Scientific Committee on Consumer Safety

SCCS

OPINION ON

Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form)

The SCCS adopted this opinion by written procedure

on 20 March 2015

Revision of 29 September 2015

About the Scientific Committees

Three 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.
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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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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, Silica, Hydrated Silica, and Silica Surface Modified with Alkyl Silylates (nano form), Regulation 1223/2009, CAS 7631-86-9 and 112926-00-8, 7631-86-9 and 112945-52-5, 68611-44-9 and 68909-20-6, 7631-86-9 and 112926-00-8, EC: 231-545-4, 271-893-4, and 272-697-1, 231-545-4.

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1. BACKGROUND

Article 2(1)(k) of Regulation (EC) No 1223/2009 establishes that "nanomaterial" means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.

That definition covers only materials in the nano-scale that are intentionally made and are insoluble/partially-soluble or biopersistent (e.g. metals, metal oxides, carbon materials, etc), and it does not cover those that are soluble or degradable/non-persistent in biological systems (e.g. liposomes, emulsions, etc). Article 16 of the Cosmetics Regulation requires any cosmetic product containing nanomaterials to be notified to the Commission six months prior to being placed on the market, and Article 19 requires nano-scale ingredients to be labelled (name of the ingredient, followed by 'nano' in brackets). If there are concerns over the safety of a nanomaterial, the Commission shall refer it to the Scientific Committee on Consumer Safety (SCCS) for a full risk assessment.

The Commission received 172 notifications as reported in the attached list of cosmetic products containing the following nanomaterials: 67 notifications for Silica (nano) CAS n.l 12945-52-5; 26 notifications for the Hydrated Silica (nano) CAS n. 112926-00-8; 12 notifications for Silica Silylate (nano) CAS n. 68909-20-6; 67 notifications for Silica Dimethyl silylate (nano) CAS n. 68611-44-9. These ingredients are not regulated in Cosmetic Regulation (EC) No 1223/2009, but they are reported in the Cosing database with several cosmetic functions. According to the Applicant the ingredients are used in nano form in leave-on and rinse-off cosmetics products, including hair, skin, lip, face, and nail products, with different concentrations and specifications as reported in the attached list.

The Commission has concerns about the use of silica in nano form because of the potential high exposure in many types of products and because concerns have been raised regarding the potential for nanoparticles of silica to break out of the agglomerates and enter cells.

Therefore, we would like to request the SCCS to provide a safety assessment of the four types of nano silica covered in the notifications listed in the annex to this mandate, in the above-mentioned categories of products, taking into account the reasonably foreseeable exposure conditions.

2. TERMS OF REFERENCE

1. In view of the above, and taking into account the scientific data provided, the SCCS is requested to give its opinion on the safety of the nanomaterials Silica, Hydrated Silica, Silica Silylate and Silica Dimethyl Silylate for use in leave-on and rinse-off cosmetics products, including hair, skin, lip, face, and nail products, taking into account the reasonably foreseeable exposure conditions.

2. *In the event the SCCS finds that the safety assessment for one or more of the nanomaterials covered by this mandate should be provided in a separate opinion, the SCCS is asked to justify its decision.*

3. The SCCS is requested to address any further scientific concerns with regard to the use of Silica, Hydrated Silica, Silica Silylate and Silica Dimethyl silylate in nano form in cosmetic products.

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

Silicon dioxide (IUPAC)

3.1.1.1 Primary name and/or INCI name

INCI name Silica
CAS No 7631-86-9; 112945-52-5

INCI name Hydrated silica
CAS No 7631-86-9; 112926-00-8

INCI name Silica dimethyl silylate
CAS No 68611-44-9

INCI name Silica silylate
CAS No 68909-20-6

3.1.1.2 Chemical names

Silica, Amorphous silica, Silicic oxide, Silicon(IV) oxide

3.1.1.3 Trade names and abbreviations

Trade names of 28 different synthetic amorphous silica (SAS) materials have been provided in the dossier. Out of these, the SCCS has considered physicochemical data on 23 materials relevant for this opinion. These have been listed in Table-1. One material (NoSAS-1) has been described as micro titanium dioxide, which is not relevant to this assessment. Another material (Py-SAS-A-9) is stated to be similar to 3 other materials, but the physicochemical data provided comprise extrapolated values, which are considered inadequate by the SCCS (see Table-1 footnotes for explanation).

3.1.1.4 CAS / EC number

The following CAS and EC numbers have been quoted for different types of SAS materials (see Table 1):

Hydrophilic precipitated silica
CAS: 7631-86-9 and 112926-00-8
EC: 231-545-4

Hydrophilic pyrogenic silica
CAS: 7631-86-9 and 112945-52-5
EC: 231-545-4

Hydrophobic pyrogenic silica
CAS: 68611-44-9 and 68909-20-6
EC: 271-893-4, and 272-697-1

Colloidal silica

CAS: 7631-86-9 and 112926-00-8
EC: 231-545-4

3.1.1.5 Structural formula

$(O=Si=O)_n$

3.1.1.6 Empirical formula

SiO_2

3.1.1.7 Coating

The SAS materials have been reported to uncoated. Hydrophobic pyrogenic materials have been surface treated with either dichlorodimethylsilane or hexamethyldisilazane to produce different alkyl silylate moieties on the particle surface.

3.1.2 Physical form

Different SAS materials have been described as white powder or fluffy white powder for amorphous materials, or as bluish white dispersion for colloidal particles. All materials included in the dossier have been claimed to be amorphous in nature. For the majority of the materials, no crystalline phase was detected by X-ray diffraction (XRD). No XRD measurements were carried out for 2 materials (C-SAS-1; C-SAS-2), but they were also expected by the Applicant to be in an amorphous state.

3.1.3 Molecular weight

Molecular weight of SiO_2: 60.08 g/mol

3.1.4 Purity, composition and substance codes

See Table 1.

3.1.5 Impurities / accompanying contaminants

See Table 1.

3.1.6 Solubility

See Table 1.

3.1.7 Partition coefficient (Log P_{ow})

See Table 1.

3.1.8 Additional physical and chemical specifications

Melting point:	1600 °C (for pure, crystalline SiO2)
Boiling point:	2230 °C
Flash point:	NA
Vapour pressure:	infinitely small at ambient conditions
Density:	See Table 1.
Viscosity:	See Table 1.

pKa: NA
Refractive index: 1.45
pH: See Table 1.
UV_Vis spectrum (Optical density of 0.05% w/v suspension in water): See Table 1.

3.1.9 Homogeneity and Stability

The powder form of the materials is stable.

SCCS comment
SiO_2 as an oxide is stable in air but can be reactive in some environments. Depending on the manufacturing and composition details, the surface termination may comprise SiOH (Silanol) groups. This termination renders the surface hygroscopic. If water is abundant, as is typical at ambient conditions or in many cosmetic formulations, multiple layers of surface water will remain present. In the presence of organosilicon compounds, for example alkyl silylates, a hydrophobic termination layer on SiO_2 prevails.

Table 1: Summary of physicochemical data provided in the submission

Material Type	Hydrophilic precipitated silica*	Hydrophilic pyrogenic silica**	Hydrophobic pyrogenic silica	Colloidal silica
Materials for which physicochemical data have been provided	• P-SAS-A-1 (batch 4747) • P-SAS-A-2 (batch 0111) • P-SAS-A-3 (batch 1007257085) • P-SAS-A-4 (batch 110428-01) • P-SAS-A-5 (batch ETWLOT107058) • P-SAS-A-6 (batch 130912)	• Py-SAS-A-1 (batch 09/08/2010) • Py-SAS-A-2 (batch 152091313)*** • PY-SAS-A-3 (batch 098D30712) • Py-SAS-A-4 (batch 1011B1073) • Py-SAS-A-5 (batch 09/08/2010) • Py-SAS-A-6 (batch 113E30751) • PY-SAS-A-7 (batch VK76195) • Py-SAS-A-8 (batch 3419861)	• Py-SAS-NA-1 (batch 3150032922) • Py-SAS-NA-2 (batch 31520315221) • Py-SAS-NA-3 (batch 30777) • Py-SAS-NA-4 (batch 3334688) • Py-SAS-NA-5 (batch 3152120235) • PY-SAS-NA-6 (batch SK33688) • Py-SAS-NA-7 (batch 3152111535)	• C-SAS-2 (batch 40J751378) • C-SAS-1 (batch 40J347511)
CAS Number(s)	Two CAS numbers provided: 7631-86-9 (amorphous silica), and 112926-00-8 (silica gel)	Two CAS numbers provided: 7631-86-9 (amorphous silica), and 112945-52-5 (amorphous fumed silica)	One CAS number provided: 68611-44-9 (silane, dichlorodimethyl-, reaction products with silica)	Two CAS numbers provided: 7631-86-9 (amorphous silica), 112926-00-8 (silica gel)
EC (EINECS) number	231-545-4	231-545-4 601-216-3 (from ECHA)	271-893-4	231-545-4
Physical form	White fluffy powder	White fluffy powder	White fluffy powder	Bluish white dispersion of colloidal particles
Purity	SiO2 content (Post calcination at 950°C) ranges from 85.5% to 98.7%. Water content (loss on drying) varies from 0.7% to 8.5% between materials. Loss on calcination at 950°C varies from 1.3 to 12.0 % (w/w) between materials.	SiO2 content (Post calcination at 950°C) ranges from 83.6% to 98.9%. Water content (loss of drying) varies from 0.09% to 10% between materials. Loss on calcination at 950°C varies from 1.0 to 16.1 % (w/w) between materials.	SiO2 content (Post calcination at 950°C) ranges from 94.3% to 99.7%. Water content (loss of drying) varies from 0.2% to 2.2% between materials. Loss on calcination at 950°C varies from 1.0 to 5.5 % (w/w) between materials.	SiO2 content (Post calcination at 950°C) ranges from 23.2% to 38.8%. Water content (loss of drying) varies from 58.8% to 69.2% between materials. Loss on calcination at 950°C varies from 60.5 to 70.7 %

				(w/w) between materials.
Significant impurities	Some materials have high levels of metal impurities, e.g. aluminium (as high as 4340 ppm) and titanium (up to 240 ppm).	Some materials have high levels of metal impurities, e.g. aluminium (as high as 350 ppm) and titanium (up to 235 ppm).	No significant impurities.	Some materials have high levels of metal impurities, e.g. aluminium (up to 990 ppm).
Solubility in water(mg/l)	Solubility values range between 54.3 and 112.9 (ICP-AES method); and between 31.3 and 48.6 (colorimetric method)	Solubility values range between 22.6 and 91.8 (ICP-AES method); and between 20.9 and 33.3 (colorimetric method)	Solubility values range between 0.4 and 15.7 (ICP-AES method); and between zero and 4.5 (colorimetric method)	Considered not relevant (aqueous dispersion)
Primary particle size	Between 10 and 30 nm	Between 10 and 50 nm	Between 10 and 50 nm	15 to 40 nm
Aggregate/ agglomerate size	Up to 5 µm	Up to 5 µm	Up to 5 µm	Loose agglomerates of primary particles
Bulk density (g/cm^3)	Between 2.0 and 2.7	Between 1.9 and 3.4	Between 2.2 and 2.7	Between 1.2 and 1.3
Volume specific surface area (m^2 cm^{-3})	Between 291 and 902	Between 303 and 1122	Between 272 and 587	NA
UV Absorption (Optical density of 0.05% w/v suspension in water)	OD (λ254nm) between 0.071 and 0.880 OD (λ320nm) between 0.095 and 0.568	OD (λ254nm) between 0.095 and 0.721 OD (λ320nm) between 0.052 and 0.605	OD (λ254nm) between 0.044 and 0.260 OD (λ320nm) between 0.035 and 0.214	OD (λ254nm) around 0.08 OD (λ320nm) around 0.01
Zeta potential (mV)	Between -24 and -56 (not measurable for some materials)	Between -11 and -44 (some suspensions slightly unstable)	Not measurable (hydrophobic silica)	-56
Dynamic viscosity (v, m^2/s)	Around $1x10^{-6}$ (at 20°C) and $0.7x10^{-6}$ (at 40°C).	Around $1.2x10^{-6}$ (at 20°C) and $0.8x10^{-6}$ (at 40°C).	Around $2.2x10^{-6}$ (at 20°C) and $1.5x10^{-6}$ (at 40°C).	Around $1.05x10^{-6}$ (at 20°C) and up to $1.78x10^{-6}$ (at 40°C).
pH value (4% w/w in water)	5.03 to 7.61	4.6 to 6.6	5.7 to 7.4	Around 9.1
Median particle size volume weighted (nm) – DLS method	125 nm to 424 nm	127 to 205 nm	112 to 275 nm	Not measurable
Median particle size number weighted (nm) – DLS method	80 nm to 403 nm	80 to 125 nm	73 to 160 nm	Not measurable
Surface modified, coated or doped	None	None	Surface treated with either dichlorodimethylsilane or hexamethyldisilazane.	No information – except that the materials are not doped
Catalytic/ Photocatalytic activity	No (not measured)	No (not measured)	No (not measured)	No (not measured)
Dustiness	NA	NA	NA	No information provided
P_{ow}	NA	NA	NA	No information provided
Redox potential	Not relevant	Not relevant	Not relevant	No information provided
Stability	Powder form stable	Powder form stable	Powder form stable	No information provided

Mercury content in all of the materials is <0.01 ppm

* Data are also provided for micro titanium dioxide coded "NoSAS-1", but it is not considered a relevant material for this assessment by the SCCS.

** Data are also provided for Py-SAS-A-9, which is stated to be similar for three other materials mentioned in the submission. However, only extrapolated values and not measured data are provided and these have been considered inadequate by the SCCS.

*** Py-SAS-A-2 (batch 152091313) has been described in the analytical dossier under hydrophilic pyrogenic material, whereas the analytical files refer to it as hydrophobic pyrogenic material.

SCCS comments to the data in the table

- It is not clear why the UV absorption at certain wavelength varies by a factor 10.
- It is not clear which of the surface treated materials contain methyl-, dimethyl- or trimethyl- silylate moieties on the surface as a result of the final reaction product.
- The solubility of SiO2 varies across a broad range and depends on the crystal phase, the temperature and pH, among other parameters. SiO2 dissociates into Si(OH)4. The presence of nanoforms as suspensions has to be ruled out when determining the solubility values for SAS materials.

