Scientific Committee on Consumer Safety

SCCS

OPINION ON

Gold (nano), Colloidal Gold (nano), Gold Thioethylamino Hyaluronic Acid (nano) and Acetyl heptapeptide-9 Colloidal gold (nano)

The SCCS adopted this document
at its plenary meeting on 24-25 June 2021

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 16 April until 14 June 2021). One comment was received during this time but it did not change the content of the Opinion.

1. ABSTRACT

The SCCS concludes the following:

1. In view of the above, and taking into account the scientific data provided, does the SCCS consider the nanomaterials Gold and Colloidal Gold, Gold Thioethylamino Hyaluronic Acid and Acetyl heptapeptide-9 Colloidal gold are safe when used in leave-on skin cosmetic products according to the maximum concentrations and specifications, taking into account reasonably foreseeable exposure conditions?

The SCCS has considered all the information provided by the Notifiers and is of the opinion that it is not possible to carry out safety assessment of the nanomaterials (Gold, Colloidal Gold and Surface Modified Gold) due to limited or missing essential information. Much of the information provided on toxicity relates to gold as such, and it is not possible to determine the relevance of the data for nano-forms of any of the materials under the current evaluation due to the absence of full study reports.

Detailed data and information need to be provided on physicochemical characterisation and toxicological evaluation, along with experiment performance to allow safety assessment of the nanomaterials.

In regard to surface modified gold, all notifications relating to Acetyl heptapeptide-9 Colloidal gold (nano) were withdrawn by the Notifiers and therefore only Gold Thioethylamino Hyaluronic Acid has been considered in this Opinion.

2. Does the SCCS have any further scientific concerns with regard to the use of materials A, B and C in nano form in cosmetic products?

The information obtained from scientific literature suggests possible systemic uptake of gold nanoparticles which may lead to accumulation in certain organs - notably the liver and spleen. In addition, the available data from literature indicate potential mutagenic/genotoxic effects of gold nanomaterials. These indications raise an alert that warrants further safety evaluation of gold nanomaterials when used as cosmetic ingredients. In the absence of sufficient data to allow safety assessment, the SCCS has considered these aspects and has concluded that there is a basis for concern that the use of Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) materials in cosmetic products can pose a risk to the consumer. The SCCS concerns for consumer safety in this regard are detailed in Annex II. The SCCS will be ready to assess any evidence provided to support safe use of the materials in cosmetic products.

Keywords: SCCS, scientific opinion, gold, colloidal gold, Gold Thioethylamino Hyaluronic Acid, Acetyl heptapeptide-9 Colloidal gold, nano, CAS No 7440-57-5, EC No. 231-165-9, CAS No. 1360157-34-1, Regulation 1223/2009

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About the Scientific Committees

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

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

SCCS

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

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Article 2(1)(k) of Regulation (EC) No 1223/2009 (Cosmetics Regulation) states 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. some metals, metal oxides, carbon materials, etc.). 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 cosmetic products containing nanomaterials other than colorants, preservatives and UV-filters and not otherwise restricted by the Cosmetics Regulation to be notified to the Commission six months prior to being placed on the market. Article 19 of this Regulation 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 notified nanomaterial, according to Article 16 of the Regulation the Commission shall refer it to the Scientific Committee on Consumer Safety (SCCS) for a full risk assessment.

(A) The Commission services received 237 notifications under Article 16 of the Cosmetics Regulation via the Cosmetic Product Notification Portal (CPNP) for cosmetic products containing Gold (68 notifications) and Colloidal Gold (169 notifications) with CAS No 7440-57-5 and EC No. 231-165-9 in nano form, as reported in the attached list. Gold, without any reference to the nano form, is reported in CosIng database as a colorant (CI 77480) and it is regulated according to entry 133 of Annex IV (IV/133) of the Cosmetic Regulation (EC) No 1223/2009. Colloidal Gold without any reference to the nano form is reported in CosIng with antimicrobial and skin conditioning functions.

According to the available notifications, both ingredients (Gold and Colloidal Gold) are used in nano form in leave-on skin cosmetic products with different concentrations and specifications as reported in the attached list.

(B) The Commission services received 11 notifications under Article 16 of the Cosmetics Regulation via the Cosmetic Product Notification Portal (CPNP) for cosmetic products containing Gold Thioethylamino Hyaluronic Acid [CAS No. 1360157-34-1, EC No. not available] in nano form, as reported in the attached list. Gold Thioethylamino Hyaluronic Acid without any reference to the nano form is reported in the CosIng database with the function of "skin conditioning". It is not regulated under the Cosmetic Regulation (EC) No 1223/2009.

According to the notifications submitted, this ingredient is used in dermal, leave-on skin care cosmetic products, with different concentrations and specifications as reported in the attached list.

(C) The Commission services received 18 notifications under Article 16 of the Cosmetics Regulation via the Cosmetic Product Notification Portal (CPNP) for cosmetic products containing Acetyl heptapeptide-9 Colloidal gold [CAS and EC No. not available] in nano form, as reported in the attached list. Acetyl heptapeptide-9 Colloidal gold (nano) is not reported in the CosIng database and is not regulated under the Cosmetic Regulation (EC) No 1223/2009.

According to the notifications submitted, this ingredient is used in dermal, leave-on skin care cosmetic products, with different concentrations and specifications as reported in the attached list.

The Commission has concerns on the use of Gold - Colloidal Gold **(A)**, Gold Thioethylamino Hyaluronic Acid **(B)** and Acetyl heptapeptide-9, Colloidal gold **(C)** in nano form because of the potential for nanoparticles to be absorbed dermally or across mucous membrane and to enter cells. Therefore, we request the SCCS to carry out a safety assessment of the nano form of Gold - Colloidal Gold **(A)**, Gold Thioethylamino Hyaluronic Acid **(B)** and Acetyl heptapeptide-9, Colloidal gold **(C)** reported in the notifications listed in the annex to this mandate.

Terms of reference

1. *In view of the above, and taking into account the scientific data provided, does the SCCS consider the nanomaterials A, B and C are safe when used in leave-on cosmetic products according to the maximum concentrations and specifications reported in the attached list, taking into account reasonably foreseeable exposure conditions?*

2. *Does the SCCS have any further scientific concerns with regard to the use of materials A, B and C in nano form in cosmetic products?*

3. OPINION

Preamble

The information provided by the Notifiers through CPNP on the materials considered in this Opinion was assessed by the SCCS, and further clarifications were asked where appropriate. Additionally, a call for information was made and a literature search performed by the Commission to obtain further information from other sources. In developing this Opinion, the SCCS has taken into account the responses received from the Notifiers, the information received from the Commission's call for information, and the results of the literature search.

Among the 266 initially received notifications, 45 notifications were withdrawn by the Notifiers. The withdrawn notifications have not been taken into account in this Opinion. These included all the notifications related to C category (Acetyl heptapeptide-9 Colloidal gold in nano form) that have not be considered in this Opinion. For one notification, the specification file was related to silver, and therefore the notification was not taken into account.

For the purpose of confidentiality, the trade names, abbreviations, and related notification reference numbers of the materials assessed in this Opinion have been coded by the SCCS and are referred to by the relevant codes (see Table 1 in Annex I).

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

A. Gold
Gold (nano) / CI 77840
Aqua, Gold (nano)
Colloidal Gold
Colloidal Gold (nano)
Colloidal Gold [nano]

B. Gold Thioethylamino Hyaluronic Acid

SCCS comment:
Some of the notifications indicated that the information related to the INCI name is "not available"

3.1.1.2 Chemical names

A. Gold (nano) and Colloidal Gold (nano)
B. Gold thioethylamino hyaluronic Acid (nano); Gold 4-deoxy-4-((2-mercaptoethyl) amino) hyaluronate complexes (nano)

3.1.1.3 Trade names and abbreviations

Gold Water, aXonnite Gold, aXonnite Gold nano-TECH, Granpowder PSQ-Au, Nanozloto, Nano gold partical, Goldex ZŁOTO NANOKOLIDALNE (H_2O Au) NIECHEMICZNE, Water&Cellulose Gum&SodiumCarbonate&Gold&Silver, ALM70c, Au@TSK1, Złota Woda nano-TECH, Gold Water nano-TECH, Phiten GWE – 1000, Gold Colloid Metalor, Lipobelle Gold, Colloid PMG-PG, PurestColloids-MesoGold, Endor-GH, Hyalgen

The Trade names and abbreviations are listed in Table 1 in Annex I.

3.1.1.4 CAS / EC number

A. Gold and colloidal gold (nano):
CAS: 7440-57-5/ EC: 231-165-9

B. Gold thioethylamino hyaluronic acid:
CAS 1360157-34-1/ EC No. not available

3.1.1.5 Structural formula

A (G-4): The structure shows a polymer with three crosslink points per monomer, thus forming an extremely tight-knit polymer solid of a distribution of very high to infinite molecular weights since the particle size is greater than 1 micrometer.

B (SMG-3): SMG-3 is composed by gold particles fully coated with modified Hyaluronan oligomers in a water-sodium citrate solution.

SCCS comment
Some information on structural formulae have only been provided for a few materials – for material category A (G-4), and B (SMG-3). Information on structural formulae for all other materials has not been provided. It needs to be clarified whether or not the provided information for one material covers the whole category.

3.1.1.6 Empirical formula

/

3.1.2 Physical form

SCCS comment
The 'physical form' has been reported as:
- a 'dispersion' A (G-1, G-2, G-3, G-4, G-5, CG-1, CG-2, CG-3, CG-8)
- a 'suspension' A(G-6, G-10, CG-10), B (SMG-2, SMG-3)
- a 'solution' A (G-7, G-8, CG-5(a), CG-6(b), CG-11)
- a 'gel' A (G-9)
- a 'solid' A (CG-5(b), CG-6(a)).

The 'crystalline shape' has been reported as:
- 'spherical, irregular' A (G-1, G-2(a), G-3, G-10, CG-1, CG-2)
- 'spherical' A (G-4, G-6, G-7, G-8, , CG-5(a), CG-6(b), CG-8, CG-10, CG-11), B (SMG-2, SMG-3)
- 'amorphous' A (G-9, CG-3)
- crystalline A (G-2(b))
- 'other' A (G-5, CG-5(b)).

The powder 'state' has been reported as:
- 'dispersed free particles, agglomerates' A (G-1(a), G-3, CG-2),
- 'dispersed free particles, aggregate' A (G-1(b), G-2(a), G-10)
- 'dispersed free particles' A (G-2(b), G-4, G-5, G-7, G-8, G-9, CG-1,CG-3, CG-5(a), CG-6(b), CG-8, CG-10, CG-11), B (SMG-2, SMG-3)
- 'aggregate' A (G-6)

- 'other' A (CG-5(b), CG-6(a))

The 'aspect ratio' has been reported as equal to '1' for A (G-1, G-2, G-3, G-4, CG-1, CG-2, CG-8). For the others, information has not been provided.

3.1.3 Molecular weight

Gold: 196.97 g/mol

Molecular weights of Gold thioethylamino hyaluronic Acid (nano) and Gold 4-deoxy-4-((2-mercaptoethyl) amino) hyaluronate complexes (nano) have not been provided.

3.1.4 Purity, composition and substance codes

Incomplete data have been provided.

SCCS comment
Notifiers should provide the SCCS with proper analytical files.

3.1.5 Impurities / accompanying contaminants

Incomplete data have been provided.

SCCS comment
Notifiers should provide the SCCS with proper analytical files.

3.1.6 Solubility

The solubility values have been reported to be less than 0.01 mg/L.

SCCS comment
Based on the solubility value reported, the materials under consideration could be considered as insoluble/practically insoluble.

For A (G-1), the SCCS has noted contradictory information related to solubility (unlimited solubility and solubility below 0.01 mg/L).

For B (SMG-3), based on the provided information by the Notifiers, the SCCS has noted contradictory information concerning the solubility of (completely soluble and solubility value below 0.01 mg/L).

3.1.7 Partition coefficient (Log P_{ow})

Octanol/water partition coefficient:
Not applicable.

3.1.8 Additional physical and chemical specifications

Table 1: Additional physicochemical specifications.

