Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC ADVICE

on the safety of nanomaterials in cosmetics

The SCCS adopted this document
by written procedure on 5 October 2020

ACKNOWLEDGMENTS

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1. ABSTRACT

The SCCS concludes the following:

1. *The SCCS is requested to determine the nanomaterials, as published in the recent catalogue of nanomaterials of 2019, for which specific concerns can be identified and justified in order to establish a priority list of nanomaterials for risk assessment (Article 16(4) Reg.1223/2009). More specifically, the SCCS is requested to provide a description of the specific concerns that have been identified for the nanomaterials mentioned above. This process should be based on the currently available scientific literature and SCCS' expert judgement.**

Through a review of the available information and expert judgment, the SCCS has identified certain aspects of nanomaterials that constitute a basis for concern over safety to consumers' health when used in cosmetic products. These include:

- Physicochemical aspects relating to: very small dimensions of the constituent particles; solubility/persistence/potential accumulation in the body; chemical nature and toxicity of the nanomaterial; physical/morphological features of the constituent particles; surface chemistry and surface characteristics (surface modifications/coatings);
- Exposure aspects relating to: the frequency and the amounts used, whether the number/type of consumer product(s) used is relatively high; and whether there is a potential for systemic exposure of the consumer to nanoparticles;
- Other aspects relating to: novel properties, activity or function, and specific concern arising from the type of application.

A detailed account of these aspects has been presented in this Opinion. Also, the nanomaterials listed in the EC catalogue of nanomaterials of 2019 have been tabulated in an order of priority according to risk potential in Annex 1 of this Opinion.

2. *For the nanomaterials with inconclusive SCCS opinions, such as [Colloidal Silver (nano) (SCCS/1596/18), Styrene/Acrylates copolymer (nano) + Sodium styrene/Acrylates copolymer (nano) (SCCS/1595/18) and Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form) (SCCS/1545/15)], the SCCS is requested to assess if a potential risk can be identified according to Article 16(6) Reg.1223/2009. Such assessment, regardless of the data previously submitted by the respective applicants, should be based on the available scientific literature and SCCS' expert judgement (i.e. systemic or local availability; harmful effects specifically related to nano-form; surface catalysed reactions in nano-form, absorption (or potential absorption) from dermal and inhalation routes, potential of nano-form to deliver ionic forms, etc.).**

The SCCS has reviewed previous inconclusive opinions on three nanomaterials (SCCS/1596/18; SCCS/1595/18 and SCCS/1545/15), in conjunction with any further relevant information available in published literature to identify whether there is a scientific basis for concern over their safety to consumers' health when used in cosmetic products. The SCCS has identified certain aspects relating to each of the nanomaterials that raise a safety concern. These have been detailed in three separate annexes (2, 3 and 4) to this Opinion.

* In the assessment of the above question and in order to avoid conflicting opinions with other bodies, SCCS is invited to consult SCHEER.

Keywords: SCCS, scientific advice, safety, nanomaterials, Regulation 1223/2009

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About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and are made up of scientists appointed in their personal capacity.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease Prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Establishing the concerns

Article 16(4) of the Cosmetics Regulation provides that '*In the event that the Commission has **concerns** regarding the safety of a nanomaterial, the Commission shall, without delay, request the SCCS to give its opinion on the safety of such nanomaterial for use in the relevant categories of cosmetic products and on the reasonably foreseeable exposure conditions*'.

Thus far, the '*concerns*' of the Commission that gave origin to previous mandates to SCCS have been based on the intrinsic properties of nanomaterials, as a category, in light notably of their nano-scale dimension, bio-persistence and insolubility.

Establishing potential risk to human health

According to the Cosmetics Regulation, once a risk assessment for a nanomaterial has been performed, the Commission shall proceed with risk management measures provided that the risk assessment has established the presence of a potential risk to human health.

In this respect, Article 16(6) of the Cosmetics Regulation states that '*taking into account the opinion of the SCCS, and where there is a **potential risk to human health**, including when there is insufficient data, the Commission may amend Annexes II and III*'. The risk of having '*insufficient data*' materialised in the recent experience with the inconclusive SCCS opinions on nanomaterials (as notified through CPNP)[1]. In these cases, due to the lack of relevant information from the applicants both in the original notifications and in the additional information requested by the SCCS the '*potential risk to human health*' could not be established nor excluded by SCCS.

In the cases mentioned above, even if the '*insufficient data*' provision is fulfilled, the '*potential risk to human health*' is not fully established and the Commission is not in a position to take potential regulatory measures, in accordance with Article 16(6) of the Cosmetics Regulation.

The general principle of precaution allows the adoption of restrictive measures even when it is not possible to determine with certainty the existence and/or extent of an alleged risk, but the likelihood of a real harm persists should the risk materialise. Consequently, even if conclusive evidence is not required, the risk addressed by the measure shall be more than hypothetical and based on a scientific risk assessment as thorough as possible.

Therefore, a key question is to determine the minimum level of '*potential risk*', which could justify a restrictive regulatory measure for those substances with inconclusive opinions issued. In view of the current situation, the Commission considers that, regardless of the data submitted by the applicants, evidence in scientific literature could be used to assess if a '*potential risk*' to human health can, nevertheless, be identified and can reasonably justify the adoption of regulatory measures in accordance with Article 16(6) of the Cosmetics Regulation.

[1] Colloidal Silver (nano) (SCCS/1596/18), Styrene/Acrylates copolymer (nano) + Sodium styrene/Acrylates copolymer (nano) (SCCS/1595/18) and Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form) (SCCS/1545/15).

Such evidence at the level of substances or group of substances may include, but are not limited to the following:

- systemic or local availability;
- harmful effects specifically related to nano-form;
- surface catalysed reactions in nano-form;
- absorption (or potential absorption) from dermal and inhalation routes;
- potential of nano-form to deliver ionic forms.

Terms of reference

1. *The SCCS is requested to determine the nanomaterials, as published in the recent catalogue of nanomaterials of 2019, for which specific concerns can be identified and justified in order to establish a priority list of nanomaterials for risk assessment (Article 16(4) Reg.1223/2009). More specifically, the SCCS is requested to provide a description of the specific concerns that have been identified for the nanomaterials mentioned above. This process should be based on the currently available scientific literature and SCCS' expert judgement.**

2. *For the nanomaterials with inconclusive SCCS opinions, such as [Colloidal Silver (nano) (SCCS/1596/18), Styrene/Acrylates copolymer (nano) + Sodium styrene/Acrylates copolymer (nano) (SCCS/1595/18) and Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form) (SCCS/1545/15)], the SCCS is requested to assess if a potential risk can be identified according to Article 16(6) Reg.1223/2009. Such assessment, regardless of the data previously submitted by the respective applicants, should be based on the available scientific literature and SCCS' expert judgement (i.e. systemic or local availability; harmful effects specifically related to nano-form; surface catalysed reactions in nano-form, absorption (or potential absorption) from dermal and inhalation routes, potential of nano-form to deliver ionic forms, etc.).**

* In the assessment of the above question and in order to avoid conflicting opinions with other bodies, SCCS is invited to consult SCHEER.

3. OPINION

PREAMBLE

The very small size and other particle features of nanomaterials may confer certain distinctive characteristics to these materials compared to conventional forms. It was noted at early stages of the development and application of nanomaterials that the same nano-scale features, that make them desirable for a wide range of industrial and consumer applications, may also render them harmful for human health and/or the environment. Whilst the science of safety assessment of nanomaterials is still evolving, and there are several knowledge gaps, a number of characteristics have been identified as important in relation to the distinctive properties, behaviour and toxicological effects of nanomaterials. These are briefly summarised and discussed in the following sections in relation to potential concerns over safety to consumers' health.

3.1 DISCUSSION

3.1.1 PHYSICOCHEMICAL ASPECTS

3.1.1.2 VERY SMALL DIMENSIONS OF CONSTITUENT PARTICLES

The single common denominator amongst the vast array of nanomaterials is that they all have primary particles in the size range of ≤100 nm in one or more dimensions. It has been known since 1950s that properties of particulate materials may change when they are manufactured at very small size dimensions (Feynman, 1959). It is also known that rules governing physicochemical properties of conventional (bulk) substances generally do not apply well to the same materials when they are in nano form (SCENIHR, 2010). However, although reducing the particle size may confer some fundamental shifts in the physicochemical properties of the materials, the nanoscale itself should not be considered as a threshold for such a phenomenon because, depending on the type of material, such changes may occur in a continuum over a wider range of particle sizes (SCENIHR, 2010).

Where lowering the particle size leads to changes in the physicochemical properties of a material, it could also lead to changes in the biokinetic behaviour, biological interactions and effects, compared to the bulk equivalents. For example, quantum effects are known to dominate on the properties of nanoparticles, especially when they are in the lower nm size range. It has been suggested that most physicochemical changes in inorganic nanoparticles occur at sizes around or below 30 nm (Auffan *et al.*, 2009a).

Another size-related aspect emanating from several studies relates to the ability of nano-sized particles to cross biological membrane barriers that protect vital organs from the entry of insoluble particles - e.g. cellular barrier, gastrointestinal barrier, blood-brain barrier, placental barrier (SCENIHR 2007, 2009). This means that nanoparticles can potentially enter those parts of the body, where larger-sized particles could not have reached. Nanoparticles are also claimed to have a greater uptake, absorption and bioavailability in the body compared to bulk equivalents (SCENIHR, 2007). For example, nano-sized carriers have been used for enhancing the delivery of nutrients and other substances in food supplements, nutraceuticals, cosmeceuticals and health-food products (e.g. Joye *et al*., 2014; EFSA Guidance, 2018).

The ability of nanoparticles to cross biological membranes and enter cells and tissues is an important factor for all toxicity endpoints, and more so for genotoxicity. Whilst the uptake of nanoparticles to the cellular nucleus may only take place for very small sized nanoparticles,

unless nanoparticles enter nucleus during cell division (mitosis). It is an important consideration for understanding the mechanism of genotoxicity to establish whether it is due to direct contact and interaction of the particles with the genetic material, or through an indirect mechanism, e.g. via oxidative stress. In this regard, the cellular uptake of nanoparticles is not only influenced by the particle size but also by other features such as charge, surface properties, etc.