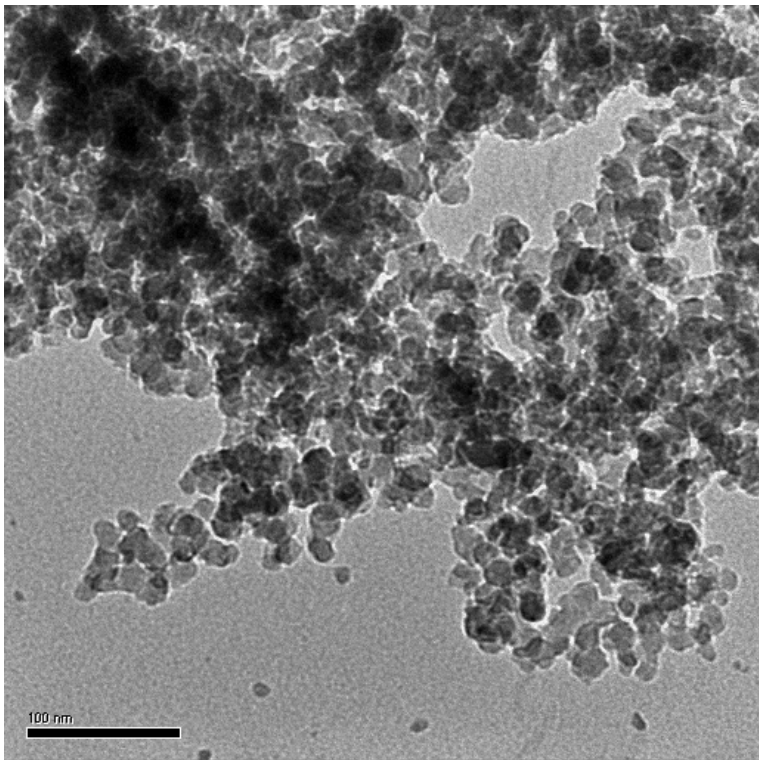

Figure 1: Typical TEM image of a hydrophilic precipitated SAS material

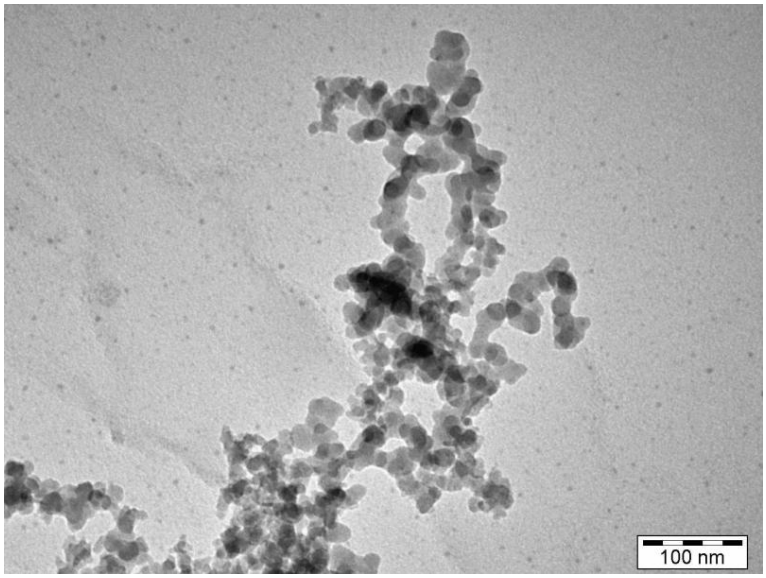

Figure 2: Typical TEM image of a hydrophilic pyrogenic SAS material

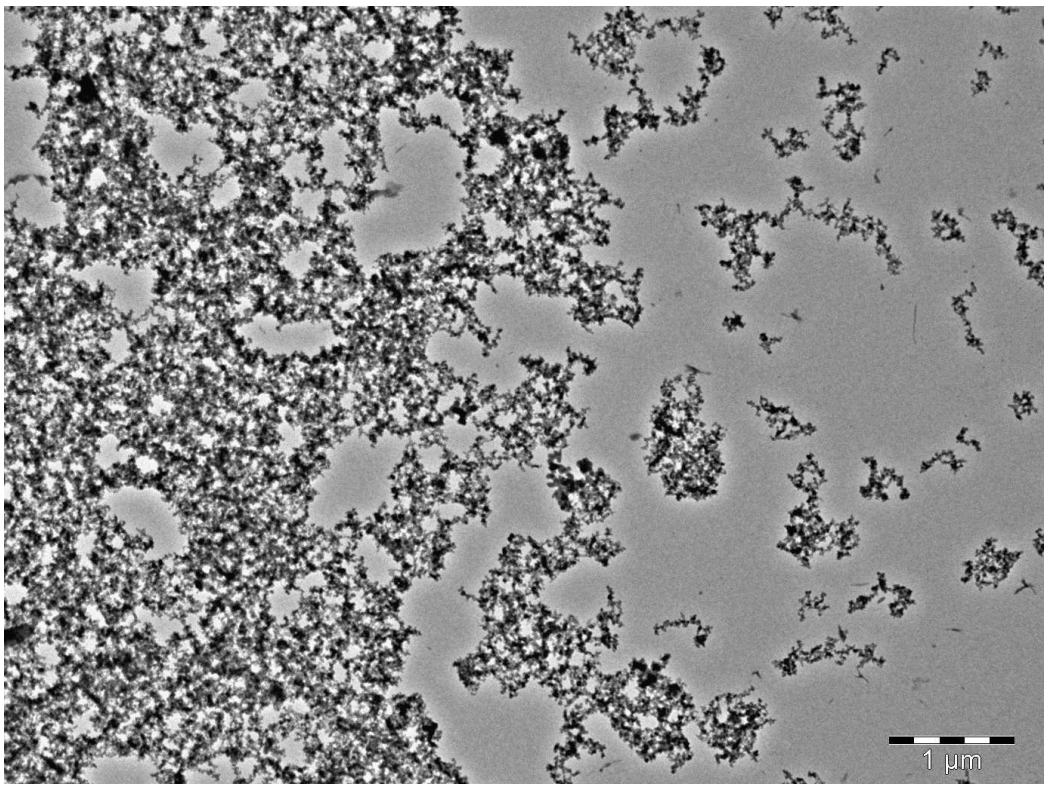

Figure 3: Typical TEM image of a hydrophobic pyrogenic SAS material

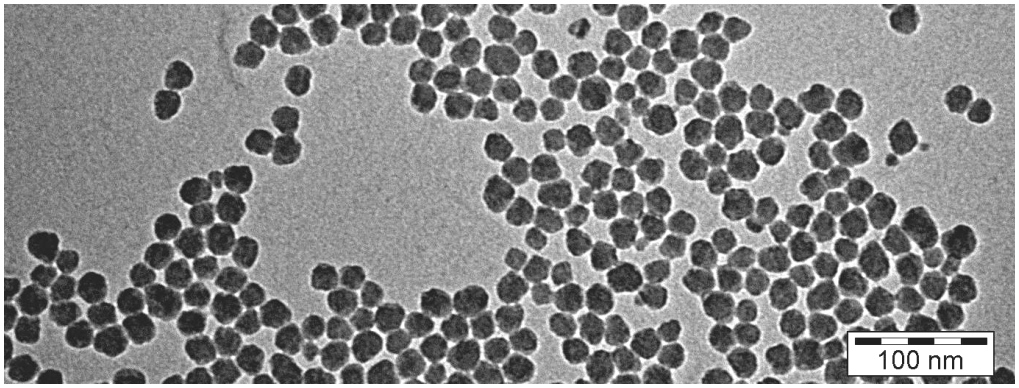

Figure 4: Typical TEM image of a colloidal SAS material

SCCS comments on physicochemical characterisation

The analytical part of the dossier provides characterisation data on the 23 SAS materials listed in Table 1. On the basis of the material types, they could be loosely categorised into the following 4 groups:

- Hydrophilic precipitated silica - 6 materials (one other material listed under this category is not an SAS material)
- Hydrophilic pyrogenic silica – 8 materials (see footnote under Table 1)
- Hydrophobic pyrogenic silica – 7 materials
- Colloidal silica – 2 materials

Within each group, the provided data for different materials show a large variation in the experimental values for volume specific surface area. The reason for this is not clear as there are no apparent corresponding large differences in the primary particle sizes of the materials to explain the anomalies. Similarly, there is a large variation in the solubility of individual materials within each group, and a large variation in the densities of the materials within the hydrophilic pyrogenic group. In the absence of a scientifically valid justification for such large variations, the SCCS has regarded the provided evidence to be insufficient to allow data on one material to be applied to other materials within the same group, or between materials in different groups. This conclusion is in line with the decision on substance evaluation pursuant to article 46(1) for silicon dioxide by ECHA in 2015. In this dossier ECHA did not agree that the sameness of SAS has been demonstrated within the specific registration dossier. They concluded that SAS is a nanomaterial that is manufactured in many types and forms, which may vary largely in physicochemical characteristics.

The SAS materials under consideration are composed of primary particles in the nanometre range, and are stated to exist in larger aggregates and agglomerates. For the SAS materials produced by a non-pyrogenic route (e.g. precipitation), additional data on secondary particle size should be provided to clearly indicate whether the primary particles are in aggregated as well as agglomerated form, or just in agglomerated form, as the latter could de-agglomerate under certain conditions to give off nanoparticles.

For most of the materials, information has been provided on whether they are surface modified, coated/uncoated, or doped. Some of the hydrophobic pyrogenic materials have been described as not having been surface modified, coated or doped, but titles of the individual analytical files indicate that they were in fact surface modified through treatment with either dichlorodimethylsilane or hexamethyldisilazane. In view of this, the SCCS has considered all of the hydrophobic pyrogenic SAS materials as surface treated. However, it is not clear from the description of the materials which ones contain methyl-, dimethyl-, or trimethyl- silylate moieties on the surface as the final reaction product.

Measurement data on catalytic/ photocatalytic activity have not been provided for any of the materials, but they have been regarded generally not to be catalytic/ photocatalytic on the basis of prior knowledge from scientific literature. The SCCS has accepted this view for the materials under consideration on the basis that they are inert and amorphous in nature.

The reported pH of the two colloidal materials (C-SAS-2 and C-SAS-1) in 4% aqueous media is quite high (around 9.0), and the materials are intended for use at even higher concentrations (up to 10%) in the final products. If pH of the materials is not adjusted/neutralised in the formulation, such materials may be corrosive/irritant.

Data on particle size distribution have been provided as median values from measurements by Dynamic Light Scattering (DLS). Particle size measurements have not been provided for the two colloidal materials, and the Applicant has reported erroneous results obtained by DLS. The SCCS has already commented on this method as not being suitable for particle size measurement of some nanomaterials, such as silica. Although the dossier also contains electron microscopy images for some of the materials under consideration, the SCCS recommends measurement of particle size distribution of all the SAS materials by a different analytical method (other than DLS) to ascertain that any small nanoparticles (10 nm and below) have also been accounted for.

Some of the materials (e.g. P-SAS-A-5, P-SAS-A-2, P-SAS-A-3) have a significantly high metal content – especially aluminium and titanium. The SCCS recommends that the aluminium and titanium contents should be reduced in the SAS nanomaterials to minimum levels - in line with many of the other materials under consideration (e.g. Py-SAS-A-1 and Py-SAS-A-2).

3.2 Function and uses

According to the Applicant, 'SAS materials' in micro and/or nanoform, have been commercialised since the 1950s and are currently used in a wide variety of industrial applications. The main use of SAS is as a reinforcement and thickening agent in various systems such as elastomers, resins and inks. SAS materials exhibit a high water absorption capacity due to their high specific surface area. Therefore SAS materials are used as adsorbing agents and in dry powder systems to enhance their flow properties. SAS materials are also used in consumer products including cosmetics, pharmaceuticals and foods (food and feed additives, beer and wine clarification, dental and medical applications).

According to the Applicant, SAS materials in general have a long history of safe use as cosmetic ingredients in a wide range of products. Typical uses of SAS in cosmetic products are in leave-on skin products (skin care and make-up), rinse-off skin products as well as hair and lip products.'
All products are claimed to be on market, except those containing Silica silylate (CAS 68909-20-6). The range of notified SAS concentrations in different product types is so variable that it is not possible to draw a narrow range. For example, the concentration of hydrated (colloidal) silica used in foundation ranges between 0.003%, 0.62%, and 8%. Similarly, the concentration of SAS materials in eye contour products ranges between 0.15%, 1.5%, and 5%. For risk assessment purposes, the SCCS has identified the highest concentration in each product category (shown in descending concentration order in Table 2) as follows:

Table 2: The maximum concentration of SAS material used in each cosmetic product category.

PRODUCT TYPE	Leave on/ Rinse off	Concentration (%)
Products for temporary hair styling	Leave on	38.0
Foundation	Leave on	10.0
Lip stick	Leave on	7.5
Toothpaste	Rinse off	7.0
Other lip make-up products	Leave on	5.3
Nail varnish / Nail make-up	Leave on	5.1
Eye contour products	Leave on	5.0
Face care products other than face mask	Leave on	5.0
Other face make-up products	Leave on	5.0
Other skin care products	Leave on	5.0
Other skin cleansing products	Rinse off	5.0
Other sun and self-tanning products	Leave on	4.3

Sun protection products	Leave on	4.3
Hair bleaching and dye remover products	Rinse off	4.2
Eye liner	Leave on	3.8
Body care products	Leave on	3.5
Face mask	Leave on	3.0
Hair relaxer / straightener products	Rinse off	3.0
Eye shadow	Leave on	2.5
Other make-up product	Leave on	2.5
Concealer	Leave on	2.0
Hand care products	Leave on	2.0
Nail care products	Leave on	2.0
Lip care products	Leave on	1.7
Eye pencil	Leave on	1.4
Oxidative hair colour products	Rinse off	1.2
Make-up remover products	Rinse off	1.0
Mascara	Leave on	1.0
Other eye make-up products	Leave on	1.0
Before and after sun products	Leave on	0.5
Hair conditioner	Rinse off	0.5
Products with antiperspirant activity	Leave on	0.1

3.3 Toxicological Evaluation

An overview on toxicological studies provided is described in the Annex-Table 1.

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline: not stated
Species/strain/sex: Rat, Sprague-Dawley
Group size: 5 / sex / dose for P-SAS-A-7
10 /sex / dose for Py-SAS-NA-2
Test substance: P-SAS-A-7
Py-SAS-NA-2
Batch: not given
Purity: not given
Dose levels: 5040, 6350, or 7900 mg/kg in 50 mL/kg in olive oil for P-SAS-A-7
2500 mg/kg or 5000 mg/kg in 50 mL/kg in peanut oil
Exposure:
GLP statement: not given

Date: report dated 1977

Fasted rats (5/sex for P-SAS-A-7, and 10/sex for Py-SAS-NA-2) were given a single dose of P-SAS-A-7 by oral gavage at 5040, 6350, or 7900 mg/kg in 50 mL/kg olive oil, or Py-SAS-NA-2 at 2500 mg/kg or 5000 mg/kg in 50 mL/kg peanut oil. The highest dose levels used represented the maximum dose level that could be administered. Rats were observed for clinical signs for 4 weeks, and food intake, body weight gain and behaviour were monitored. All animals killed at the end of the observation period were subjected to macroscopic examination.
No deaths occurred and no signs of toxicity were seen during the 4-week observation period. Food intake and body weight gain were not influenced as a result of the administration of P-SAS-A-7 or Py-SAS-NA-2. No macroscopic lesions were observed at necropsy.
No batch number is given.

Conclusion
Under the conditions of this study, the maximum non-lethal doses of P-SAS-A-7 (hydrophobic precipitated SAS) and Py-SAS-NA-2 (hydrophobic pyrogenic SAS) following single oral gavage to rats were above 7900 and 5000 mg/kg, respectively.

SCCS comment
P-SAS-A-7 is not one of the materials included in the submission and should either not be addressed here or justification should be given why results from P-SAS-A-7 are relevant for the materials covered in the submission. Information on material characterisation for Py-SAS-NA-2 batch 31520315221 dates from 2013 and was provided to the SCCS. The toxicological study however was performed in 1977, and no information on the batch of Py-SAS-NA-2 used in the toxicity study is given. Thus, it is not clear whether the material used for the acute toxicity study exhibits the same physicochemical properties as the material used for material characterisation.
The study report was not provided for evaluation.

Ref.: Pr-B (1977) in IUCLID (2003)
Lewinson *et al.* (1994)

Guideline:	not stated
Species/strain/sex:	Rat, Sprague-Dawley
Group size:	10 /sex
Test substance:	Py-SAS-A-1 (hydrophilic pyrogenic SAS)
Batch:	not given
Purity:	not given; SiO_2 content > 98 %
Dose levels:	2000 or 3300 mg/kg
Exposure:	oral, gavage
Vehicle;	1 % methylhydroxyethylcellulose in water
GLP statement:	not given
Date:	report dated 1977

Rats (10/sex) were given a single dose of Py-SAS-A-1 by oral gavage at 2000 or 3300 mg/kg, suspended in 1% methylhydroxyethylcellulose in water. The highest dose represents the maximum achievable dose level. Rats were observed for clinical signs for 4 weeks. All animals killed at the end of the observation period were subjected to macroscopic examination.
There were no clinical signs or gross observations at autopsy.
Under the conditions of this study, the maximum non-lethal dose of Py-SAS-A-1 following single oral gavage to rats was above 3300 mg/kg.

SCCS comment
It is not clear whether the material used for the acute toxicity study dated 1977 exhibits the same physicochemical properties as the material used for material characterisation performed in 2013 on a batch dated 09/08/2010.
It is stated that a single group of 10 rats/ sex was used in the main study. It is not clear which dose level was considered as main study.
The study report was not provided for evaluation.

Ref.: Pr-B (1977a) in OECD (2004)

Guideline:	OECD TG 401
Species/strain/sex:	Rat, Wistar
Group size:	5 /sex in the main study
Test substance:	P-SAS-A-8 (hydrophilic precipitated SAS)
Batch:	not given
Purity:	not given; SiO_2 > 98 %, Na_2O < 1 %, Al_2O_3 < 0.2 %; SO_3 < 0.8 %, Fe_2O_3 < 0.03 %
Dose levels:	5110 mg/kg
Exposure:	oral, gavage
Vehicle;	1 % carboxymethylcellulose in water
GLP statement:	yes
Date:	report dated 1990

Rats (5/sex) were given a single dose of P-SAS-A-8 by oral gavage at 5110 mg/kg. Test item was suspended in 1% carboxymethylcellulose in water and given at a dosing volume of 21.5 ml/kg. Rats were observed for clinical signs and their bodyweight was recorded. All animals killed at the end of the observation period were subjected to macroscopic examination.
There were no clinical signs and no necropsy findings related to the administration of P-SAS-A-8.
Under the conditions of this study, the maximum non-lethal dose of P-SAS-A-8 following single oral gavage to rats was above 5110 mg/kg.

SCCS comment
P-SAS-A-8 does not belong to the materials of the submission. Justification should be given for why results obtained from P-SAS-A-8 could be used for the materials covered by the submission.

Ref.: Pr-B (1990) in OECD (2004)

3.3.1.2 Acute dermal toxicity

No data provided

3.3.1.3 Acute inhalation toxicity

No data provided

3.3.1.4 Acute intraperitoneal toxicity

No data provided.

SCCS conclusion on acute toxicity

Two nanomaterials covered by the submission were investigated for acute oral toxicity (Py-SAS-NA-2 and Py-SAS-A-1). There is no statement on guideline adherence and GLP. Only summaries of the reports were presented and it is not clear to the SCCS whether the materials used for the acute toxicity studies exhibit the same physicochemical properties as the materials used for material characterisation.
Two further materials not covered by the submission were also tested for acute oral toxicity. Justification is lacking why results obtained from these materials could be used for the materials covered by the submission.
No information was provided on acute toxicity by other routes of administration (e.g. the inhalation or dermal uptake route).
In summary, no conclusion on acute toxicity can be drawn based on the information provided by the Applicant.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline: /
Species/strain: New Zealand white rabbits;
Group size: 3 animals per sex
Test substance: P-SAS-A-7
Py-SAS-NA-2
Batch: /
Purity: /
Vehicle: a) olive oil (0.5 g P-SAS-A-7 suspended at 50 %)
b) 1% aqueous suspension of methylhydroxyethyl cellulose (0.5 g Py-SAS-NA-2 suspended at 6%)
Dose level: area 6.25 cm²
Dose volume: Volume applied was not reported
Observation:/
GLP:/
Study period: study performed in 1978

Applied intact and scarified skin, in contact with the skin for 24 hours by occlusive patch. Skin irritation was scored immediately after removal of the patch and 48 h later (i.e. 72 h after application). Changes in behaviour, food intake, and body weight gain were monitored during a 14-day observation period.

