4. OPINION

Cosmetic products[3] are primarily intended for use on skin, hair or oral mucosa (76/786/EEC). Products may contain particulate matter with dimension(s) below 100 nm, henceforth called nanoparticles. The nanoparticles are claimed to serve various purposes: enhancing the formulation properties and acceptability, a direct effect on skin and hair, e.g. moisturizing or anti-aging formulations, make-ups and hair-conditioners or protect the skin e.g. UV-filters in sunscreens.

A crucial factor in assessing possible risks associated with nanoparticles is their possible uptake. For topical applications, the route of exposure is essentially transdermal including follicular and other transadnexal pathways. In addition to this route, exposure via inhalation, ingestion, conjunctival and mucosal surfaces may have to be considered, e.g. nanoparticles from hairsprays, lipsticks, or toothpastes. Although pigmentary grades of TiO_2 are usually considered to consist of micron sized particles, particles below 100 nm may be present in such grades. Information on the particle size distribution is required for all micro- and/ or nanosized materials.

Nanoparticles can be divided into two groups: i) soluble and/or biodegradable nanoparticles which disintegrate upon application to skin into molecular species (e.g. liposomes, microemulsions, nanoemulsions), and ii) insoluble and/or biopersistent particles (e.g. TiO_2, fullerenes, quantum dots). For the first group, conventional risk assessment methodologies based on mass metrics *may* be adequate, whereas for the latter other metrics such as the number of particles, and their surface area as well as their size distribution are also required.

It is primarily for the insoluble particles that health concerns related to possible uptake arise. Should they become systemically available, translocation/transportation and eventual accumulation in secondary target organs may occur. This feature does become important with repeated application of cosmetic products. Inevitably, insoluble nanoparticles do represent a burden for the environment and a complete life cycle analysis is required. These analyses would need to be made on a case by case basis. If the technical specifications of nanomaterials are maintained from batch to batch, no toxicological assessment of different batches is required.

When characterising nanoparticles for risk assessment, in general the following properties need to be considered:

physical characteristics:

- size,
- shape (e.g. spherical or fibrous),
- surface area,
- surface charge,
- surface morphology,
- rheology,
- porosity,
- crystallinity and amorphocity,
- primary nanoparticles, agglomerates and/or aggregates.

[3] (76/768/EEC) Article 1: A 'cosmetic product' shall mean any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition.

chemical characteristics**:**

- chemical composition,
- surface chemistry,
- oxidative capacity,
- catalytic activity
- stoichiometry (may change for large surface to volume ratios),
- dissolution kinetics and solubility,
- hydrophilicity or hydrophobicity,
- surface coating,
- impurities (foreign elements, chemical by-products or degradation products etc),
- intentional or unintentional surface adsorbents, (both of which determine the reactivity).

In other words, the characterisation of the properties of nanoparticles themselves is insufficient. The interactions of the nanoparticles within a given environment must be examined including solubility/insolubility, free radical formation etc.

There are major data gaps in the assessment of the exposure and the uptake of nanoparticles via dermal absorption, inhalation, oral ingestion and eye contact.

The actual situation for dermal exposure is as follows:

1) There is evidence of some skin penetration into viable tissues (mainly into the *stratum spinosum* in the epidermal layer, but eventually also into the dermis) for very small particles (less than 10 nm), such as functionalised fullerenes and quantum dots.

2) When using accepted skin penetration protocols (intact skin), there is no conclusive evidence for skin penetration into viable tissue for particles of about 20 nm and larger primary particle size as used in sunscreens with physical UV-filters.

3) The above statements on skin penetration apply to healthy skin (human, porcine). There is an absence of appropriate information for skin with impaired barrier function, e.g. atopic skin or sunburned skin. A few data are available on psoriatic skin.

4) There is evidence that some mechanical effects (e.g. flexing) on skin may have an effect on nanoparticle penetration.

5) There is no information on the transadnexal penetration for particles under 20 nm. Nanoparticles of 20 nm and above penetrate deeply into hair follicles, but no penetration into viable tissue has been observed.

The situation for inhalation of nanoparticles can be summarized as follows:

1) During inhalation nanoparticles in a size range of 10 -100 nm deposit predominantly in the alveolar region of the respiratory tract and also in small bronchioles with deposition probabilities of 0.2 – 0.6. In contrast, nanoparticles < 10 nm will deposit predominantly in upper airways.