Code No SCCS	pH value	Conductivity	Density	Turbidity	Viscocity	Colour
A(G-1)	6-7.5	5-50 µS	0.990 – 1,010	max. 8 NTU	1000 x 10^{-6} Pa x s	max.5 Pt/l
A(G-2 and G-3)	6.5 ± 1	2-50 µS	0.990 – 1,010	max. 8 NTU	1000 x 10^{-6} Pa x s	colourless to pale pink
A(G-6)	Not provided	2 - 50 µS	19.3 g/mL at 25 °C	max. 8 NTU	Not provided	Additional information: bp* 2808°C (lit.), mp* 1063°C (lit.), Resistivity 2.05 µΩ cm
A(CG-1)	6.5 ± 2	2-50 µS/cm	0.990 – 1.010	max. 8 NTU	1000 x 10^{-6} Pa x s	light purple
A(CG-6)	5.5-8.5	Not provided	1.20 to 1.24 g/mL	Not provided	Not provided	Not provided
A(CG-8)	Not provided	Not provided	Not provided	Not provided	Not provided	ruby red to reddish purple colour called "purple of Cassius"
B(SMG-2)	Not provided	Not provided	1.0027 mg/mL	Not provided	Not provided	Not provided

* bp: boiling point, mp: melting point

SCCS comment

Limited information has been provided only for some of the substances listed in Table 1:

- no complementary information has been provided for A(G-4, G-5, G-7, G-8, G-10, CG-5), B(SMG-3)
- complementary information provided is related to the cosmetic product for A(CG-3, G-9)
- For B(SMG-2), the density should have been expressed in g/mL, and not mg/mL.

The indicated density (close to 1 g/mL) is noted to correspond to the density of water.

3.1.9 Particle size

Table 2: Particle size as reported in notifications

	Code	Lowest cut off level (nm):	Primary Particle size (Volume weighted) Min – Max (nm)	Primary Particle size (Number weighted) Min – Max (nm)	Secondary Particle size (Volume weighted) Min – Max (nm)	Complementary information obtained by EM/spICPMS
A. Gold (nano) and Colloidal Gold (nano)	G-1	1	1 – 100	1 - 100	1 - 100	Size of nanoparticles: 3-5 nm(80-85 %)- 5-100 nm (15-20%)
	G-2 (a)	1	1 - 100	1 - 100	1 - 100	Average particle size: 2-5 nm (70-75%) and 5-100 nm (25-30%)
	G-2 (b)	100	1 - 100	1 - 100	1 - 100	
	G-3	1	1 - 100	1 - 100	1 - 100	Average particle size: 2-5 nm (70-75%) and 5-100 nm (25-30%)
	G-4	8	8 - 15	8 - 15	8 - 15	Average Particle Size: 3 – 10 µm
	G-5	2	7 - 18	2 - 16	/ - /	//
	G-6	8	8 - 12	8 - 12	7 - 50	//
	G-7	10	12 - 48	14 - 41	/ - /	//
	G-8	5	5 - 10	5 - 10	/ - /	//
	G-9 (a)	1	2 - 3	2 - 3	4 – 5	Average: 171.0 nm, SD = 5.7 nm
	G-9 (b)	2	2 - 3	2 - 3	4 – 5	
	G-9 (c)	51	245 - 490	309 - 517	52 - 117	
	G-10	35	111 - 119	111 - 119	/ - /	primary particles of about 35 nm (spICPMS and TEM).
	CG-1	1	1 - 100	1 - 100	1 - 100	Average particle size - 2-4 nm
	CG-2	1	1 - 100	1 - 100	1 - 100	Size of nanoparticles -3-5 nm(80-85 %)- 5-100 nm (15-20%)
	CG-3 (a)	10	25 – 30	17 - 21	// - //	Average diameter of primary particles: 15 nm. Secondary Particle Sizes: Mean Volume Diameter: 0.03 µm Mean Area Diameter: 0.016 µm Mean Number Diameter: 0.02 µm
	CG-3 (b)	10	15 - 30	17 - 21	// - //	
	CG-5 (a)	2	10 - 40	10 - 40	// - //	Available particle sizes 10 nm, 20 nm, 30 nm and 40 nm Size distribution 80% of the particles within ±2.5 nm
	CG-5 (b)	2	1 - 4	1 - 4	// - //	
	CG-5 (c)	20	10 - 30	10 - 30	// - //	
	CG-6 (a)	20	10 - 30	10 - 30	// - //	//
	CG-6 (b)	2	10 - 40	10 - 40	// - //	
	CG-6 (c)	2	1 - 4	1 - 4	// - //	
	CG-8	8	8 - 15	8 - 15	8 - 15	//

	CG-9	**4**	4 - 12	**3** - 12	// - //	//
	CG-10	2	2 – 6	2 - 6	// - //	//
	CG-11	//	11 - 85	// - 90	// - //	//
B. Gold Thioethylamino Hyaluronic Acid (nano)	SMG-2	7	13 - 16	10 - 14	23 - 29	Size /TEM (AunNP): 12 ± 3 nm Size / DLS (HA-AuNP): 26 ± 3 nm
	SMG-3	7	13 - 16	10 - 14	23 - 29	20 ± 5 nm (DLS)

SCCS comment
For some notifications A(G-2(b), CG-5 (b), CG-5 (c), CG-6 (a), CG-6 (b), CG-6 (c), CG-8, CG-9), the lowest limit of the particle size range has been reported as being lower than the lowest cut off level. This should be explained or corrected.

3.1.10 Microscopy

TEM images have been provided for A(G-1, G-6, CG-5, CG-11), B(SMG-2, SMG-3), supporting the determination of the nanoparticle size distribution for A (G-1, CG-5) and B(SMG-2). SEM images have been provided for A (CG-3).

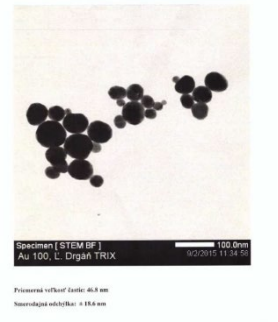

Fig. 1a: TEM image for A(CG-11)

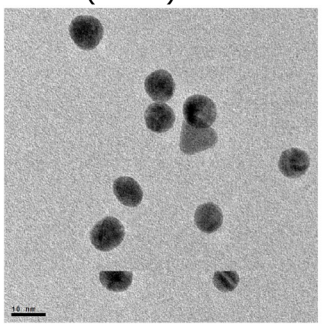

Fig. 1b : TEM image for B(SMG-2)

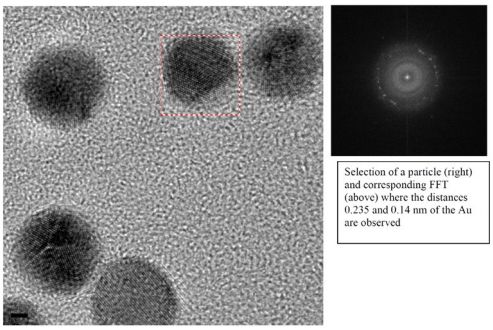

Fig. 1c : HR-TEM image for B(SMG-2)

SCCS comment
EM images have been provided for A(G-1, G-6, CG-5, CG-11), and B(SMG-2, SMG-3). For A(G-6), a TEM image has been provided without any scale.

3.1.11 Crystal structure

SCCS comment
Information was not provided on the crystal structure of all the materials. The provided information related to specific materials (i.e. A(G-1, G-6, CG-5, CG-11), and B(SMG-2, SMG-3), for which the electron microscopy images show spherical, triangular/pyramidal or irregular shaped particles.

3.1.12 UV absorption

B(SMG-3): In order to characterise conjugation of hyaluronic to gold, absorbance spectrum (UV-Vis) has been obtained (Figure 2). The first image represents the absorbance spectrum of isolated gold nanoparticles. The second one corresponds to conjugated gold nanoparticles. The maximum level of absorbance has been shifted to higher wavelengths. Gold Particles localized surface plasmon resonance (LSPR) is located at 519.5nm and Golden Hyaluronan is red-shifted to 521.5nm.

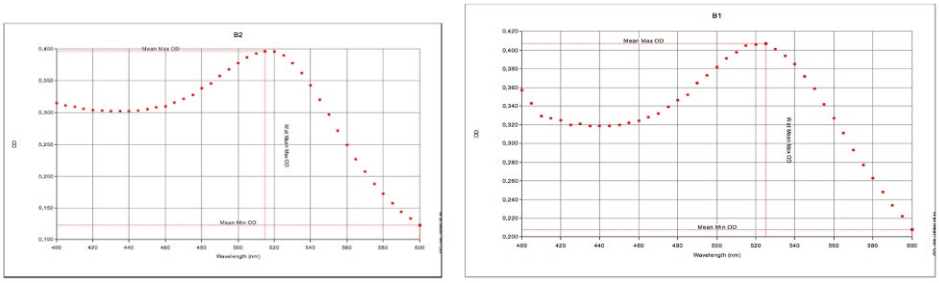

Figure 2: Absorbance spectrum – Left: gold nanoparticles, Right: B(SMG-3)

SCCS comment
Information related to UV absorption was provided for A(G-10, CG-3) and B (SMG-2 and SMG-3)

3.1.13 Surface characteristics

The 'surface charge' (Zeta potential) has been reported to be equal to -66.3 mV for A(G-9(b)), -40 mV for A(G-8), -38.7 for B (SMG-3), -34 for A(G-10), -32 mV for A(G-7), -2.69mV for A(G-9(a)), 0 mV for A(G-3) and B (SMG-2), 20 for A(CG-3), 30 mV for A(CG-9), 40 mV for A(CG-11(a)). The 'surface charge' (Zeta potential) has been noted as being not measurable for A(G-1, G-2, G-4, G-5, G-6, CG-1, CG-2, CG-5, CG-6, CG-8, CG-10, CG-11(b)).

A 'surface modification' or 'functionalization' has been reported for A (G-4, G-6, CG-8), B (SMG-2, SMG-3). For the others, nor 'surface modification' nor 'functionalisation' has been reported.

Surface 'coating" has been reported for A(G-4, G-8, G-10, CG-5(a), CG-6(b), CG-8), B (SMG-2, SMG-3). For the others, no coating has been reported.

The specific surface area (SSA/BET) has been reported to be equal to '1 m^2/g' for A(G-1, G-2, G-3, CG-1, CG-2, CG-11(b)), to '6 m^2/g' for A(G-5, G-9(b)), '66 m^2/g' for A(G-9(a)). For the others, no information has been provided.

The volume specific surface area (VSSA) has been reported to be equal to '1 m^2/cm^3' for A(G-1, G-2, G-3, CG-1, CG-2, CG-11(b)), to '18 m^2/cm^3' for A(G-9(b)), '180 m^2/cm^3' for A(G-9(a)). For the others, no information has been provided.

For one notification, the nanoparticle surface has been reported to be equal to 78.8 and 113.1 nm^2 B (SMG-3).

SCCS comment
Only cursory information on coating/ surface modification has been provided for A(G-4, G-6, G-8, G-10) without any indication of the nature of coating or surface modification.

3.1.14 Droplet size in formulations

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3.1.15 Homogeneity and stability

Information was provided for the shelf life of the notified materials (up to 18, 24 or 36 months).

SCCS comment
The information provided was inadequate. In the specific case of A(G-10), the SCCS has noted that the gold nanoparticles are considered as being stable up to 4 hours. The SCCS further noted that after 2 weeks storage, structural associations/ changes were reported to occur between gold nanoparticles and elements of organic origin.

3.1.16 Other parameters of characterisation

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3.1.17 Summary on supplementary physicochemical characterisation

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3.2 FUNCTION AND USES

The functions, uses and the use conditions of the various Gold(nano), Colloidal Gold(nano) and Surface modified Gold (nano) notifications have been reported in Table 1, Annex I.

SCCS comment
The concentration reported in some notifications is related to the dispersion/solution concentration, not the content of gold nanoparticles. Further information received in response to the SCCS query also did not provide clarification in this regard.

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

A(CG-3)

Guideline:	OECD 423 (2001); Acute Toxic Class Method
Species/Strain/Sex:	Rat, Sprague-Dawley, female
Group size:	3
Test substance:	A(CG-3)
Test batch:	PW-01

Purity:	not given
Dose:	2000 mg/kg bw
Dose Volume	2 ml/kg
Application:	single
Route:	oral (gavage)
Observation period:	15 days
GLP:	In compliance
Study period:	14 August 2003 – 23 September 2003

The acute oral toxicity of A(CG-3) was investigated in two groups of three female Sprague-Dawley rats at a dose of 2000 mg/kg bw. The starting dose was selected based on the assumption that it was most likely to produce mortality in some of the dosed animals. Overnight fasted animals received the test substance at 2000 mg/kg bw by oral gavage. After the first three animals, an additional group of three animals was treated. Animals were observed for 14 days after administration and killed on day 15 after administration. Mortality and general clinical observations were checked twice per day, body weights were determined on day 1 (day of administration) and on days 7, 14 and 15 after administration. On day 15, animals were killed, subjected to gross necropsy and organs (liver, spleen, kidneys, stomach, intestines, gonads, lungs and heart) were examined macroscopically.
The report states that body weights of the first group of animals were lower than normal during the second week, whereas mean weight gain in the second group was normal. The report further states that no organ or tissue gross findings were observed. The study authors concluded that the LD50 of the material investigated is higher than 2000 mg/kg bw.