Results
No signs of cutaneous erythema and oedema were observed.

Conclusions
When tested diluted at 50% under occlusive conditions for 24 h, P-SAS-A-7 was non-irritant to rabbit skin.
When tested diluted at 6% under occlusive conditions for 24 h, Py-SAS-NA-2 was non-irritant to rabbit skin.

SCCS comment
The material P-SAS-A-7 does not belong to the submission. It should be justified whether the results from P-SAS-A-7 are relevant for the materials covered by the submission. The material Py-SAS-NA-2 does belong to the submission.

The toxicological study was performed in 1978 and it is not clear whether the batches in these tests are the same as those in the characterisation tests. In other words, does the material used in the irritation tests have the same physico-chemical properties as the material used for material characterisation?

The study report was not provided for evaluation.

Ref.: Pr-B (1978) in IUCLID (2003)
Lewinson *et al.* (1994)

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline:	/
Species/strain:	New Zealand white rabbits;
Group size:	5 in first group; 3 in second group
Test substance:	P-SAS-A-7 (used with vehicle) Py-SAS-NA-2 (used without vehicle)
Batch:	Not specified
Purity:	Not specified
Vehicle:	olive oil (100 Mg P-SAS-A-7 suspended at 50%) Dose level: 100 mg
Administration	Instillation into the conjunctival sac of the eyes of test animals
Dose volume:	not reported
Observation:	/
GLP:	/
Study period:	study performed in 1978

First group (5 animals): 5 minutes after instillation of the test item, the eyes were rinsed-off for 2 minutes with 300 ml water.
Second group (3 animals): eyes were rinsed-off 24 h after instillation.
Ocular reactions were scored 1, 24, 48, and 72 h after instillation, according to Draize scoring.

Results
P-SAS-A-7
No signs of ocular irritation 5 minutes after instillation of the test item.
When test substances was rinsed-off after 24 h, a slight erythema (score 1) of the conjunctiva was observed up to 48 h after application, and was completely reversible within 72 h of application.

Py-SAS-NA-2
No ocular reactions were observed for any of the animals treated in either group.

Conclusions
Under the conditions of this study, P-SAS-A-7 was considered to be non-irritant to rabbit eyes when tested diluted at 50%.
Under the conditions of this study, Py-SAS-NA-2 was considered to be non-irritant to rabbit eyes when tested undiluted.

SCCS comment
The material P-SAS-A-7 does not belong to the submission. It should be justified whether the results from P-SAS-A-7 are relevant for the materials covered by the submission. The material Py-SAS-NA-2 does belong to the submission.

The toxicological study was performed in 1978 and it is not clear whether the batches in these tests are the same as those in the characterisation tests. In other words, does the material used in the irritation tests have the same physico-chemical properties as the material used for material characterisation?

The study report was not provided for evaluation.

Ref.: Pr-B (1978) in IUCLID (2003)
Lewinson *et al.* (1994)

Relevant Open Literature on irritation (provided by the Applicant), but not considered to be adequate for regulatory submission because of some shortcomings.

SKIN
No signs of irritation in experimental studies under occlusive conditions in intact or abraded skin (ECETOC, 2006) were identified for one (Py-SAS-A-1) hydrophilic pyrogenic silica; 5 (not included in the submission) hydrophilic precipitated silica; One hydrophilic gel; 7 (of which one is included in the submission) hydrophobic pyrogenic silica.

Slight and reversible erythema on intact and/or abraded sites: One (Py-SAS-A-8) hydrophilic pyrogenic silica; and 2 (not included in the submission) hydrophilic precipitated silica (ECETOC, 2006; OECD, 2004).

EYE
The acute ocular irritation potential of various SAS materials, either neat or suspended at 50% in olive oil, was evaluated following instillation in rabbit eyes (ECETOC, 2006; OECD, 2004).
No sign of irritation was found for one (Py-SAS-A-1)Hydrophilic pyrogenic silica ; 5 (not included in the submission) Hydrophilic precipitated silica, one (not included in the submission) hydrophilic gel; and 4 (of which 2 are not included in the submission) Hydrophobic pyrogenic silica.
Slight, reversible conjunctival erythema in unwashed eyes for 3 (of which 1 was not included in the submission) Hydrophilic pyrogenic silica; one (not included in the submission) Hydrophilic precipitated silica; and 3 (not included in the submission) Hydrophobic pyrogenic silica.
Mixed, reversible observations
One (not included in the submission) **Hydrophilic pyrogenic silica** produced slight conjunctival erythema or chemosis in some animals within 72 h of ocular instillation, and a transient corneal opacity was observed in 2 animals 4 h after instillation.
One (not included in the submission) **Hydrophilic precipitated silica** produced no signs of irritation when instilled at 40 mg but when applied at 100 mg, slight redness of the conjunctiva was observed within 72 h of instillation. These minor changes were readily reversible.
One (not included in the submission) **Hydrophobic pyrogenic silica** silane-treated SAS produced a slight to moderate conjunctival erythema within 4 h of instillation into the eyes of rabbits: the effects were reversible within 24 h of instillation.

Conclusions
Skin irritation of various silica materials was evaluated in a number of studies. No or limited, reversible cutaneous reactions were observed. Eye irritation potential of various silica materials was evaluated in a number of studies. No or limited, reversible ocular reactions were observed.

SCCS comment
As indicated above, these studies were not considered adequate for safety assessment of the materials in the regulatory submission – but contain some valid information.
It is noted that the materials causing slight skin irritation generally also caused (if tested) slight eye irritation. Therefore, some caution should be taken for those not tested in the eye but showing some irritation potential on the skin.
Among different types/categories of SAS materials, some have shown no adverse effect whereas others have shown limited and reversible irritation, making it impossible to differentiate between the SAS materials on the basis of type/category.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

No data.

Other literature/tests on sensitisation.

A single study on the skin sensitisation potential of a SAS material was identified, which was reported by the Cosmetic Ingredient Review Expert Panel (CIR, 2009). This skin sensitisation study was performed in guinea pigs with a 20% aqueous suspension of hydrated silica (exact type was not reported).
No skin reactions were observed in either the test group (n=10) or the control group (vehicle, distilled water; n=5), indicating the absence of skin sensitisation potential of the material evaluated.

Additionally, multiple Human Repeat Insult Patch Tests (HRIPT) have been conducted on cosmetic products containing SAS materials at concentrations of up to 7% hydrated silica (CIR, 2011). No cases of skin sensitisation were reported in these studies.

Finally, no cases of skin sensitisation have been reported in workers exposed to SAS materials in spite of the wide use and large production (current production of above 1 Million t/year, see section 2) of these materials for decades (Fruijtier-Pölloth, 2012).

Conclusions
SAS materials in general can be considered to be devoid of skin sensitising potential. These views are consistent with the long history of safe use of SAS materials in cosmetic products.

SCCS comment
From the available information, it is not clear which materials were used in the different reported sensitisation tests. It is therefore not clear whether they are the same materials included in the dossier and/or are similar in terms of physicochemical properties.
The study report was not provided for evaluation.
The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.
Although no skin sensitisation is reported, no conclusions can be drawn from the provided information.

3.3.4 Dermal / percutaneous absorption

***In vitro* Percutaneous Absorption Study– Imaging Method**

Guideline/method: OECD testing guideline 428 (2004)
Species: Human breast, abdomen and back skin samples
Test substances:

- Hydrophobic pyrogenic SAS (Py-SAS-NA-2), (particles <20 nm, larger particles 40-70 nm)
- Hydrophilic pyrogenic SAS (Py-SAS-A-1), (particle size <20 nm)
- Hydrophilic precipitated SAS (P-SAS-A-4)
- Aqueous dispersion of colloidal SAS at 30% (C-SAS-1)

Formulation: For comparative purposes hydrophilic precipitated silica with a wide range of particle sizes (up to 3 µm) was also used. The tests were carried out using three typical skin-care formulations:

- hydrophobic pyrogenic SAS (Py-SAS-NA-2) at 3% in a typical cream formulation (Formulation A),
- hydrophilic pyrogenic SAS (Py-SAS-A-1) at 3% in a typical cream formulation (Formulation B),
- hydrophilic precipitated SAS (P-SAS-A-4) at 2.5% and colloidal SAS particles at 1.8% in a typical cream formulation (i.e. 6% of C-SAS-1, containing 30% active matter, yielding 1.8% of silica (Formulation C)

Cells per application: n = 4 per formulation (12 in total)
Untreated control cells: n =2
Particle size: not provided for the nano-materials tested. Group sizes: Intact human dermatomed skin (breast, abdomen and back) samples from two different donors. Where possible the same donors were used for all three formulations.
Dose applied: About 2mg/cm² of 3 typical skin care formulations containing either 3% of hydrophobic pyrogenic SAS, or 3% of hydrophilic pyrogenic SAS, and 2.5% of hydrophilic precipitated SAS and 1.8% of colloidal SAS, respectively (corresponding to exactly 60, 60 and 86 µg/cm² of silica respectively) were applied onto the skin surface of treated cells and left for 24 hours, simulating use conditions.
Skin area exposed: 2.54 cm^2
Skin temperature: 32°C ± 1°C
Diffusion cell type: Static; Test chamber: Receptor fluid: Phosphate-buffered saline (pH 7.4)
Exposure period: 24 hours
GLP: Yes – a deviation noted that purity and expiry date of the materials is not certified.
Published: No
Study period: 2013
References: Johnson IR (2013a)

Method:
The skin samples, stored on ice and kept frozen at –20°C until use, were dermatomed (500 µm in thickness) and mounted in diffusion cells, using phosphate-buffered saline (pH 7.4) as the receptor fluid.
Skin integrity was checked by measurement of electrical resistance across the membrane. Membranes with a resistance <10 kΩ are regarded as lower than normal and not used for exposure to the test material. Twenty-four (24) hours after application, remaining formulation on the skin surface was removed using a standardized washing procedure and

the percutaneous absorption of SAS particles was investigated by Transmission Electron Microscopy (TEM) examining ten sections of each exposed skin sample. In parallel, ten sections of each unexposed skin sample were examined by TEM. Samples of the receptor fluids were also examined by TEM.

Results
TEM images showed no SAS particles or material on the areas examined of the processed skin samples from the cells 23, 19 (untreated controls), 34, 35 (formulation A), 62, 68 (formulation B) and 14 (formulation C) suggesting that the silica particles did not penetrate through the various layers to the dermis.

TEM images captured for the processed skin samples from cells 21 (formulation A), 63, 69 (formulation B), 12, 17 and 18 (formulation C) all showed varying degrees of delamination of the *stratum corneum* and epidermis. SAS particles and remaining test formulation were observed on the skin areas examined of cells 21 (formulation A), 63, 69 (formulation B), 12, 17 and 18 (formulation C). From these observations, the degree of penetration of the SAS particles and test formulation into the *stratum corneum* of the areas examined appears to be variable, but SAS particles were not observed beyond the uppermost layers of the *stratum corneum*.

There was also no evidence of SAS particles or material in the receptor fluid samples examined.

Conclusions
As silica particles were not observed by TEM in the receptor fluid, or in the regions beyond the *stratum corneum*, such as the viable epidermis or dermis, following application of any of the three formulations, it can be concluded that the silica material in these representative skin-care formulations would not be considered as bioavailable.

SCCS comment
The dermal absorption study has not been performed according to the basic criteria described by the SCCS (NoG, 8th revision (SCCS/1501/12) and SCCS basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10) with respect to the number of donors and number of diffusion cells per formulation. The concentration of SiO2 in the formulation used does not represent the maximum used concentration in cream products (Table 2).
This study is based on transmission electron microscopy imaging of dermatomed human skin after application of formulations containing four types of SAS materials (hydrophobic pyrogenic, hydrophilic pyrogenic, hydrophilic precipitated, aqueous dispersion of colloidal SAS). The particle size distribution has not been provided for all the test materials, but it is assumed that they correspond with the materials for which data are shown in Table 1. It is most likely that the particles were present as agglomerates as the test items were used in cream formulations. The results showed a lack of detectable penetration of any of the SAS materials tested through skin beyond the upper most layers of *stratum corneum*. This study is only based on TEM imaging, which is subject to certain limitations arising from sample preparation, number of frames investigated, etc. The procedure described as "…ten sections of each exposed skin sample…" seems inappropriate to provide firm evidence of the absence of skin penetration for this system.

***In vitro* Percutaneous Absorption Study using Human Dermatomed Skin – Analytical Approach**

Study Design:
Guideline/method: OECD testing guideline 428 (2004)
Species: Human dermatomed breast and abdomen skin samples
Test substances: Four different forms of SAS material were used in this study:
- Hydrophobic pyrogenic SAS (Py-SAS-NA-2)

	• Hydrophilic pyrogenic SAS (Py-SAS-A-1) • Hydrophilic precipitated SAS (P-SAS-A-4) • Aqueous dispersion of colloidal SAS at 30% (C-SAS-1) Three typical skin care formulations were used, containing respectively: • Hydrophobic pyrogenic SAS (Py-SAS-NA-2 and Py-SAS-NA-1) at 3% in a typical cream formulation (Formulation A), • Hydrophilic pyrogenic SAS (Py-SAS-A-1) at 3% in a typical cream formulation (Formulation B), hydrophilic precipitated SAS (P-SAS-A-4) at 2.5% and colloidal SAS particles at 1.8% in a typical cream formulation (i.e. 6% of C-SAS-1, containing 30% active matter, yielding 1.8% of silica (Formulation C)
Particle size:	not provided for the nano-materials tested
Group sizes:	10 intact samples from four different donors
Cells per application:	10 + 4 untreated controls
Dose applied:	2 mg formulation/cm^2
Diffusion cell type:	Static; 2.54 cm^2 exposure area
Skin temperature:	32 ± 1°C
Receptor fluid:	Phosphate buffered saline (pH 7.4)
Exposure period:	24 hours
GLP:	yes – a deviation noted that purity and expiry date of the materials is not certified.
Published:	no
Study period:	2013
References:	Johnson IR (2013b) Heylings J (2013)

Method

The skin was stored on ice and kept frozen at –20°C until use. Skin samples were dermatomed (500 µm in thickness) and mounted in diffusion cells, using phosphate-buffered saline (pH 7.4) as the receptor fluid. Skin integrity was checked by measurement of electrical resistance across the membrane. Membranes with a resistance <10 kΩ were regarded as lower than normal. Ten diffusion cells (treated cells) and four diffusion control cells (untreated cells) per skin care formulation were used, and skin was maintained at approximately 32°C.

About 2mg/cm² of three typical skin care formulations containing 3% of hydrophobic pyrogenic SAS, 3% of hydrophilic pyrogenic SAS, and 2.5% of hydrophilic precipitated SAS and 1.8% of colloidal SAS, respectively (corresponding to actual silica contents of 90, 68 and 120 µg/cm² respectively) was applied onto the skin surface of treated cells and left for 24 hours, simulating use conditions. After this time period, the remaining formulation on the skin surface was removed using a standardised washing procedure. Twenty-four (24) hours after application, the percutaneous absorption of SAS particles was evaluated by monitoring Si contents in the different skin compartments. Si contents were measured by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), using direct ashing and gravimetric analysis. A correction factor of [x 2.14] was applied to convert silicon (Si) contents into Silica-eq [SiO_2] contents. Si contents were measured in the following compartments: skin washes, *stratum corneum* (isolated by tape strippings), epidermis/dermis, unexposed skin and receptor fluid.

Results
Table 3: SAS absorption using Human Dermatomed Skin.

Application	Dose applied (µg silica-eq)	Mean Silica-eq recovered (µg)	Amount of Silica-eq in *stratum corneum* (µg)	Amount of Silica-eq in Receptor fluid (µg)	Amount of Silica-eq in Epidermis (µg)	Amount of Silica-eq in Dermis (µg)	Amount of Silica-eq in Epidermis, Dermis, and Receptor fluid (µg)
Skin care formulation A	228.6	1191.3	72.1	1.9	15	6.5	23.4
Skin care formulation B	172.7	1201.4	39.1	0.3	7.4	7	14.7
Skin care formulation C	304.8	1178.6	40.1	0.5	7.3	6	13.8
Untreated controls	0	777.2	38.6	0.9	4.7	7.9	13.5

Results showed that mass balance for the three formulations evaluated was unexpectedly high, this high level being essentially due to the high recovery observed in the sponges wash (between 385% and 697% of the dose applied; absolute amounts of silica in the sponge washes were similar between the 3 formulations). In the untreated control cells, the levels of silica in the sponges were high and only slightly lower than the levels observed with the formulations, probably due to the lower number of sponges used to wash the skin surface of the untreated cells. Complementary analyses confirmed that these high silica levels in sponge washes were due to the silica contents of the natural sponges used (Heylings, 2013).