The very small size of the constituent particles also leads to a huge increase in ratio between surface area and volume of a nanomaterial, compared to conventional (bulk) form. Thus, on a weight per weight basis, surface reactivity of a nanomaterial can potentially be much greater than its conventional bulk equivalent. Particles at the nano-scale are also known to have large free energy at the surface (Simon and Joner, 2008). This not only increases the chances of agglomeration of nanoparticles, but may also lead other substances to bind to particle surfaces. The latter raises the possibility that nanoparticles may 'transport' other potentially harmful substances adsorbed on their surfaces into cells and tissues – a phenomenon termed as 'Trojan horse' effect (EFSA Guidance, 2018). Such alterations in physicochemical properties and biokinetic behaviour may also result in changes in the interaction of a nanomaterial with its known biological target, or with a different target, that could lead to adverse effects, compared to bulk form of the same material.

The current scientific knowledge indicates that particulate materials composed or comprised of small particles may differ from conventional (bulk) form of the same materials in terms of certain physicochemical properties. For example, they may have much greater surface reactivity due to increased surface areas. Particles in the nanoscale (≤100 nm in one or more dimension), may also have a different biokinetic behaviour and may reach those organs that are normally protected from entry of the particles by membrane barriers. Such changes in physicochemical properties and biokinetic behaviour may lead to toxicological effects that are either atypical, or manifest in unexpected organs, compared to conventional (bulk) form of the same material. Therefore, composition of a particulate material in or around nanoscale should raise the trigger for a risk assessor in the first place to further evaluate safety data in consideration of the nano-scale properties of materials.

As a general rule, the lower the size of a nanoparticle is within the nano-scale, the higher the concern should be for its safety to the consumer health.

3.1.1.3 SOLUBILITY/PERSISTENCE/POTENTIAL ACCUMULATION IN THE BODY

A crucial aspect to consider when assessing the potential risk of nanomaterials is that they are composed or comprised of particles in the nanoscale. Any particle size related change in a material's properties, behaviour, or toxicity can only be expected with the existence of such a particle configuration. Where a nanomaterial loses particulate composition, e.g. due to immediate particle dissolution/breakdown, its subsequent risk will not be different from conventional form, and risk assessment for the dissolved chemical form is generally sufficient.

For partially or slowly dissolving nanomaterials, however, the risk of both the particles and the dissolved substance needs to be considered. The dissolution rate in relevant media can provide information on the forms and speciation in the nanomaterial, as well as toxicokinetics when it comes into contact with relevant areas of the human body (Dekkers *et al.*, 2016). This may also result in the particles delivering a relatively higher concentration of the solubilised material in certain organs, which would not occur if the material was fully solubilised. Thus, solubility and dissolution rate of a nanomaterial are

important criteria that can help establish whether there is the likelihood of exposure to insoluble, biopersistent nanoparticles.

Due to the very small size, insoluble/poorly soluble and persistent particle nature, and potentially reactive particle surfaces, the interaction of nanoparticles with biological entities may take place close to the molecular level. Unlike conventional soluble forms, the biokinetics of insoluble particles is not driven by a concentration gradient. Instead, nanoparticles are generally actively removed from systemic circulation by phagocytising cells of the mononuclear phagocytic system (MPS), and thus mainly end up in liver and spleen – the organs rich in phagocytic cells (De Jong *et al.*, 2013; Geraets *et al.*, 2014; Lankveld *et al.*, 2010; Lankveld *et al.*, 2011; Yuan *et al.*, 2019). Also, nanoparticles may be absorbed via different exposure routes and their adverse effects may be at local and/or systemic levels. If elimination of nanoparticles from the body is limited, they may also accumulate in the body over time. As an example, the distribution and accumulation of nano-iron can be different from that of non-nano-iron, which can result in altered toxicity (Brand *et al.*, 2017; Alphandery, 2019).

Solubility and dissolution rate of a nanomaterial in relevant media are important criteria for deciding whether a risk assessment carried out for the conventional chemical form would be sufficient, or whether it poses the risk of exposure to insoluble/poorly-soluble and persistent nanoparticles. In the latter case, consideration of the data on toxicokinetics becomes essential for risk assessment.

As a general rule, the lower the solubility and dissolution rate of a nanomaterial are, the higher the concern should be for its safety to the consumer health.

3.1.1.4 CHEMICAL NATURE AND TOXICITY OF THE NANOMATERIAL

The chemical nature of nanomaterials can be as diverse as that of conventional chemicals, and they may comprise inorganic, organic, or composite/hybrid substances. It is therefore important that chemical nature of the substance(s) constituting a nanomaterial is also taken into consideration in safety assessment for any inherent toxicological hazard of the constituting chemical(s). The chemical nature of a nanomaterial is also important in considering the form of any ions/molecules that may be released as a result of particle dissolution/disintegration. The information on the potential toxicity of chemical components of a nanomaterial is generally obtained by searching different databases; for example, Risctox (https://risctox.istas.net/en/); ECHA database for REACH registered substances (https://echa.europa.eu/information-on-chemicals/registered-substances); TOXNET database (available as part of ChemIDPlus: https://chem.nlm.nih.gov/chemidplus/). A database of nanomaterial safety (eNanoMapper: https://data.enanomapper.net/) is also available that may provide relevant toxicity information on some of the already tested nanomaterials.

As discussed before, the properties/effects of nanomaterials are driven both by chemical nature and physical form of the constituent particles. The information on chemical toxicity therefore needs to be combined with any physical characteristics of the particles that may lead to a different biological outcome (e.g. toxicokinetics). It is also possible that the chemical nature of each of the components that make up a nanomaterial is safe individually, but may pose a hazard when put together in the form of a nanoparticle as such, or cause indirect effects by delivering the components to unintended places in the body.

It has been suggested that chemically stable metallic nanoparticles have no significant cellular toxicity, whereas nanoparticles that are able to undergo oxidation, reduction or

dissolution can be cytotoxic and even genotoxic for cellular organisms (Auffan *et al.*, 2009b).

In regard to the potential toxicity of a nanomaterial, a particular focus is on identifying whether or not the nanomaterial or the constituting chemical(s) have CMR (carcinogen, mutagen or reproductive toxicant) properties. A nanomaterial should be assigned the highest priority for a further follow up for safety assessment if there are indications of potential CMR property from either chemical composition or the available data on the nanomaterial.

Especially when toxicity is evaluated in *in vitro* test systems specific considerations apply. One issue may be whether the tests had been carried out ensuring good stability of the test suspension and exposure of the test system to nanoparticles is established. Interactions of the nanomaterial with test media/environment can also pose problems when testing nanomaterials *in vitro* because potential interaction with the test systems may lead to unreliable outcomes (Kroll *et al.*, 2012; Guadagnini *et al.*, 2015). The presence of the particles alone could be a source for interference with readout systems that use an optical method (e.g. light scattering and absorbance). In addition, nanomaterials may interfere with assay components. For example, colorimetric assays may be prone to interference due to the interaction between the dye and nanoparticles, and washout of the nanomaterials can be difficult due to such interactions (Guadagnini *et al.* 2015, Dusinska *et al.*, 2015). Guadagnini *et al.* (2015) showed that many nanoparticle characteristics (composition, size, coatings, and agglomeration) can interfere with a range of *in vitro* cytotoxicity assays (WST-1, MTT, LDH, neutral red, propidium iodide, 3H-thymidine incorporation, and cell counting), proinflammatory response evaluation (ELISA for GM-CSF, IL-6, and IL-8), and oxidative stress detection (monoBromoBimane, dichlorofluorescein, and NO assays). The interferences were found to be specific for both the assays, as well as the type of nanoparticle.

In vitro systems, generally used for testing conventional chemicals, may not be applicable to test nanomaterials, or may need to be modified for the purpose. For example, *in vitro* genotoxicity data are not acceptable if derived from AMES test, because nanoparticle uptake is not likely to take place in bacteria. Similarly, the timing of administration of cytokinesis blocking agent (cytochalasine B) is critical in the micronucleus test using the cytokinesis-blocked micronucleus (CBMN) method because as it can also block the cellular uptake of nanoparticles.

Data on chemical composition provide another trigger for safety concern to establish whether the constituent chemical(s) pose a toxicological hazard, either individually or when in the form of a nanomaterial. The toxicity data need to be assessed with consideration of the chemical nature as well as the potential changes in properties of the particles at the nano-scale. Testing of nanomaterials also needs to take into consideration the limitations of certain test methods and the potential interaction of nanoparticles with assay components or the test systems.

As a general rule, where chemical component(s) are toxic, as such or when put together in the form of a nanomaterial, they should constitute a trigger for concern over safety to the consumer health.

3.1.1.5 PHYSICAL/MORPHOLOGICAL FEATURES OF THE CONSTITUENT PARTICLES

Nanomaterials may be comprised of, or contain, free nanoparticles and/or larger-sized agglomerates and aggregates. Depending on the type of application, a nanomaterial may be present in the final product in the form of free nanoparticles, and/or larger sized clusters of particles. In the aggregated form, primary particles are strongly bound together and are

therefore not likely disaggregate under normal condition. Compared to this, primary particles are only held together by weak van der Waals forces in agglomerates, and may de-agglomerate under certain conditions of pH, ionic strength, etc. Therefore, nanomaterials that are composed of free nanoparticles or agglomerates (and nano-sized aggregates) are of more concern for safety than the same materials in which particles are present in the form of larger-sized aggregates.

Among the nanomaterial-containing products, those that can lead to inhalation exposure of nanoparticles are considered as being of the highest risk because particulate materials generally tend to induce more harm to the respiratory system (Donaldson and Seaton 2012). Among these, needle, tube and fibre shaped nanomaterials pose an even more severe risk due to the particular morphologies. Certain fibre characteristics like fibre length, rigidity and lack of degradation can result in the induction of inflammatory processes similar to those induced by asbestos (Donaldson *et al.* 2010).

It has been shown for carbon nanotubes, that mechanistically, a number of mediators, signaling pathways, and cellular processes can be identified as major mechanisms that underlie the interplay among inflammation, fibrosis, and malignancy, and serve as pathogenic basis for such diseases in CNT-exposed animals. This also raises concern for similar disease conditions in humans (Dong and Ma, 2019).