Ref.: 1

SCCS comment
From the study report, it can be deduced that an aqueous liquid was used, which appears to be a dilution. The complete description of the chemical and physical properties of A(CG-3), including stability and certificate of analysis was not provided. Therefore, the actual dose applied is unclear. No conclusion on acute oral toxicity can be derived from that study in the absence of analytical details on the test material used.

SCCS overall comment on acute oral toxicity
Apart for one specific material for which a study was provided A(CG-3), only statements were provided for other materials (A(G-4, CG-8)) to say that pure gold is non-toxic and that gold is approved as a food additive in the EU. However, such statements are only relevant for the bulk form of the material and not the nano-forms. Thus, no conclusion on acute oral toxicity can be drawn from the information provided in the notifications.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin Irritation

A(G-4)– Skin irritation
See the results of the Human Repeat Insult Patch Test study on sensitisation: the data do not indicate an irritant potential of the test article on the human skin.

A(G-10)
Skin irritation - *In vitro* test

Guideline:	OECD 439 (2015)
System:	Reconstructed human epidermis ('EpiSkin')
Principle:	Colorimetric assessment of MTT reduction
Test substance:	Nanoparticles colloidal gold A(G-10) 66.6 mg/kg in water
Batch:	161017
Vehicle:	Water

Test concentrations:	50 µL undiluted on each tissue
Positive control:	Sodium Dodecyl Sulphate (SDS) 5% (w/v) in water
Negative control:	Dulbecco's Phosphate-Buffered Saline (D-PBS)
Runs:	Triplicate tissues, simultaneously, for test item and controls
GLP:	Yes
Date:	2017
Published:	No

According to the notification, preliminary tests were performed to detect the ability of the test item to directly reduce MTT as well as its colouring potential.
Following the preliminary tests, the skin irritation potential of the test item was tested in the main test. The test item and both the negative and positive controls were applied topically on triplicate tissues and incubated at room temperature for 15 minutes. At the end of the treatment period, each tissue was rinsed with D-PBS and incubated for 42 hours at +37°C, 5% CO_2 in a humidified incubator. The cell viability was then assessed by means of the colorimetric MTT reduction assay.
Relative viability values were calculated for each tissue and expressed as a percentage of the mean viability of the negative control tissues, which was set at 100%.

Results, according to the notification:
In the preliminary tests, the test item was found to have neither direct MTT reducing properties nor colouring potential.
Main test: All acceptance criteria for the negative and positive controls were fulfilled. The study was therefore considered to be valid.
Following 15 minutes exposure and 42 hours of recovery period, the relative mean viability of the tissues treated with the test item was 95% with a standard deviation of 3%. As the mean viability was > 50% after the MTT reduction, the results met the criteria for a non-irritant response.

Conclusion from Notifier
Under the experimental conditions of this study, the test item, A(G-10), is considered to be non-irritant to skin.
According to the results of this study, the classification of the test item should be No Category (UN GHS and Regulation (EC) No. 1272/2008).

Ref.: 2

SCCS comment
The test results indicate that the tested material has no skin irritant properties. However, the *in vitro* skin irritation test using RhE has not yet been validated/ evaluated for nanomaterials.

A(CG-3) batch PW-01
Acute skin irritation test according to OECD 404

Guideline/method:	OECD TG 404 (April 2002)
Species/strain:	Rabbit (New Zealand Albino)
Sex:	Male 2.9 – 3.1 kg at start of study
Housing conditions:	Individual in standard cages, RT 17-21^{0}C, humidity 45% - 65%.
Group size:	3
Test substance:	A (CG-3) (batch PW-01)
Batch:	PW-01 (Certificate of analysis not provided)
Appearance:	Slight crimson liquid
pH:	7 (as measured with pH paper)
Vehicle:	Not provided
Concentration:	0.02%

Application:	Single application of 0.5 mL of G-water on an area of approximately 6 cm^2.
Exposure time:	4 hours
Route:	Topical application on skin (semi-occluded). Test substance directly added to skin and covered with a gauze. The gauze was protected by a pad.
Read out:	One hour, 24, 48 and 72 hours after removal of the dressing
GLP:	Yes
Date:	August 2003. Report November 2003
Published:	No

Study results:
Mean indices were calculated from results obtained from each rabbit at times 24, 48, and 72 hours. The non-treated area of the test animal serves as negative control (OECD TG 404). Results obtained were as follows:

Table 3: Mean index

Treatment	Animal number	Erythema	Oedema
A(CG-3)	20030469	0	0
	20030470	0	0
	20030471	0	0

Conclusion provided by Notifier:
Under the experimental conditions adopted, A(CG-3) (batch PW-01) was found to be non-irritant for the skin of the rabbit.

Ref.: 3

SCCS comment
The concentration of gold particles in the A(CG-3) used in the tests was not stated in the study report. From an accompanying document it can be deduced that it is probably 0.02 % (w/w).
The application of the A(CG-3) liquid was directly on the skin. OECD TG404 indicates for liquids to be first applied to the gauze patch which is then applied to the skin.
Information concerning the vehicle was not provided.

A(CG-3): batch PW-01
Local tolerance after repeated daily application

Guideline/method:	-
Species/strain:	Rabbit (New Zealand Albino)
Sex:	Male and female between 2.1 – 2.4 kg at start of study
Housing conditions:	Individual in standard cages, RT 17-21°C, humidity 45% - 65%.
Group size:	6 (3 males and 3 females)
Test substance:	A(CG-3) (batch PW-01)
Batch:	PW-01 (Certificate of analysis not provided)
Appearance:	Slight crimson liquid
pH:	8.4
Vehicle:	not provided
Concentrations:	Repeated application of 0.5 mL of A(CG-3)on right scarified flank and left non scarified flank.
Application:	Once a day for 14 consecutive days
Route:	Topical application on scarified and non-scarified skin.
Read out:	Once a day one hour after treatment day 1 to day 9, and from day 10 before the next treatment
GLP:	Yes
Date:	September 2003. Report February 2004

Published: No

No mortality occurred during the study.
No clinical signs were seen during the study.
Body weight changes were normal during the course of the study.
Oedema and erythema scores were 0.00 for all six rabbits from day one to the day of necropsy.
Stomach: White spots on the fundic zone in male No. 20030627, red zone on the fundic zone in female No. 20030628, white zones on the glandular zone in female No. 20030629 and white spots and white points on the glandular zone in female No. 20030630 were noted.
Lungs: Presence of white raised zones on two lobes was observed in female No. 20030630. These observations were not treatment-related.
There were no other observations at the necropsy of the rabbits.

Conclusion from Notifier
Under the experimental conditions adopted, A(CG-3) (batch PW-01) was found to be non-irritant on scarified and non-scarified skin in the rabbit after repeated daily application during 14 days.

SCCS comment
The concentration of gold particles in the A(CG-3) used in the tests was not stated in the study report. From an accompanying document it can be deduced that it is probably 0.02 % (w/w). The lack of irritation at the tested low concentration does not exclude the possibility of irritation at higher concentrations.

Ref.: 4

A(CG-8) - Skin Irritation
See the results of the Human Repeat Insult Patch Test study on sensitisation: the data do not indicate an irritant potential of the test article on the human skin.

SCCS comment
The limited data provided indicate that the skin irritating effect at the use concentrations is unlikely.

B(SMG-2) - Skin irritation - *In vitro* test

Guideline:	OECD 439 (2015)
System:	Reconstructed human epidermis ('Skinethic')
Principle:	Colorimetric assessment of MTT reduction
Test substance:	Nanoparticles gold conjugated to hyaluronic acid in citric acid and water, concentration not specified
Batch:	B (SMG-2) GH-53
Vehicle:	not specified, probably water
Test concentrations:	16 µL at concentration of 1.0078 mg/ml
Positive control:	Sodium Dodecyl Sulphate (SDS) 5% (w/v) in water
Negative control:	Phosphate-Buffered Saline (D-PBS)
Runs:	Triplicate tissues, simultaneously, for test item and controls
GLP:	Yes
Date:	2020
Published:	No

According to the notification, the skin irritation of the HA-Au-NPs has been newly assayed by measuring the viability in the Skinethic™ reconstructed human dermal epidermis (Skinethic™ RhE). The Test Item was applied for 41 minutes, the inserts were washed, and the plate was incubated for 41 hours. Once the period of incubation ended, MTT was applied to the inserts

in order to quantify their viability spectrophotometrically at 570 nm. The inserts treated with the Test Item showed a mean viability of 59.81%. Therefore, according to the international guidelines DB-ALM Method Summary no. 117, DB-ALM Protocol no. 135 and OECD TG 439, the Test Item can be considered as a non-irritant agent that classifies as no category, confirming the results derived from the human Patch test.

Ref.: 5

SCCS comment
The concentration of the nanoparticles in the test sample was not specified in the study report. The results indicate that the tested article has no skin irritant properties. However, the RhE model has not (yet) been validated/ evaluated for nanomaterials.

General SCCS comments on irritation and corrosivity test results provided
The notification dossiers include study reports on A(G-4, G-10, CG-3 and CG-8) and B(SMG-2).
For these materials, the tests do not indicate a skin irritation potential. It should be noted that only one concentration was tested in each test, apparently corresponding to the concentration in the unformulated ingredient. However, except for A(G-10), these concentrations are not clearly specified in the test reports.

3.3.2.2 Eye Irritation

A(G-4) Eye irritation

SCCS comment
Only global statements on gold are provided for material A (G-4), not specifically addressing the nano-form. No conclusions on eye irritation can be drawn from this information.

A(G-10)
Mucous membrane irritation/eye irritation

Guideline:	OECD 492 (2015)
Cells:	Reconstructed human Cornea-like Epithelium (tissues)
Material:	A(G-10)
Solvent:	/
Batch:	161017
Composition:	66.6 ± 1.4 mg/kg
Concentrations:	Two experiments: 0, 588.63, 1265.55, 2720.93, 5850, 12577.51, 27041.64, 58139.53 and 125000 µg/mL
GLP compliance:	Yes
Study Period:	December 2017

Preliminary tests were performed to detect the ability of the test item to directly reduce MTT as well as its colouring potential. Following the preliminary tests, the eye irritation potential of the test item was assessed in the main test. The test item and both negative and positive controls were applied topically on duplicate tissues and incubated at +37°C for 30 minutes. At the end of the treatment period, each tissue was rinsed with D-PBS, incubated for 12 minutes at room temperature to remove any remaining test item from the tissue, blotted on absorbent material, and then incubated for another 2 hours at 37°C, 5% CO_2 in a humidified incubator. The cell viability was then assessed by means of the colorimetric MTT reduction assay. Mean viability values were calculated for each tissue and expressed as a percentage of the mean viability of the negative control tissues, which was set at 100% (as reference viability).

Results

Preliminary test

In the preliminary tests, the test item was found to have neither direct MTT-reducing properties, nor colouring potential.

Main tests

All acceptance criteria for the negative and positive controls were fulfilled. The study was therefore considered to be valid.

The relative mean viability of the tissues treated with the test item was 96% with a difference of 4% between duplicate tissues. As the mean viability was > 60% after the MTT reduction, the results met the criteria for a non-irritant response.

Conclusion from Notifier

Under the experimental conditions of this study, the test item, A(G-10), is considered to be non-irritant to reconstructed human Cornea-like Epithelium.
According to the results of this study, the classification of the test item should be No Category (GHS 2015 and Regulation (EC) No. 1272/2008).

Ref.: 6

SCCS comment

The SCCS assumes that A(G-10) (Batch 161017) is a mixture containing colloidal gold (CAS number 7440-57-5) nanoparticles in suspension at 66.6 mg/kg (± 1.4 mg/kg) in water (determined by ICP-MS method) with traces of plant extracts (*Hubertia ambavilla*). It can be calculated that the highest concentration tested of 125000 µg/mL (prepared from nanogold stock solution) corresponded to the final concentration of gold nanoparticles of 8.325 µg/mL. Such low concentrations might not correspond with conditions of a valid study.
The SCCS notes that the test has not yet been adopted for nanomaterials. Further details on demonstration of assay interference (as recommended in SCCS/1611/19) have not been provided.

