the lowest dose investigated (5 mg/kg body weight)."*
- Local effects in the lung (p. 57)
- Human cell genotoxicity (p. 62)
- Cytotoxicity (p.87)
    - Oxidative stress markers were increased at a dose of 0.008 µg/mL exposure.
- Liver toxicity (p. 93)
    - *"it can be concluded that based on the observations on serum liver enzyme levels and histopathology, the systemic availability of either ZnO nanoparticles or Zn ions has the potential to induce liver toxicity."*

It appears from the evidence presented that micro-scale ZnO, whilst demonstrating some similar toxic effects to nano ZnO, has lower overall toxicity than nano-ZnO:

> *"As far as evaluated in the toxicity testing, micro-sized ZnO has been shown to induce either similar toxic effects (in terms of general toxicity, lung toxicity after inhalation, uptake from gastroIntestinal-tract, serum liver enzyme presence) or lower toxic effects (in terms of genotoxicity, liver histopathology) when compared to nano-sized ZnO."* (p. 98)

Free radical production

The capacity for nanomaterials to create free radicals (reactive oxygen species) is well recognised.[1] As we reduce the size of particles, the larger relative surface area increases the potential for free radical production which can damage proteins and DNA. A study cited in the SCCS Opinion found that when zinc oxide was applied topically to rats over a 28 day period there was:

> *"a significant decrease in the collagen content of the skin and the tail in all the nano ZnO treated groups of rats compared to the control, as well as with the micro-sized zinc oxide treated groups."* (p.59)

It was suggested that this effect was due to:

> *"potential skin penetration of ZnO nanoparticles due to partial dissolution, followed by induction of reactive oxygen species"* (p.59)

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[1] Tran D and Salmon R. (2010) Potential photocarcinogenic effects of nanoparticle sunscreens. *Austral J Dermatol* **52**(1):1-6; Newman M, Stotland M, EllisJ. 2009. The safety of nanosized particles in titanium dioxide and zinc oxide based sunscreens. *J Am Acad Dermatol* **61**: 685-92, http://www.idlc.com.au/pdf/IDLC-Final-nanoparticle-sunblock-lecture.pdf

Nano ZnO was also found to induce DNA damage in human epidermal cells.[2] Another study found that nano zinc oxide induced DNA damage in human nasal mucosal cells whilst bulk zinc oxide didn't.[3] Another study concluded that:

> *"repetitive exposure to low concentrations of ZnO nanoparticles results in persistent or ongoing DNA damage."*[4]

Phototoxicity/photoirritation and photosensitisation

The SCCS Opinion notes that a 2006 study using Chinese Hamster lung cells found that treatment with UV light resulted in an increased susceptibility of the cells to DNA damage by nano zinc oxide.[5] Similar results were observed using Chinese Hamster ovary cells (p. 75). FoEA is therefore concerned that no further studies on phototoxicity/photoirritation and photosensitisation have been submitted since (p. 69).

Carcinogenicity

The leader of CSIRO's Nanosafety group warned in 2008 that in a worst-case scenario, nano-ingredients in sunscreens could cause skin cancer.[6] It is therefore deeply concerning that no data was available regarding the carcinogenicity of ZnO, given the known capacity of nanomaterials to damage DNA.[7]

NTN is deeply concerned about the data gap regarding the carcinogenicity of nano ZnO and believes there is inadequate evidence to support the SCCS's conclusion that:

> *"In view of the occurring dissolution of the ZnO nanoparticles it can be assumed that the carcinogenic risk is similar to the conventionally manufactured ZnO preparations."*(p. 95)

Inhalation

NTN shares the SCCS's concerns about the use of ZnO nanoparticles in spray application (p.93).

Mutagenicity

NTN strongly disagrees with the SCCS's conclusion that:

> *"where ZnO nanoparticles are applied on the skin in a sunscreen formulation, there is sufficient evidence to conclude that due to the very low if any systemic exposure, the risk to the consumer is negligible."* (p. 95)

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[2] Sharma *et al* (2009) cited in SCCS (2012) Opinion on zinc oxide (nano form) p.62; SCCS (2012) p. 94.

[3] Hackenberg et al (2011a) cited in SCCS (2012) p.64.

[4] Hackenberg et al (2011b) cited in SCCS (2012) p.66.