Depending on the conditions during manufacturing, processing and handling, nanoparticles may exist in different physical and morphological forms in a nanomaterial. As a general rule, safety concerns should increase in the order from larger sized aggregates<agglomerates<free-nanoparticles. Also certain morphological forms should raise more safety concerns than the others (e.g. needle shape, rigid long fibres, etc).

3.1.1.6 SURFACE CHEMISTRY

Due to the relatively large surface-to-volume ratio, the reactivity of nanomaterials can be enhanced compared to non-nanomaterials. The reactivity of such enlarged surfaces inside living cells may interfere with biological processes and trigger, for example, the generation of reactive oxygen species (ROS), which could lead to oxidative stress and inflammatory outcomes in biological tissues.

The surface redox state of metal oxide nanomaterials was considered relevant for induction of in vitro cytotoxicity. Nanomaterials with an overlap of conduction band energy (Ec) levels with the cellular redox potential were found to be cytotoxic while nanomaterials with a redox potential outside this level were less toxic (Zhang *et al.* 2012). The toxicity was ascribed to the induction of oxidative stress in the cells.

Nanomaterial surface chemistry has significant effect on interactions at the nano-bio interface, with important toxicological consequences. Recent data has shown complexity in the dynamic relationship between the composition of the biological environment and the physico-chemical properties of the nanomaterials (Lundqvist *et al*., 2008, Walkey *et al*, 2012, Wang *et al*., 2013; Yallapu *et al*., 2015, Lynch *et al*., 2015; Khan *et al*., 2020). Physiological environments, such as blood, interstitial fluid, and cellular cytoplasm, contain complex protein mixtures. When nanoparticles enter the physiological environment, they adsorb proteins to form protein corona (Cedervall *et al*., 2007a, b; Lundqvist *et al*., 2008). Protein corona that forms around nanoparticles alter the physicochemical properties of nanoparticles (Glancy *et al*., 2019; Marichal *et al*., 2019, Khan *et al*., 2020), and is a critical factor that affects their physiological response, influences the interactions between nanoparticles and biological systems and modulates the kinetics, transport, and reactivity of

nanoparticles (Monopoli *et al*., 2011; Walkey *et al*., 2012, Khan *et al*., 2020, Clemments et al., 2017, Cai *et al*., 2020, Khan *et al*., 2020).

Surface characteristics of nanoparticles determine the reactivity of a nanomaterial, such as (photo)catalytic activity, potential for radical formation, biokinetic behaviour, and potential transport of other substances into the systemic circulation. Surface chemistry is a vital component which impacts the corona composition and subsequent distribution, uptake, toxicity and clearance of nanomaterials. These should be considered in conjunction with other confounding factors in safety assessment.

3.1.1.7 SURFACE CHARACTERISTICS (SURFACE MODIFICATIONS/COATINGS)

Particle surfaces can be chemically/biochemically modified to suit a particular function or property for some applications. For example, nanoparticles may be made more hydrophobic or hydrophilic through surface modification. This could have a profound effect on the ADME properties (e.g. increasing or decreasing systemic bioavailability) than the same nanoparticles without surface modification. The systemic availability of nanoparticles with surface modified with a protein or peptide may have immunological effects.

Any surface modification of a nanomaterial needs to be considered carefully in regard to potential changes in the biokinetic behaviour of the nanoparticles, in conjunction with other confounding factors in safety assessment.

3.2 EXPOSURE ASPECTS

3.2.1 SYSTEMIC EXPOSURE OF THE CONSUMER TO NANOPARTICLES

As mentioned above, due to nano-scale dimensions, the ADME (absorption, distribution, metabolism, excretion) characteristics of nanoparticles may be different from bulk equivalents. As a result, systemic exposure of the consumer to nano-form of a material may be different compared to bulk form of the same material. As a general rule, exposure to particles with sizes in the lower range (1-30 nm) of the nano-scale increases the chances of systemic exposure. The exposure assessment for such particles also need to consider other confounding factors, such as coatings or other surface modifications, solubility and dissolution rate in the exposure vehicle and the biological phases.

The route of exposure to nanomaterials is equally important in risk assessment. Studies have indicated that exposure to nanomaterials via inhalation route carries a relatively greater potential for risk to human health. However, depending on the absorption of nanoparticles and systemic availability, exposure from other routes (oral, dermal) may also be of similar safety concern.

As a general rule, safety concerns should by higher for those nanomaterials (or nanomaterial applications) that may lead to systemic exposure of the consumer to nanoparticles.

3.3 OTHER ASPECTS

3.3.1 NOVEL PROPERTIES, ACTIVITY OR FUNCTION

Another aspect that could lead to safety concerns is that a nanomaterial may be smart/functionalised to have a novel property, activity, or function that was not present in conventional form of the same material. Also, it is possible a nanomaterial is designed in such a novel way that it does not have a conventional comparator for assessment of changes in the properties, activity or function.

Novel nanomaterials designed for a specific activity or function should trigger a concern for safety as the activity/function may lead to adverse outcomes in an unintended part of body due to the altered biokinetic behaviour of nanoparticles.

3.3.2 SPECIFIC CONCERN ARISING FROM THE TYPE OF APPLICATION

The type and frequency of application of a nanomaterial containing product may also raise a safety concern. For example, application of a nanomaterial in loose powder or sprayable products may pose a risk of inhalation of respirable particles into the respiratory tract and expose the consumer's lung. Similarly, there will be more safety concerns if nanomaterials are used in products that are more frequently used, used in relatively large amounts, or intended for use by certain more vulnerable people or young children.

Certain type of products containing nanomaterials, and those used more frequently, or used by more vulnerable consumers may further increase the concerns over safety of the consumer's health.

3.4 OVERALL SUMMARY

In regard to the safety of nanomaterials, in the first place, the presence of small particles (in the nanometer range) in an ingredient should draw attention of the risk assessors/managers to look more closely to the information on physicochemical characterisation of the nanomaterial. In particular, the presence of any significant proportion of nano-sized particles in consumer products should raise the first alert for potential concerns over safety.

Although there are currently no hard and fast rules for working out the safety concerns for nanomaterials, as a general principle, each of the following attributes should add a further degree of safety concern. For example, where:

1. The nanomaterial has constituent particles that have sizes in the lower range of the nano-scale (1-100 nm),
2. The nanomaterial is insoluble, or only partially-soluble,
3. The chemical nature of the nanomaterial suggests the potential for a toxicological hazard,
4. The nanomaterial has certain physical/morphological features (e.g. needle shape, rigid long fibres) that point to potential for harmful effects,
5. The nanomaterial has surface reactivity in terms of catalytic (including photocatalytic) activity, potential for radical formation, or other surface properties (e.g. that can enhance cellular uptake, or confer allergenicity due to proteinaceous surface),
6. The nanomaterial has a different biokinetic behaviour than the conventional equivalent. For example, on the surface a modification/coating (e.g. hydrophobic coatings, encapsulation) has been applied to the core nanoparticles to alter their ADME properties and as a result make them more accessible systemically, compared to the neat nanoparticles and/or their conventional chemical forms,
7. The nanomaterial is used as vehicle to carry other substances - which have not been assessed for safety as individual components, or together in the nano-scale entity,
8. There is a likelihood of systemic exposure of the consumer to nanoparticles through the use of final products, that enhance absorption in the skin (skin penetration) by a surface modification,
9. The frequency of use, and/or the amounts of the consumer product are relatively high,
10. There is evidence for persistence/accumulation of nanoparticles in the body,
11. Nanoparticles have other distinctive properties not present in conventional form of the same material or a new activity/function (e.g. a smart/functional nanomaterial),
12. The nanomaterial is so novel that it does not have a conventional comparator to allow assessment of changes in properties, behaviour or effects,
13. The nanomaterial is used in a product that is inhalable (taken up by inhalation into respiratory tract and lung), and the particles are respirable (can reach respiratory epithelium i.e. alveoli),
14. The assessment of genotoxicity is inadequate, e.g. in vitro studies are without information on stability of the test suspension, or evidence of cell exposure (internalisation).

The different aspects discussed above provide a basis for safety concerns that may arise from each individual aspect of nanomaterials. However, the overall concern for consumer safety will be a combination of all the aspects that are relevant to a specific nanomaterial.

In this regard, there are no agreed rules on how to combine all the individual 'alerts' to obtain an overall concern for safety. This is where expert judgement has been used to prioritise nanomaterials for safety assessment. Recently, a relevant scoring system has been proposed by Brand *et al.* (2019) that combines consideration of the key aspects of nanomaterials that can trigger a 'signal' for risk, which when combined with expert judgment can help assign an arbitrary score for prioritisation on the basis of risk potential for human health. Table-1 below has been adapted from Brand *et al.* (2019) in view of the

potential usefulness in identifying priority nanomaterials for further action regarding safety assessment.

It needs to be noted that the outcome of such a scoring system is not meant to be an alternative to evidence-based safety assessment, but to provide a means for prioritising nanomaterials so that they can be subjected to proper safety assessment.

Table 1. Scoring system with key questions to assess a selected signal for prioritisation on risk potential for human health (adapted from Brand *et al.*, 2019).

Descriptor	Question	Answer[a] *(score)*		
		Yes *(3)*	No *(0)*	? *(1)*
Physico-chemical properties[b] *(max 12 pts)*	Indication of low or no dissolution or degradation rate in physiologically relevant media?			
	Indication of reactivity? E.g. due to surface area, type of chemical, surface treatment.			
	Indication of release of toxic ions or molecules?			
	Indication that the nanomaterial is persistent and rigid, i.e. a High Aspect Ratio Nanoparticle (HARN)[c] ?			
Hazard *(max 12 pts)*	Is the chemical itself a substance of very high concern, relating to human health hazard[d] ?			
	Indication of mutagenicity/carcinogenicity (of the material)?			
	Indication of immunotoxicity (of the material)?			
	Indication of other toxicity (of the material)?			
Kinetics *(max 12 pts)*	Indication of absorption?			
	Indication of distribution to brain or reproductive organs?			
	Indication of accumulation in any tissue?			
	Indication of change in kinetic profile compared to non-nano situation?			
Exposure[e] *(max 12 pts)*	Products used or likely to be used much or in many products and/or by wide population?			
	Is exposure of sensitive subgroups anticipated? (e.g. babies or elderly people)			
	Is exposure likely to occur frequently (more than a few incidental times)?			
	Is there potential for nanomaterial exposure likely, based on the product use description?			
	Total marks	…	…	…
		x 3	x 0	x 1
	Sub-score	…	0	…
	[2]Total score		…	

2[a] An indication for a specific physicochemical property, hazard, (toxico)kinetic behaviour or exposure is sufficient to attribute the maximum score of 3. Unknown (=?) can also be interpreted as 'maybe', in case the indications are weak.