For formulations B and C, the sum of the silica amounts found in the epidermis, the dermis and the receptor fluid were similar to the amounts found in the same skin/receptor fluid compartments for untreated controls (14.7, 13.8 and 13.5 µg, respectively, see Table 3). For formulation A, these combined Silica-eq amounts were slightly higher than those observed for untreated controls (23.4 vs. 13.5 µg, see Table 3). However, since they were of minimal magnitude and since they did not reach statistical significance, these differences were considered of no biological relevance.

Conclusions
The results obtained in this study indicate that a significant background quantity of Silica-eq was found in the natural sponges used to decontaminate the skin.
The combined amounts of Silica-eq measured in the epidermis, dermis and receptor fluid of the control cells were broadly equivalent to those measured for all the three test formulations, indicating that these Si/silica amounts correspond to background levels. Accordingly, it can be concluded that the SAS materials in these three representative skin care formulations have no potential to penetrate into or through living skin, thus no potential to produce systemic exposure after dermal application.

SCCS comment
The dermal absorption study has not been performed according to the basic criteria described by SCCS (NoG, 8th revision (SCCS/1501/12) and SCCS basic criteria for the *in*

vitro assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10) with respect to the number of donors and number of diffusion cells per formulation. The concentration of SiO2 in the formulation used does not represent the maximum concentration used in cream products (Table 2).
The results of this study are also not valid because of the major artefacts caused by large amounts of silica in the sponges used to rinse-off the skin. As a consequence, ICP-OES analyses showed recovery of much greater levels of silica than the actual amounts applied. In terms of mass balance, such a huge artefact would mask any small differences in the amounts of silica recovered/absorbed between controls and treated samples.
The Applicant has concluded that the slightly higher amounts of silica found in the epidermis, the dermis and the receptor fluid in sample from formulation-A treatment were of minimal magnitude and did not reach statistical significance and therefore were of no biological relevance. However, no statistical indicators (e.g. stdDev, p-values) have been quoted along with the values in Table 3 to support this conclusion.
The results of Data from chemical analysis of the skin samples and the receptor fluid in this study would have provided supporting evidence to the TEM imaging study of Johnson, (2013a). However, the results of this study do not allow a conclusion to be drawn on the penetration, or the lack of it, of silica nanoparticles through the various layers of the skin.

***In vitro* Percutaneous Absorption Study using Human Skin Imaging Approach**

Study Design:

Guideline/method: OECD testing guideline 428

Species: Human abdominal skin samples were obtained from a single donor subjected to plastic surgery.

Test substances: The following SAS materials were used in this study:

- Hydrophobic pyrogenic SAS (Py-SAS-NA-2 and Py-SAS-NA-1)
- Hydrophilic pyrogenic SAS (Py-SAS-A-1)
- Hydrophilic precipitated SAS (P-SAS-A-4)
- Colloidal SAS (C-SAS-1, i.e. a 30% aqueous dispersion of SAS particles).

Five different typical cosmetic products were tested in this study:

I. A leave-on skin care product containing colloidal SAS particles at 1.8% (i.e. 6% of C-SAS-1), and Hydrophilic precipitated SAS at 2.5% (P-SAS-A-4)
II. A rinse-off skin care product containing hydrophobic pyrogenic SAS at 2% (Py-SAS-NA-1) and hydrophilic pyrogenic SAS at 1% (Py-SAS-A-1)
III. A make-up remover containing hydrophilic pyrogenic SAS at 2% (Py-SAS-A-1)
IV. An oxidative hair colouring product containing hydrophobic pyrogenic SAS at 1.2% (Py-SAS-NA-2), yielding a final on-head concentration of 0.48% after mixing with the oxidizer
V. A hair relaxer cream containing hydrophilic pyrogenic SAS at 3% (Py-SAS-A-1), yielding a final on-head concentration of 0.34% after mixing

Particle size: not provided for the nano-materials tested

Group sizes: Skin samples from a single donor.

Dose applied: Nominal application rates were 10 mg/cm^2 for skin care and make-up remover products, and 20 mg/cm^2 for the hair relaxer and oxidative hair colouring products. Actual application rates onto skin samples were 9.14 mg/cm^2, 10.77 mg/cm^2, 9.97

	mg/cm^2, 19.49 mg/cm^2 and 19 mg/cm^2 for products I to V respectively
Skin area:	Exposed surface was 2 cm² and the receptor fluid volume was 3 ml
Skin temperature:	31.1-31.3°C
Test chamber:	Static diffusion cells
Receptor fluid:	0.9% saline
Exposure period:	24 hours for products I, II and III, 30 minutes for product IV, and 25 minutes for product V.
GLP:	No (Not mentioned)
Published:	No
Study period:	2011
Reference:	Hallegot P and Grégoire S (2011)

Method
Skin samples were mounted intact (758-1260 µm in thickness) in diffusion cells, using sodium chloride (pH 7.4) as the receptor fluid. Six diffusion cells (five cells exposed with the five different formulations and one unexposed cell) were used and skin was maintained at approximately 31°C in static mode.
About 10 mg/cm² (skin care and make-up remover products) or 20 mg/cm² (oxidative hair colouring and hair relaxer products, which were mixed with the oxidizer or relaxer, respectively) were applied to the skin surface. Skin care and make-up products were applied for 24 h whereas hair products were applied for 25 to 30 minutes in order to mimic actual use conditions. After this time period, the remaining formulation on the skin surface was removed using a standardised washing procedure. Skin samples treated with products I, II or III were washed with 0.6 ml of 5% sodium lauryl ether sulfate and dried with a cotton swab. The skin was rinsed a second time with 0.6 ml of 5% sodium lauryl ether sulfate, then three times with 0.6 ml of water and subsequently dried with two cotton swabs.
Skin samples treated with product IV and V were washed twice with 3 ml of water then washed again with 0.6 ml of 5% sodium lauryl ether sulfate and dried with a cotton swab for 30 seconds. The samples were finally rinsed 5 times with 0.3 ml of water and subsequently dried with two cotton swabs. A control sample (770 µm in thickness) was also processed at 31.1°C, with no application of product and no rinsing.
Twenty-four (24) hours after application, the percutaneous absorption of SAS particles was estimated by TEM examination of multiple skin sections. In addition, toluidine blue-stained semi-thin sections from control and treated skin samples were examined at light microscopy.

Results
TEM examination of multiple sections of the skin samples treated with the different skin care, make-up and hair care products containing up to 4.3% SAS identified the presence of exogenous particles interpreted as SAS material in the outer layers of the s*tratum corneum*. No such particles were observed in the deeper layers of the s*tratum corneum* or in the living epidermis/dermis of treated skin samples, or on sections obtained from the unexposed skin sample.

Conclusions
The results obtained in the present study suggest that SAS materials used in typical cosmetic products have no potential to penetrate into or cross through the skin, thus no potential to produce systemic exposure of consumers after dermal application.

SCCS comment
The dermal absorption study was not performed according to the basic criteria described by the SCCS (NoG, 8th revision (SCCS/1501/12) and SCCS basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10) with respect to

the number of donors, characterisation of the skin samples and number of diffusion cells per formulation. The concentration of SiO_2 in the formulation used does not represent the maximum used concentration in the tested products (Table 2).

The study is based on TEM imaging of ultrathin sections of human skin after application of the 5 different formulations to intact skin samples comprising leave-on skin care product with colloidal silica; rinse-off skin care product containing hydrophobic pyrogenic silica and hydrophilic pyrogenic silica; make-up remover containing hydrophilic pyrogenic silica; oxidative hair colour product containing pyrogenic hydrophobic silica; and hair-relaxer cream containing pyrogenic hydrophilic silica. The particle size distribution of the test materials has not been provided, but it is assumed that they correspond with the materials for which data are shown in Table 1. In all of the types of materials studied, the results showed a lack of detectable penetration of SAS particles through skin beyond the upper most layers of *stratum corneum*. The study is however only based on TEM imaging and no chemical analysis of the skin samples or the receptor fluid was performed which could have supported the findings of this study.
A particular disadvantage of the TEM cross-section analysis is that only a subset of the principally detectable amount can be picked up. This depends on the number of cuts and analysed. Another known artefact is the possible migration of particles between different compartments of the dermis induced by the cutting procedure, which has led to extended controversies in the scientific community in the past.

Relevant Open Literature (provided by the Applicant)

Study 1
Boonen *et al.* (2011) evaluated the percutaneous penetration potential of silica particles (3 µm) suspended in water and in 65% ethanolic plant extract on ex vivo dermatomed human skin by means of scanning electron microscopy (SEM). Characterisation of the silica material used was very limited, with even the chemical identity of the test material being unclear (no CAS number, no information on amorphous character). Unlike the silica particles in water, which did not penetrate deeper than the epidermis, the microparticles formulated in the ethanolic plant extract reached the dermis. The Applicant has considered this study of very poor scientific relevance due to the absence of sufficient information on the test material as well as on the testing procedure used.

SCCS comment
Whilst the study has not reported characterisation data, it is worth noting that ethanolic preparations are reported to have enhanced dermal penetration of microsilica particles. This possibility cannot be discounted as the Applicant has not provided any data on dermal penetration of silica nanoparticles in ethanolic preparations.

Study 2
Rancan *et al.* (2012) investigated on human skin explants the skin penetration of silica particles of fluoresceine isothiocyanate (FITC)-labeled silica particles of four different sizes (42, 75, 200 and 300 nm) prepared by a seeded growth process. These silica nanoparticles were not SAS materials as they were prepared according to a specific synthesis process, the Stöber process, not relevant to SAS (Wang *et al.*, 2010). Skin explants were pre-treated, with a cyanoacrylate stripping of the skin surface performed before application of the test materials, which induces disruption of the *stratum corneum*. After application of the particle dispersion (0.4%), skin was incubated in a humidified chamber at 37°C for 16 h. The analysis of the skin surface by confocal laser microscopy showed that silica particles were observed mostly in skin furrows, in the hair follicle openings, and also adsorbed on the hair shafts. The 42 nm particles were also observed within the *stratum corneum*. After topical application of nanoparticles on human skin explants with partially disrupted *stratum corneum*, only the 42±3 nm particles were found to be associated with epidermal cells and

especially dendritic cells, independent of their surface functionalisation. The Applicant has regarded the results as supporting the theory that human skin can efficiently block the penetration of silica particles, even after disruption of the skin barrier.

SCCS comment
The results of the study actually indicate that amorphous silica particles with a size around 40 nm can penetrate across a slightly damaged skin barrier and be internalised by skin cells (included dendritic cells). However, the nanoparticles used in this study were FITC-labelled and may have different physicochemical properties than those intended for use in cosmetic formulations.

Study 3
Staronova *et al.* (2012) used 1000 µg/ml Rhodamine-labelled fluorescent silica nanoparticles with a mean size of 170 nm in the saline media (0.9% NaCl), applied on the surface of human skin at 50 µl/cm². No silica particles were visualised in the receptor fluid. Particles were observed in depth from 10 to 160 µm by confocal microscopy. However, according to the authors of the study, these observations require confirmation as the presence of natural and narrow ridges in the skin might have caused nanoparticles present on the surface of *stratum corneum* or in the upper epidermis to show up on images at lower depths. Pictures were taken in depths from 10 to 160 µm from the defined surface layer. NPs were not visible below 160 µm. Inferred from the intensity of red colour; the amount of SiO_2 NPs deposited in the skin is reduced with increasing depth. A quantification of NPs in the different strata of the skin was not possible. The Applicant has regarded this study of poor relevance to the cosmetic use, as the silica was applied at a very high amount.

SCCS comment
The SCCS agrees with Applicant's conclusions on this study.

Study 4
A recent study by Nabeshi *et al.* (2011) used suspensions of fluorescent (red-F)-labelled amorphous silica particles (25 mg/ml and 50 mg/ml). The test materials with particle size diameters 70, 300 and 1000 nm were used in this study following 5 min sonication and 1 min vortexing to disperse the particles. The silica nanoparticles of 70 nm (nSP70) were applied to the inner side of both ears of BALB/c mice for 28 days. Twenty-four hours after the last administration, skin, lymph nodes and brain were excised and prepared for transmission electron microscopy (TEM) evaluation. The results of 28-day application of nSP70 to mice showed that the particles entered the skin, the regional lymph nodes and hepatocytes, as well as the cerebral cortex and the hippocampus. Nuclear localisation of nSP70 was also detected in the skin and the lymph node. The Applicant has regarded the silica nSP70 used in this study as different from the SAS materials used in cosmetic products, and speculated whether the results could be explained by unintentional oral exposure, resulting from grooming after application (no information on animal housing or protection of application sites was provided in the article), or artefacts resulting from the fixation or cross contamination from the tissue preparation (Prentice, 2011).

Study 5
In another study by the same group, Hirai *et al.* (2012) evaluated the percutaneous absorption of well dispersed nSP70 particles following repeated (three days) application on the inner side of the ears of female BALB/c mice. The ears and regional lymph node were excised 24h after the last administration and then analysed by TEM. The authors concluded that nSP70 particles entered epidermal Langerhans cells, the dermis and regional lymph nodes. The Applicant regarded this study as suffering the same shortcomings as the study by Nabeshi *et al.* (2011), and considered it of no relevance for the safety evaluation of SAS materials used in cosmetic products.

SCCS comment
Whether or not this study can be discredited for its shortcomings would need further analysis beyond the present lines and/or more stringent experimental evidence. A critical and in-depth analysis of the corresponding studies reported in published literature would also be required.

Overall SCCS comments on Dermal Absorption of SAS Materials
Two of the three studies provided in the submission are based on imaging of silica particles by TEM, and one on chemical analysis of silica (as silicon) by ICP-OES. Although the results have been regarded by the Applicant to show a lack of detectable penetration of any of the SAS materials tested through skin beyond the upper most layers of *stratum corneum*, TEM imaging can be subject to a number of limitations and results from TEM alone would not suffice to draw a conclusion. The analytical study, which could have provided supporting evidence to TEM results, has also suffered from serious artefacts. This has made it difficult to draw any conclusions from these studies.
To confirm the relevance of dermal absorption studies with respect to cosmetic applications, the following issues need to be addressed:

1) The solubility of the test item in the test vehicle should be determined
2) The concentration of SiO2 in the formulation used should represent the maximum used concentration in the tested products (see Table 2).
3) The physicochemical characteristics of the tested materials and surface moieties should be adequately characterised and tested in all representative categories of cosmetic products in which the Applicant intends to use SAS materials.

A number of studies in the published literature have indicated the possibility of penetration of amorphous silica particles through skin after repeated administration (Nabeshi *et al.*, 2011; Hirai, *et al.*, 2012)– especially when skin barrier is slightly 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, if 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.
It is noted by the SCCS that the particles used in many of the published studies were different from those intended for use in cosmetic products; for example, they were labelled with fluorescent dyes which might have changed their properties/behaviour. On the other hand there is also solid counter evidence on the uptake of nanoparticles via the skin barrier from theoretical considerations [Watkinson, *et al.* Pharm Res (2013) 30:1943–1946 DOI 10.1007/s11095-013-1073-9] and from studies on nanomaterials different from silica (e.g. TiO_2: Nanoderm), which suggests that some experimental studies need revisiting. A recent 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 use of such products on flexed, cut, compromised and diseased skin also remains to be clarified in this context. In view of the cumulative evidence from the studies provided as part of the submission, and the information from the open literature, the SCCS regards the evidence for the lack of skin penetration of silica nanoparticles/clusters as insufficient and inconclusive.

Ref.: Boonen J. *et al.* (2011)
Rancan *et al.* (2012)
Hirai *et al.* (2012)
Nabeshi *et al.* (2011)
Nafisi *et al.* (2014)
Prentice D. (2011)
Staronova *et al.* (2012)

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose sub-chronic (90 days) oral toxicity

Study 1:

Guideline:	/
Species/strain/sex:	Rat, Wistar
Group size:	10 /sex/dose
Test substance:	P-SAS-A-9
Batch:	not given
Purity:	97 – 98 %
Dose levels:	0; 0.4-0.7; 1.7-1.9- and 6.5-7 % in diet (0; 300-330; 1200-1400 and 4000-4500 mg/kg bw/day)
Exposure:	oral, diet
GLP statement:	not stated
Date:	report dated 1981

The subchronic toxicity of P-SAS-A-9 was investigated in Wistar rats (10/sex/group) after in-diet administration at target concentrations of 0 (controls), 0.5, 2 and 6.7% for 13 weeks. Evaluations and measurements included mortality, clinical signs, body weight and food intake, haematology, blood chemistry and urinalysis. At the end of the dosing period, animals were killed and subjected to macroscopic examination, selected organs were weighed, and a wide range of organs/tissues were preserved. Microscopic examination was performed for specified tissues/organs.
The chemical analysis of the diet administered during the study showed that actual mean silica concentrations were about 0.4-0.7, 1.7-1.9, and 6.5-7%. Accordingly, mean dose levels achieved during the study were about 300-330, 1200-1400 and 4000-4500 mg/kg/day.
There were no deaths, no adverse clinical signs attributed to P-SAS-A-9 and no other findings at haematological, blood-chemical, urinary and histopathological examinations. Mean food intake was slightly higher in the female high dose group when compared to controls, with no associated changes in body weight gain. These minor, isolated changes were not observed in males and considered to be non-toxicologically significant.

Conclusions
The No Observed Adverse Effect Level (NOAEL) of P-SAS-A-9 in the present study was above 6.7% in-diet i.e. above 4000-4500 mg/kg.