[5] Shiseido (2006). Photo-chromosomal aberration test of FINEX-50 ZnO with cultured mammalian cells. Safety Assessment Group, Quality Assessment Center, Shiseido Co. Ltd., Japan, 31 August 2006, cited in SCCS (2012)

[6] Safety concerns over high-tech sunscreens, http://www.abc.net.au/7.30/content/2008/s2449409.htm

7 Tran D and Salmon R. (2010)

As mentioned above, the vast majority of skin penetration studies have been extremely short term and *in vitro* -including the Nanoderm Project cited by the SCCS, where the maximum exposure time was 48 hours.[8]

NTN believes that there is insufficient evidence regarding the fate of absorbed nano zinc oxide in the body to warrant the SCCS's conclusion that nano zinc oxide in sunscreens is safe.

3. Unrealistic dermal/percutaneous absorption

The SCCC opinoin relies almost exclusively on *in vitro* human and non-human skin studies. There have still been no long-term studies examining the extent to which nano zinc oxide penetrates the skin and remains in the particle form. A precautionary approach should dictate that until these studies are performed, nano zinc oxide should not be deemed 'safe'. Data gaps should not be used as an excuse for regulatory inaction.

Existing skin penetration studies are inadequate

NTN is concerned about the SCCS's over-reliance on ZnO skin penetration research data provided by industry groups with a vested interest in negating concerns. A literature review by the Australian Therapeutic Goods Administration in 2009 found that most studies to date have found no or limited skin penetration by nano-ingredients. However, serious limitations in these studies prevent us concluding that skin absorption does not occur. The European Union's Scientific Committee on Consumer Products has warned that existing research into skin penetration by nano-ingredients is inadequate and that further studies "taking into account abnormal skin conditions and the possible impact of mechanical effects on skin penetration need to be undertaken"[9], yet no research data addressing the role of abnormal skin condition in potential ZnO penetration is presented in this opinion.

Many existing skin penetration studies are deficient because they:

- **Are overwhelmingly short term, often 24 hours**
- **Mostly based on excised skin *in vitro***, where there is no movement or blood circulation
- **Fail to consider the role of skin condition** (e.g. eczema, acne, sunburn, children with thinner skin) and,
- **Do not assess the role of penetration enhancers**, despite the prevalence of these substances in sunscreens, cosmetics and workplaces

Recent peer-reviewed literature reviews have emphasised that these deficiencies limit the relevance of earlier skin penetration studies to 'real life' scenarios and prevent any reliable conclusion about skin penetration by nano-ingredients.[10] These shortcomings were present in most of the skin penetration studies presented in the Opinion.

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[8] Butz, T. et al (2007) NANODERM: Quality of Skin as a Barrier to ultra-fine Particles Final Report, http://www.uni-leipzig.de/~nanoderm/Downloads/Nanoderm_Final_Report.pdf

9 SCCP (2007) Opinion on safety of nanomaterials in cosmetic products. European Commission. Available at: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf

10 Tran D &Salmon R. (2010); Newman M, Stotland M, EllisJ. (2009) The safety of nanosized particles in titanium dioxide and zinc oxide based sunscreens. *J Am Acad Dermatol* **61**: 685-92.

Peer-reviewed studies now demonstrate that skin penetration can occur

The SCCS opinion did not include a recent research publication (Gulson 2010) that demonstrates that skin penetration occurs. The reason for this is unclear, however the study has been available online from 7 February, 2012.

The Gulson 2010 study found small amounts of zinc from sunscreen in the blood and urine of human trial participants[11]. The study used live human volunteers and was carried out over 5 days, with follow up testing for at least six days. This study shows that skin uptake of ingredients from sunscreen applied to intact skin does occur in some form. The results of a separate pilot study conducted as a prelude to this study have since been published. This new publication also showed that small amounts of zinc from sunscreen were detectable in blood and urine[12].

Several other peer-reviewed studies have shown skin penetration by other types of nanomaterials. Quantum dots and fullerenes can penetrate skin[13], especially if skin is flexed[14] (as during exercise) or exposed to 'penetration enhancers' which can be found in some cosmetics.[15]. A 2003 study by the United States National Institute for Occupational Safety and Health[16] found that when accompanied by repetitive skin flexing, inert fluorospheres 1000 nm in size could reach living cells in the dermis. Particles were also found to be concentrated under torn skin, suggesting that compromised skin is more vulnerable to penetration. Despite the relevance of these studies, and the SCCS's claims that "the applicants have performed and provided a comprehensive review and assessment of the available *in vivo* and *in vitro* dermal penetration studies," (p. 92) none of these studies are referred to in the SCCS Opinion.