[b] Take into account that outer layers may not be stable and therefore consider changes in surface properties.

The scoring system uses descriptors relating to physicochemical properties, hazard, (toxico)kinetics and exposure aspects of nanomaterials. Expert judgement is needed to answer the questions (yes, no or unknown) to assign a score (3, 0, or 1, respectively).

It needs to be noted while considering such a scoring system that there will also be certain 'exit' routes for a nanomaterial from needing nano-specific safety assessment. For example, where the data indicate that:

1. a nanomaterial is completely dissolved or loses its nano-structure[3]
2. there is no systemic exposure to particulate form of the material
3. the nanoform of the material has been shown to be non-toxic

In such cases, nano-specific risk assessment may not be needed and conventional risk assessment should be sufficient.

4. CONCLUSION

1. *The SCCS is requested to determine the nanomaterials, as published in the recent catalogue of nanomaterials of 2019, for which specific concerns can be identified and justified in order to establish a priority list of nanomaterials for risk assessment (Article 16(4) Reg.1223/2009). More specifically, the SCCS is requested to provide a description of the specific concerns that have been identified for the nanomaterials mentioned above. This process should be based on the currently available scientific literature and SCCS' expert judgement.**

Through a review of the available information and expert judgment, the SCCS has identified certain aspects of nanomaterials that constitute a basis for concern over safety to consumers' health when used in cosmetic products. These include:

- Physicochemical aspects relating to: very small dimensions of the constituent particles; solubility/persistence/potential accumulation in the body; chemical nature and toxicity of the nanomaterial; physical/morphological features of the constituent particles; surface chemistry and surface characteristics (surface modifications/coatings);
- Exposure aspects relating to: the frequency and the amounts used, whether the number/type of consumer product(s) used is relatively high; and whether there is a potential for systemic exposure of the consumer to nanoparticles;
- Other aspects relating to: novel properties, activity or function, and specific concern arising from the type of application.

A detailed account of these aspects has been presented in this Opinion. Also, the nanomaterials listed in the EC catalogue of nanomaterials of 2019 have been tabulated in an order of priority according to risk potential in Annex 1 of this Opinion.

2. *For the nanomaterials with inconclusive SCCS opinions, such as [Colloidal Silver (nano) (SCCS/1596/18), Styrene/Acrylates copolymer (nano) + Sodium*

[c] HARN = a material that has a diameter <100 nm and a length many times greater than its diameter (aspect ratio greater than 3 or 5:1), as defined by ECHA (2017) [11].
[d] Reference to ZZS list: www.rivm.nl/rvs/Stoffenlijsten/Zeer_Zorgwekkende_Stoffen, only substances on this list that relate to human health hazards are considered.
[e] Restricted to exposure of consumers.
[3] e.g. in a formulation, a test medium, or biological surface/environment, due to solubilisation, breakdown or degradation, or interactions with other substances (see SCCS/1611/19).

*styrene/Acrylates copolymer (nano) (SCCS/1595/18) and Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form) (SCCS/1545/15)], the SCCS is requested to assess if a potential risk can be identified according to Article 16(6) Reg.1223/2009. Such assessment, regardless of the data previously submitted by the respective applicants, should be based on the available scientific literature and SCCS' expert judgement (i.e. systemic or local availability; harmful effects specifically related to nano-form; surface catalysed reactions in nano-form, absorption (or potential absorption) from dermal and inhalation routes, potential of nano-form to deliver ionic forms, etc.).**

The SCCS has reviewed previous inconclusive opinions on three nanomaterials (SCCS/1596/18; SCCS/1595/18 and SCCS/1545/15), in conjunction with any further relevant information available in published literature to identify whether there is a scientific basis for concern over their safety to consumers' health when used in cosmetic products. The SCCS has identified certain aspects relating to each of the nanomaterials that raise a safety concern. These have been detailed in three separate annexes (2, 3 and 4) to this Opinion.

* In the assessment of the above question and in order to avoid conflicting opinions with other bodies, SCCS is invited to consult SCHEER.

5. MINORITY OPINION

None.

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ANNEX 1: THE LIST OF PRIORITY NANOMATERIALS IN THE EC CATALOGUE OF NANOMATERIALS (2019) ON THE BASIS OF RISK POTENTIAL

Category/ Nanomaterial	**CAS Number**	**CosIng Entry**	**Already assessed by SCCS ?**	**SCCS Concerns over Potential Risk to the Consumer**	**Priority for Risk Potential** (according to Brand *et al.*, 2019)
Methylene Bis Benzotriazolyl Tetramethylbutyl phenol **(UV Filter)**	103597-45-1	Nano: VI/23a Specific use conditions (column h and i)	Yes, MBBT SCCS/1460/11 and SCCS/1546/15	Methylene bis benzotriazolyl tetramethylbutylphenol (MBBT) is an insoluble and persistent/bioaccumulative material. There is a positive SCCS Opinion for the use of uncoated form of MBBT as a UV filter with certain specified characteristics in dermally-applied products, mainly on the basis of a lack of dermal absorption in insoluble particulate form. However, the Opinion noted inflammatory effects via the inhalation route, and also a lack of clarity in regard to potential genotoxicity/ carcinogenicity. Some applications of MBBT listed in the EC catalogue may lead to oral or inhalation exposure, which raises concern over safety of the consumer from the use of such applications.	34
Colloidal Silver **(Other Functions)**	7440-22-4		SCCS opinion available – SCCS/1596/18	Silver (Ag) is a slowly solubilising material under physiological conditions with the release of silver ions. Depending on the concentration and site of release, silver ions may be harmful because of the ability to bind with other moieties (e.g. proteins, enzymes). There are indications for genotoxicity, immunotoxicity, developmental toxicity of nano silver. Oral applications of colloidal silver are also listed in the catalogue (toothpaste, mouth wash, oral hygiene products). Such uses are of concern in regard to safety of the consumer due to potential for systemic exposure to silver nanoparticles.	34
Silver **(Other Functions)**				Silver (Ag) is a slowly solubilising material under physiological conditions with the release of silver ions.	34

				Depending on the concentration and the site of release, silver ions may be harmful because of the ability to bind with other moieties (e.g. proteins, enzymes). There are indications for genotoxicity, immunotoxicity, developmental toxicity of nano silver. Oral applications of silver are listed in the EC catalogue (toothpaste, mouth wash, oral hygiene products). Such uses are of concern in regard to safety of the consumer due to the potential for systemic exposure to silver nanoparticles.	
Tris-Biphenyl Triazine **(UV Filter)**	31274-51-8	VI/29	Yes SCCS/1429/11	Tris-biphenyl triazine (ETH50) is an insoluble material that is not absorbed via dermal or oral routes. There is a positive SCCS Opinion on the use of uncoated form of ETH50 with a median primary particle size > 80 nm as UV filter in dermally-applied products, mainly on the basis of a lack of dermal absorption of the material in insoluble particulate form. However, the Opinion does not recommend use in products that could lead to inhalation exposure because of the potential to cause strong inflammatory response in the lung. Therefore, the use of ETH50 in products that could lead to inhalation exposure, as listed in the catalogue, raise concern over safety of such applications to the consumer.	30
Colloidal Copper **(Other Functions)**	7440-50-8			Copper (Cu) is an insoluble material that may degrade to ionic form of copper under certain conditions. Colloidal copper is apparently toxic by oral route, and there are indications that it leads to the formation of reactive oxygen species. Dermal penetration and systemic availability of copper nanoparticles is currently unclear. As oral uses are also reported in the EC catalogue (mouth wash), there is a concern over its safety to the consumer due to the potential for systemic exposure to copper nanoparticles. Colloidal copper is currently under evaluation for consumer	30

				safety by the SCCS.	
Platinum/ platinum powder **(Other Functions)**	7440-06-4			Platinum (Pt) is an insoluble, and persistent material, which in non-nano form is inert and is not likely to degrade/ionise. However, Pt nanoparticles may surface-catalyse oxidative reactions, which under biological conditions may lead to harmful effects. Colloidal platinum is currently under safety evaluation by the SCCS. Non-nano form is also used as antimicrobial in cosmetics. Due to insoluble, persistent and surface-reactive nature, the use of nano-form of platinum in cosmetic product is of concern in regard to consumer safety due to the potential for systemic exposure to Pt nanoparticles.	30
Colloidal Platinum **(Other Functions)**	7440-06-4			Platinum (Pt) is an insoluble and persistent material, which in non-nano form is inert and is not likely to degrade/ionise under physiological conditions. However, due to surface reactivity, Pt nanoparticles may surface-catalyse oxidative reactions, which under biological conditions may lead to harmful effects. Colloidal platinum is currently under safety evaluation by the SCCS. Non-nano form of Pt is also used as antimicrobial in cosmetics. Due to insoluble, persistent and surface-reactive nature, the use of colloidal platinum in cosmetic product is of concern in regard to consumer safety due to the potential for systemic exposure to Pt nanoparticles.	30
Styrene/Acrylate Copolymer **(Other Functions)**			Yes SCCS/1595/18	There is an inconclusive SCCS opinion on the safety of styrene/acrylate copolymer, which contained other cosmetic ingredients packaged inside the encapsulates. Such a nano-scale packaging of bioactive substances is of a concern regarding consumer safety because of the potential for nano-scale delivery and the resulting effect of the encapsulated substances to unintended parts of the body.	30