A(CG-3) - batch PW-01
Acute eye irritation study (OECD TG 405)

Guideline/method:	OECD TG 405 (April 2002)
Species/strain:	Rabbit (New Zealand Albino)
Sex:	Male between 3.6 – 3.9 kg at start of study
Housing conditions:	Individual in standard cages, RT 17-21˚C, humidity 45% - 65%.
Group size:	3 (3 males)
Test substance:	A(CG-3) (batch PW-01)
Batch:	PW-01 (Certificate of analysis not provided)
Appearance:	Slight crimson liquid
pH:	7 (as measured with pH paper)
Vehicle:	not provided
Concentrations:	Application of 0.1 mL of undiluted G-water.
Application route:	Conjunctival sac of the left eye.
Control:	Contralateral eye
Read out:	Conjunctival, iris and corneal lesions at one hour, 24, 48, and 72 hours after application
Scoring:	Chemosis 0-4, redness 0-3, appearance iris 0-2, cornea opacity 0-4, cornea area of involvement 0-4
GLP:	Yes
Date:	August 2003. Report November 2003
Published:	No

Study results:
Mean indices were calculated from results obtained from each rabbit at times 24, 48, and 72 hours. The non-treated right eye of the test animal serves as negative control (OECD TG 405). Results obtained were as follows:

Table 4: Mean index.

Treatment	Animal number	Erythema	Oedema
G-Water	20030364	0	0
	20030365	0	0
	20030366	0	0

The individual scores for Chemosis, Redness, Iris, Cornea opacity and Cornea involvement were all negative.

Conclusion from Notifier
Under the experimental conditions adopted, A(CG-3) (batch PW-01) was found to be non-irritant for the eye of the rabbit.

SCCS comment on A(CG-3) test performed
The concentration of gold particles in the A(CG-3) used for the tests was not stated in the study report. From an accompanying document, it can be deduced that it is probably 0.02 % (w/w).
The characterisation of the chemical and physical properties of the test sample was not provided and described to be the responsibility of the Sponsor. An analysis certificate of the test substance was not provided by the Sponsor.
Information concerning the vehicle was also not provided.

Ref.: 7

CG-8 – Colloid PMG-PG (silk)

SCCS comment
Only general statements on gold are given, not specifically addressing the nano-form. No conclusions on eye irritation can be drawn from this information.

Ref.: 8

SCCS overall comment on eye irritation
Study reports were provided for A(G-10) and A(CG-3).
For these materials, the tests do not indicate an eye irritation potential of the tested solutions. However, there is no clear information on the actual gold concentration tested, which appears to be very low. The assays used have not been demonstrated to be valid for nanomaterials.
Therefore, no conclusion on the irritation potential can be drawn based on the notified information.

3.3.3 Skin sensitisation

A(G-4)
Method: Human Repeat Insult Patch Test study
Subjects: 51 humans (39 women, 12 men)
Test substance: A(G-4)
Batch: Lot No. 1031005316
Concentrations: 'As is', undiluted
Route: topical on the back, under occlusive patch for 24 hrs

Induction:	3x per week during 3 weeks with 0.2 ml or 0.2 g
Challenge:	10-14 days after last induction (week 6)
Control:	None
GCP:	Reviewed by Institutional Review Board
Date:	2007
Published:	No

No adverse reactions of any kind were noted during the course of this study. The test material when tested under occlusion as described may be considered as a non-primary irritant and a non-primary sensitizer to the skin according to the reference.

Ref.: 9

SCCS comment on A(G-4) skin sensitisation
Predictive human sensitisation tests of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients should not be undertaken (SCCNFP/0120/99, SCCS/1576/15). Historical data may be considered.
It is not clear whether the test article was equivalent to the undiluted/undispersed raw material (apparently a powder) and, consequently, whether the amount of test article applied in ml is similar to the amount in grams.
The limited information from the submitted study does not indicate a sensitising potential of the test article. With a completely negative test among 50 participants, the sample size is considered too small to yield an acceptable confidence interval.

A(G-10)
***In vitro* sensitisation tests**
A

Guideline/method:	ARE-Nrf2 luciferase ('KeratinoSens') OECD 442D
System:	HaCaT cell line transfected with luciferase gene
Test substance:	Nanoparticles colloidal gold A(G-10) 66.6 mg/kg in water
Batch:	161017
Vehicle:	Water
Test concentrations:	0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, 200, 400 µg/mL
Positive control:	Cinnamic aldehyde in DMSO, conc. ranging from 4 to 64 µM
Negative control:	DMSO 1%
Runs:	4
GLP:	Yes
Date:	2018
Published:	No

According to the notification, since no precipitate/emulsion was observed in the test item-treated wells at the end of the treatment period, the absence of Log P value is no longer considered to be a limitation for the applicability of this test.
Furthermore, during this study, highly heterogeneous results were obtained since the first run was considered as negative, the second as inconclusive, the third as positive, and finally the fourth as negative. Therefore, only two of the four runs performed gave concordant negative results. Nevertheless, the final outcome is negative, in agreement with the OECD Guideline. This negative result can be used to support the discrimination between skin sensitizers and non-sensitizers in the context of an integrated approach to testing and assessment. It cannot be used on its own to conclude on a skin sensitisation potential.

It can be noted that during the only positive run (third run), an induction < 1.5 was observed at the highest but non cytotoxic concentration, while statistically significant gene-fold inductions above the threshold of 1.5 were noted at lower concentrations. This unexpected result (decrease of the induction not related to cytotoxicity) can be due to a weak potential

of the Test Item, A(G-10), to activate the Nrf2 transcription factor, supported by the low induction values not substantially higher than 1.5.

Conclusion, as reported in the notification:
Under the experimental conditions of this KeratinoSens assay, the test item, A(G-10), was found to be negative in two concordant runs out of the four performed. Therefore it was considered to have no potential to activate the Nrf2 transcription factor.

Ref.: 2

B

Guideline/method:	Human cell line activation test – hCLAT. Pre-OECD 442E
System:	Human monocytic leukaemia cell line, THP-1 cells
Test substance:	Nanoparticles colloidal gold A(G-10) 66.6 mg/kg in water
Batch:	161017
Vehicle:	Water
Test concentrations:	0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, 200, 400 µg/mL
Positive control:	DNCB with DMSO diluted to 8µg/ml in the culture medium and $NiSO_4$ with 0.9% NaCl diluted to 200 µg/ml in the culture medium.
Negative control:	no vehicle control, culture medium used as control
Runs:	4, of which 1 inconclusive
GLP:	Yes
Date:	2018
Published:	No

Table 5: Results and conclusion as reported in the notification:

Test item Name	Conc. (µg/mL)	RFI for CD86				RFI for CD54				Viability (%)				Run conclusion				General conclusion
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
A(G-10)	1395.4	112	111	91	105	98	104	136	104	95.4	96.0	97.4	96.0	I	N	P2	P2	Positive
	1674.5	94	101	89	95	106	84	154	142	94.9	95.6	97.2	95.8					
	2009.4	96	92	109	92	112	148	129	167	94.6	95.2	97.6	96.4					
	2411.3	107	93	100	100	90	112	104	113	94.7	94.7	97.5	96.3					
	2893.5	108	103	98	88	108	92	125	167	95.4	96.0	97.3	95.3					
	3472.2	110	87	88	86	132	146	125	204	95.3	95.4	97.2	95.5					
	4166.7	121	93	106	107	104	130	146	154	95.6	96.0	97.9	95.7					
	5000.0	101	92	97	91	154	172	346	213	94.9	94.9	97.2	95.0					

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N = run with negative outcome
P_1 = run with positive outcomefor CD86
P_2 = run with positive outcome for CD54
P_{12} = run with positive outcome for CD86 and CD54

I = Invalidated run
Inc = Inconclusive run

Conc. = concentration
RFI = Relative Fluorescence Index
I = Invalidated run

Under the experimental conditions of this study, the test item, A(G-10), was found to be positive in the h-CLAT assay.

Ref.: 2

C

Guideline/method:	Gene upregulation in RHE: SENS-IS. Under ECVAM validation
System:	Reconstructed Human epidermis (RHE) - EpiSkin
Test substance:	Nanoparticles colloidal gold A(G-10)
Batch:	NPTSK1MBH310718
Vehicle:	PBS and DMSO
Test concentrations:	1%, 10% and 50% in PBS and 10% in DMSO
Positive control:	TNBS 1M
Negative control:	DMSO 100%
Nr of experiments:	2
GLP:	Yes
Date:	2018

Published: No

Results and conclusion as reported in the notification:
In the first experiment, the test item "Nanoparticules d'or" induced less than 7 genes in the "SENS-IS" and "ARE" gene groups when tested at 1, 10 and 50% (v/v) in PBS and at 10% (v/v) in DMSO. In the second experiment, the test item "Nanoparticules d'or" induced less than 7 genes in the "SENS- IS" and "ARE" gene groups when tested at 50% (v/v) in PBS and at 100% (not diluted).
Considering the number of over-expressed gene in the "SENS-IS" and "ARE" gene groups, the test item " Nanoparticules d'or" gave negative result (less than 7 genes induced) when it was tested diluted at 1,10 and 50% (v/v) in PBS and at 10% (v/v) in DMSO. Moreover, negative results were also obtained when the test item was tested at 100% (not diluted). In conclusion, under the experimental conditions of this SENS-IS assay, the test item" Nanoparticules d'or" can be classified as a non-sensitizer.

Ref.: 2

SCCS overall comment on the sensitisation studies performed on A(G-10)
Although sensitisation to ionised gold has been documented, the limited information from the submitted studies on colloidal gold does not indicate a sensitising potential.

For A and B reported studies, the SCCS assumes that A(G-10) (Batch 161017) is a mixture containing colloidal gold (CAS number 7440-57-5) nanoparticles in suspension at 66.6 mg/kg (± 1.4 mg/kg) in water (determined by ICP-MS method) with traces of plant extracts (Hubertia ambavilla). It can be calculated that the highest concentration tested of 400 µg/mL (prepared from nanogold stock solution) corresponded to the final concentration of gold nanoparticles of 0.02664 µg/mL.

The notification indicates that the composition of the initial sample (A(G-10) undiluted) of the test article is moderately polydispersed (dispersion between 0.25 and 0.38). The test article used in the SENS-IT assay seems to be representative of the abovementioned test article used in the other tests. The report on SENS-IT indicated a solubility of the test article in PBS and in DMSO at 10% and 50%, which seems unlikely in view of its physicochemical properties.

Although testing with a stable dispersion is according to the OECD guideline acceptable for the KeratinoSens ARE-Nrf-2 luciferase assay, there is very limited experience with the testing of nanoparticles in the *in-vitro* assays. It is as yet unknown whether the tested gold nanoparticles can undergo haptenation (covalent binding to proteins) as a key event in the sensitisation process.

A(CG-3)

Guideline/method:	OECD 406
Species/strain:	Guinea Pig (Albino Hartley)
Group size:	10 males treated, 5 males negative control, 5 males positive control, 6 males preliminary test
Test substance:	A(CG-3)Negative controls: water (with and without FCA in isotonic saline)
Batch:	PW-01 (Certificate of analysis not provided)
Vehicle:	Water with and without FCA in isotonic saline
Concentrations:	100% and 50% for induction, 100% and 50% for challenge
Positive controls:	DNCB in alcohol (with and without FCA in isotonic saline)
Route:	Injection and topical
Induction:	On Day 1 injection of test items with and without FCA On Day 8 irritation with 10% SDS, on Day 9 topical application of test items

Challenge: On Day 22 on the flank topical application of test items, reading on Day 23 and 24
GLP: Yes
Date: 2003
Published: No

Table 6: Summary of results from challenge on days 22/23/24

Treatment	Time	number of animals score 0	number of animals score I	number of animals score 2	number of animals score 3	% of sensitised animals
Pos control	24 h	0	3	2	0	100
	48h	3	2	0	0	40
Neg control	24h	5	0	0	0	0
	48 h	5	0	0	0	0
Testsubstance	24 h	10	0	0	0	0
	48h	10	0	0	0	0

Negative control = solvent of study test substance.
Positive control= 1% dinitrochlorobenzene (DNCB) in alcoholic solution.

The sensitising capacity of A(CG-3) was studied in the male Guinea pig, in comparison with a negative control group receiving only sterile water during the induction phases. The sensitivity and the reliability of the experimental method were verified, using a positive control group in which animals were treated with dinitrochlorobenzene (D.N.C.B., 1%).
Under the experimental conditions adopted, the test substance A(CG-3) (batch PW-01) showed no allergenicity at 24 and 48 hours. According to the terminology employed, it is considered that the test substance is free of any sensitising capacity in the male Guinea pig.