Studies do not adequately consider the role of penetration enhancers and other additives

Commentators have suggested that since nanomaterials are highly reactive, they may react with other sunscreen ingredients and become coated with proteins or lipids. For example, the rapid formation of a 'protein corona' is well recognised.[17] A number of papers have demonstrated the

11 Gulson B *et al* (2010)

12 Gulson B *et al* (2012)

13 Ryman-Rasmussen J, Riviere J, Monteiro-Riviere N. 2006. Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicol Sci* **91**(1):159-165.

14 Rouse J, Yang J, Ryman-Rasmussen J, Barron A, Monteiro-Riviere N. 2007. Effects of mechanical flexion on the penetration of fullerene amino acid derivatized peptide nanoparticles through skin. *Nano Lett* **7**(1):155-160.

15 Monteiro-Riviere N, Yang J, Inman A, Ryman-Rasmussen J, Barron A, Riviere J. 2006. Skin penetration of fullerene substituted amino acids and their interactions with human epidermal keratinocytes. *Toxicol* **168** (#827).

16 Tinkle S, Antonini J, Rich B, Roberts J, Salmen R, DePree K, et al. 2003. Skin as a Route of Exposure and Sensitization in Chronic Beryllium Disease. *Environ Health Perspect* **111**:1202-1208.

[17] Turney, J. (2009) Nanomaterials, Section 4.1: How do nanoparticles interact with proteins? http://ec.europa.eu/health/scientific_committees/opinions_layman/nanomaterials/en/l-2/4.htm

strong affinity that nanomaterials have with proteins.[18] It is therefore possible that nanomaterials in sunscreens will be coated in proteins (picked up either in sunscreens or from the skin surface). This could make it less likely that zinc oxide in nanoparticle form will ionise when in contact with the skin. A protein coating may also affect the extent of skin penetration[19], as well as the toxicity of individual nanoparticles.[20] However, the key implication is that if skin penetration does occur, it could be in nanoparticle form, rather than as ions.

The SCCS notes that:

> *"Any cosmetic products containing ZnO particles (nano or non-nano) with coatings that can promote dermal penetration will also be of concern."* (p. 98)

The use of penetration enhancers is extremely widespread in the cosmetics industry. US researchers have found that penetration enhancers "greatly enhance" the uptake of carbon fullerene nanoparticles through skin.[21] However, to the best of our knowledge the influence of penetration enhancers hasn't been explored in relation to the vast majority of nano-ingredients now used in cosmetics.

Given the prevalence of penetration enhancers in the cosmetics industry, NTN believes that is premature for the SCCS to conclude that skin penetration by nano zinc oxide does not occur, before the impact of these chemicals on skin penetration has been properly assessed.

Lack of long-term *in vivo* studies looking at the extent of penetration into the basal skin layer

Consideration of skin basal layer absorption is important, because if zinc oxide nanoparticles are absorbed into the basal skin layer, the photocatalytic potential of ZnO to generate free radicals and damage active proteins and DNA will be dramatically increased.

Given the limitations of the data regarding skin penetration NTN believes that it is premature for the SCCS to conclude that:

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[18] Linse, S. e*t al* (2007) Nucleation of protein fibrillation by nanoparticles, *PNAS*, **104**(21):8691-8696, http://www.pnas.org/content/104/21/8691; Cedervall, T. e*t al* (2007) Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, *PNAS*, **104** (7):2050-2055, http://www.pnas.org/content/104/7/2050

[19] See for example Zheng, D. *et al* (2012) Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation, *PNAS,* July 6, 2012 http://www.pnas.org/content/early/2012/07/03/1118425109.abstract

[20] Webb, S. (2012) Coatings Influence Nanoparticle Toxicity, *Chemical & Engineering* News, January 18, 2012, http://cen.acs.org/articles/90/web/2012/01/Coatings-Influence-Nanoparticle-Toxicity.html

[21] Monteiro-Riviere N, Yang J, Inman A, Ryman-Rasmussen J, Barron A, Riviere J. (2006) Skin penetration of fullerene substituted amino acids and their interactions with human epidermal keratinocytes. *Toxicol* **168** (#827).

> *"It is worth highlighting that this opinion has considered the small proportion of the absorbed Zn following dermal application of nano ZnO to most likely be a solubilised ionic form. This is in consideration of the solubility and dissolution aspects of ZnO described in section 3.1.6. However, if any new evidence emerges in the future to show that the translocating species were in the form of insoluble and potentially persistent nanoparticles, then the SCCS may consider revising the safety assessment of nano ZnO."* (p. 97)

NTN believes that there is sufficient evidence to warrant a precautionary approach and that the SCCS should revisit this issue immediately.

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