2) Nanoparticles will cross the epithelial barrier into interstitial spaces.

3) Translocation studies after inhalation and instillation were performed which show that nanoparticles translocate to various organs and are able to cross the blood-brain-barrier.

4) Inhalation of ultrafine particles has been linked to thrombotic (blood clotting) effects,

due to direct effect or via pulmonary inflammation.

5) Clearance from the lung can be slow, because of
   a. lower phagocytic activity of macrophages;
   b. poor clearance from the alveoli;
   c. retention in the interstitium.

   and therefore nanomaterials may persist in the lung.

6) Possible local effects in the lung are
   d. Oxidative stress, induced by the particle itself and/or by the activation of phagocytotic and epithelial cells;
   e. Inflammatory response ;
   f. Mutagenity/genotoxicity: whilst there is evidence of such effects in cultured lung cells, there are no data on the genotoxicity of nanomaterials after inhalation exposure;
   g. Cytotoxicity.

The situation for intestinal and eye exposure to nanoparticles may be summarized as follows:

- There is absence of information.

The situation for cellular studies may be summarised as follows:

1. Various skin cells have been studied *in vitro* for their cellular responses to nanoparticles. The observed effects have been diverse, ranging from internalization via endocytotic and non-endocytotic mechanisms, increased calcium and reactive oxygen species concentration within cells, and an effect on cell proliferation and cell viability.

2. Although *in vitro* tests may be useful for hazard identification, no alternative methods, validated or optimized by using nanomaterials exist. Nanoparticles diffuse, settle and agglomerate in cell culture media as a function of the environment (media, ionic strength, acidity, and viscosity) and particle properties (size, shape and density). Consideration of these properties and effects would significantly improve the basis for nanoparticle toxicity testing.

3. *In vitro* assays (validated and non-validated) are at present unavailable for appropriate risk assessments.

The overall situation may be summarised as follows:

At present, there is concern about insufficient knowledge in the following areas:

- Hazard identification;
- Exposure assessment;
- Uptake (including physiologically compromised human skin);
- The role of physico-chemical parameters of nanoparticles determining absorption and transport across membranes in the gut and lungs;
- The role of physico-chemical parameters of nanoparticles in systemic circulation determining biokinetics and accumulation in secondary target organs;
- Possible health effects (including susceptible individuals);
- Translocation of nanoparticles via the placenta to the foetus;
- *In vitro* and *in vivo* test methods validated or optimized for nanomaterials.

Responses to the questions in the Terms of Reference

Question 1

In view of the concerns recently raised about the use of nanomaterials in cosmetics the SCCP is requested to review and, if appropriate, to amend its notes of guidance for the testing of cosmetic ingredients and their safety evaluation as concerns cosmetic ingredients in the form of nanomaterials, including nanoparticles and nanoliposomes, and in particular as regards skin absorption and resorption of these substances. In assessing this, regards should be made to differing skin conditions, different sizes of particles and to question whether mass unit is the appropriate basis for regulating the exposure to nanomaterials. Possible implications on animal testing of nanoparticles and nanoliposomes should be addressed.

In the safety evaluation of nanomaterials, actual or intended marketed nanomaterials should be used for material characterisation and hazard identification. Furthermore, distinction should be made between soluble and/or biodegradable versus insoluble and/or biopersistent nanomaterials. Nanoparticles which disintegrate into molecular species upon application have to be distinguished from insoluble particles. For the former, conventional risk assessment methodologies based on mass metrics *may* be adequate for their use in cosmetic products, whereas for the latter (e.g. TiO_2, ZnO, fullerenes, carbon nanotubes, and quantum dots) other metrics are needed. A complete characterisation of physico-chemical characteristics and properties is required for these nanomaterials. Particle size, particle number, shape and surface characteristics are considered essential additional metrics. Consideration should also be given to certain moieties (e.g. surface modifications) on nanomaterials which could possibly enhance or reduce potential adverse health effects.