Ref.: 10

A(CG-3) : Human data on sensitisation
Method: Human Repeat Patch test study (Marzulli & Maibach)
Subjects: 50 humans (46 women, 4 men)
Test substance: A(CG-3)
Batch: PW-01 (Certificate of analysis not provided)
Concentrations: As is
Route: topical for induction, topical for challenge, both with Finn chambers
Induction: 3x per week during 3 weeks with 25 microliter
Challenge: single application at day 40 (week 6)
Control: Blank Finn chamber
GCP: Yes
Date: 2004
Published: No

No significant clinical manifestation of intolerance or allergy was observed by the investigator. In the conditions of the study, this product presents no sensitizing potential.

Ref.: 11

SCCS comment on A(CG-3) skin sensitisation (*in vivo* and human data)
The concentration of gold particles in the 'A(CG-3)' used for the tests was not stated in the study report. From an accompanying document it can be deduced that it is probably 0.02 % (w/w). Only a Guinea pig maximisation study and a human repeat patch test study were available to evaluate the sensitising properties of A(CG-3). Although sensitisation to metallic gold and gold salts has been documented, the limited information from the submitted studies on colloidal gold does not indicate a sensitising potential. While the concentrations of the colloidal gold in the test article is unclear, it may have been too low to detect a sensitising

potential. Predictive human sensitisation tests of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients should not be undertaken; historical data may be considered. (SCCNFP/0120/99, SCCS/1576/15).

A(CG-8)

Method:	Human Repeat Insult Patch Test study
Subjects:	51 humans (39 women, 12 men)
Test substance:	A(CG-8)Batch: Lot No. 1031005316
Concentrations:	'As is', undiluted
Route:	topical on the back, under occlusive patch during 24 hrs
Induction:	3x per week during 3 weeks with 0.2 ml or 0.2 g
Challenge:	10-14 days after last induction (week 6)
Control:	None
GCP:	Reviewed by Institutional Review Board
Date:	2007
Published:	No

Results and conclusion according to the Notifier

No adverse reactions of any kind were noted during the course of this study. The test material when tested under occlusion as described may be considered as a non-primary irritant and a non-primary sensitizer to the skin according to the reference.

Ref.: 8

SCCS comment on A(CG-8) skin sensitisation

The test report included in the safety file is exactly the same as the report of the HRIPT that was performed for A(G-4). The composition of the material to which the safety report on A(CG-8) refers appears to be different: besides colloidal gold it contains pentylene glycol (0.09 – 1.10 %) and hydrolysed silk (0.04-0.08 %).

The limited information from the submitted study does not indicate a sensitising potential of the test article. A total of 50 participants had completely negative test results, but the sample size is considered too small to yield an acceptable confidence limit. In addition to the objection raised above, in view of the above, the test cannot be accepted because of a discrepancy between the material/ingredient and the test article.

Predictive human sensitisation tests of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients should not be undertaken; historical data may be considered (SCCNFP/0120/99, SCCS/1576/15).

B(SMG-2) - **Skin sensitisation – *In vitro***

Guideline/method:	Human cell line activation test – hCLAT. OECD 442E
System:	Human monocytic leukaemia cell line, THP-1 cells
Test substance:	Gold nanoparticles conjugated to hyaluronic acid with sodium citrate in water, concentration not specified.
Batch:	B(SMG-2) GH-53
Vehicle:	Unclear, probably water
Test concentrations:	10%, 8.3%, 6.9%, 5.8%, 4.8%, 4%, 3.3%, 2.8%
Positive controls:	DNCB 4 µg/mL and $NiSO_4$
Negative control:	apparently the culture medium was used as control
Runs:	2
GLP:	Yes
Date:	2020
Published:	No

Results and conclusion as reported in the notification:

The cells treated with eight concentrations of Test Item at 2.8-10% showed a viability >96.2% and RFI values <150 for CD54 and < 200 for CD86. Therefore, HA-Au-NPs were shown to be non-sensitising agents in an *in vitro* skin sensitization human cell line activation test (h-CLAT).

SCCS comment
There is very limited experience with the testing of nanoparticle dispersions in the *in-vitro* sensitisation assays. It is as yet unknown whether the tested gold nanoparticles can undergo haptenation (covalent binding to proteins) as a key event in the sensitisation process. It is also unclear which vehicle was used for the serial dilutions of the test item. The concentration of the nanoparticles in the original test item was not provided in the study report.

Ref.: 12

General SCCS comments on skin sensitisation test results provided
Study reports are available for A(CG-3), A(G-4) and A(G-10) and B(SMG-2).
Sensitisation to metallic gold (postulated to originate from released ions) has been documented. Although the limited information from the submitted studies on nano-gold do not indicate a sensitising potential of the test articles, the SCCS regards these studies as inconclusive, with the exception of the study on A(G-10).
It should be noted that there is as yet very limited experience with the testing of gold nanoparticles for sensitisation. And it is as yet unknown whether the tested gold nanoparticles can undergo haptenation (covalent binding to proteins) as a key event in the sensitisation process. A recent *in viv*o study [Roach *et al.* (2020)] with nano-gold particles did not indicate a sensitising potential.

Ref.: 13

3.3.4 Dermal/percutaneous absorption

A (G-10)

Tissue:	Human skin explant from a Caucasian woman of 27 years old (ref. P2172-AB27)
Group size:	3 explants per group and 4 groups plastic control, untreated control, P1 group and P2 group
Skin integrity:	Microscopic examination on paraffin sections after staining with Godner's variant Masson trichrome
Test items:	NP COS 090719 A(G-10)(P1) Oils TSK20190715 batch 20190715 (P2)
Replicates:	3
Controls:	untreated skin and "controle plastie"
Nanoparticle concentrations:	P1 = 1.97 g/ml and P2 = 3 mg/ml
Method of analysis:	Transmission Electron Microscopy (TEM)
GLP compliance:	No
Period:	July – September 2019

The test items investigated were final cosmetic product formulations and it was stated that gold concentration (no further information on characterisation of gold NPs) is 0.15 %. The final product formulations were applied onto skin explants prepared from one female volunteer which were cultivated in BIO-ECs Explant Medium at 37°C and in an atmosphere of 5 % CO_2.
On days 0, 1, 2 and 3, the products P1 and P2 were applied morning and evening, topically, at a rate of 2 µl per explant (2 mg/cm^2) and spread using a spatula.
Control explants received no treatment except for renewal of the medium.
Half of the medium was renewed (1 ml per well) on day 1 and day 2.
On day 5 (D5), explants were divided and fixed differently for microscopic analysis (cell viability) and transmission electron microscopy (to determine skin penetration).

Results
Cellular viability of the different groups is reported below.

Table 7: Cellular viability

Group	Cellular viability	
	Epidermis	Dermis
Control group Day 0	Good	Good
Control group Day 5	Reasonable	Good
P1 group Day 5	Reasonable	Good
P2 group Day 5	Reasonable	Good

Legend: Good, Reasonable, Slightly altered, Moderately altered, Quite clearly altered, Significantly altered, Very significantly altered.

Gold nanoparticle skin penetration is reported in the table below.

Table 8: Gold nanoparticle skin penetration

Group	Gold nanoparticle skin penetration			
	Stratum corneum	Stratum granulosum	Stratum spinosum	Dermo-epidermal junction / papillary dermis
Control group Day 5	ND	ND	ND	ND
P1 groupe Day 5	D	ND	ND	ND
P2 groupe Day 5	ND	ND	ND	ND

Legend: Not detected = ND, D = Detected
At D5, on the control group, no nanoparticle was detected regardless of the skin compartment.

At D5 after application of P1 (nanoparticle), gold nanoparticles were systematically and easily observed at the surface of the upper layer of the stratum corneum. Nanoparticles are found either in clusters of different sizes, or individually. The distribution is random and non-continuous. No nanoparticle was detected in the other skin compartments.

At D5, no nanoparticle was detected regardless of the skin compartment for group P2 (oil).

Conclusion from the Notifier
Products NP COS 090719 A(G-10) (P1) and Oils TSK20190715 (P2) are well tolerated. They do not induce any morphological alteration.

The product NP COS 090719 A(G-10) (P1) is associated with the systematic presence of gold nanoparticles on the surface of the upper layer of the Stratum corneum. Nanoparticles are found either in clusters of different sizes, or individually. Their distribution is random and non-continuous. No trace of nanoparticles was visualized in the other skin compartments.

The product TSK20190715 (P2) oils is not associated with the presence of gold nanoparticles on the surface or within the cutaneous tissue. No trace of nanoparticles was visualized in the other skin compartments.

Ref.: 14

SCCS comment
The SCCS notes that contradicting information on gold concentration was presented in the submission file and in the original study report, and information is missing on the material characterisation. The exact concentration of the gold nanoparticles in the test material is not clear and this should be provided.

The study performed was not a dermal penetration study as recommended by the SCCS (SCCS/1602/18 and SCCS/1611/19). Furthermore, only one human donor was used. Although this study points to the absence of dermal penetration, in view of the comments above, it is of limited relevance to assess dermal penetration of the material under investigation.

Other studies on toxicokinetics

B(SMG-2)
***In vitro* permeability across Caco-2 cell monolayers**

Guideline:	/
System:	Caco Ready™ Caco—2 Cells
Principle:	Measurement of permeation through Caco-2 cell layer in the absence or presence of a P-Glycoprotein inhibitor (Valspodar)
Test substance:	B(SMG-2) (gold nanoparticles conjugated with Hyaluronic acid)
Batch:	GH-53
Vehicle:	HBSS (Hank's Balanced Salt Solution) containing 1.3 mM $CaCl_2$ and 0.5 mM $MgCl_2$
Test concentrations:	25, 50 and 100 µg/ml
Duration:	2 hr
Positive controls:	(±)-Propranolol Hydrochloride for high permeability Atenolol, 98% for low permeability
Replicates:	3
GLP:	No
Date:	2020
Published:	No

The permeability of B(SMG-2) across Caco-2 cell monolayers was investigated *in vitro*. B(SMG-2) was applied to the apical chamber of the Caco-2 transwells at three concentrations in the presence and absence of the P-glycoprotein inhibitor Valspodar. The content in gold as a marker for the Test Item was measured by IPC-MS in samples from the apical chamber at start (T=0) and after 2 hr and in samples from the basal chamber after the 2-hours incubation period at 37°C.

All the controls applied to the assay demonstrated the correct barrier functionality of the Caco-2 cells and validated the experiments: i.e. TEER measurement (pre-assay control), high and low permeability positive controls and post-assay permeability of Lucifer Yellow.
The results of the assay showed that the levels of gold were below the limit of detection (< 2.5 ng) in the basal chamber at all the concentrations tested (25, 50 and 100 µg/mL) at both experimental conditions, strongly suggesting that the apparent permeability of B(SMG-2) was very low or negligible, although some low permeability of B(SMG-2) across the Caco-2 monolayer cannot be fully discarded.

Ref.: 15

SCCS comment
While the Caco-2 cell assay addresses intestinal permeability, it does not inform about skin absorption and uptake by other organs. It has not been validated for nanomaterials. Therefore this study has not been considered in this Opinion.

3.3.5 Repeated dose toxicity

A(G-10)
Repeated dose (8 days) oral / dermal / inhalation / intraperitoneal toxicity
Guideline: Not reported

Species/Strain: Male C57/BL6 mice
Route: Intraperitoneal
Group size: n = 8
Material: AuNPs citrate surface coating (please see below characteristics)
Dose: 0, 40, 200, 400 µg/kg/day
Exposure: 8 days
GLP compliance: No
Study Period: 2010

Table 9: Composition and characteristics of gold nanoparticles (GNPs) used in this study.

Coating	Citrate surface coating
Morphology and primary size	12.5 nm ± 1.7 nm with regular shapes and narrow size distribution
Resonance peak	520 nm
Zeta potential	- 53 mV

Results
The gold levels in blood did not increase with the dose administered, whereas in all the organs examined there was a proportional increase of gold, indicating efficient tissue uptake. Although brain was the organ containing the lowest quantity of injected GNPs, our data suggest that GNPs are able to cross the blood–brain barrier and accumulate in the neural tissue. Importantly, no evidence of toxicity was observed in any of the diverse studies performed, including survival, behaviour, animal weight, organ morphology, blood biochemistry and tissue histology. The results indicate that the tissue accumulation pattern of GNPs depend on the doses administered and the accumulation of the particles does not produce subacute physiological damage.

Ref.: 16

SCCS comment
This is a literature study, which was not carried out according to the official guidelines, and the route of administration is intraperitoneal. Also, there is uncertainty about whether the used gold nanoparticles are the same as notified A(G-10) material or other notified materials.