SCCS comment
No information on the Batch of P-SAS-A-9 used in the toxicity study is given.
No information on OECD and GLP-adherence is given. Tissues/organs investigated were not specified.
Further, only a study summary is given which is considered as second hand information. In order to be able to properly evaluate the subchronic toxicity of P-SAS-A-9, the original study report is required.
No conclusions can be drawn from the information provided.

Ref.: Pr-B (1981) in OECD (2004)

Study 2:

Guideline:	study precedes guidelines
Species/strain/sex:	Rat, strain not specified
Group size:	15/sex/dose
Test substance:	P-SAS-A-10 (fluffy) (hydrophilic pyrogenic SAS)
Batch:	not given

Purity:	SiO_2 > 99 %
Dose levels:	0, 1, 3 and 5 % in diet (0, 700, 2100 and 3500 mg/kg bw/day)
Exposure:	oral, diet
GLP statement:	study precedes GLP
Date:	report dated 1958

The subchronic toxicity of P-SAS-A-10 was investigated in rats after in-diet administration for 13 weeks at 0 (controls), 1, 3 and 5% (corresponding to mean dose levels of 700, 2100 and 3500 mg/kg). Evaluations and measurements included mortality, clinical observations, body weight and food intake, haematology, and blood chemistry. A subgroup of 3 animals/sex/group was killed after 45 days of dosing. At the end of the respective dosing period, animals were killed and subjected to macroscopic examination, selected organs were weighed, and a wide range of organs/tissues were preserved. Microscopic examination was performed for specified tissues/organs.
There were no clinical signs of toxicity, and body weight changes; food consumption and survival rates were similar between groups including controls. No gross observations or histopathological changes that could be attributed to administration of P-SAS-A-10 were observed. Following ingestion of the diet containing high SAS levels, the SiO_2 contents of liver, kidney, spleen, blood, and urine for the period of 45 and 90 days showed no deposition of SiO_2 when compared to the controls.

Conclusions
Under the conditions of the present study, No Observed Adverse Effect Level (NOAEL) of P-SAS-A-10 fluffy was above 5% in-diet (i.e. 3500 mg/kg).

SCCS comment
The study preceded OECD guidelines and GLP. The material used was not specified and it is not clear whether the study material was comparable to Py-SAS-A-8; Py-SAS-NA-4, the two similarly named materials (but different in terms of surface characteristics) covered by the submission. Further, only a study summary was provided.
No conclusions can be drawn from the information provided.

Ref.: Pr-A (1958) in OECD (2004)

Relevant Open Literature

Reuzel et al (1991) studied the 13 weeks inhalation toxicity in rats of three amorphous silicas (Py-SAS-A-1, Py-SAS-NA-1 and P-SAS-A-2) and quartz dust. Rats were exposed to I, 6 or 30 mg Py-SAS-A-1/m³, 30 mg Py-SAS-NA-1/m³, 30 mg P-SAS-A-2/m³ or 60 mg quartz/m³ for 6 hr/day, 5 days/wk for 13 wk. Some rats were killed at the end of the exposure period and some were killed 13, 26, 39 or 52 wk later
Rats exposed to Py-SAS-A-1, Py-SAS-NA-1 or quartz developed granulomatous lesions. Silicosis was observed only in quartz-exposed animals. At the end of the exposure period, Py-SAS-A-1 and quartz had induced the most severe changes. Quartz dust was hardly cleared from the lungs and the changes in the lungs progressed during the post-treatment period, and eventually resulted in lesions resembling silicotic nodules and in one squamous cell carcinoma. Although Py-SAS-A-1 was very quickly cleared from the lungs and regional lymph nodes, the changes in these organs were only partly reversed during the post-exposure period in rats exposed to 30 mg/m³. Py-SAS-NA-1 and the lower levels of Py-SAS-A-1 resulted in less severe, and mostly reversible, changes. The slightest changes were found after exposure to P-SAS-A-2, notwithstanding the persistence of this silica in the lungs during the major part of the post-treatment period. The results of this study revealed that only quartz induced progressive lesions in the lungs resembling silicotic nodules. Of the amorphous silicas examined Py-SAS-A-1 induced the most severe changes in the lungs, which only partly recovered, whereas P-SAS-A-2 induced the least severe, completely reversible lung changes.

SCCS comment
The study shows that different types of SAS can result in somewhat different health outcomes. Notwithstanding this, the data has to be read with some caution since this study was not performed under GLP, nor any specific characterization of the material was performed.

3.3.5.2 (Sub) chronic (6 months)

Study 1
A 6-month chronic toxicity study was performed with SAS-G-1, a gel SAS.

Ref.: Pr-C (1975) in OECD (2004)

SCCS comment
This material is not covered by the submission because it is outside the 4 types of SAS categories considered in this Opinion (see Table 1). Justification is lacking why results obtained from this material could be transferred to the materials covered by the submission.

Study 2

Guideline:	study precedes guidelines
Species/strain/sex:	Rat, Wistar
Group size:	20/sex/dose
Test substance:	Py-SAS-NA-2
Batch:	not given
Purity:	not given
Dose levels:	0 and 500 mg/kg bw/day
Vehicle:	diet
Exposure:	oral
GLP statement:	study precedes GLP
Date:	report dated 1965

The chronic toxicity of Py-SAS-NA-2, hydrophobic pyrogenic SAS, was investigated in Wistar rats (20/sex/group) after in-diet administration for 6 months at 0 (controls) and 500 mg/kg/day. Five animals per sex and per group were observed for an additional 3-week recovery period. Evaluations and measurements included mortality, clinical observations, body weight (weekly), food intake (daily), and haematology (monthly). At the end of the dosing period, animals were killed and subjected to macroscopic examination; selected organs were weighed, and a wide range of organs/tissues were preserved. Microscopic examination was performed for specified tissues/organs.
There were no significant changes in food consumption, body weight gain and haematological parameters when compared to controls. There were no deaths, no changes in organ weight and no macroscopic findings attributed to the administration of the test item. At histopathological examination, increased lipid contents in the fasciculate zone of adrenal glands were observed in some animals given 500 mg/kg. These minor changes were no longer observed after the 3-week recovery period. These slight progressive changes in the adrenals were therefore regarded as reversible, attributed to chronic stress and considered to be non-adverse.
Under the conditions of the present study, the No Observed Adverse Effect Level (NOAEL) of Py-SAS-NA-2 was above 500 mg/kg.

SCCS comment
The study preceded OECD guidelines and GLP.
Information on material characterisation for Py-SAS-NA-2 batch 31520315221 dates from 2012 – 2013 and was provided to the SCCS. The study report of this 6-month repeat-dose toxicity study however dates from 1965 and no information on the batch of Py-SAS-NA-2 used in the toxicity study is given. Thus, it is not clear whether the material used for the 6-month toxicity study exhibits the same physico-chemical properties as the material used for material characterisation.
Tissues/organs investigated were not specified.
Furthermore, only a study summary is given which is considered as second hand information. In order to be able to properly evaluate acute toxicity, the original study report is required.

Ref.: Pr-B (1965) in IUCLID (2003)
Lewinson *et al.* (1994)

SCCS conclusion on short term repeated dose toxicity
Repeated dose toxicity was investigated by the oral route only. Information on two 13-week studies and two 6-month-studies was provided.
Only one of the 6-month studies used material covered in the submission, i.e. in total three oral repeat dose toxicity studies were available for evaluation.
The studies partly preceded OECD and GLP. Only summaries of the reports were presented and it is not clear to the SCCS whether the materials used for the repeat toxicity studies exhibit the same physico-chemical properties as the materials used for material characterisation.

In summary, no conclusion on repeat-dose toxicity can be drawn based on the information provided in the submission. Other information available in the open literature (Van der Zande, 2014) has highlighted the potential harmful effects of hydrophilic pyrogenic SAS materials via the oral route (see section 3.3.1.2).

3.3.5.3 Chronic (> 12 months) toxicity

See Carcinogenicity.

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial reverse mutation assay

Study 1

Guideline:	OECD 471
Species/Strain:	*Salmonella typhimurium* (5 strains: TA98, TA100, TA1535, TA1537, TA1538)
Replicates:	Three plates for condition
Test substance:	"Silane, dichloromethyl-treated SAS", a hydrophobic pyrogenic SAS
Batch:	/
Purity:	>99%
Particle size:	Not specified
Vehicle:	Dimethyl sulphoxide (DMSO)
Concentration:	100, 333, 1000, 3333, 5000 µg/plate

Treatment: Treatment was performed without and with S9-mix plus negative (vehicle) and positive controls (unspecified "known mutagens"). Other details not specified
GLP: In compliance
Published: No
Study period: Test results were reported in 1995

Procedure
The test substance was tested for gene mutation in five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) at the concentrations detailed above, using triplicate plates.
The experiment was performed in the absence or in the presence of metabolic activation (S9-mix from the livers of Aroclor-treated rats).
Negative (vehicle) and positive controls were included.
The experiments were conducted according to the direct plate incorporation method.
Details of the procedure are not given.

Results
No biologically relevant increase in the number of revertants was observed with any bacterial strain either with or without S9-mix. No evidence of toxicity was observed up to 5000 μg/plate. Precipitation was observed at 3333 μg/plate
All solvent and positive controls gave numbers of revertants within the expected ranges. Therefore, the experiment was considered to be valid.

Conclusion
Under the conditions used, the test substance was not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 either in the presence or absence of metabolic activation.

Ref.: Pr-A (1995) in IUCLID (2003)

SCCS comment
The identification of the material tested is not precisely defined.

Study 2
Guideline: /
Species/Strain: *Salmonella typhimurium* TA98, TA100, TA1537*); Escherichia coli* (WP2 uvrA)
Replicates: duplicates (TA98 and TA100) or triplicates (TA1537) for condition
Test substance: Py-SAS-NA-2 (a dichloromethyl-treated hydrophobic pyrogenic SAS)
Batch: /
Purity: /
Particle size: Median size 275 nm (volume), 160 nm (number).
Vehicle: Dimethyl sulphoxide (DMSO)
Concentration: 5 (without S9 only), 15.8, 50, 158, 500 and 1580 μg/plate.
Treatment: Treatment was performed without and with S9-mix plus negative (vehicle) and positive controls (Unspecified "known mutagens"). Other details not specified
GLP: Not declared
Published: Yes
Study period: Test results were reported in 1983

Procedure
Py-SAS-NA-2 was extracted with toluene and the mutagenic potential of the extracts was evaluated in the absence and presence of metabolic activation (S9-mix from the livers of Aroclor 1254-treated rats). The strain TA98 was tested with S9-mix in the presence of the

epoxide hydrolase inhibitor and GSH depletory 1,1,1-trichloropropene-2-3-oxide in order to increase the sensitivity of the test. Negative (vehicle) and positive controls were included.
The experiments were conducted according to the direct plate incorporation method.
Details of the procedure are not given.

Results
The maximum concentration tested (1580 μg/plate) was selected on the basis of precipitation criteria. Toxicity was not reported.
No biologically relevant increase in the number of revertants was observed with any bacterial strain either with or without S9-mix. All solvent and positive controls gave numbers of revertants within the expected ranges. Therefore, the experiment was considered to be valid.

Conclusion
Under the conditions used, Py-SAS-NA-2 was not mutagenic in bacteria in both the absence and presence of metabolic activation.

Ref.: Pr-B (1983) in IUCLID (2003)
Lewinson *et al.* (1994)

SCCS comment
It is not clear if Py-SAS-NA-2 has ever been in direct contact with bacteria. The experiment seems to have been performed with extracts rather than with nanoparticles.

Study 3

Guideline:	Not declared
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538*)*
Replicates:	/
Test substance:	Py-SAS-A-10 (a hydrophilic pyrogenic SAS)
Batch:	/
Purity:	/
Particle size:	/
Vehicle:	/
Concentration:	From 667 to 10000 μg/plate (intermediate concentrations not specified)
Treatment:	Treatment was performed without and with S9-mix. Negative (vehicle) and positive controls not specified. Other details not specified
GLP:	/
Published:	No
Study period:	Test results were reported in 2004

Procedure
The mutagenic potential of Py-SAS-A-10 was evaluated in the absence and presence of metabolic activation (S9-mix from the livers of Aroclor-treated rats).
Details of the procedure were not given.

Results
No biologically relevant increase in the number of revertants was observed with any bacterial strain either with or without S9-mix. Toxicity was not reported.
No information was given on revertant counts with solvent and positive controls.

Conclusion
Under the conditions used, Py-SAS-A-10 was not mutagenic in bacteria both in the absence and the presence of metabolic activation.

Ref.: Pr-A (1989) in OECD (2004)

SCCS comment
Py-SAS-A-10, in contrast to Py-SAS-A-8 and Py-SAS-NA-4, is not included in the materials listed in the physico-chemical characterisation.

No information was given on batch, purity, particle size, vehicle, cytotoxicity, negative or positive controls. Therefore this test has no or limited value.

Study 4

Guideline:	/
Species/Strain:	*Salmonella typhimurium* (5 strains: TA98, TA100, TA1535, TA1537, TA1538*); Escherichia coli* (1 strain: WP2 uvrA)
Replicates:	/
Test substance:	SAS-G-2 (a hydrophilic gel SAS)
Batch:	/
Purity:	/
Particle size:	/
Vehicle:	Dimethyl sulphoxide (DMSO)
Concentration:	From 33 to 10000 μg/plate.
Treatment:	Treatment was performed without and with S9-mix plus negative (vehicle) and positive controls (Unspecified "known mutagens"). Other details not specified
GLP:	Not declared
Published:	Yes (see the comment)
Study period:	Test results were reported in 1981 and 1991

Procedure
The mutagenic potential of SAS-G-2 was evaluated in the absence and presence of metabolic activation (S9-mix from the livers of Aroclor 1254-treated rats). Negative (vehicle) and positive controls were included. The experiments were conducted according to the direct plate incorporation method. Details of the procedure were not given.

Results
No biologically relevant increase in the number of revertants was observed with any bacterial strain either with or without S9-mix. Toxicity was reported as absent at the highest concentration.
No information on revertant counts with solvent or positive controls is given.

Conclusion
Under the conditions used, SAS-G-2 was not mutagenic in bacteria in both the absence and presence of metabolic activation.

Ref.: Mortelmans and Griffin (1981) in OECD (2004)
Prival *et al.* (1991)

SCCS comment
SAS-G-2 is not included in the materials cited in the physico-chemical characterisation.
No information is given on batch, purity, particle size nor in the results obtained with negative or positive controls (although these have been reportedly performed). Therefore, the test has no or limited value. It is unclear if the publication cited only reports the results of tests performed in 1981 or new results.

SCCS overall comment on the gene mutation tests in bacteria
The bacterial reverse mutation test was performed on four types of SAS, three pyrogenic SAS (two dichloromethyl-surface treated, one untreated hydrophilic) and one hydrophilic gel SAS. No genotoxic potential was reported for the SAS tested

Only one of the studies (Study 1) seems to have been performed according to OECD guideline 471 and GLP. However, even in this study the precise identification of the material tested is missing.
The materials SAS-G-2 (Study 4) and Py-SAS-A-10 (Study 3) do not belong to the submission. It should be justified whether the results with these materials are relevant for the materials covered by the submission. The material Py-SAS-NA-2 does belong to the submission, but a toluene extract rather than the nano-material itself seems to have been used for the test.
The results of negative and positive controls have not been reported in two studies, thus preventing the assessment of the validity of the tests.

The SCCS notes that, although the bacterial reverse mutation test is a reliable genotoxicity screen test for the analysis of soluble chemicals, it does not appear to be suitable for the assessment of nano-materials. This may in part be related to the size of bacteria, presence of bacterial cell wall and the limited or no uptake of nanoparticles by bacteria (Doak *et al.*, 2012; Magdolenova *et al.,* 2014). Consequently, the results obtained with the gene mutation test in bacteria have no value for the assessment of the genotoxic potential of the compound(s) tested.

Mammalian Cell Gene Mutation Test in CHO Cells (*hprt* locus)

Guideline:	/
Species/Strain:	Chinese hamster ovary (CHO) cells
Replicates:	/
Test substance:	Py-SAS-A-10 (hydrophilic pyrogenic SAS)
Batch:	/
Purity:	/
Particle size:	Median size 127.4 nm (volume), 80 nm (number)
Vehicle:	Dimethyl sulphoxide (DMSO)
Concentration:	From 10 to 250 μg/ml in the absence of metabolic activation; from 100 to 500 μg/ml in the presence of metabolic activation
Treatment:	Treatment was performed without and with S9-mix plus negative (vehicle) and positive controls (benzo(a)pyrene and ethyl methane sulfonate). Other details not specified.
GLP:	In compliance
Published:	No
Study period:	Test results were reported in 2004
Reference:	Pr-A (1990) in OECD 2004

Procedure
Py-SAS-A-10 was tested for mutation in the mammalian gene mutation test in CHO cells (*hprt* locus).
The experiment was performed in the absence and in the presence of metabolic activation (S9-mix from the livers of Aroclor-treated rats).
Negative (vehicle) and positive controls (Benzopyrene, either in the absence or in the presence of S9-mix and ethyl methanesulfonate) were included.
Cells were suspended in culture medium and exposed to the test item, negative or positive controls for 5h and then resuspended in culture medium. They were transferred to flasks for growth through the expression period (not specified) or were diluted to be plated for survival.
At the end of the expression period, acceptable cultures were then plated for viability or 6-TG resistance.