CI 77891 (Titanium dioxide) **(Colorant)**	13463-67-7 1317-70-0 1317-80-2	Non-Nano: IV/143	Assessed as UV-Filter	Titanium dioxide (TiO2) is a practically insoluble and persistent material that is inert in non-nano form. There is a positive SCCS Opinion for the use of its nano-form as a UV filter in dermally applied products, mainly on the basis of a lack of dermal absorption of TiO2 nanoparticles. However, the Opinion did not recommend use of nano forms of TiO2 in cosmetic products that could lead to inhalation exposure because of the potential to cause inflammatory response in the lung. There is also a safety concern (potential carcinogenicity) when exposure is via the inhalation route. The non-nano form of TiO2 (that contain a significant fraction in the nano-scale) as pigment/colorant in cosmetic products is currently under assessment by the SCCS.	29
Titanium Dioxide **(UV Filter)**		Nano: VI/27a Specific use conditions (column h and i)	Yes SCCS/1516/13 SCCS/1580/16	Titanium dioxide (TiO2) is a practically insoluble and persistent material that is inert in non-nano form. There is a positive SCCS Opinion for the use of its nano-form as a UV filter in dermally applied products, mainly on the basis of a lack of dermal absorption of TiO2 nanoparticles. However, the Opinion did not recommend use of nano forms of TiO2 in cosmetic products that could lead to inhalation exposure because of the potential to cause inflammatory response in the lung. There is also a safety concern (potential carcinogenicity) when exposure is via the inhalation route. The non-nano form of TiO2 (that contain a significant fraction in the nano-scale) as pigment/colorant in cosmetic products is currently under assessment by the SCCS.	29
Silica Dimethyl Silylate **(Other Functions)**	To be checked			Same concerns as under silica, except that with hydrophobic modification to make dimethylated particle surface, the absorption and systemic availability may be higher compared to neat silica and this raises a concern over consumer safety due to greater risk of	29

				internal exposure to the nanoparticles.	
Silica Dimethicone Silylate **(Other Functions)**	CAS not given			According to CosIng, this is a reaction product of silica with polydimethylsiloxane. There are same concerns associated with this nanomaterial as under silica above, except that, with surface modification with simethicone silylate, the absorption and systemic availability may be higher compared to neat silica and this raises a concern over consumer safety due to greater risk of internal exposure to the nanoparticles.	29
Silica Silylate **(Other Functions)**	68909-20-6		Yes SCCS/1545/15	Same concerns as under silica, except that with hydrophobic modification to make trimethylated particle surface, the absorption and systemic availability may be higher compared to neat silica and this raises a concern over consumer safety due to greater risk of internal exposure to the nanoparticles.	28
Fullerenes **(Other Functions)**	99685-96-8			Fullerene is composed of extremely small particles (around 1 nm) made of carbon lattice. Due to the extremely small size, fullerene particles have the potential to penetrate biological membrane barriers when exposed via dermal, oral or inhalation routes. The use of fullerenes as antimicrobial in cosmetics has been reported but it has not yet been evaluated for safety of the SCCS. Due to the extremely small particle size and persistent nature, the use of fullerene in cosmetic products is of concern in regard to consumer safety due to the potential for systemic exposure to fullerene nanoparticles.	26
Silica **(Other Functions)**	7631-86-9 112945-52-5		Yes SCCS/1545/15 SCCS/1606/19	Silica (SiO_2) is an insoluble and potentially persistent material, which in non-nano form is inert and is not likely to degrade/ionise. Different forms of the nano-structured synthetic amorphous silica (SAS) have been evaluated by the SCCS. The Opinion (SCCS/1545/15) however could	26

				not draw any firm conclusion either for or against the safety of SAS materials because of the inadequacy of safety data. Another SCCS opinion (SCCS/1606/19) assessed the solubility of SAS materials to conclude that hydrophilic and hydrophobic SAS materials could be regarded as insoluble and very-slightly-soluble respectively. In the absence of conclusive evidence for safety, the use of nano-structured forms of silica in cosmetic products, especially those that may lead to oral or inhalation exposure to nanoparticles, raises concern over safety of the consumer.	
Hydrated Silica **(Other Functions)**	7631-86-9 112926-00-8		Yes SCCS/1545/15	Same concerns as under silica, except that hydrated silica particles are likely to be relatively larger in size than other silica particles.	26
Gold Thioethylamino-Hyaluronic Acid **(Other Functions)**	CAS/Identity unclear			Gold thioethylamino-hyaluronic acid is an insoluble and persistent material. Several studies are available that point to dermal penetration of colloidal/nano gold, and surface modification with thioethylamino-hyaluronic acid may further increase absorption of the nanoparticles through skin and other exposure routes than neat gold nanoparticles. This material has yet not gone through SCCS evaluation for safety. Some applications mentioned in the catalogue (hair relaxer/hair straightener products) may lead to inhalation exposure. Thus, consumer safety concerns from the use of gold thioethylamino-hyaluronic acid is the same as for colloidal gold – i.e. due to the potential for systemic exposure to the nanoparticles.	25
Carbon Black/ CI 77266 **(Colorant)**	1333-86-4, 7440-44-0	Nano: IV/126a Specific use conditions (column h and i)	Yes, SCCS/1515/13	Carbon black is an insoluble nanostructured material that is used as a colorant in many cosmetic products. There is a positive SCCS Opinion for its use in dermally-applied products. However, the opinion did not recommend applications that might lead to inhalation exposure of the consumer to	25

				carbon black nanoparticles due to the likelihood of harmful effects, including the potential to induce genotoxic effects. The Opinion also did not cover oral uses (such as tooth whitener) that are listed in the EC catalogue. Therefore, there is a safety concern over the use of carbon black in applications that may give rise exposure of the consumer to nanoparticles via oral or inhalation routes. The SCCS also noted in the Opinion SCCS/151/13 that the lowest particle size for which data were available was 20 nm. Additional information would be required on the use of any carbon black material intended for use in cosmetic products with particles size smaller than 20 nm. Furthermore, the Opinion specified that the purity of carbon black nanomaterials used in cosmetic products should be >97%, with a comparable impurity profile with the material(s) tested for toxicity in the submission, and the material(s) should comply with FDA specifications with respect to carbon black produced by furnace method.	
Colloidal Gold **(Other Functions)**	7440-57-5			Gold (Au) is an insoluble and persistent material, which in non-nano form is inert and is not likely to significantly degrade/ionise under physiological conditions. Colloidal gold is currently under evaluation by the SCCS. Several studies are available that point to dermal penetration of colloidal/nano gold. Some in vivo information on toxicity of colloidal/nano gold is also available. Some applications mentioned in the EC catalogue (hair relaxer/hair straightener products) may lead to inhalation exposure to gold nanoparticles, which raises a concern over the safety of colloidal gold due to the potential for systemic exposure of the consumer to gold nanoparticles.	24
Gold **(Other Functions)**				Gold (Au) is an insoluble and persistent material, which in non-nano form is inert and is	23

				not likely to degrade/ionise under physiological conditions. Colloidal gold is currently under evaluation by the SCCS. Several studies are available that point to dermal penetration of colloidal/nano gold. Some in vivo information on toxicity of colloidal/nano gold is also available. Some applications mentioned in the catalogue (hair relaxer/hair straightener products) may lead to inhalation exposure, which raises concern over safety of the consumer due to the potential for systemic exposure to gold nanoparticles.	
Alumina (Aluminium oxide, Al_2O_3) **(Other Functions)**				Alumina (Al_2O_3) is an insoluble and potentially biopersistent material, which is not likely to degrade/ionise easily. In non-nano form, the material is considered relatively inert. However, the use of a nano form of alumina in cosmetic products has not yet gone through SCCS evaluation. Like other insoluble/persistent nanomaterials, the use of nano-forms of alumina in cosmetic products raises a concern over safety of the consumer due to the potential for systemic exposure to the nanoparticles.	23
Hydroxyapatite **(Other Functions)**			Yes SCCS/1566/15	Hydroxyapatite in non-nano form is a natural material that is a component of bones and teeth. The nano-form of hydroxyapatite is currently under safe ty evaluation by the SCCS for oral applications (mouthwash, toothpaste). There are concerns in relation to the potential absorption of hydroxyapatite nanoparticles in the oral mucosa and the potential for harmful effects in the consumer.	21
Lithium Magnesium Sodium Silicate **(Other Functions)**	CAS 53320-86-8			Little relevant information is available in published literature regarding both non-nano and nano forms of lithium magnesium sodium silicate. Therefore, the same safety concerns apply to this nanomaterial as described under silica.	20

Sodium Propoxyhydroxypropyl Thiosulfate Silica **(Other Functions)**	CAS unclear			Little information is available in published literature regarding both non-nano and nano forms of sodium propoxyhydroxypropyl thiosulfate silica. Therefore, the same concerns apply to this nanomaterial as described under silica, except that, with such a surface modification, the absorption and systemic availability may be higher compared to neat silica particles, which raises a concern over consumer safety due to greater risk of internal exposure to the nanoparticles.	20
Sodium Magnesium Fluorosilicate **(Other Functions)**	85085-18-3			Sodium magnesium fluorosilicate is a soluble material that in non-nano form has low/no toxicity. The nano-form of the material has not yet been safety assessed by the SCCS.	17
Sodium Magnesium Silicate **(Other Functions)**	101659-01-2			Sodium magnesium silicate is a soluble materials, that in non-nano form has low/no toxicity. The nano-form of the material has not yet been safety assessed by the SCCS.	17
CI 77947 (Zinc Oxide) **(Colorant)**		Non-Nano: IV/144	Assessed as UV-filter	Zinc oxide (ZnO) is an insoluble material, which under non-static biological environments keeps on releasing Zn ions until the particles are completely solubilised. At low concentrations Zn ions are not considered of any concern because of the essential biological function of zinc, and the existence of a large pool of Zn in the body. There is a positive SCCS Opinion on the use of certain nanoforms as UV filter in dermally-applied products on the basis of a lack of dermal absorption in insoluble particulate form. Oral applications are also mentioned in the EC catalogue (lipstick and lip care products). The use of nanoforms of ZnO with different coatings as UV filter is currently being assessed by the SCCS.	15
Zinc Oxide **(UV Filter)**	1314-13-2	Nano: VI/30a Specific use conditio	Yes SCCS/1489/12	Zinc oxide (ZnO) is an insoluble material, which under non-static biological environments keeps on releasing Zn ions until the particles are completely	13

		ns (column h and i)		solubilised. At low concentrations Zn ions are not considered of any concern because of the essential biological function of zinc, and the existence of a large pool of Zn in the body. There is a positive SCCS Opinion on the use of certain nanoforms as UV filter in dermally-applied products on the basis of a lack of dermal absorption in insoluble particulate form. Oral applications are also mentioned in the EC catalogue (lipstick and lip care products). The use of nanoforms of ZnO with different coatings as UV filter is currently being assessed by the SCCS.	