A(G-10)
Subchronic (90 days) oral / dermal / inhalation / intravenous toxicity
Guideline: Not reported
Species/Strain: Male Sprague-Dawley rats
Route: Intravenous
Group size: n = 9
Material: AuNPs citrate surface coating (please see below characteristics)
Dose: 0, 0.9, 9, 90, µg per rat
Exposure: 7 days per week for 7 weeks followed by a 14-day washout period
GLP compliance: No
Study Period: 2016

Table 10: Composition and characteristics of gold nanoparticles used in this study.

Coating	Citrate surface coating		
Morphology and primary size	14 ± 1.2 nm and a spherical shape		
Resonance peak	520 – 530 nm		
Dispersion	Monodispersity		
Zeta potential	- 47 mV		
Hydrondynamic size	25 nm		
Administered mass of AuNPs (mg) per rat	90	9	0.9

Administered number of AuNPs (10^{12}) per rat	3.3	0.33	0.033
Administered surface area (cm^2)	20.2	2.02	0.202

Results
After sacrificing, the amount of gold was quantified in the liver, lungs, spleen, skeleton and carcass using neutron activation analysis (NAA). During the study, pre and post (24 h) administration blood samples were collected from both the test and control groups, the latter which received an equal injection volume of normal saline. General health indicators were monitored together with markers of kidney and liver damage for acute and subchronic toxicity assessment. Histopathological assessments were done on the heart, kidneys, liver, lungs and spleen to assess any morphological changes as a result of the exposure to AuNPs. The mass measurements of all the groups showed a steady increase with no signs of overt toxicity. The liver had the highest amount of gold (µg) per gram of tissue after 56 days followed by the spleen, lungs, skeleton and carcass. Markers of kidney and liver damage showed similar trends between the pre and post samples within each group and across groups. The histopathological examination also showed no hepatotoxicity and nephrotoxicity. There was accumulation of Au in tissues after repeated dosing, albeit with no observable overt toxicity, kidney or liver damage.

Ref.: 17

SCCS comment
This is a literature study, which was not carried out according to the official guidelines, and the route of administration is intravenous. Also, there is uncertainty about whether the used gold nanoparticles are the same as notified A(G-10) material or other notified materials.
The study is not acceptable because data derived from animal studies carried out after 11 March 2013 cannot be used to support safety of a cosmetic ingredient/product due to the EU ban on animal testing under the Cosmetic Regulation (EC) No 1223/2009.

General SCCS comments on repeated doses toxicity test results provided:
The provided information is from literature studies that have not been carried out according to the official guidelines. Also, there is uncertainty about whether the used gold nanoparticles are the same as the notified materials. Furthermore, studies carried out after 11 March 2013 cannot be used to support safety of a cosmetic ingredient/product due to the EU ban on animal testing under the Cosmetic Regulation (EC) No 1223/2009.

3.3.6 Mutagenicity/genotoxicity

Table 11: Overview of genotoxicity tests provided by the Notifiers and SCCS comments on results

Nanomaterial tested	Cytotoxicity/cell type	Mutagenicity endpoint/cell type	Result Comments from SCCS Cytotoxicity / mutagenicity	Reference
A(G-3)	Agar diffusion test/ mouse fibroblast cells NCTC clone L929	Micronucleus test/ mouse fibroblast cells NCTC clone L929	Inconclusive / inconclusive	18
A(G-3)	-	Ames test/ strains TA1535, TA1537, TA98 and TA100 and Escherichia coli strain WP2uvrA-	Ames test is not considered appropriate for NM mutagenicity assessment	22
A (G-4)	-	Ames test/ TA97a, TA98, TA100, TA102 and TA1535	Ames test is not considered appropriate for NM mutagenicity assessment	9
A(G-10)	-	Micronucleus test/ L5178Y $Tk^{+/-}$ Mouse lymphoma cells	Inconclusive	20

A(G-10)	-	gene mutation test (tk-locus)/ L5178Y $Tk^{+/-}$ Mouse lymphoma cells	Inconclusive	20
A(CG-8)	Agar diffusion test/ cell line not indicated	-	Inconclusive	8
B(SMG-2)	MTT reduction test/ - human hepatocarcinoma HepG2 - mouse fibroblast Balb/c 3T3 Clone A31 - human colorectal carcinoma CaCo-2 - human lung carcinoma A549	-	Negative up to 10% on all cell lines after 24 h exposure	23
B(SMG-2)	-	Micronucleus test/ Chinese Hamster Ovary cell line (CHO)	Inconclusive	23
B(SMG-2)	(CHO cells)	*In Vitro* Mammalian Cell Hprt Gene Mutation Assay	Inconclusive	24

A(G-3)
Cytotoxicity and micronucleus test
The following information is provided for A(G-3), Sample code: NI-0776-17. Both, a cytotoxicity test *in vitro* (agar diffusion) and a genotoxicity test (micronucleus test) have been performed (Table 12).
Table 12: Design and summary of the results of the cytotoxicity and genotoxicity test for colloidal gold dispersion A(G-3), Sample code: NI-0776-17

Translated Table

	Parameter assessed	**Test Method**	**Requirement**	**Result**
1(*)	Cytotoxicity *in vitro*	Diffusion on agar according to PN-EN ISO 10993-5: 2009	--	Degree of cytotoxicity – 0 Interpretation - no cytotoxicity Final result - a non-cytotoxic sample
2	Genotoxicity	Micronucleus test according to PN-EN ISO 10993-3:2014 PN-EN ISO 10993-12:2012	--	non-genotoxic sample

(*) method included in the scope of PCA accreditation No.AB774

For both cytotoxicity and genotoxicity testing, the mouse fibroblast cells NCTC clone 929 ATCC were tested. The results of the genotoxicity study are presented in Table 13.

Table 13: Results of the micronucleus test *in vitro* (given as % of binucleated cells with micronuclei in population of binucleated cells):

Translated Table

Test without metabolic activation, short-term		
Control cells	**Positive control**	**Test sample**
1.71 % ± 0.50%	26.31 % ± 2.78 % (YES)	2.58 % ± 0.53 (NO)

Translated Table

Test without metabolic activation, long-term		
Control cells	**Positive control**	**Test sample**
3.36 % ± 0.59%	89.17% ± 1.73 % (YES)	2.86 % ± 0.63% (NO)

Translated Table

S9 metabolic activation study, short-term		
Control cells	**Positive control**	**Test sample**
2.27 % ± 0.99%	14.38 % ± 1.45% (YES)	1.51 % ± 0.31% (NO)

Conclusion by the Notifier
The conclusion from the study is that the sample is not cytotoxic nor genotoxic.

Ref.: 18

SCCS comment on A(G-3)
The information provided in the study on cytotoxicity and genotoxicity is neither acceptable nor sufficient. The results of the whole study (on both cytotoxicity and genotoxicity testing) are not reliable for the following reasons:

Cytotoxicity study
- According to the data provided, only one concentration was tested and no cytotoxicity was observed.
- There is a discrepancy concerning the actual concentration tested. On the 1st page of the report, there is information indicating that a concentration of 100 ppm was tested while on the 2nd page (paragraph 6) it is stated that a concentration of 50 ppm was tested. From the information available in published literature, it is known that the EC_{50} for gold nanoparticles may vary and can be below 100 µg/mL, depending on cell types and particle sizes (Ref. 19).
- No information on control substances used was given, neither positive nor negative.
- No data are provided on stability of the gold nanoparticle suspension and how it was applied on the agar.
- No information on number of replicates is given.
- The agar diffusion test used is not considered suitable to determine cytotoxic properties of nanoparticles. According to PN-EN ISO 10993-5:2009 ('8.4.1 Agar diffusion 8.4.1.1), the test allows only a qualitative assessment of cytotoxicity. Also, ISO 10993-5 is dedicated mainly to the testing of extracts of medical devices and not pure chemicals.
- More specifically for nanomaterials, ISO 19007 describes an *in vitro* MTS assay for measuring cytotoxic effects of nanomaterials. Also, other tests for quantitative assessments of cytotoxicity might be used such as the Colony forming efficiency test or colorimetric assays (the NRU, the MTT and the XTT tests under the condition that assay interference is considered).
- The SCCS is therefore of the opinion that a method that is not prone to interference should be preferably used, such as colony-forming efficiency. The cytotoxicity test should be carried

out at different concentrations to enable calculation of EC_{50} to compare the relative toxicity of the various colloidal gold dispersions in nano form.

Genotoxicity study

- It is not clear to the SCCS why an ISO guideline for testing of medical devices was followed, when cosmetic ingredients should be tested using OECD TG test guidelines or EU methods (See SCCS 1611/19)
- L929 fibroblasts are not suggested in OECD TG 487: the choice of the cell line was not justified by the study authors
- No data on positive control substances were given (concentrations, vehicles, etc.)
- No historical control data were provided
- No data on cell proliferation have been provided. Such information is necessary to demonstrate that the cells in culture have divided, to indicate that a substantial proportion of the cells scored had undergone division during or following treatment with the test substance. The measurement of Relative Population Doubling (RPD) or Relative Increase in Cell Count (RICC) is recommended to estimate the cytotoxic and cytostatic activity of a treatment – apparently no such parameters were assessed.
- In the study, only one concentration has been evaluated (10 ppm, page 3 of the report). At least three test concentrations (not including the solvent and positive controls) that meet the acceptability criteria (appropriate cytotoxicity, number of cells, etc.) should be evaluated.
- No data on nanoparticle internalisation by the cells have been provided. This is particularly important considering the negative results obtained.

Overall SCCS comment on genotoxicity/mutagenicity of A(G-3)

The SCCS is of the opinion that mutagenicity/genotoxicity data on gold nanoparticles provided by the Notifiers are not sufficient. Only results on chromosomal aberrations have been provided and these are not acceptable. Assessment of mutagenicity by bacterial Ames test is not acceptable due to the size of bacteria and limited or no uptake of nanoparticles by the bacteria (SCCS/1611/19). According to the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS 1611/19), results on gene mutation in mammalian cells are required. Additionally, information on uptake of nanoparticles by cell should be provided. The provided studies were not performed or reported according to GLP system.

A(CG-8)

Cytotoxicity assessment

AGAR DIFFUSION CYTOXICITY TEST (ISO METHOD)

The Agar Diffusion Test is an *in vitro* procedure designed to determine the biological reactivity of mammalian cell cultures following indirect contact with the test material that has been labelled as follows: K-9799, A(CG-8) Lot. No. 1031005316.

A(CG-8) is polymethylsilsequioxane (an inert solid support), coated with colloidal gold.

The cell culture test system is suitable if the observed responses to the negative control is a grade 0 (no reactivity) and to the positive control is at least a grade 3 (moderate reactivity). The test article meets the requirements of the test if the response to the test article is not greater than grade 2 (mildly reactive). The test must be repeated if the suitability of the test system is not confirmed. If there are evident differences in the test result for replicate culture vessels, then the test is either inappropriate or invalid.

Table 14: Explanation of biological reactivity

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extends 0.5 to 1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond specimen

Table 15: Results

Sample description	Sample Identification	Grade (Plates 1,2,3)	Reactivity
Test – 1, 2, 3	K-9799	0,0,0	None
Negative – 1,2,3	G1D115	0,0,0	None
Positive – 1,2,3	8622609189	3,3,3	Moderate
Filter Paper Blank–1,2,3	6H0034	0,0,0	None
Blank – 1,2,3	N/A	Normal Healthy Cells	

Suitability of the test system was confirmed. The test results were consistent among all replicates.

Summary/conclusion by the Notifier
The test article: K-9799 A(CG-8) exhibited no reactivity (Grade 0) after the 24 hour observation point. The test article K-9799 (A(CG-8), Lot No. 1031005316) does meet the criteria of the test since no reactivity was observed.

Ref.: 8

SCCS comment on cytotoxicity of material A(CG-8)
The study report does not contain sufficient information to draw any conclusions on the cytotoxicity of the test material.
For colorimetric assays, the potential interference of the nanomaterial with the assay components and the optical read out system needs to be evaluated and information on interference controls should be provided.

A(G-4)
Mutagenicity assessment
The bacterial reverse mutation (Ames) test was used to evaluate mutagenic potential of the test sample G-4 at concentrations 5, 1, 0.5, 0.1 and 0.05 mg/plate in five strains TA97a, TA98, TA100, TA102 and TA1535) in the presence and absence of S9 mix with negative results. There was no detectable genotoxic activity associated with the five tested concentrations either in the presence or absence of S9 enzyme activation.

Ref.: 9

SCCS comment on mutagenicity of material A (G-4)
Although A(G-4) has been tested negative in the Ames test, the test is not considered appropriate for mutagenicity assessment of nanomaterials due to the size of bacteria and limited or no uptake of nanoparticles by the bacteria (SCCS/1611/19).