Results
No increase in mutant frequency was observed either with or without S9-mix as compared with the data found for the untreated controls.

Negative controls gave mutant frequencies within normal ranges and positive controls yielded distinct increases in mutant frequencies. Therefore the study was considered to be valid.

Conclusion
Under the test conditions used, Py-SAS-A-10 was considered to be non-mutagenic in the mammalian gene mutation assay (*hprt* locus) either in the presence or in the absence of metabolic activation.

Ref.: Pr-A (1990) in OECD 2004

SCCS comment
Py-SAS-A-10, in contrast to Py-SAS-A-8 and Py-SAS-NA-4, is not included in the materials listed in the physico-chemical characterisation.
No apparent data on cytotoxicity is provided and the number of biological replicates included in the study is not evident.

Chromosome Aberration Test in Cultured CHO cells

Study 1

Guideline/method:	OECD 473
Cells:	Chinese Hamster Ovary (CHO) cells
Replicates:	Duplicate cultures
Test substance:	silane, dichlorodimethyl-treated SAS, a hydrophobic pyrogenic SAS (purity >99%)
Batch:	/
Purity:	/
Particle size:	/
Vehicle:	DMSO
Test concentration:	63, 125, 250 and 500 µg/ml with and without metabolic activation
Treatment time:	4 and 12 h with and without S9-mix, respectively
GLP:	In compliance
Year:	1995
Reference:	Pr-A (1995) in IUCLID (2003)

Methods
The test item was evaluated for potential cytogenetic effects in Chinese hamster ovary (CHO) cells in the absence or presence of S9-mix. The S9-mix was prepared from livers of rats treated with Arochlor 1254. Positive controls were mitomycin C and cyclophosphamide in the experiments without and with S9-mix respectively. Cytogenetic analyses were performed at four treatment concentrations of 63, 125, 250 and 500 µg/ml. Treatment time was 4 and 12 h with and without S9-mix, respectively. Cultures were treated with colcemid to induce metaphase arrest two hours before harvesting. One hundred well spread metaphases per concentration were evaluated for cytotoxicity and chromosomal aberrations where possible.

Results
In the absence of a significant cytotoxicity, the highest concentration of 500 µg/ml was selected on the basis of solubility criteria.
Silane, dichlorodimethyl-treated SAS did not induce a biologically relevant increase in the number of cells with structural chromosomal aberrations.

Conclusions
Under the conditions of the present study, silane, dichlorodimethyl-treated SAS was not genotoxic (clastogenic) in this chromosomal aberration test.

Study 2

Guideline/method:	/
Cells:	Chinese Hamster Ovary (CHO) cells
Replicates:	Duplicate cultures
Test substance:	Py-SAS-A-10 (purity >99%), a hydrophilic pyrogenic SAS
Batch:	/
Purity:	/
Particle size:	/
Vehicle:	DMSO
Test concentration:	19 to 300 µg/mL without metabolic activation, and from 250 to 1000 µg/mL with metabolic activation
Treatment time:	2 and 16 h with and without S9 mix, respectively
GLP:	In compliance
Year:	1990
Reference:	Pr-A (1990) in OECD 2004

Methods
The test item was evaluated for potential cytogenetic effects in Chinese hamster ovary (CHO) cells in the absence or presence of S9-mix. The S9-mix was prepared from livers of rats treated with Arochlor 1254. Positive controls were triethylene melamine and cyclophosphamide in the experiments without and with S9-mix, respectively. Cytogenetic analyses were performed at 19 to 300 µg/mL without metabolic activation, and from 250 to 1000 µg/mL with metabolic activation. Treatment time was for 2 and 16 h with and without S9-mix, respectively. Cultures were treated with colcemid to induce metaphase arrest, two hours before harvesting. One hundred well spread metaphases per concentration were evaluated for cytotoxicity and chromosomal aberrations where possible.

Results
Toxicity (reduction in the mitotic index) was 92% (without S9-mix) and 63% (with S9-mix). This is much higher toxicity than the required level of around 50% reduction as compared to the untreated controls. Py-SAS-A-10 did not induce a significant increase in the number of cells with structural chromosomal aberrations.

Conclusions
Under the conditions of the present study, Py-SAS-A-10 was not genotoxic (clastogenic) in this chromosomal aberration test

Ref.: Pr-A (1990) in OECD 2004

SCCS comment
It is noted that the tested SAS do not induce significant increases in genotoxicity in either the cytokinesis-blocked micronucleus assay or *hprt* gene mutation assay in mammalian cells. This conclusion has to be seen in the light of 2 important conditions that are essential for the SCCS to come to a definitive conclusion. Firstly, direct exposure of the test cells to the nanomaterials and/or particle uptake into exposed cells have not been demonstrated. The Applicants occasionally demonstrate cytotoxicity. However, cytotoxicity is not a specific indicator of cell uptake as it can be induced through a variety of other mechanisms. Secondly, only pyrogenic silica materials have been tested. Therefore, the SCCS cannot make conclusion about any other material form.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Bone Marrow Chromosome Aberration Test

Guideline:	/
Species/Strain:	Rats, Sprague-Dawley albino

Replicates:	Five animals per group per treatment time
Test substance:	SAS-G-1, a gel SAS
Batch:	FDA 71-48, Lot Number 2
Purity:	/
Particle size:	/
Vehicle:	0.85% saline
Concentration:	1.4, 14, 140, 500, 5000 mg/kg (single doses or 5 doses, 24h apart)
Treatment:	Male rats received the test substance by the oral route (gavage). A negative control group consisted in animals treated with vehicle only. A positive control group (5 animals) was given a single dose of triethylene melamine (0.3 mg/kg intraperitoneally).
GLP:	/
Published:	No
Study period:	Test results were reported in 1974

Procedure
Rats were treated with single doses of SAS-G-1. for 6, 24 or 48 hours after dosing. Animals from SAS-G-1 or vehicle control groups that were treated daily for 5 days were killed 6 hours after the last dosing.
Two hours before sacrifice, the animals were treated with colchicine by the intraperitoneal route in order to block the cells at the metaphase stage. The animals were killed by CO_2 inhalation in excess, and slides were prepared from femoral bone marrow. For each animal, slides were scored blind for mitotic index (500 cells) and chromosome aberrations.

Results
When compared to controls, the incidence of cells with chromosome aberrations was markedly and statistically significantly increased in animals given the positive control triethylene melamine, showing the adequate sensitivity of the test system and procedure used.
A biologically relevant increase in cells with structural aberrations was not found for SAS-G-1-treated rats as compared to untreated control animals.

Conclusion
Under the conditions of this study, SAS-G-1 was not genotoxic in this chromosome aberrations study and therefore not considered to have clastogenic or aneugenic potential.

Ref.: OECD (2004)
US-FDA (1974)

SCCS comment
The bone marrow chromosome aberration test was performed on rats treated with SAS-G-1, a gel SAS, through oral gavage. No clastogenic or aneugenic activity was noted.
The SCCS notes that the test material, SAS-G-1 is not included in the list of SAS that have undergone physico-chemical characterisation. It should be justified whether the results with this material are relevant for the materials covered by the submission.

The SCCS notes that no associated toxicokinetic studies have been conducted to demonstrate that the material administered by oral gavage reached bone marrow cells. Without evidence of the materials coming into contact with the target tissue, the SCCS does not consider the evidence sufficient to reach a definitive conclusion on genotoxicity of SAS materials.

Ref.: OECD (2004)
US-FDA (1974)

3.3.6.3 Mutagenicity / Genotoxicity in open literature

A number of studies in the open literature have assessed the mutagenic/ genotoxic potential of SAS materials. Detailed analysis of these studies is provided in Tables 4 and 5 below.

In summary, a number of SAS nanomaterials (NM) from the JRC reference materials repository (NM 200, NM 201, NM 202, and NM 203) were tested for genotoxicity using the Comet assay, micronucleus test, and mammalian gene mutation test within the NANOGENOTOX project. The results of these tests showed that:

- The SAS materials were positive in Caco2 cells, BEAS2B, and A549 for the comet assay.
- Mammalian gene mutation test was negative for all the SAS materials tested.
- Micronucleus test data showed positive effect of some of the SAS materials for Caco2, and A549 (NM201, NM 202), but mostly negative in other cells.

Other studies in the open literature also showed positive results for some of the SAS materials in Caco2, primary mouse embryo cell, mouse fibroblasts and weakly positive for V79 cells. Comet assay results were negative in human lymphocytes and TK6 cells. There is also an indication that smaller nanoparticles are more genotoxic in the Comet assay and sister chromatid exchange and micronucleus tests (Tarantini *et al.*, 2015, Battal *et al.*, 2015, see Table 4) than the larger sized nanoparticles.

To conclude, there is evidence from *in vitro* studies reported in the open literature (see Tables 4 and 5) that indicates the possibility for SAS materials to cause DNA breaks and oxidised DNA lesions in some cells. These reported effects are also consistent with findings of the NANOGENOTOX project. However, negative results have been reported for mammalian gene mutation test and data from micronucleus test also show both positive and negative results depending on the cell type and the type of silica materials used.

The *in vivo* results from micronucleus and comet assays reported so far are also inconclusive as there are both positive and negative outcomes in the *in vivo* micronucleus data (Downs, 2012).

Table 4: Genotoxicity of silica - *In vitro* studies from open literature

Type of silica (Character. method)	Uptake	Conc.	Genotoxicity methods, cells, treatment	Results	Reference	Notes
Mesoporous silica NPs (MSNs) (TEM)	labelled NP TEM	120 µg/ mL	human embryonic kidney (HEK293) cells, mRNA expression, fluorescent in situ hybridization (FISH) (chromosome changes and gene amplification). Mutations in the EGFR1 and KRAS	altered gene expression negative mutations negative FISH	Zhang et al, 2015	

			genes by DNA sequencing, 24h treatment,			
15 and 55 nm colloidal SiO_2 (Levasil) (TEM, DLS, in medium, stability)	uptake in cytoplasm not in nucleus	4,16, 32, 64 ug/ml	Caco2, γH2Ax and MN, apoptosis (caspase 3), 24 treatment	15 nm silica MN and other tests positive, 55 nm negative	Tarantini et al 2015	Treatment in serum free medium (more genotoxic) than in medium with 2% serum oxidative stress (DCFH-DA), proinflammatory effects (IL-8)
SiO2 nanoparticles, 6 nm, 20 nm, 50 nm (TEM, DLS; agglomerates filtered and monodispersion checked by DLS)	No	150 ug/ml	human peripheral blood lymphocytes sister chromatid exchange (SCE), MN, Comet assay 72h	Positive Comet and SCE. Not significant in MN	Battal *et al.* 2014	Freshly synthesised; Size dependent genotoxicity
Amorphous silica (SiO2) (Specific surface area BET, XRD, X-ray diffraction; DLS; PDI, ζ, zeta-potential by DLS)	No	5, 10, 20 µg/mL	TK6, high throughput comet assay 4h treatment	Negative	Watson *et al.* 2014	
Silica NPs			Human lymphocytes, MN HTP	Negative	Vecchio *et al.*, 2014	Only abstract of the article is reviewed.
Coloid SiO2, 20 and 100nm (size, morphology, surface charge by DLS, Field emission SEM,	No	175, 350, 700, and 1,400 µg/mL	Ames test, Chromosomal aberrations (Chinese hamster lung fibroblast cells), Treatment 6h with and without S9	Negative	Kwon *et al.*, 2014	GLP, following OECD guideline

Zetasiser)						
Mezoporus silica (Zeta potential)	No	3.91 µg/mL	CHO cells MN (without CytB) 24h	Negative	Shah et al 2013	MN tested in one nontoxic concentration
Mezoporus SiO2-25nm and SiO2-100nm (TEM, EDS,DLS, zeta sizer)	Confocal microscope	10, 50, and 150ug/ml	Human HT-29 cells γ-H2Ax foci Treatment 24h	Positive	Sergent et al 2012	inverse dose-dependent relationships for SiO2-100nm nanoparticles .
Amorphus silica nanoparticles between 15 and 300 nm NM-200, NM-203, NRT-808, NRT-817, NRT-820, NRT-944 (DLS, SEM)	Intracellular localization by 85 nm fluorescently-abelled SiO2 NPs	1,10,100 ug/mL	Balb/3T3 mouse fibroblasts Cell Transformation Assay (CTA) MN Treatment up to 72 h,	Negative	Uboldi et al 2012	Non cytotoxic by MTT, colony forming assay Particles internalised and localised in cytoplasma
Amorphous silica NPs (15 and 55 nm) Fully characterised	(*in vivo* uptake-histopatology)	Up to 1000 ug/ml	human peripheral blood cells MN (OECD protocol 2010) Treatment 24, (CytB both at the treatment or added after 4h)	Negative	Downs *et al.*, 2012	Study performed together with *in vivo* (see Table)
Amorphous silica, 10, 30, 80 and 400 nm (TEM, DLS, Malvern 3000HS Zetasizer)	(in 3T3-L1 cells by TEM)	4,40,400 µg/mL	MN (3T3-L1 mouse fibroblast cells) lacZ gene mutation assay (MEF-LacZ cells)	MN positive - 80nm Mutation positive-80nm	Park et al 2011	ROS. Particles synthesized by Stöber method without any stabiliser
Amorphous silica NPs, 16, 60 and 104 nm (ICP-MS)	Yes		A549 cells MN, FISH, Comet assay 40 hours	Negative	Gonzalez at al 2010	particle number and total surface area appeared to account for

						MN Only abstract of the article is reviewed.
Silicon dioxide NPs	No		primary mouse embryo fibroblast cells Comet assay	Positive (weak)	Yang et al 2009a	Only abstract of the article is reviewed.
Amorphous silica: alumina Coated, sodium counter ion stabilised and nonstabilized (TEM, DLS, zeta potential)	No	4 and 40 μg/ml	3T3-L1 mouse fibroblasts Comet assay Treatment 3, 6, and 24 h	Negative	Barnes et al 2008	
Non-crystalline silica, Spherisorb® (Not reported)	No	80 and 160 μg/cm^2),	V79 cells MN	Positive at toxic concentrations	Liu et al 1996	Pulmonary surfactant – similar dispersion=lower genotoxicity
Four different sized SAS particles (nominal sizes: 10, 30, 80 and 400 nm; actual sizes: 11, 34, 34 and 248 nm) synthesized with the Stober-method without stabiliser and were endotoxin-, bacteria- and fungi-free.	Micronucleus 3T3-L1 mouse fibroblasts			Positive 80 nm silica NPs a weak, but significant increase in the number of cells with micronuclei	Park *et al.*, 2010a,b	
	gene mutations in mouse embryonic fibroblasts carrying the lacZ reporter gene			30 and 80 nm silica NP positive, the increases were at most three-fold but only for the 80 nm	Park *et al.*, 2010a,b	

	(4, 40 or 400 mg/L)			significant		
Alumina coated SAS particles of SiO2 particles in a "a crystal structure with an average size of 20.2 nm	Comet assays performed on a human breast cell line, hamster and human embryonic lung fibroblasts, in a neuronal cell line			slight positive effect, significant	Kim *et al.*, 2010; Pacheco *et al.*, 2007; Zhong *et al.*, 1997). Yang *et al.* (2009a,b)	Concern about cytotoxic doses
Luminiscent silica	A549 cells, Comet assay, Puls field gel electrophoresis, western blot Repair			negative	Yuhui Jin *et al.*, *Chem. Res. Toxicol* 2007	Comet parameter tail length does not say much
amorphous silica NPs (Ludox SM-30) from Ludox	HaCat and A549 cells, The comet assay Colloid Silica cytotoxicity was observed at nanoparticle concentrations ≥ 1 µg/ml, but DNA damage was evident at 0.1 µg/ml and above.			Positive	Mu *et al.*, 2012 Particle and fibre toxicity	Detailed characterisation and uptke
Amorphous silica fluorescent (red-F)-labeled amorphous silica particles (Micromod Partikeltechnologie GmbH) (25 mg/ml and 50 mg/ml) 70, 300, 100nm	HaCat cells, primary hepatocytes Comet assay and Ames			Ames positive in smaller sizes and the highest concentrations Comet data positive	Nabeshi *et al.*, 2011	Detailed characterisation and uptake. Interesting that Ames positive, logically only small size
Amorphous silica fluorescent (red-F)-labeled amorphous	Comet assay, keratinocytes			positive	Nabeshi *et al.*,Particle and Fibre Toxicology 2011	ROS production as well DNA damage, smallest NPs the most

silica particles (Micromod Partikeltechnologie GmbH) (25 mg/ml and 50 mg/ml) 70, 300, 100nm						genotoxic

NM= nanomaterial, NPs = Nanoparticles, MN=Micronucleus; FISH=Fluorescent *in situ* hybridisation; HPRT=Hypoxanthine phosphoribosyltransferase-encoding gene