ANNEX 2: SAFETY CONCERNS ON NANOMATERIALS – COLLOIDAL SILVER (NANO)

The SCCS has recently evaluated the safety of colloidal silver (nano) when used in cosmetics including toothpastes and skin care products with a maximum concentration limit of 1%, taking into account the reasonably foreseeable exposure conditions (SCCS/1596/18). From this evaluation, and other relevant information from published literature, the SCCS has concluded that there is a basis for concern that the use of colloidal silver (nano) in cosmetic products can pose a risk to the consumer because of the following considerations:

PHYSICOCHEMICAL ASPECTS

1. Colloidal silver (nano) is comprised of primary particles that are in the nano-scale. The particle sizes are reported to range from the lowest cut-off size of 1.56 nm to 100 nm (Table 2, SCCS/1596/18).

2. Colloidal silver is a slow dissolving material, composed of particles that liberate silver ions dependent on the conditions of the media/environment. In the 2018 evaluated dossier, the solubility was reported by the Applicants as either 'unlimited solubility', or 'solubility below 0.01 mg/l and no further dissolution in aqueous media' (Section 3.1.6, SCCS/1596/18).

TOXICOLOGICAL ASPECTS

3. The chemical and particulate nature of colloidal silver (nano) suggests a potential for toxicological hazard, as detailed below:

Genotoxicity: The SCENIHR, 2014 Opinion indicates that several in vitro studies have reported genotoxic effects of nanosilver. Any contradicting results may be explained by differences such as in coating of silver nanoparticles (AgNPs), cell type used, the cellular uptake, intracellular dissolution, the genotoxicity endpoint chosen, and the way the cells were exposed. For example, pre-dispersion in a medium before cellular exposure may result in initial dissolution of the AgNP, so that Ag+ is present from the beginning, contributing to (geno)toxic effect, especially in short-term exposure assays (e.g. for two hours).

Literature on AgNP genotoxicity published after the SCENIHR 2014 opinion confirms these conclusions. There are many positive results on genotoxicity which cannot be ignored although there are variations in the results from different studies (Rodriguez-Garraus *et al.*, 2020). Published studies with positive results generally show that the cytotoxic and genotoxic effects of AgNPs in vitro depend on size, shape, coating, concentration, duration of treatment and cell type. Some in vitro and in vivo studies also show that the effects are not size-dependent but more related to surface properties (Huk *et al.*, 2014, Li *et al.*, 2014, Nallanthighal *et al.*, 2017). There are several mechanisms that could lead to genotoxicity: direct damage by AgNPs (several studies show their presence in the cell nucleus); AgNP-induced oxidative stress and inflammatory response; release of ions from the NPs surface. A 'Trojan-horse' effect may also explain the genotoxic effects of AgNPs, where their uptake would be followed by a release of silver ions. The extent of silver ion release from the nanosilver however depends on the type of AgNP. Some studies show that silver ion release does not significantly impact the genotoxicity of AgNPs (Huk *et al.*, 2015, Li *et al.*, 2017) but rather the surface properties of AgNPs and coating are important. Although it is likely that the genotoxicity associated with AgNPs toxicity occurs either directly, or indirectly via oxidative stress, AgNPs also have high affinity for thiol groups, which are important for protein folding and for function as ROS (reactive oxygen species) scavengers (Chen *et al.*, 2020). As currently many different AgNPs have been tested for genotoxicity under highly variable test settings and conditions it is not possible to group AgNPs with respect to genotoxicity. Rather, each material needs to be evaluated individually.

General toxicity: The SCENIHR, 2014 Opinion states that silver and nanosilver are clearly shown to have toxic potential, although toxicity in general seems to be low in humans. In in-vitro studies, AgNPs have been shown to be cytotoxic and with genotoxic DNA-damaging

capacity. Although Ag uptake and possible persistence in the testes has been observed, histopathology did not reveal specific testicular toxicity. Liver toxicity is indicated by the effect of AgNPs on various liver enzymes. In vivo effects on the immune system were observed both regarding allergy to Ag itself, but also in repeated dose toxicity studies in terms of effects on cytokine production and on non-specific immune responses like natural killer cell activity. SCENIHR (2014) stated that these immune effects warrant further studies to the functionality of the immune system after exposure to AgNPs.

Literature published after the SCENIHR 2014 opinion confirms the persistence in testes after oral administration of nano-silver and indicate effects on Leydig cells, spermatogenesis, sperm quality as well as histopathological changes in testes. However, male fertility was not affected (Ema *et al.*, 2017). In addition, the review paper by Ema *et al.* (2017) indicated that maternal oral exposure might lead to apoptosis and neuronal degeneration in the brain of the offspring via oxidative stress and that nano-silver might affect embryonic/fetal survival and growth. However, such effects were reported to have not led to adversity in regard to morphological development of the offspring.

A further study focussed on kidney effects after repeated (60 d) oral administration of nano-silver to female Wistar rats. Nano-silver treatment led to a decrease in kidney weights, some loss of renal functions and ultrastructural changes in the kidneys (Tiwari *et al.*, 2017).

Dabrowska-Bouta *et al.* (2018) have reported that both nano-silver and ionic silver induce morphological disturbances in myelin ultrastructure and alter the expression of myelin-specific proteins, suggesting that the CNS may be a target of low-level toxicity of nano-silver. There are other reports that nano-silver might alter gut microbiota (Dahiya *et al.*, 2018), and that nano-silver might damage epithelial cell microvilli and intestinal glands (Duran *et al.*, 2020).

Bianco *et al.* (2015) investigated the skin penetration of Ag nanoparticles using intact skin. The Ag nanoparticles were derived from soaking three different textiles in a synthetic sweat solution in the donor fluid of the Franz diffusion cell for 24h. The resulting aggregates consisted of silver and silver chloride, indicating that the silver was released from the textiles mostly in ionic form. Released Ag concentrations in the soaking solutions (i.e. exposure concentration) ranged from 0.7 to 4.7 µg/mL (0.6–4.0 µg/cm2), fitting the bactericidal range. Silver and silver chloride aggregates at sizes of up to 1 µm were identified both in the epidermis and dermis. The large size of these particles suggests that the aggregation had occurred in the skin.

Another study by the same group with the same experimental set up confirmed that silver percutaneous absorption occurs after exposure to polyvinylpyrrolidone coated silver (~19 nm) in three human skin graft samples (fresh, glycosylated and cryopreserved skin) (Bianco *et al.*, 2014). The silver particles aggregated significantly in the artificial sweat, but silver content was detected in the receptor fluid. After 24 h, the silver penetration was 0.2 ng/cm2,h for fresh skin, 0.3 ng/cm2,h for cryopreserved skin, and 3.8 ng/cm2,h for glycerolized skin. Since there were no differences between fresh and cryopreserved skin, silver permeation through the skin could be through passive diffusion rather than active uptake.

EXPOSURE ASPECTS

4. The frequency of use of the products containing colloidal silver (nano) can be relatively high as it is in widespread use as antimicrobial agent in a variety of consumer products (clothing, food container, refrigerators, environmental exposure, cosmetics, etc)

5. The material poses the likelihood of systemic exposure of the consumer through the use of final products:

Oral:

'bioavailability of silver after oral administration of AgNPs was shown in one rat study; it was suggested that 1-4 % of the oral dose of silver was taken up systemically.' (SCENIHR, 2014).

Dermal:

Experimental data on intact and damaged skin in vitro using the Franz diffusion method has shown that silver nanoparticle absorption was very low but detectable (Larese *et al.*, 2009). The experiment was performed with full thickness human skin obtained as surgical waste using electro-thermal AAS for Ag determination. Silver nanoparticles were observed by TEM in the stratum corneum of the skin (SCHENIHR, 2014). The absorption of silver through damaged skin has been reported as a result of application as an antimicrobial agent in wound dressings (Trop *et al.* 2006, Vlachou *et al.* 2007, Larese *et al.* 2009).

George *et al.* (2014) studied dermal application of Acticoat® dressings with silver crystal particles (10-40 nm) to 16 patients for 4-6 days. Skin samples were obtained from 8 patients, serum samples obtained from all samples. The results showed staining throughout the superficial stratum corneum, and in 25% of the samples, staining of deeper layers of the epidermis. Ag nanoparticle could penetrate as deep as the reticular dermis. In skin, Ag most probably reacts with tissue components or precipitates. There may also be diffusion of Ag+ ions and secondary aggregation in the dermis. However, there was no increase in serum silver levels after application of the dressings containing silver crystal particles with a size of 10-40 nm.

Tak *et al.*, 2015 used a stable colloidal dispersion of rod-, spherical- and triangle shaped Ag nanoparticles to study skin penetration in vivo in hairless mice as well as in vitro in the skin from hairless mice. The results showed that, amongst the tested materials, the in vitro and in vivo penetration was the highest for rod shaped nanoparticles. After in vivo dermal application the presence of silver could be detected in blood by ICP-MS and the amount of silver detected was dependent on particle shape.

Kraeling *et al.* (2018) investigated skin penetration of commercially available 20 nm silver nanoparticles with three different coatings from an aqueous solution or simple cosmetic oil-in-water (O/W) emulsion formulation at two consumer relevant dosing concentrations. Skin penetration studies were conducted for 24 h in viable weanling pig skin, and excised human cadaver skin using an in vitro flow through diffusion cell system. The three surface coatings were chosen for their electrical charges: citrate (CIT, negative; 19.9 ± 2.4 nm, median particle size distribution of 21 nm), polyethylene glycol (PEG, neutral; 22.87 ± 2.8 nm, median particle size distribution of 24 nm), and branched polyethyleneimine (bPEI, positive; 21.5 ± 2.12 nm, median particle size distribution of 21 nm; 22.3 ± 3.5 nm, median particle size distribution of 22.5 nm). Human full thickness skin from 3 caucasian female donors, age 28-75 years was used. After application the procedure used tape stripping, separation of epidermis and dermis, and analysis of fractions by ICP-MS. The results indicated penetration of very low amounts into viable epidermis. It was however not determined whether the amounts referred to were Ag nanoparticles or silver ions.