A(G-10)
Mutagenicity assessment
Micronucleus Test
Guideline: OECD 487 (2014)
Cells: L5178Y $Tk^{+/-}$ Mouse lymphoma cells
Material: A(G-10)

Solvent:	Water for injections
Batch:	161017
Composition:	66.6 ± 1.4 mg/kg
Concentrations:	312.5, 625, 1250, 2500 and 5000 µg/mL with and without S9-mix
Treatment:	3 h treatment with and without S9 mix followed by a 24 h recovery period or 24 h treatment without S9 mix with no recovery period
GLP compliance:	Yes
Period:	November – December 2017

After a preliminary cytotoxicity test, the test item A(G-10), diluted in water for injections, was tested in a single cytogenetic experiment, with and without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254, as follows:

Without S9 mix:	3h treatment + 24h recovery
	24h treatment + 0h recovery
With S9 mix:	3h treatment + 24h recovery

Each treatment was coupled to an assessment of cytotoxicity at the same dose levels. Cytotoxicity was evaluated determining the PD (Population Doubling) of cells.

After the final cell counting, the cells were washed and fixed. Then, cells from three dose levels of the test item-treated cultures were dropped onto clean glass slides. The slides were air-dried before being stained in 5% Giemsa. Slides from vehicle and positive control cultures were also prepared as described above. All slides were coded before analysis, so that the analyst was unaware of the treatment details of the slide under evaluation ("blind" scoring). For each main experiment (with or without S9 mix), micronuclei were analysed for three dose levels of the test item, for the vehicle and the positive controls, in 1000 mononucleated cells per culture (total of 2000 mononucleated cells per dose).

The number of cells with micronuclei and the number of micronuclei per cell were recorded separately for each treated and control culture.

Results

Since the test item was found freely soluble and non-cytotoxic in the preliminary test, the highest dose level selected for the main cytogenetic experiment was 5000 µg/mL, according to the criteria specified in the international regulations.

The mean population doubling and the mean frequencies of micronucleated cells for the vehicle controls were as specified in the acceptance criteria. Also, positive control cultures showed clear statistically significant increases in the frequency of micronucleated cells. The study was therefore considered to be valid.

Using a test item stock solution at the concentration of 500 mg/ml in the vehicle and a treatment volume of 1% (v/v) in culture medium, the selected dose levels were: 312.5, 625, 1250, 2500 and 5000 µg/mL for the 3-hour treatments with and without S9 mix, as well as for the 24-hour treatment without S9 mix.

No precipitate was observed in the culture medium at any dose levels, either at the beginning or the end of the treatment periods.

Cytotoxicity

No noteworthy cytotoxicity was induced at any dose levels, either following the 3-hour treatments with and without S9 mix or the 24-hour treatment without S9 mix, as shown by the absence of notable decrease in the PD.

Micronucleus analysis
For the three experimental conditions, the dose levels selected for the micronucleus analysis were: 1250, 2500 and 5000 µg/ml, the latter being the highest recommended dose level.

Following the 3-hour treatments with and without S9 mix or the 24-hour treatment without S9 mix, neither statistically significant nor dose-related increase in the frequency of micronucleated cells was noted at any of the analyzed dose levels relative to the corresponding vehicle control. Moreover, none of the analyzed dose levels showed frequency of micronucleated cells of both replicate cultures above the corresponding historical range.

Thus, these results met the criteria of a negative response.

Conclusion by the Notifier
Under the experimental conditions of the study, the test item, A(G-10), did not induce any chromosome damage, or damage to the cell division apparatus, in cultured mammalian somatic cells, using L5178Y TK ± mouse lymphoma cells, either in the presence or absence of a rat liver metabolizing system.

Ref.: 20

SCCS comment on A(G-10)
The SCCS assumes that A(G-10) (Batch 161017) is a mixture containing colloidal gold (CAS number 7440-57-5) nanoparticles in suspension at 66.6 mg/kg (±1.4 mg/kg) in water (determined by ICP-MS method) with traces of plant extracts (Hubertia ambavilla). It can be calculated that the highest concentration tested of 5000 µg/mL (prepared from nanogold stock solution) corresponded to the final concentration of gold nanoparticles of 0.333 µg/mL. Such low concentrations might not correspond to the conditions of a valid genotoxicity study. Although detailed characterisation and stability of dispersion in different media was provided, this did not include any information on cellular or nuclear uptake that is essentially required to support the results of genotoxicity tests on nanomaterials. Therefore, the SCCS considers the study as inconclusive.

A(G-10)
Mammalian cell gene mutation test (tk-locus)

Guideline:	OECD 490 (2015)
Cells:	L5178Y $Tk^{+/-}$ Mouse lymphoma cells
Material:	A(G-10)
Solvent:	Water for injections
Batch:	161017
Composition:	66.6 ± 1.4 mg/kg
Concentrations:	Experiment I: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL, 3 hours treatment with and without S9-mix
GLP compliance:	Yes
Period:	November 2017 – January 2018

Two known mutagens, dissolved in water for injections, were used to check the sensitivity of the test system:
- Without S9 mix: methylmethane sulfonate (MMS), used at a final concentration of 25 µg/mL,
- With S9 mix: cyclophosphamide (CPA), used at a final concentration of 3 µg/mL.

A(G-10) was assayed for gene mutations at the tk locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on toxicity, measuring relative suspension growth.

Results
Since the test item was found freely soluble and non-cytotoxic in the preliminary test, the highest dose level selected for the main experiment was 5000 µg/ml, according to the criteria specified in the international guidelines.

The cloning efficiencies, the mutation frequencies and the suspension growths of the vehicle controls were as specified in the acceptance criteria.

For the positive control cultures, the increase in the mutation frequencies met also the acceptance criteria. In addition, the upper limit of cytotoxicity observed in the positive control cultures had an Adj. RTG (Adjusted Relative Total Growth) greater than 10%. The study was therefore considered to be valid.

Using a test item stock solution at the concentration of 500 mg/mL in the vehicle and a treatment volume of 1% (v/v) in culture medium, the selected dose levels were 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL, both with and without S9 mix.

No precipitate was observed at any dose levels, in any conditions, as shown by the absence of notable decreased in the Adj. RTG relative to the corresponding vehicle control.

No noteworthy increase in the mutation frequency was noted relative to the corresponding vehicle control, at any dose levels with or without S9 mix (IMF < GEF of 126 x 10-6). Moreover, no dose-response relationship was demonstrated by the linear regression.
Thus, these results met the criteria for a negative response.

Conclusion by the Notifier
The authors concluded that under the experimental conditions reported, the test item A(G-10) did not show any mutagenic activity in the mouse lymphoma assay, either in the presence or absence of a rat liver metabolizing system.

Ref.: 21

SCCS comment
The SCCS assumes that A(G-10) (Batch 161017) is a mixture containing colloidal gold (CAS number 7440-57-5) nanoparticles in suspension at 66.6 mg/kg (±1.4 mg/kg) in water (determined by ICP-MS method) with traces of plant extracts (Hubertia ambavilla). It can be calculated that the highest concentration tested of 5000 µg/mL (prepared from nanogold stock solution) corresponded to the final concentration of gold nanoparticles of 0.333 µg/mL. Such low concentrations might not correspond with conditions of a valid genotoxicity study. Although detailed characterisation and stability of dispersion in different media was provided, this did not include any information on cellular or nuclear uptake that is essentially required to support the results of genotoxicity tests on nanomaterials. Therefore, the SCCS considers the study as inconclusive.

A(CG-3)
SCCS comment
Only a summary (no detailed data) of an Ames test performed according to OECD TG 471 is given, stating that the test material (which was not further described) was not mutagenic. Although A(CG-3) has been tested negative in the Ames test, the test is not considered appropriate for mutagenicity assessment of nanomaterials, due to the size of bacteria and limited or no uptake of nanoparticles by the bacteria (SCCS/1611/19).

A(CG-8)
Cytotoxicity assessment / AGAR DIFFUSION CYTOXICITY TEST (ISO METHOD)
The Agar Diffusion Test is an *in vitro* procedure designed to determine the biological reactivity of mammalian cell cultures following indirect contact with the test material that has been labelled as follows: K-9799, A(CG-8) Lot. No. 1031005316.
A(CG-8) is polymethylsilsequioxane (an inert solid support), coated with colloidal gold.

The cell culture test system is suitable if the observed responses to the negative control is a grade 0 (no reactivity) and to the positive control is at least a grade 3 (moderate reactivity). The test article meets the requirements of the test if the response to the test article is not greater than grade 2 (mildly reactive). The test must be repeated if the suitability of the test system is not confirmed. If there are evident differences in the test result for replicate culture vessels, then the test is either inappropriate or invalid.

EXPLANATION OF BIOLOGICAL REACTIVITY

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extends 0.5 to 1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond specimen

Table 16: Results

Sample description	Sample Identification	Grade (Plates 1,2,3)	Reactivity
Test – 1, 2, 3	K-9799	0,0,0	None
Negative – 1,2,3	G1D115	0,0,0	None
Positive – 1,2,3	8622609189	3,3,3	Moderate
Filter Paper Blank–1,2,3	6H0034	0,0,0	None
Blank – 1,2,3	N/A	Normal Healthy Cells	

Suitability of the test system was confirmed. The test results were consistent among all replicates.

Summary/conclusion by the Notifier:
The test article: K-9799 exhibited no reactivity (Grade 0) after the 24-hour observation point. The test article K-9799 (A(CG-8), Lot No. 1031005316) does meet the criteria of the test since no reactivity was observed.

Ref.: 8

SCCS comment
The agar diffusion test is not considered suitable to determine cytotoxic properties of nanoparticles. According to PN-EN ISO 10993-5:2009 ('8.4.1 Agar diffusion 8.4.1.1) the test allows only a qualitative assessment of cytotoxicity. Additionally, the study report does not contain sufficient information to draw any conclusions on cytotoxicity of the test material.

A(CG-3)
Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2uvrA$^-$ were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors).
The Ames test was performed to conform the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471

"Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

SCCS comment
Although A(CG-3) (material was not further described) has been tested negative in the Ames test, the test is not considered appropriate for nanomaterial mutagenicity assessment, due to the size of bacteria and limited or no uptake of nanomaterials by the bacteria (SCCS/1611/19). According to the SCCS Guidance On the Safety Assessment of Nanomaterials in Cosmetics (SCCS 1611/19) results on gene mutation in mammalian cells are also required. Additionally, results on chromosomal aberrations need to be provided along with the evidence for cellular uptake.

Ref.: 22

B(SMG-2)

Micronucleus Test

Guideline:	OECD 487 (2016)
Cells:	Chinese Hamster Ovary cell line (CHO)
Test Material:	B(SMG-2)-GH53, liquid
Particle size:	20±5 nm
Solvent:	water, culture medium
Batch:	GH-53
Composition:	H_2O + sodium citrate + Gold particles (12 nm) + Hyaluronic acid (5-10 kDa)
Concentrations:	preliminary test: 10, 2, 0.4, 0.08, 0.016% v/v main test: 10, 5, 2.5% v/v
Treatment:	4 h ± S9 mix; 24 h –S9 mix
Positive control:	vinblastine at 0.2 µg/mL for the short exposure and 0.1 µg/mL for the long exposure; cyclophosphamide at 4 µg/mL for the short exposure
Negative control:	negative control was culture medium without any treatment; the solvent control was 10% of water in culture medium
GLP compliance:	Yes
Period:	August – December 2020

The aim of the study was to determine the genotoxic potential of the test item by assessing its ability to induce cytogenetic damage and/or effect on chromosomes or mitotic apparatus in cultured cells by detecting micronuclei. These micronuclei are residual fragments of genetic material formed at a short exposure time of 4 hours and a long exposure time of 24 hours.
The assay consisted in two phases: a preliminary cytotoxicity test and a main micronucleus test. In the preliminary cytotoxicity test, the Test Item was applied for 3h 30 min with and without metabolic activation at 10, 2, 0.4, 0.08 and 0.016 %. As no cytotoxicity was observed at any concentration, the main test was conducted by applying 10, 5 and 2.5 % of Test Item with and without metabolic activation at two incubation times, 4h 17 min and 23 h 18 min. Once the period incubation times ended, the mono-, bi- and polynucleated cells were counted and binucleated cells with micronucleus were compared between the negative control and the treatments.

Results
In the main assay none of the test item concentrations exhibits a statistically significant increase in micronuclei ($p<0.05$) compared with the concurrent negative control. Furthermore, when a concentration-related increase was evaluated ($r2= 0.9689$), no signification was observed according to r-Pearson coefficient (>0.997).

Conclusion by the Notifier
In accordance with the OECD TG 487 *In Vitro* Mammalian Cell Micronucleus Test and the test experimental conditions of this study the Test Item B(SMG-2) is not genotoxic.

