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THINKING AHEAD IN TOXICOLOGY

INSIGHTS INTO THE NEW INFORMATION REQUIREMENTS TRIGGERED BY THE REVISED SCCS GUIDANCE ON THE SAFETY ASSESSMENT OF NANOMATERIALS IN COSMETICS: ENSURING NANO-SAFETY AND COMPLIANCE WITH THE EU COSMETIC PRODUCT REGULATION (CPR)

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Introduction

Recent advances in Nanotechnology have opened new opportunities for innovation in cosmetics. In Europe, the use of Nanomaterials (NM) in cosmetics is regulated under the Cosmetic Regulation (EC) No 1223/2009 (CPR). When the CPR was adopted in 2009, there was no internationally agreed definition of NM. Consequently, the CPR provided a sector-specific definition of NM in Article 2(1)(k), which slightly deviates from the first Commission Recommendation issued in 2011 regarding the particle size distribution thresholds and the concept of natural, incidental or engineered NM.

The EU Scientific Committee on Consumer Safety (SCCS) requires a thorough risk assessment for demonstrating the safety of NM uses in cosmetics. Its recommendations and criteria evolved over time, key elements being the 2017 checklist for the use NM in cosmetics (SCCS/1588/17) and the 2nd revision of the SCCS guidance on the safety assessment of NM in cosmetics in 2023 (SCCS/1655/23) which should be considered alongside with the latest revision of the SCCS Notes of Guidance (SCCS/1647/22). Revised guidance SCCS/1655/23 includes new sections (e.g., solubility/dissolution rate, aspect ratio, uptake into blood cells, endcorine disruption), provides a comprehensive overview of the key aspects triggering potential safety concerns and a revision of section on read-across/grouping. It emphasises the importance of apprpriate characterisation of NM and the submission of a relevant data set considering the specific properties of NM.

This poster aims to provide practical insights into the analytical, physico-chemical and toxicological data required to assess the safety of NM used in cosmetic products, allowing the development of SCCS-guideline compliant safety dossiers.

Key aspects of the latest SCCS Guidance on Nanomaterials (SCCS/1655/23)

SAFETY ASSESSMENT FRAMEWORK

- **Comprehensive safety evaluation** considering potential size-related effects (e.g., physico-chemical properties, biokinetic behavior, toxicological impact).
- **Definition and material specification** (e.g., particle size distribution, solubility, persistence).

KEY RECOMMENDATIONS FOR SAFETY ASSESSMENT

- **Material characterisation**: Ensure unambiguous identification and detailed characterization of the NM.
- **Exposure assessment**: Evaluate both local and systemic exposure, considering the potential translocation of NM across skin, lung or gastro-intestinal barriers, whilst mimicking real-use scenarios.
- **Hazard identification and dose-response characterisation**: Conduct toxicological studies addressing local toxicity, skin sensitisation, genotoxicity and systemic effects (if absorbed). Consider nano-specific factors such as particle solubility, aggregation and agglomeration behaviour, penetration through biological membranes, interactions with biological entities at local and systemic levels, surface adsorption /binding of other substances, surface catalysed reactions and persistence.
- **Risk assessment**: Due to the EU ban on animal testing, rely on alternative methods and existing data. Integrate various sources to build a weight of evidence, use *in vitro* to *in vivo* extrapolation (IVIVE) when necessary, and apply additional uncertainty factors if data are insufficient or inadequate.

FUTURE PERSPECTIVES

- **Evolving science:** guidance may change as scientific understanding evolves.

Evolution of SCCS opinions on Nanomaterials over time

Following the mandates provided by the Commission, in the last 10 years, SCCS issued 14 opinions covering over 20 nanomaterials used in cosmetic products. In March 2024, Annexes II and III to Regulation (EC) No 1223/2009 were updated, prohibiting the use of 13 NM (styrene/acrylates copolymer, sodium styrene/acrylates copolymer, copper, colloidal copper, hydroxyapatite, gold, colloidal gold, gold thioethylamino hyaluronic acid, acetyl heptapeptide-9 colloidal gold, platinum, colloidal platinum, acetyl tetrapeptide-17 colloidal platinum and colloidal silver) and restricting the use of hydroxyapatite in cosmetics products[1].

NM SCCS Opinions conclusions	Inconclusive due to insufficient information	No safety concerns identified except for those with inhalation exposure potential	Typical SCCS concerns
2014-2017	-	4	• Deficiencies in compositional, structural and physico-chemical characterization (e.g., level of impurities, manufacturing methods, particle size analysis, homogeneity / stability), function and uses • Insufficient data for safety assessment on: ➢ Toxicokinetics, dermal absorption and systemic availability ➢ Genotoxicity ➢ Systemic toxicity especially through inhalation ➢ DART
2018-2022 – acc. Checklist SCCS/1588/17	6	1	
2023-2024 – acc. Revision SCCS/1655/23	2	1	

Addressing some key SCCS concerns: Practical insights and experience

Importance of compositional, structural and physico-chemical characterisation

BACKGROUND

- NM exhibit a wide variety of properties due to differences in size, composition, morphology, and surface chemistry (**Figure 1**). These variations can significantly impact their safety profiles.