6. As noted by SCENIHR (2014), the bioavailability of silver after oral administration of Ag nanoparticles has been shown in one rat study, which suggested that 1-4% of the oral dose of silver may be taken up systemically. The main target organs for Ag nanoparticle distribution after systemic availability were the spleen, liver and kidney while there was less distribution to other organs. Also in the testes, high levels of silver were sometimes noted. Recent studies have indicated that some persistence of Ag may occur in the brain and testes (SCENIHR, 2014; Ema *et al.*, 2017), although it is not clear whether the silver was present in the brain tissue or limited to the endothelium of the brain. There is also some evidence that ionic Ag may also form silver structures at the nanoscale in vivo. Presence of Ag in faeces after intravenous and subcutaneous administrations indicates biliary excretion of Ag originating from parentally administered Ag nanoparticles.

Although most toxicokinetic studies have used chemical analyses to detect silver in different organs, without establishing its ionic or particulate nature, there is evidence to suggest that systemically available nano-silver could be distributed to, and might accumulate in, kidneys, liver, spleen, brain, lungs, and testes, and persist in some organs for several weeks (Mercier-Bonin *et al.*, 2018). A gender-specific difference in nano-silver accumulation has been observed in a 90-day oral exposure study with ~60 nm nano-silver, where it was found that female Fischer 344 rats accumulated twice the amount of silver in their kidneys as male rats (reported in Cameron *et al.*, 2018).

It appears from these studies that, compared to conventional silver compounds, AgNPs release Ag+ ions slowly, and may thus act as a reservoir releasing silver ions inside the body over long periods if taken up and transported to distant tissues (e.g. brain, testes).

CONCLUSION

With a collective consideration of the physicochemical, toxicological and exposure aspects noted above, the SCCS is of the view that there is a basis for concern that the use of colloidal silver (nano), as notified through CPNP for use in cosmetic products, can pose a health risk to the consumer. The SCCS will be ready to assess any evidence provided to support safe use of the material in cosmetic products

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ANNEX 3: SAFETY CONCERNS ON NANOMATERIALS – STYRENE/ACRYLATES COPOLYMER (NANO)

The SCCS has previously evaluated the safety of styrene/acrylate copolymer (nano) intended for use in leave-on cosmetics products up to a concentration of 0.06% (SCCS/1595/18). The material was notified as a nanomaterial in the form of nano beads that contained different encapsulated substances (e.g. methylsilanol mannuronate and dimethylsilanol hyaluronate), meant to be antistatic, humectant, moisturising and skin conditioning.

The SCCS has found that the published literature is scarce on the safety aspects of nano-scale styrene/acrylates as such or when used as a carrier for other (bioactive) substances. The SCCS therefore considered other relevant information on micro/nanoplastics as such and when used for encapsulating other substances.

On the basis of evaluation of the available information, the SCCS has concluded that the use of nano beads made of styrene/acrylate copolymer, containing other encapsulated substances for use in cosmetic products, constitutes a concern for consumer safety on the basis of the following:

PHYSICOCHEMICAL ASPECTS

1. The styrene/acrylate copolymer (nano beads) containing other substances is comprised of primary particles that are in the nano-scale (20-160 nm) (SCCS/1595/18).

2. The styrene/acrylate co-polymer is composed of non-dissolving particles in the nanoscale, with the reported solubility of less than 0.01 mg/L and no further dissolution in aqueous media (SCCS/1595/18).

3. Due to the insoluble polymeric nature, styrene/acrylate co-polymer bears similarities with other micro/nano plastics that are generally insoluble, non-degradable and persistent in nature (Ganesh Kumar *et al.*, 2020). The SCCS has therefore also looked into the available data on physiochemical and toxicological aspects of other micro/nano plastics for possible use in the safety assessment of styrene/acrylate co-polymer.

TOXICOLOGICAL ASPECTS

4. As detailed below, micro/nano plastics (including styrene/acrylate copolymer) have been reported for potential toxicological hazards:

Genotoxicity:

Polystyrene nanoparticles (100 nm) have been shown to induce DNA damage in the cytokinesis-block micronucleus (CBMN) assay in vitro in human fibroblast cells (Poma *et al.*, 2019). The presence of protein corona on the surface of polystyrene nanoparticles (~100 nm) has been reported to increase DNA damage in lymphocytes in a Comet assay (Gopinath *et al.*, 2019). However, negative results have also been reported from micronucleus assay of polystyrene nano- (47-64 nm) and micro- (565-597 nm) particles in CHO-K1 cells (Hesler *et al.*, 2019).

General Toxicity:

Most concerns regarding nanoplastics are related to their persistence and effects on the environment (Ng *et al.*, 2018, Alimba and Faggio 2019, Stapleton 2019, Yong *et al.* 2020,

Ganesh Kumar *et al.*, 2020). More recently concerns for mammalian and human toxicity have gained more attention, although data are generally scarce (reviewed in Lehner *et al.*, 2019, Chang *et al.*, 2020, Stapleton 2019, Yong *et al.*, 2020, Allan *et al.* 2020). The possible toxic effects of plastic particles have been attributed to the potential toxicity of plastics themselves, and their combined toxicity with leachable additives and adsorbed contaminants (Chang *et al.*, 2020).

In an in vitro study, polystyrene particles were not acutely toxic for a coculture of Caco-2 and HT29-MTX-E12 or BeWo b30 cells, and did not cross intestinal and placental barriers, but both the polystyrene nano- (47-64 nm) and micro- (565-597 nm) particles showed cellular uptake and intracellular accumulation (Hesler *et al.*, 2019). In the same studies, cytotoxicity of polystyrene microparticles was observed at doses above 25 µg/mL for NIH/3T3 and murine embryonic stem cells, and myocard cell differentiation in embryonic stem cells was hampered after exposure to doses at 1 µg/mL. The microparticles were found to be more toxic than the nanoparticles, both in terms of cytotoxicity and embryotoxicity (nanoparticles IC50 >100 µg/mL, microparticles IC50 >12.6 µg/mL), although both were indicated as weakly toxic.

Considerable cytotoxicity and hemolysis was observed for polystyrene nanoplastics (particle size ~100 nm) at an exposure dose of 10 µg/mL that was dramatically increased after protein corona formation on the particle surface (Gopinath *et al.*, 2019).

5. Toxicity data on the two substances assessed in SCCS/1595/18 (methylsilanol mannuronate and dimethylsilanol hyaluronate) are not available. However, silanols consist of compounds of variable complexity in which a silanol group ((≡Si-OH; =Si (OH)2) has been incorporated in the chemical structure. Silanols are present as chemical functionalities on the surface of silica particles determining the hydrophilicity of silica nanoparticles (Napierska *et al.* 2010). Long chain silanol terminated compounds were found to be more toxic than short chain silanol terminated compounds for corneal toxicity (Green *et al.* 1992).

EXPOSURE ASPECTS

6. The purpose of the use of styrene/acrylate co-polymer nano beads loaded with other compounds is stated to offer slow release of the compounds at cutaneous level with controlled diffusion. The SCCS considers it a test case for the novel way of using a substance at the nano-scale in cosmetics products. This type of application can potentially open up the opportunity for the use of numerous other (bioactive) substances in a large number of applications resulting in a wider exposure of the consumers to nano-encapsulated materials, the safety of which has not yet been assessed.

OTHER ASPECTS

7. Although the information on the substances encapsulated in styrene/acrylate co-polymer nano beads is virtually non-existent, it can be envisaged that encapsulation of a substance in a nano-sized carrier, made of a hydrophobic plastic, may alter its properties and biokinetic behaviour that may further alter its toxicological effects, compared to the same substance in non-encapsulated form. Because of the potential of such a nano-carrier to deliver substances deeper into the skin or other systemic organs, this type of application may be used for encapsulating a multitude of other substances for a variety of cosmetic applications. It is however important to note that, even if safety of a polymer and the encapsulated substance can be shown individually, this cannot be taken as an evidence for the safety of the two when put together in the form of a nano-scale entity. In this context, the SCCS is of the view that, in the absence of sufficient data to demonstrate the safety of compounds nano-encapsulated in the polymer matrix, such an application constitutes a concern for the safety of the consumer.

CONCLUSION

With a collective consideration of the physicochemical, toxicological and exposure aspects noted above, the SCCS is of the view that there is a basis for concern that the use of nano beads of styrene/acrylate copolymer encapsulating other substances, as notified through CPNP for use in cosmetic products, can pose a health risk to the consumer. The SCCS will be ready to assess any evidence provided to support safe use of the material in cosmetic products.

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ANNEX 4: SAFETY CONCERNS ON NANOMATERIALS – SILICA, HYDRATED SILICA, AND SILICA SURFACE MODIFIED WITH ALKYL SILYLATES (NANO)

In 2015, the SCCS evaluated the safety of synthetic amorphous silica (SAS) materials intended for use in cosmetic products (SCCS/1545/15, Revision of 29 September 2015). The Opinion considered the available evidence to be insufficient to allow drawing a conclusion on the safety of any of the SAS materials evaluated (i.e. silica, hydrated silica, and silica surface modified with alkyl silylates).

In 2019, the SCCS evaluated the solubility aspects of SAS materials intended for use in cosmetic products (SCCS/1606/19). The Opinion concluded that none of the SAS materials (hydrophilic or hydrophobic) could be regarded as soluble to merit exclusion from the definition of nanomaterial as provided in Cosmetic Regulation.

Although the SAS materials are amorphous and largely comprise of aggregated particles, they are composed of primary nanoparticles of very small dimensions (as low as 10 nm). They also contain a fraction of small sized aggregates and potentially free particles that are below 100 nm in size. In view of this, the SCCS considers it relevant to look into the potential toxicological effects of nanoparticles (in addition to the data on SAS materials) to identify the risk potential of the nano-scale fraction of the SAS materials.