In traditional risk assessment, skin penetration studies are carried out using healthy or intact skin. Possible enhanced uptake in the case of impaired skin is considered to be covered in the Margin of Safety (MoS) approach. However, in the case of nanomaterials the conventional MoS may not give an adequate expression of the safety. If there is any penetration into the vital layers of the skin there may be a transfer to the systemic circulation. It may be anticipated that any systemic absorption will be more likely in conditions of abnormal skin e.g. sunburnt, atopic, eczematous, psoriatic skin. There is evidence that physical, in particular mechanical, and/or chemical action on the skin may have an effect on nanoparticle penetration.

At present, the *in vitro* diffusion cell chamber is the standard device for estimating percutaneous absorption. However, because mechanical factors may be important in potential penetration/absorption of nanoparticles, this standard model may not be ideal. Therefore, modified or new optimized methodologies to assess percutaneous penetration pathways are required.

There are large data gaps in risk assessment methodologies with respect to nanoparticles in cosmetic products. To evaluate possible pulmonary effects (and the linked systemic effects), simple *in vitro* systems exist, e.g. to study cytotoxicity, pro-inflammatory effects. However, these are not suitable for studying effects that reflect the complexicity of the lung. *In vitro* models for systemic and (sub-)chronic toxicity do not yet exist and need to be developed, in particular for translocation, biodistribution, accumulation and clearance studies. Therefore, *in vivo* studies on potentially toxic nanomaterials are still necessary.

Size dependence of the deposition probability of inhaled nanoparticles is reasonably understood in the respiratory tract of healthy subjects; however, for individuals with respiratory disorders, predictions for nanoparticle deposition probability are limited.

The biodistribution (toxicokinetics) of nanomaterials has not been studied in detail. Therefore, it is impossible to model, *in silico*, hazard characterization and the distribution of nanomaterials. In particular, there is limited information on the role of physico-chemical parameters of nanoparticles determining their absorption and transport across barriers, e.g. skin, gut, lungs and eye, and their subsequent uptake in the systemic circulation, metabolism, potential accumulation in secondary target organs and excretion.

Mutagenicity/genotoxicity testing is required in general for cosmetic ingredients, including nanomaterials, but the specific characteristics of nanoparticles may require further consideration. The mutagenic/genotoxic *potential* of nanoparticles could probably be assessed in mammalian cells *in vitro*. The presently validated *in vivo* genotoxicity tests, however, do not cover the expected target organs of nanoparticles (particularly the respiratory tract) and have not been validated or optimized for reference substances including nanomaterials for cosmetics.

All *in vivo* and *in vitro* risk assessment methods for nanomaterials are still under development. Although some validated *in vitro* methods do exist they have not yet been validated and/or optimized with nanoparticles as reference compounds. This implies that for safety assessment of cosmetic ingredients, there are no validated *in vitro* methods available for nanoparticles.

Although animal testing can be largely reduced for skin penetration studies, it remains essential for translocation and accumulation studies as well as for chronic toxicity studies. Finally, the SCCP emphasizes that for the safety assessment of cosmetics, the 7th Amendment of the Cosmetic Directive (76/678/EEC) imposes animal testing and marketing bans, which will soon prohibit *in vivo* testing of cosmetic ingredients. Only validated *in vitro* methods are to be used for risk assessment.

Each safety dossier concerning nanomaterials needs to be evaluated on a case by case basis.

Question 2

In the light of the findings under question 1, does the SCCP consider it is necessary to review existing opinions on nanosized TiO_2 and ZnO as cosmetic ingredients and if appropriate to identify which additional elements are required for the submission of a safety file?

A complete safety dossier on micronsized and nanosized ZnO was requested by SCCNFP in its opinion on ZnO in 2003 (SCCNFP/0649/03). An opinion on the safety of such materials will be dependent on the availability of on an adequate dossier.

The SCCNFP opinion from 2000 (SCCNFP/0005/98) is on micro-crystalline preparations of TiO_2 and preparations of coarse particles. However, since this opinion new scientific data on nanosized particles, including TiO_2 has become available. Therefore, the SCCP considers it necessary to review the safety of nanosized TiO_2 in the light of recent information. Also, a safety assessment of nanosized TiO_2, taking into account abnormal skin conditions and the possible impact of mechanical effects on skin penetration need to be undertaken.

5. MINORITY OPINION

None