Table 5: Genotoxicity of silica - *In vivo* studies from open literature

Type of silica (Character. Method)	Uptake	Conc.	In vitro/ In vivo/ genotox method - cells	Results	Reference	Notes
SAS 6, 15, 30 and 55 nm, Microsized Silica dioxide (TEM for size and morphology, DLS, LDV)	No	0.1, 1, 5 and 10mM	In vivo, Drosophila melanogaster Comet assay with and without enzyme Wing-spot test for somatic and recombination mutation Treatment at third instar larvae	Positive Comet assay (both +/ - FPG), Size dependent, Negative in somatic and recombination mutations silica dioxide-negative	Demir *et al.* 2015	
SAS precipitated NM-200,NM-201, pyrogenic NM-202, NM-203 (Full characterisation)	histopathology	cumulative doses 9, 18, 36 mg/kg (instillation), cumulative dose 15, 30, 60 mg/kg (iv)	Male Sprague Dawley rats, intratracheal instillations Intravenous injection with NM-203. Fpg-modified comet assays in bronchoalveolar lavage (BALF), lung, blood, spleen, liver, bone marrow, kidney MN in bone marrow Treatment 48, 24, and 3 hrs	Negative CA Negative MN after instillation, Week positive/equivocal MN (iv –MN-203)	Guichard et al 2015	*in vivo* Positive trend, concluded as negative NANOGENOTOX www.nanogenotox.eu
pyrogenic SAS (NM-202, NM- 203) Precipitated SAS (NM-200, NM -201)	histopathology	5, 10, or 20 mg/kg b.w./day	Male Sprague Dawley rats DNA strand breaks and oxidised DNA bases in blood, bone marrow, liver, spleen, kidney, duodenum, and colon	Negative in comet assay Negative in bone marrow MN Weak positive MN in colon (pyrogenic	Tarantini *et al.* 2014	No dose response, the lowest dose significant MN in colon NANOGENOTOX www.nanogenotox.eu suggestion that bone

(TEM, EDS, BET, DLS)			with the comet assays, MN in bone marrow and in colon Exposure for three days by gavage,	SAS)		marrow are not good model for MN.
Coloid SiO2, 20 and 100nm (size, morphology, surface charge by DLS, Field emission SEM, Zetasiser)	No	500, 1,000, and 2,000 mg/kg	Male Sprague Dawley rats Comet assay in liver, stomach, Treatment by gavage at 0, 24, and 45 hours. ICR mice MN bone marrow Treatment orally in 10 mL/kg	Negative	Kwon *et al.*, 2014	GLP, following OECD guideline
Amorphous silica NPs (15 and 55 nm) (Well characterised)	histopatology	25, 50, 125 mg/kg	male Wistar rats Comet assay in liver, lung and blood MN in reticulocytes intravenous injections to at 48, 24 and 4h before sacrifice.	Weak positive (liver) CA Positive MN	Downs *et al.*, 2012	Primary and secondary mech, inflammatory markers secondary genotoxic effects through release of inflammatory cell-derived oxidants (threshold manner action)
Amorphous Silica (SiO2 NPs) 37 and 83 nm (Characterisation in aerosol (size), concentration)	histopatology	3.7 × 107 or 1.8 × 108 NPs/cm3) mass concentrations 1.8 or 86 mg/m3	male Crl:CD (SD)IGS BR rats MN in reticulocytes Inhalation 6h/day, 1 and 3 days, sampling 24h after exposure	Negative	Sayes et al 2010	
Silica (Aerosolised but no data)	No	50mg/m^3	Rats, HPRT mutations in alveolar	Negative	Johnston et al, 2000	Whole body chamber; Positive with crystalline

			epithelial cells Exposure. Inhallation 6h/day for 13 weeks			silica

NPs = Nanoparticles, MN=Micronucleus; FISH=Fluorescent in situ hybridisation; HPRT=Hypoxanthine phosphoribosyltransferase-encoding gene

Note: Any *in vivo* study done after March 2013 for cosmetic ingredients is not acceptable under the EU's Cosmetic regulation. However, the studies listed above were not done specifically for cosmetics use and hence have been taken into consideration by the SCCS.

SCCS conclusion

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.

3.3.7 Carcinogenicity

The carcinogenic potential of SAS-G-1, silica gel, was investigated in an oral study in mice and rats after in-diet administration at 0, 1.25%, 2.5% and 5% for at least 21 and 24 months, respectively. These concentrations correspond approximately to dose levels of 0, 1800, 3500 and 7000 mg/kg/day for mice, and 0, 625, 1250 and 2500 mg/kg/day for rats. The administration of SAS-G-1 was well tolerated. There were no biologically or toxicologically significant changes, and SAS-G-1 was therefore considered to be devoid of toxic or carcinogenic potential (Takizawa *et al.*, 1988).

The carcinogenic potential of Py-SAS-NA-2, surface-treated hydrophobic SAS material, was investigated in a 2-year oral toxicity study performed in rats after in-diet administration at the single dose level of 100 mg/kg/day. There were no deaths and no treatment-related changes in the various parameters monitored and examinations performed including blood clinical chemistry, haematological and histopathological examinations. There were no toxicological or carcinogenic effects attributed to Py-SAS-NA-2, the nature and incidence of tumours observed in treated animals being similar to those observed in untreated rats of this strain and age. Accordingly, Py-SAS-NA-2, hydrophobic pyrogenic SAS, was considered to have no carcinogenic potential

In the Applicant's conclusion, the carcinogenic potential of SAS materials was investigated in dedicated rat and mouse studies with two forms of SAS materials (hydrophilic gel and hydrophobic pyrogenic). These studies showed the absence of toxicological and carcinogenic effects associated with lifetime administration of SAS materials to rodents. Materials of all SAS forms were not evaluated in dedicated carcinogenicity studies.

Ref.: Pr-B (1969) in IUCLID (2003)
Lewinson *et al.* (1994)

SCCS comment

In both the provided studies, material tested was not properly identified/characterised, the relevance of the test material to those included in the submission was not provided except for one material (Py-SAS-NA-2); no information was provided on the GLP status, (One study was performed before GLP become mandatory), and no information was provided on whether the study was performed according to appropriate OECD guideline. Therefore, neither carcinogenicity study can be considered as adequate.

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

The rats exposed to Py-SAS-NA-2, a hydrophobic pyrogenic SAS, at dose levels of 0 or 100 mg/kg/day in the aforementioned carcinogenicity study (see section 3.3.7) were mated. The offspring was adjusted to 5 pups/sex/litter and allowed to mature. After 7 months, they were mated and their litters were also adjusted to 5 pups/sex/litter. No effects on reproductive performance, pre- and post-natal development were observed

Conclusion
On the basis of the validity criteria outlined in section 4 of the submission dossier, this study was not considered to be adequate for regulatory submission because of some shortcomings. They were however considered to represent additional, collateral evidence of the safety of SAS materials.

Ref.: Pr-B (1965) in IUCLID (2003)
CIR (2011)
Lewinson *et al.* (1994)

3.3.8.2 Other data on fertility and reproduction toxicity

Guideline:	/
Species/strain/sex:	Rat, Wistar, males and females
Group size:	10 females per dose group
Test substance:	Py-SAS-NA-2
Batch:	/
Purity:	/
Dose levels:	0 and 500 mg/kg/day
Exposure:	oral, diet
GLP statement:	not stated
Date:	report dated 1965

In a combined fertility, prenatal and post-natal developmental toxicity study, female rats were given Py-SAS-NA-2, a hydrophobic pyrogenic SAS, in-diet at dose levels of 0 or 500 mg/kg/day for 8 or 17 weeks before mating with males. They were then treated over mating, gestation and lactation periods, while they were allowed to litter and rear their pups. Males were exposed to Py-SAS-NA-2 under similar conditions for 4.5 months before mating. They were randomly selected from animals treated in the 6-month repeated dose toxicity study described above (see section 3.3.5.2).
This study represented an additional arm of a 6-month toxicity study on Py-SAS-NA-2 (Pr-B, 1965; in ECETOC, 2006, see section 3.3.5.2).
Female Wistar rats (10/group) were given Py-SAS-NA-2 in-diet at dose levels of 0 or 500 mg/kg/day for 8 or 17 weeks before mating with males (5 Females/1 Male). Males were similarly exposed for 4.5 months and were selected from the males in the parallel 6-month repeated dose study described above (see section 3.3.5.2). Animals were administered Py-SAS-NA-2 over the mating, gestation and lactation periods, after dams were allowed to litter and rear their pups until week 4 of lactation. Maternal evaluations and measurements included daily clinical signs and food intake, and body weight recorded weekly. Reproductive performance (mating and gestation indices), haematological parameters, organ weights, macroscopic and microscopic observations were monitored. Pups were examined for gross external anomalies immediately after birth. Pup evaluation and measurements included

clinical signs and body weight gain during the lactation period. At the end of the 4-week lactation period, all pups were killed and subjected to macroscopic examination.

Results
After the first mating period in week 8, 9/10 females in the treated group and 6/10 in the control group were pregnant. Seven additional females from each group were pregnant after the second mating period in week 17. There were no changes in appearance and behavior and no changes in body weight gain and food consumption in parental animals. Reproductive performance was overall similar for control and treated animals and there were no effects on male and female reproductive organ weights. Similarly, there were no microscopic findings attributed to the administration of Py-SAS-NA-2. There were no treatment-related effects on litter size, pup weight at birth and pup appearance and behaviour. Pup development over the 4-week lactation period was not affected by administration of Py-SAS-NA-2, and no salient macroscopic findings were noted in pups at the end of the study.

Conclusion
In summary, the oral administration of Py-SAS-NA-2 at 500 mg/kg/day to rats during the mating, gestation and lactation periods produced no changes attributed to test item. Accordingly, under the conditions of the present study, the No Observed Effect Level (NOEL) was above 500 mg/kg/day for male and female reproductive performance as well as pre-natal and post-natal development.

SCCS comment
Information on material characterisation for Py-SAS-NA-2 batch 31520315221 dates from 2013 and was provided to the SCCS. The toxicological study however was performed in 1965 and no information on the batch of Py-SAS-NA-2 used in the toxicity study is given. Thus, it is not clear, whether the material used for the reproductive toxicity study 4 decades ago exhibits the same physico-chemical properties as the material used for material characterisation. Therefore, apart from other shortcomings already considered in the Applicant's conclusion, the study cannot be considered as adequate.

Ref.: Pr-B (1965) in IUCLID (2003)
Lewinson J *et al.* (1994)

3.3.8.3 Developmental Toxicity

The potential effects of SAS-G-1, a silica gel material, on embryo-foetal development were evaluated through daily oral gavage at 0, 13.4, 62.3, 289, and 1340 mg/kg/day to pregnant female mice during the sensitive period of organogenesis (gestation days 6 to 15). The administration of SAS-G-1 did not affect foetal survival and produced no changes in dams. The NOEL for maternal and developmental toxicity was therefore above 1340 mg/kg/day (US-FDA, 1973).

Likewise, the administration of SAS-G-1 to pregnant rats at the dose levels 0, 13.5, 62.7, 292, and 1350 mg/kg/day by gavage during the sensitive period of organogenesis produced no changes in dams and did not affect fetal survival rate. The soft tissue and skeletal abnormalities observed in animals given SAS-G-1 were similar in nature and incidence to those observed in the control group. Therefore, the NOEL for both maternal and developmental toxicity was above 1350 mg/kg/day (US-FDA, 1973).

Finally, the absence of maternal and developmental toxicity potential of SAS-G-1 was confirmed in two studies conducted in rabbits and hamsters. SAS-G-1 was administered at the dose levels 0, 16, 74.3, 345, and 1600 mg/kg/day by gavage to pregnant rabbits and hamsters during the sensitive period of organogenesis. The test material had no toxic potential in dams and conceptuses, and specifically no teratogenic potential. The NOELs for

both maternal and developmental toxicity were therefore above 1600 mg/kg/day in both studies (US-FDA, 1973).

Ref.: US-FDA (1973)

SCCS comment
SCCS notes that the test material, SAS-G-1, a gel SAS, is not included in the list of SAS materials submitted that were undergone physicochemical characterisation.

SCCS overall conclusion on reproductive toxicity
For Py-SAS-NA-2, a hydrophobic pyrogenic SAS, two old studies on reproductive toxicity were provided, a two-generation study with various shortcomings, and a combined fertility prenatal and post-natal developmental toxicity study.
The studies were conducted 4 decades ago and preceded OECD and GLP. Only summaries of the reports were presented. It is not clear to the SCCS whether the materials used for these studies exhibit the same physicochemical properties as the Py-SAS-NA-2 batch 31520315221 used for material characterisation.

The potential effects of SAS-G-1, a silica gel material, on embryo-foetal development were investigated in 4 developmental toxicity studies in rats, mice, rabbits and hamsters.
This material is not covered by the submission. Justification is lacking on how results obtained from this material can be transferred to the materials covered by the submission.

In summary, no conclusion could be drawn on reproductive toxicity of the materials covered by the submission.

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

In summary of the submission, SAS materials have been regarded to be absorbed under particulate or dissolved form, and there is a consistent body of evidence that shows that SAS materials are eliminated by the renal route. After administration by the inhalation, oral, and intravenous routes, SAS particles are readily eliminated with no indication of accumulation, even after prolonged exposure to high doses or concentrations. It is only after repeated administration at high dose levels by the parenteral route that Si was evidenced. However these findings are of limited relevance to SAS-containing cosmetic products applied dermally that produce no systemic exposure to SAS material.

SCCS comment
The submission has not provided the SCCS with any specific study on the toxicokinetic profile of SAS materials that might be considered to be adequate for regulatory assessment. Studies from the literature described in the dossier also have some limitations.
In the study of Cho *et al.* (2009) SAS nanoparticles were not properly characterised; only size of particles was confirmed with TEM. In the study of Kumar et al (2010) they used multimodal organically modified silica nanoparticles with diameters of 20-25 nm, which is not included in the materials cited in the physicochemical characterisation.
It is important to note that SAS may be absorbed after administration by the inhalation and oral routes. However, submission has not provided any relevant data to these exposure routes.

3.3.9.2 Toxicokinetics in humans

No data provided.

3.3.10 Photo-induced toxicity

Measurement data on photocatalytic activity have not been provided for any of the materials, but they have been regarded as generally not photocatalytic on the basis of prior knowledge from scientific literature. The SCCS has accepted this view for the non-surface modified materials under consideration on the basis that they are inert, amorphous in nature, and have not been reported in scientific literature as photocatalytic or phototoxic.

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

/

3.3.10.2 Photomutagenicity / photoclastogenicity

/

3.3.11 Human data

According to the Applicant, multiple Human Repeat Insult Patch Tests (HRIPT) have been conducted on cosmetic products containing SAS materials at concentrations of up to 7% (CIR, 2011). No cases of skin sensitisation were reported in these studies. Also, no cases of skin sensitisation have been reported in workers exposed to SAS materials in spite of the wide use and large-scale production (current production of above 1 Million ton/year) of these materials for decades (Fruijtier-Pölloth, 2012).

SCCS comment
The study of Fruijtier-Pölloth (2012) is a review on SAS material in general. The SCCS did not identify any original epidemiological study concerning SAS materials.
The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

3.3.12 Special investigations

Recently, systematic studies have been undertaken to re-evaluate the safety of SAS in food (additive E551). A few observations are worth noting:
In the study of Van der Zande et al (2014) two types of nanosilica were orally dosed to rats for 84 days. NM-202 is representative hydrophilic, pyrogenic synthetic amorphous silica nanostructured silica, selected by OECD; the second SAS material in the study was a commercially available food-grade, hydrophilic, pyrogenic synthetic amorphous silica.

The most important observations from this study were:

(a) effects on the liver; NM-202 induced stronger effects in the liver (fibrotic liver) compared to SAS (only mild changes found).
(b) a reverse dose dependency.

It was also noted that silica probably accumulated in the liver (both NM 202 and SAS particles), and that in SAS exposed rats possible accumulation in the spleen was also observed.

In the report of deliverable 7 of Nanogenotox (EU-project) tissue specific accumulation has been reported of SAS.

Based on this data it can be concluded that:

- high oral dosing regimens are not preferable since intake seems to be impaired due to agglomeration of the SAS in intestinal track;
- similar (in this case hydrophilic pyrogenic) SAS give different outcomes;
- accumulation of SAS in the body has not been previously reported.

3.3.13 Safety evaluation

Given the limited evidence, safety evaluation on any of the SAS materials included in the submission cannot be performed.

3.3.14 Discussion

Physicochemical properties

The analytical part of the dossier provides characterisation data on the 23 SAS materials listed in Table 1. On the basis of the different synthesis methods and the material characteristics, they could be loosely categorised into hydrophilic precipitated silica; hydrophilic pyrogenic silica; hydrophobic pyrogenic silica and colloidal silica materials.

The SAS materials under consideration are composed of primary particles in the nanometre range, and are stated to exist in larger aggregates and agglomerates.

Within each group, the data provided for different materials show a large variation in the experimental values: in volume specific surface area, in the solubility of individual materials, and in the densities of the materials within the hydrophilic pyrogenic group. In the absence of a scientifically valid justification for such large variations, the SCCS has regarded the provided evidence as insufficient to allow the use of data on one material to other materials within the same group, or between materials in different groups.

For most of the materials, information has been provided on whether they are surface modified, coated/uncoated, or doped. Some of the hydrophobic pyrogenic materials have been described as not having been surface modified, coated or doped, whereas titles of the individual analytical files indicate that they were in fact surface modified through treatment with either dichlorodimethylsilane or hexamethyldisilazane. In view of this, the SCCS has considered all of the hydrophobic pyrogenic SAS materials as surface treated. Information is however needed on which of these materials have methyl-, dimethyl-, or trimethyl- silylate moieties on the surface as the final reaction product.

The SCCS has accepted the Applicant's reasoning that the SAS materials as such (i.e. without surface modification) are inert, amorphous in nature, and generally lack catalytic or photocatalytic activity. The SCCS recommends testing of surface-modified SAS materials for reactivity and photocatalytic activity as they may behave differently from unmodified SAS materials.