In consideration of all the relevant information provided in safety dossiers, and from published literature, the SCCS is of the view that the use of SAS materials in cosmetic products constitutes a concern for consumer safety on the basis of the following:

PHYSICOCHEMICAL ASPECTS

1. SAS materials are comprised of primary particles that are in the nano-scale, ranging between 10 and 50 nm in size (SCCS/1545/15; SCCS/1606/19). Depending on the manufacturing process, nanoparticles in the SAS materials may exist in the form of larger sized agglomerates and aggregates, but also as free particles as well as agglomerates and aggregates that are within the nano-scale (i.e. 1-100 nm) (Fruijtier-Polloth, 2012; Fruijtier-Polloth, 2016).

2. The solubility of hydrophilic SAS materials in water is reported to range from 22 mg/L to 225 mg/L, and that of hydrophobic SAS materials from 0.4 up to 180 mg/L. According to the definitions of solubility terms provided in the USP 38/USP 38–NF33 and the European Pharmacopeia, these materials can only be regarded as being very slightly soluble and insoluble, respectively (SCCS/1606/19).

3. Although no data were provided for the previous SCCS evaluations, the physicochemical nature of the SAS materials suggest that they are likely to be persistent in biological environments. This is underlined by the conclusions of a nano-specific risk assessment, which highlighted SAS as a biopersistent material prone to accumulation in tissues upon long-term exposure with daily consumption (Van Kesteren *et al.*, 2015).

5. The SAS materials are produced by different processes and surface treatments, and may exist in hydrophilic, hydrophobic or colloidal form - each with a different surface characteristics (SCCS/1545/15; SCCS/1606/19). The physicochemical properties and biokinetic behaviour of these different SAS materials is likely be differ depending on the type of surface characteristics.

6. The SAS materials could potentially adsorb other chemical moieties that have an affinity towards hydroxyl groups on the surface of SAS particles. Therefore, formulation of SAS materials with other chemical and biochemical moieties may further modulate their toxicokinetics, or this may lead to unexpected effects due to nano-scale delivery of other substances.

TOXICOLOGICAL ASPECTS

7. The chemical and insoluble particulate nature of SAS nanoparticles suggests a potential for toxicological hazard, as detailed below:

In vitro toxicity:

In general, aggregation of primary nanoparticles can be expected to reduce the chances of systemic toxic effects of a nano-structured material. However, a review of the published studies has indicated that all types of SAS nanoparticles (SAS NPs) can induce cytotoxicity (Murugadoss *et al.*, 2017), and that cytotoxicity of the aggregates of >100 nm size is not always less than that of the nano-sized counterparts (Murugadoss *et al.*, 2020). The in vitro toxic effects of SAS NPs have been reported in several cell types lines to be through the induction of oxidative stress and/or pro-inflammatory responses and mediation of apoptosis, mainly via the intrinsic or mitochondrial pathway (caspase-dependent pathway) in a size- and dose-dependent manner.

Nanoparticle mediated production of reactive oxygen species (ROS) is believed to be an important mechanism of toxicity, including the nano forms of silica. Cytotoxicity and genotoxicity induced by Stöber-manufactured and colloidal SAS NPs have been strongly correlated with the induction of oxidative stress. Precipitated SAS NPs have also been associated with cytotoxicity due to oxidative stress but not with genotoxicity. Interestingly, pyrogenic SAS NPs have been shown to cause cytotoxicity, mostly without involving oxidative stress (Murugadoss *et al.*, 2017). In contrast, other studies have shown that pyrogenic SAS NPs are biologically more reactive than colloidal SAS NPs (Zhang *et al.*, 2012) and precipitated SAS NPs (Di Cristo *et al.*, 2016) of the same composition and size.

Genotoxicity:

An overview on the genotoxicity of SAS materials has been given in SCCS/1545/15 (section 3.3.6.3), leading to the conclusion that 'There is evidence for in vitro and in vivo genotoxicity of SAS nanomaterials in the open literature as demonstrated by several studies in terms of positive Comet and micronucleus assays. It has also been noted by the SCCS that the particles used in most of these studies were probably different from those intended for use in cosmetic products. Nevertheless, these studies indicate the potential mutagenic/genotoxic effects of SAS materials if there is an internal exposure.'

Genotoxicity of amorphous silica nanoparticles has recently been reviewed by Yazdimamaghani *et al.* (2019). The authors analysed 106 publications describing experimental studies on SAS NPs genotoxicity. Although there were negative and inconsistent reports on genotoxicity, a number of studies showed that exposure to SAS NPs could lead to genotoxicity through direct or indirect mechanisms.

Immunotoxicity:

Chen *et al.* (2018) reviewed in vitro and in vivo studies on the effects of silica nanoparticles to the immune system. Proinflammatory responses, ROS formation and autophagy were considered as the main mechanisms for the immunotoxicity of SAS NPs, which can also induce autophagy even at subtoxic levels (Kretowski *et al.*, 2017, Wang *et al.*, 2017).

A recent review by Sharma and Jha (2020) has summarised the possible toxic mechanisms of SAS NPs on the cellular and biochemical processes as well as on the innate immune responses, inflammation, and immune related dysfunctions.

In vivo toxicity:

Based on the available literature, and unpublished studies reviewed by OECD (2016) and ECHA (2019), there are no indications for an association between dermal exposure and adverse effects of amorphous or crystalline form of silica either in humans or animals (ATSDR, 2019). The same ATSDR review also reported that no adverse effects were associated with oral amorphous silica exposures ranging from acute to chronic duration. However, other recent publications have indicated systemic toxicity (mainly liver fibrosis or vacuolisation of tubular epithelial cells in kidney) after repeated oral exposures to pyrogenic silica (Zande *et al.*, 2014; Tassinari *et al.*, 2020) and precipitated SAS (Boudard *et al.* 2019, 2020).

EXPOSURE ASPECTS

8. Amorphous silica (as well as crystalline forms) is found in many commercial products (e.g., bricks, mortar, plaster, caulk, granite and engineered stone kitchen counter tops, roofing granules, wallboard, concrete cleansers, art clays and glazes, talcum powder) (NTP 2009, SCCS, 2015). SAS materials are used in a wide range of consumer and industrial applications. The frequency of use of the products containing SAS materials can also be relatively high. The general population is therefore exposed to silica (crystalline and amorphous) through air, indoor dust, food, water, soil, and various consumer products (ATSDR, 2019).

9. SAS is an authorised food additive (E551) in 22 categories of food and food supplements (in solid or liquid form), as well as in a number of food-grade components (additives, enzymes, flavorings, nutrient sources) at levels ranging from 2000 to 30,000 mg/kg or quantum satis (Younes *et al.*, 2018). Exposure of the general public to silica is also expected to occur through the diet. In addition to use as a food additive, E551 is also used in cosmetics (notably as an abrasion additive in toothpastes), in pharmaceuticals (as a free-flow additive, carrier, retardant agent and tableting aid) (Fruijtier-Polloth, 2016), and in food packaging. Typical cosmetic uses of SAS materials are in leave-on skin products (skin care and make-up), rinse-off skin products, as well as hair and lip products (SCCS/1545/15).

10. The widespread use of SAS materials poses the likelihood of consumer exposure via food and use of consumer products through different routes:

Dermal Uptake:

The dermal uptake of SAS materials has been discussed in the SCCS Opinion (SCCS/1545/15). A number of studies in the published literature have indicated the possibility of penetration of amorphous silica particles through skin after repeated applications (Nabeshi *et al.*, 2011; Hirai, *et al.*, 2012) – especially when skin barrier is damaged (Rancan *et al.*, 2012). One study (Boonen *et al.*, 2011) has indicated the possible skin penetration of even larger (micron) sized silica particles when applied in ethanolic formulations. Therefore, where SAS materials are intended for use in ethanolic formulations for cosmetic applications, the penetration potential of the nanoparticles should also be assessed in ethanolic media.

The SCCS noted in the Opinion (SCCS/1545/15) that the particles used in many of the published studies were different from those intended for use in cosmetic products; for example, some were labelled with fluorescent dyes that might have changed their properties/behaviour. A review by Nafisi *et al.* (2014) has also highlighted the need for more, properly designed, studies on the dermal penetration of silica nanoparticles. The situation with the use of such products on flexed, cut, compromised and diseased skin also remains to be clarified in this context. Having considered all the aspects, the SCCS concluded in SCCS/1545/15 that the evidence for the lack of skin penetration of silica nanoparticles/clusters was insufficient and inconclusive and there was a need for further evidence from more properly designed studies.

Oral uptake:

Oral toxicokinetic studies in rat reported in OECD (2016) have pointed to a very low absorption of silica from the gastrointestinal tract as indicated by the slightly increased levels in liver, spleen and kidneys. Two other more recent in vivo studies, focusing on longer term exposure (3–18 months) at doses in the expected range of dietary intake, have reported adverse effects in the liver, kidney and thyroid (Boudard *et al.*, 2019); Boudard *et al.*, 2020, Tassinari *et al.*, 2020), indicating systemic exposure. Furthermore, systemic availability of particulate SiO_2 has recently been reported from post-mortem tissue samples from 15 deceased persons (Peters *et al.*, 2020). All tissue samples investigated (liver, spleen, kidney and the intestinal tissues - jejunum and ileum) contained particles consisting of SiO_2 (and silicates) as confirmed by electron microscopy analysis. The SiO_2 particle mass concentrations in the tissues ranged from 0.2 to 25 mg Si/kg tissue with an average of 1.2 ± 3.1 mg Si/kg tissue, with a particle size ranging between 150–850 nm.

Influence of Coating:

Some SAS materials used in cosmetic products are also surface-treated to confer hydrophobic properties. Examples include silica dimethyl silylate, silica silylate, silica dimethicone silylate, silica caprylyl silylate and silica cetyl silylate (SCCS, 2019). The hydrophobic surface treatments have been found to strongly decrease solubility of the materials, and consequently increase the likelihood of greater persistence of the SAS materials (Hardy *et al.*, 2018; SCCS, 2019). In addition, such surface modifications can also affect ADME (absorption, distribution, metabolism, and excretion) behaviour of the particulate materials – especially of the nano-scale particles (Hardy *et al.*, 2018).

CONCLUSION

With a collective consideration of the physicochemical, toxicological and exposure aspects noted above, the SCCS is of the view that there is a basis for concern that the use of SAS materials, as notified through CPNP for use in cosmetic products, can pose a health risk to the consumer. The SCCS will be ready to assess any evidence provided to support safe use of the material in cosmetic products.

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