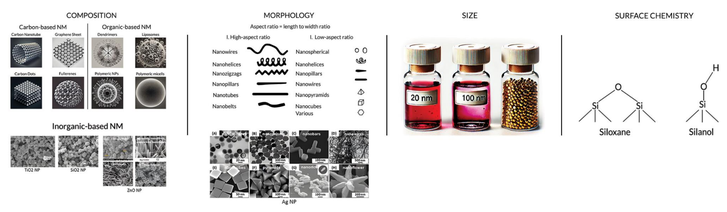

Figure 1: Large diversity of NM

RECOMMENDATIONS

To accurately predict the safety profile of NM, a comprehensive physicochemical characterization should be conducted at the raw material stage, within the cosmetic formulation, and in the test system. This characterisation should include the following analyses:

- **Chemical identify and composition** using methods such as UV-Vis, HPLC, GC/LC-MS, AAS, ICP-MS, FTIR, NMR, XRD and Mössbauer spectroscopy.
- **Particle size distribution** of primary and secondary particles on at least five batches, including mean, median, number, and mass size distributions which must be measured by more than one method such as EM (SEM or TEM), CLS or PTA.
- **Solubility or dissolution rate** in water and other relevant media.
- **Aspect ratio**, especially for biopersistent materials, with number-based distribution.
- **Surface modification types** providing details on coatings, surface moieties, doping materials, and encapsulating materials.
- **Stability and homogeneity**

Watch out on analytics for percutaneous absorption study

BACKGROUND

- Insoluble and persistent NM present a potential risk of penetrating the outer layers of the skin through various pathways (**Figure 2**). This could lead to unintended systemic distribution and interactions with biological entities at the molecular level.

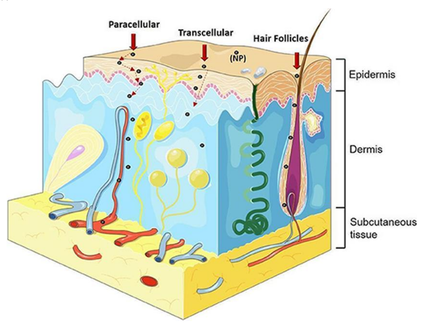

Figure 2: Schematic representation of different potential pathways of NM penetration into the skin: paracellular (between cells), follicular (transport by hair follicles), and transcellular (inside the cells)[2]

RECOMMENDATIONS

- **Analytical techniques and sampling methods:**
  - Use appropriate methods to determine the possible adsorption of substances on NM surfaces.
  - Provide qualitative and quantitative of dermal penetration / absorption
- **Systemic absorption:** If *in vitro* absorption tests indicate potential systemic absorption, the integrity of the NM needs to be confirmed.
- **Test system:** The standard *in vitro* diffusion cell chamber, used for non-nano ingredients, may not be ideal for testing NM due to potential mechanical interference.

Experimental design considerations for genotoxicity assays

BACKGROUND

- In many inconclusive opinions of the SCCS, it has been highlighted that the assessment of genotoxicity is often inadequate.
- A common issue in *in vitro* studies is the lack of information on the stability of the test suspension or the evidence of cellular exposure or internalisation (**Figure 3**).

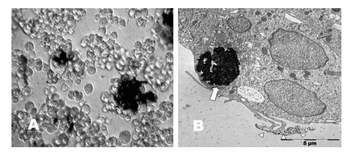

Figure 3: Uptake of carbon nanotubes by NR8383 alveolar macrophages: (A) light microscopy image (magnification: 800x) and (B) TEM image (magnification: 3000x)[3]

RECOMMENDATIONS

- **Protocol selection:** Appropriate protocols should be used to assess the genotoxicity of NM. For example, the bacterial Ames test is not suitable due to the limited uptake of NM by bacteria through endocytosis. In contrast, more appropriate alternatives include mammalian cell gene mutation assay and micronucleus test. These recommendations align with the key concern of ensuring cellular uptake of nanoparticles in genotoxicity testing. It is further recommended to test for NM-induced intracellular reactive oxygen species (ROS), potential formation of free radicals and oxidative damage to cells and tissues.
- **Characterisation and stability:** Proper characterisation of NM, along with an analysis of its stability in test media, is crucial. Specifically, the stability of NM dispersion in cell culture medium should be assessed both before and after the experiment to ensure reliable results. The studies should be performed along with the characterisation of the test material in culture media and its uptake by the cells.
- **Cellular uptake:** This step is essential for validating the results, especially when negative findings are reported. *In vitro* genotoxicity studies for NM should always include an assessment of cellular uptake, preferably nuclear uptake, to confirm target exposure.

Conclusions and future perspectives

- This poster provides practical insights into the analytical, physico-chemical and toxicological data necessary to assess the safety of NM used in cosmetics.
- Given the evolving nature of NM safety research, future revisions of the SCCS Guidance are likely as new scientific knowledge emerges.
- In line with the EU Chemicals Strategy for Sustainability (Ref. Ares(2021)6011962 - 04/10/2021), it is anticipated that the definition of NM in the CPR will soon align with this strategy. Consequently, a change in the safety assessment framework within the cosmetics industry is expected in the near future.

References

[1] Commission Regulation (EU) 2024/858. 14 March 2024. https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=OJ:L_202400858

[2] Zaiter W *et al.,* 2022. Toxicity assessment of nanoparticles in contact with the skin. *Journal of Nanoparticle Research* 24:149.

[3] Pulskam K, Diabaté ., Krug H, 2007. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicology Letters* 168 : 58-74.

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