Data on particle size distribution have been provided as median values from measurements by Dynamic Light Scattering (DLS) (section 3.1.9). As indicated in the SCCS preliminary comments (March 2014), measurement of number size distribution is sub-optimal, since particles in the lower nanometre scale can be underestimated (or missed). Although the dossier also contains electron microscopy (EM) images for the materials under

consideration, TEM is subject to certain limitations that may arise from sample preparation, number of frames investigated, etc. The SCCS therefore recommends measurement of particle size distribution, including median value and d0.1 and d0.9, by a different method so that particles in the lower nanometre range are also fully accounted for.

Some of the materials (e.g. P-SAS-A-5, P-SAS-A-2, P-SAS-A-3) have a significantly high metal content – especially aluminium and titanium. The SCCS recommends that the aluminium and titanium contents should be reduced in the SAS nanomaterials to minimum levels - in line with many of the other materials under consideration (e.g. Py-SAS-A-1 and 2).

Function and uses

According to the Applicant 'SAS materials have been commercialized since the 1950s and are currently used in a wide variety of industrial applications. The main use of SAS is as a reinforcement and thickening agent in various systems such as elastomers, resins and inks. SAS materials exhibit a high water absorption capacity due to their high specific surface area. Therefore SAS materials are used as adsorbing agents and in dry powder systems to enhance their flow properties.

Typical uses of SAS in cosmetic products are in leave-on skin products (skin care and make-up), rinse-off skin products as well as hair and lip products.'

Toxicological Evaluation

Acute toxicity

Two nanomaterials covered by the submission were investigated for acute oral toxicity (Py-SAS-NA-2 and Py-SAS-A-1). There is no statement on Guideline Adherence and GLP. Only summaries of the reports were presented and it is not clear to the SCCS whether the materials used for the acute toxicity studies exhibit the same physicochemical properties as the materials used for material characterisation (the acute toxicity study dated 1977, material characterisation was performed in 2013).
Two further materials, not covered by the submission, were also tested for acute oral toxicity. Justification is lacking how results obtained from these materials could be used for the materials covered by the submission.
No information was provided on acute toxicity by other routes of administration (e.g. the inhalation or dermal uptake route).

In summary, no conclusion on acute toxicity can be drawn based on the information provided by the Applicant.

Local toxicity

Skin & eye irritation

No significant skin or eye irritation was reported for the SAS tested.
One nanomaterial covered by the submission was investigated for local toxicity (Py-SAS-NA-2). There is no statement on Guideline Adherence and GLP. Only summaries of the reports were presented and it is not clear to the SCCS whether the materials used for the local toxicity studies exhibit the same physicochemical properties as the materials used for material characterisation (the local toxicity study dated 1978, material characterisation was performed in 2013).
Another material (P-SAS-A-7), not covered in the submission, was also tested for local toxicity. Justification is lacking how results obtained from this material could be used for the materials covered by the submission.
A number of studies in the published literature have shown no significant skin or eye irritation response.

It is noted that the materials causing slight skin irritation generally also caused (if tested) slight eye irritation. Therefore, some caution should be taken for those not tested in the eye but showing some irritation potential on the skin.
Among different types/categories of SAS materials, some have shown no effect whereas others have shown limited and reversible irritation making it impossible to differentiate among between the SAS materials on the basis of type/category.

Sensitisation
From the available information, it is not clear which materials were used in the different reported sensitisation tests. It is therefore not clear whether they are the same materials included in the dossier and/or are similar in terms of physicochemical properties.
The study report was not provided for evaluation.
Although no skin sensitisation is reported, no conclusions can be drawn from the provided information.
Although the Applicant has reported neither skin nor eye irritation, nor sensitisation potential for the SAS materials, the SCCS cannot draw a conclusion on either of the endpoints on the basis of the provided information.

Dermal absorption
Two of the three studies provided in the submission are based on imaging of silica particles by TEM, and one on chemical analysis of silica (as silicon) by ICP-OES. Although the results have been regarded by the Applicant as showing a lack of detectable penetration of any of the SAS materials tested through the skin beyond the upper most layers of *stratum corneum*, TEM imaging can be subject to a number of limitations, and results from TEM alone would be insufficient to draw a conclusion. The analytical study, which could have provided supporting evidence to TEM results, also suffered from serious artefacts. This made it difficult to draw any conclusions from these studies.
To confirm the relevance of dermal absorption studies with respect to cosmetic applications, the following issues need to be addressed:

1) The solubility of the test item in the test vehicle should be determined.
2) The concentration of SiO2 in the formulation used should represent the maximum used concentration in the tested products (see Table 2).
3) The physicochemical characteristics of the tested materials and coatings should be adequately characterised and tested in all representative categories of cosmetic products in which the Applicant intends to use SAS materials.

A few published studies have pointed to the possibility of skin penetration of SAS materials through slightly damaged skin, and even larger (micron) sized particles when applied in ethanolic formulations. Therefore, the SCCS recommends measurement of skin absorption of silica nanoparticles in the relevant media if SAS materials are intended for dermal application in an ethanolic formulation. It has been noted by the SCCS that the particles used in many of the studies in open literature were different from those intended for use in cosmetic products in that they were surface modified with fluorescence dyes which might have changed their properties/behaviour. Nevertheless, these studies indicate that surface modification may have a role in enhancing the dermal penetration of SAS particles. Surface modified SAS materials therefore merit a closer scrutiny in relation to dermal absorption. The situation with flexed, cut, compromised and diseased skin also remains to be clarified in this context.

In view of the cumulative evidence from the studies provided in the submission, and the information from open literature, the SCCS regards the evidence for the lack of skin penetration of silica nanoparticles/clusters as insufficient and inconclusive. The SCCS recommends a well-planned study that incorporates both TEM imaging and chemical analyses on the same samples of receptor fluid and skin treated with different formulations containing SAS materials.

Repeated dose toxicity
Repeated dose toxicity was investigated by the oral route only. Information on two 13-week studies and two 6-month-studies were provided.
Only one of the 6-month studies used material covered by the submission, out of the total three oral repeat dose toxicity studies that were available for evaluation.
These studies partly preceded OECD and GLP. Only summaries of the study reports were presented and it is not clear to the SCCS whether the materials used for the repeat toxicity studies exhibit the same physicochemical properties as the materials used for physicochemical characterisation.

In summary, no conclusion on repeat-dose toxicity can be drawn based on the information provided by the Applicant.

Mutagenicity
Insufficient genotoxicity evaluation of SAS has been presented because valid approaches have not been used for the three genotoxicity endpoints (gene mutations, chromosome aberrations and aneuploidy). These studies show that SAS materials did not induce gene mutations in bacteria. However, the SCCS is of the opinion that, although the gene mutation test in bacteria is reliable for the analysis of soluble chemicals, it does not appear to be suitable for the assessment of nanomaterials. Consequently, even without considering the limitations of the studies provided, the negative results presented with this test have no value for the assessment of the genotoxic potential of SAS. SAS treatment did not result in a biologically relevant increase in the mutant frequency at the *hprt* locus nor in the number of chromosome aberrations in mammalian cells. However, cellular uptake, and therefore, exposure of test cells to the nanomaterials, have not been demonstrated. Consequently, the value of these tests is also limited.
The negative finding of the *in vitro* experiments was confirmed in an *in vivo* chromosome aberration test in bone marrow cells of rats. After treatment with SAS, an increase in cells with chromosome aberrations was not observed. However, associated toxicokinetics was not conducted. Without evidence of the materials coming into contact with the target tissue, the value of this negative result is limited.
The SCCS also notes that the materials SAS-G-2 and Py-SAS-A-10 that were evaluated in some of the genotoxicity tests, do not belong to the submission, as physicochemical characterisation data for these materials have not been provided in the submission.

The SCCS carried out a search for publications relevant to SAS materials in the open literature and also launched a call for information[1]. The evidence available from the several open literature studies indicated positive results for *in vitro* and *in vivo* genotoxicity of SAS nanomaterials from Comet and micronucleus assays. It has 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.

Carcinogenicity
Two "old" studies have been included. In both studies, the material tested was not properly identified/characterised, the relevance of the test materials to the SAS materials under consideration was not proven, there was no information on GLP status, (One study was performed before GLP become mandatory) and no information was provided on whether the study was performed according to appropriate OECD guideline. Therefore, both carcinogenicity studies were considered by the SCCS to be inadequate.

[1] See call for information on SCCS website:
http://ec.europa.eu/health/scientific_committees/consultations/calls/sccs_call_info_03_en.htm

Reproductive toxicity
Summaries of reports on developmental and combined fertility, prenatal and post-natal developmental toxicity studies on rats, hamsters and rabbits by oral route were provided. It is not clear to the SCCS whether the materials used for the studies exhibit the same physicochemical properties as the materials used for physicochemical characterisation.
No conclusion on reproductive toxicity can be drawn based on the information provided.

Toxicokinetics
The submission has not provided the SCCS with any specific study on the toxicokinetic profile of SAS materials that may be considered to be adequate for regulatory assessment. Studies from the literature described in the dossier also have some limitations.
In the study of Cho *et al.* (2009), SAS nanoparticles were not properly characterised; only size of particles was confirmed with TEM. The study by Kumar et al (2010) used multimodal organically-modified silica nanoparticles with diameters of 20-25 nm, which is not included in the materials for which physicochemical characterisation data have been provided.
It is important to note that SAS may be absorbed after administration by the inhalation and oral routes. However, submission has not provided any relevant data about these exposure routes.

In humans
The lack of reported adverse effects from historic food use of SAS materials has been quoted by the Applicant as an indirect evidence for the lack of toxicity of SAS materials. However, it is worth noting that the type of SAS approved for use in food applications relates only to the neat (i.e. not surface modified) pyrogenic amorphous silica, and not to the different SAS types included in the current submission. Also, the SCCS could not identify any original epidemiological study either in the submission or in the open literature that could be considered relevant to the current evaluation of SAS safety in cosmetic products.

Safety Assessment
In the absence of a No Observed Adverse Effect Level (NOAEL) value and exposure estimates, calculation of margin of safety (MOS) is not possible.

4. CONCLUSION

Q1. In view of the above, and taking into account the scientific data provided, the SCCS is requested to give its opinion on the safety of the nanomaterials Silica, Hydrated Silica, Silica Silylate and Silica Dimethyl Silylate for use in leave-on and rinse-off cosmetics products, including hair, skin, lip, face, and nail products, taking into account the reasonably foreseeable exposure conditions.

The submission comprises safety assessment of 28 SAS materials, of which the SCCS has considered 23 materials relevant for this opinion (Table 1). These materials could be categorised into four categories: hydrophilic precipitated silica; hydrophilic pyrogenic silica; hydrophobic pyrogenic silica, and colloidal silica materials.

The physicochemical and safety data provided for the materials under consideration have been regarded by the Applicant as representative for the different types of SAS materials intended for use in cosmetic products. However, the SCCS evaluation has shown that, even within an SAS category, different materials have large differences in the experimental values for some of the physicochemical properties (section 3.1.9). No justification has been provided to explain such large variations. CAS/EC numbers for hydrophobic and hydrophilic materials are also different, suggesting that they are indeed different materials. In the absence of further justification for read-across, this has not allowed the SCCS to use data from one material to another, either within a given SAS category or between different categories. The SCCS has therefore used a case-by-case approach to assess each material

against the physicochemical and toxicological safety data provided for that particular material. In doing so, the SCCS has identified a number of inadequacies and gaps in the data relating to physicochemical properties, toxicological data and exposure assessment – i.e. in all three elements essentially required for risk assessment (see Annex-Table 1 and section 3.3.14). This is despite the fact that these issues had already been pointed out to the Applicant by the SCCS in the preliminary comments on the original submission in 2014.

After detailed evaluation of the current submission, the SCCS has concluded that the evidence, both provided in the submission and that available in scientific literature, is inadequate and insufficient to allow drawing any firm conclusion either for or against the safety of any of the individual SAS material, or any of the SAS categories, that are intended for use in cosmetic products.

Q2. In the event the SCCS finds that the safety assessment for one or more of the nanomaterials covered by this mandate should be provided in a separate opinion, the SCCS is asked to justify its decision.

As the SCCS has not been able to conclude on the safety of the SAS materials included in the current submission, the Applicant is advised to follow the SCCS Notes of Guidance (SCCS/1501/12), the SCCS Guidance on Risk Assessment of Nanomaterials (SCCS/1484/12), and the SCCS Memorandum on Data Quality (SCCS/1524/13) for any future evaluation of the SAS materials.
As mentioned before, if the SCCS opinion is to be sought again for more than one material in a single submission, a scientifically valid reasoning would be required to justify a 'read-across' between different materials/ categories. In the absence of such a justification, the SCCS will use a case-by-case approach, which will inevitably require adequate data on each individual material under evaluation.
From the current evaluation, the SCCS has noted a number of inadequacies and data gaps. Whilst more detailed analysis of these is presented in relevant sections (e.g. see Annex-Table 1 and section 3.3.14), the following brief summary is provided to enable/facilitate future evaluation of the SAS materials in cosmetic products:

- Adequate dermal absorption data would be of paramount importance for safety assessment of all types of SAS materials intended for use in cosmetic products.
    - For neat (i.e. not surface-treated) SAS materials, that are produced by a pyrogenic process and have physicochemical profile(s) similar to those used for food applications, adequate physicochemical characterisation and dermal absorption data would be particularly important for the SCCS evaluation.
    - For surface modified (hydrophobic) SAS materials, a clear identification of the surface moieties on each SAS material should also be provided; and the dermal absorption data should cover each type of the surface modification used;
    - If any of the SAS materials is intended for use in ethanolic formulation for cosmetic applications, the penetration potential of the nanoparticles should also be assessed in ethanolic media.
- For the SAS materials produced by a non-pyrogenic route (e.g. precipitation), additional data on secondary particle size should be provided to clearly indicate whether the primary particles are in aggregated as well as agglomerated form, or just in agglomerated form, as the latter could de-agglomerate under certain conditions to give off nanoparticles;
- When using data in support of an SAS category, scientific reasoning should be provided to explain any large variation in the physicochemical properties for the materials within the category;

- In toxicological tests, it is often indispensable to show the associated toxicokinetics *in vitro*, e.g. in genotoxicity/mutagenicity tests, as an evidence of the materials coming into contact with the target tissues/cell to validate the negative results.
- For all types of SAS, data on particle size distribution should be provided from a method other than DLS.
- The material examples/ categories provided with physicochemical characterisation and those provided with toxicity data should be overlapping.

Q3. The SCCS is requested to address any further scientific concerns with regard to the use of Silica, Hydrated Silica, Silica Silylate and Silica Dimethyl silylate in nano form in cosmetic products.

- Data from appropriately designed studies are needed to exclude the toxicity of the SAS materials, in particular the mutagenic/ genotoxic potential, considering the different possible exposure routes, the SAS concentration, and actual use conditions of the final products;

- Further studies are also needed to exclude the possibility of dermal penetration of SAS materials, especially the surface modified hydrophobic types, in the media/formulations that are relevant to the final product.

5. MINORITY OPINION

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6. REFERENCES

Supporting documents:

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Annex-Table 1: Summary of toxicological data provided in the submission

Type	Material/ Type (Analysis date)	Ph-ch data?	Acute	Repeated dose	Geno T Bacteria	Geno T other	Cancer	Repro tox	Irritation Skin	Irritation eye	Absorption	Kinetics
phobic pyro	Py-SAS-NA-1	YES *	X	X	X	X	X		X	X	YES *	X
phobic pyro	Py-SAS-NA-2	YES *	Oral	YES *	NA **	X	YES *	YES *	YES *	YES *	YES *	X
Hydrphil prec	P-SAS-A-4	YES *	X	X	X	X	X		X	X	YES *	X
Hydrphil prec	P-SAS-A-7	X	Oral	X	X	X	X		YES *	YES *	X	X
Hydrphil prec	P-SAS-A-8	X	Oral	X	X	X	X		X	X	X	X
Hydrphil prec	P-SAS-A-9 & P-SAS-A-2	X	X	YES *	X	X	X		X	X	X	X
Hydrphil prec	P-SAS-A-10	X	X	YES *	X	X	X		X	X	X	X
Hydrphil pyro	Py-SAS-A-1	YES *	Oral	X	X	X	X		X	X	YES *	X
Hydrophilpyro	Py-SAS-A-2	YES *	Oral	X	X	X	X		X	X	YES *	X
Hydrphil pyro	Py-SAS-A-10	X	X	X	YES *	YES *	X		X	X	X	X
Colloidal silica	C-SAS-1	YES *	X	X	X	X	X		X	X	YES *	X
?	silane, dichlorodimethyl	X	X	X	YES *	YES *	X		X	X	X	X
Gel	**SAS-G-1**	X	X	YES *	X	X	YES *	YES *	X	X	X	X
Gel	SAS-G-2	X	X	X	YES *	X	X		X	X	X	X

X: no data provided; Yes: data provided; NA: data provided but judged as confounded
Hydrphil: hydrophilic; Phobic: hydrophobic; pyro: pyrogenic; Prec: precipitated; ?: non-specified
* Data in table only indicates whether a study was provided, it does not indicated whether the study was sufficient for risk assessment.
** Study of a toluene extract not on the materials themselves (see section 3.3.6.1)