Ref.: 23

SCCS comment
Based on the study report, the exact concentration of the gold nanoparticles used for cell exposure cannot be ascertained. According to the SCCS calculation (assuming the concentration of 0.005-0.01% B(SMG-2), in the test suspension (D-Safety Report) the final maximum concentration of gold nanoparticles used for cell exposure would be 0.001% (10 µg/mL).
No information on cellular or nuclear uptake was provided in the target CHO cells. This information is particularly important considering the negative result of the study.
Therefore, the SCCS considers the study as inconclusive.

B(SMG-2)
***In Vitro* Mammalian Cell Hprt Gene Mutation Assay**

Test Item identification	Gold Nanoparticles Conjugated to Hyaluronic Acid
Description	It is an active ingredient designed to be used in cosmetic applications as a skin regenerator and anti-age treatment. The active ingredient has been developed by using nanotechnology. It is composed by an inorganic core which is a pure gold nanoparticle of 10-12 nm of diameter and an organic shell formed by low molecular weight hyaluronic acid oligomers that are covalently linked to the nanoparticle.
Reference	B(SMG-2)-GH53, liquid
Particle size (DLS)	20 ± 5 nm
Formula/Chemical group	Gold, 4-deoxy-4-((2-mercaptoethyl) amino) hyaluronate complexes
Composition	H_2O + Sodium citrate + Gold particles (12 nm) + Hyaluronic acid (5-10 kDa)
Concentration	Five concentrations of Test Item will be prepared by two-fold serial dilutions: 2, 1, 0.5, 0.25 and 0.125 µL/mL.
Negative controls:	- Medium - Solvent: 10% of water in culture medium according to the Test Item composition
Positive controls:	- EMS: 0.4 µl/mL for absence of exogenous metabolic activation - BαP: 0.01 mg/mL for presence of exogenous metabolic activation

The aim of the study was to assess if the test item can induce the *Hprt* gene mutations in CHO cells. Two assays were conducted as the first assay unexpectedly suffered a general contamination of plates in the phenotypic expression. The second assay consisted in a 3 hours. Test item treatment of cell line and a subsequently subcultured in order to obtain data for relative survival and phenotypic expression. Cells were treated at 2, 1, 0.5, 0.25 and 0.125 µL/mL of Test item in presence and absence of metabolic activation in duplicates for 3 hours at 37ºC in humidified atmosphere. Once the period incubation ended, cells were harvested, and counted, and cells were reseeded at 2 x 10^2 cells/plate (relative survival) and 1 x 10^6 cells/flask (phenotypic expression). The phenotypic expression flasks were incubated for 7 days, and after that, cells were harvested, counted and reseeded in two conditions, cloning efficiency and mutant frequency. In the cloning efficiency assay, cells were seeded at 2 x 10^2 cells/plate in a non-selective media and incubated for 7 days. In the mutant frequency, cells were seeded at 2 x 10^5 cells/plate in a selective media and incubated for 7 days. Once the period incubated ended, plates were stained and counted.

Results
Results show that there is no dose-dependent concentration of Test Item ($r2<0.9$) and none of the Test Item concentrations exhibits a statistically significant compared with negative control (P value >0.05). In contrast, positive control induced statistically significant responses in front of the negative (P value <0.05).

Conclusion by the Notifier
In accordance with the OECD 476 *In Vitro* Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes and the test experimental conditions of this study, the Test Item Gold Nanoparticles Conjugated to Hyaluronic Acid is not mutagenic.

Ref. 24

SCCS comment
- Though authors investigated gene mutation endpoints in the section on Test System, they incorrectly refer to the guideline OECD TG 476 *In Vitro* Mammalian Cell Micronucleus Test. They also refer to the SOP TOX-EXP-023 Micronucleus test *in vitro*, however no SOP on Mammalian gene mutation assay is provided.
- The concentration should be expressed in µg/mL or number of Au particles/mL to make clear what was the actual concentration range of AuNPs in treatment medium
- 3h cell exposure as used in the study may not be sufficient for Au-NPs internalization, therefore, 24-h treatment –S9 should be considered based on the fact of the negative results obtained after 3h exposure,
- Mutant Frequency should be reported as the number of mutants per 10^6 cells
- Both historical negative and positive control ranges and distributions should be provided
- According to SCCS/1611/19, a proof of Au-NPs cell internalization should be provided to demonstrate that nanoparticles in tested conditions reached the cells. This is especially important considering the fact that a negative result was obtained.
Therefore, the SCCS is of the opinion that the study should be considered as inconclusive.

The overall SCCS comment on genotoxicity/mutagenicity
The SCCS is of the opinion that the data on the different gold nanoparticles provided by the Notifiers are not sufficient to exclude mutagenicity/genotoxicity. The assessment of mutagenicity by bacterial Ames test is not acceptable due to the size of bacteria and limited or no uptake of nanoparticles by the bacteria (SCCS/1611/19). The results provided on chromosomal aberrations are neither sufficient nor acceptable. The results provided on gene mutation in mammalian cells have some limitations and are inconclusive.
Therefore, the SCCS is of the opinion that a genotoxic potential of the notified gold nanoparticles cannot be excluded based on the data provided.

3.3.7 Carcinogenicity

Information on carcinogenicity has not been provided.

SCCS comment
As described in the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19), if significant systemic exposure or genotoxicity cannot be excluded, information on carcinogenicity is required.
The SCCS notes that information has not been provided on the lack of systemic availability via the relevant uptake route(s) or genotoxicity to allow discounting the need for information on carcinogenicity.

3.3.8 Reproductive toxicity

Information on reproductive toxicity has not been provided.

SCCS comment
As described in the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19), if considerable systemic exposure cannot be excluded, information on reproductive toxicity is required.

The SCCS notes that information has not been provided on systemic availability via the relevant uptake route(s) that would allow drawing conclusions on reproductive toxicity.

3.3.9 Photo-induced toxicity

3.3.9.1 Phototoxicity/photoirritation and photosensitisation

A(G-10)

Guideline:	OECD 432 (2004): 3T3 NRU Phototoxicity test
Cells:	mouse fibroblast cell line, Balb/c 3T3, clone A31
Material:	A(G-10)
Solvent:	Hank's Balanced Salt Solution (HBSS)
Batch:	161017
Composition:	66.6 ± 1.4 mg/kg
Concentrations:	0, 67.42, 99.11, 145.69, 214.16, 314.81, 462.77, 680.27 and 1000 µg/mL
GLP compliance:	Yes
Period:	November 2017 – January 2018

The assay compares the cytotoxicity of chemicals applied to mouse fibroblasts (Balb/c 3T3, clone A31) in the presence or absence of exposure to a non-cytotoxic level of UVA light (5 J/cm²). Cytotoxicity is measured as the inhibition of the capacity to take up the vital dye, Neutral Red (NR), one day after UVA treatment.

Results

Preliminary test

A preliminary test was performed with the following test item concentrations: 0.32, 1.00, 3.17, 10.03, 31.69, 100.15, 316.46 and 1000 µg/mL in HBSS (serial dilution factor of 3.16). The following results were obtained: no change in cell morphology was observed and there was no decrease in viabilities (NR uptake) at any tested concentrations in the irradiated and non-irradiated plates.

Main test

The acceptance criteria were fulfilled and the study was therefore considered to be valid. According to the results obtained in the preliminary test, the following concentrations were used for the main test: 67.42, 99.11, 145.69, 214.16, 314.81, 462.77, 680.27 and 1000 µg/mL (dilution factor of 1.47).
The following results were obtained: no change in cell morphology was observed and there was no decrease in viabilities (NR uptake) at any tested concentrations in the irradiated and non-irradiated plates.

The main phototoxicity findings for NR uptake following analysis with the Phototox software are presented in the table below.

Table 17: Summary of main test results following analysis with the Phototox software

Parameter	Value	Conclusion
Test Item A(G-10)	IC_{50} Irr+ = not reached IC_{50} Irr- = not reached > PIF = 1.000 (by default) MPE = 0.065	Not phototoxic

Conclusion according to the Notifier
Under the experimental conditions of this study, the test item, A(G-10), tested at up to 1000 µg/mL, was determined to be not phototoxic according to the classifications presented in the OECD guideline 432.

Ref.: 25

SCCS comments on A(G-10) phototoxicity
The full study report was not made available. According to the OECD guideline the compatibility of the test substance with the assay may be questioned if poor solubility limited the concentrations that could be tested and confirmatory testing should be considered using another model. It is as yet unknown whether the 3T3 NRU phototoxicity test is suitable for testing nanoparticles.
The SCCS assumes that A(G-10) (Batch 161017) is a mixture containing colloidal gold (CAS number 7440-57-5) nanoparticles in suspension at 66.6 mg/kg (±1.4 mg/kg) in water (determined by ICP-MS method) with traces of plant extracts (Hubertia ambavilla). As can be calculated, the highest concentration tested of 1000 µg/mL (prepared from nanogold stock solution) corresponded to the final concentration of gold nanoparticles of 0.0666 µg/mL. Such low concentrations might not correspond with conditions of a valid phototoxicity study.

A(CG-3)
Phototoxicity study in Guinea pigs

Guideline/method:	not specified
Species/strain:	Guinea Pig (Albino Hartley)
Group size:	10 (5 males, 5 females), preliminary test 4 animals
Test substance:	A(CG-3)
Batch:	PW-01 (Certificate of analysis not provided)
Route:	Topical, on clipped dorsal skin, with and without UV irradiation
Irradiation:	UV-B 0.15 J/cm^2 and UV-A 4.5 J/cm^2
Negative control:	Irradiation on unexposed skin
Positive control:	8-MOP (8-methoxy-psoralen) 0.5 mg/ml in acetone
Vehicle:	Test substance applied undiluted
Test concentration:	Test item undiluted
GLP:	Yes
Date:	2003
Published:	No

According to the notification, under the experimental conditions adopted, 8-methoxy-psoralen manifested a phototoxic potential: 100 % of animals showed an erythematous reaction at time 24 and 48 hours after exposure. Under the experimental conditions adopted, animals treated with the undiluted test substance G-Water showed no erythematous reaction at times 24 and 48 hours after exposure. Under the experimental conditions adopted, the undiluted test substance A(CG-3)(batch PW-01) was found to be non-phototoxic in the Guinea pig.

Ref.: 26

A(CG-3): Photosensitisation
Photosensitisation study in Guinea Pigs

Guideline/method:	not specified
Species/strain:	Guinea Pig (Albino Hartley)
Group size:	15 (10 males, 5 females), preliminary test 4 animals
Test substance:	A(CG-3)
Batch:	PW-01 (Certificate of analysis not provided)
Route:	Topical, on clipped dorsal skin, with UV irradiation
Irradiation:	UV-B 0.2 J/cm^2 followed by UV-A 4 J/cm^2
Negative control:	5 males, exposed to test item without UV irradiation
Vehicle:	Test substance applied undiluted

Test concentration:	Test item undiluted
Induction:	FCA injection on D1. Test item epicutaneous with or without UV on Day 1, 3 and 5
Challenge:	Epicutanous with or without UV on Day 21, Reading at 24 and 48 after UV exposure
GLP:	Yes
Date:	2003
Published:	No

According to the notification, under the experimental conditions adopted, results were as follows:
At times 24 hours and 48 hours, the negative control animals did not show cutaneous reaction.
At times 24 hours and 48 hours, all animals treated with the undiluted test substance A(CG-3) and the test substance diluted at 50% in sterile water showed no cutaneous reaction.
Under the experimental conditions adopted, the undiluted test substance G-Water (batch PW-01) found to be non-photosensitising in the Guinea pig.

Ref.: 27, 28

SCCS comment
The concentration of gold particles in the A(CG-3) used for the tests was not stated in the study report. From an accompanying document it can be deduced that it is probably 0.02 % (w/w). The tests indicate that G-Water does not have phototoxic or photosensitising properties.

B(SMG-2) - Phototoxicity – *In vitro*
UV/Vis spectra absorption test

Guideline:	OECD-101
Test item:	Gold nanoparticles conjugated to hyaluronic acid
Batch:	B(SMG-2)-GH53
Composition:	Nano gold particles with hyaluronic acid in water + sodium citrate, concentration not specified
Test concentrations:	10% in acidic, neutral and basic medium
Control:	Potassiumdichromate 0.09 mg/mL
GLP:	Yes
Period:	2020

According to the notification's study report, the purpose of this test was to determine the UV absorption spectrum on wavelengths from 190 up to 400 nm of the Test Item to know the wavelengths at which the Test Item was susceptible to photochemical reactivity and with the subsequent evaluation of the phototoxicity of B(SMG-2) when tested in the presence and in the absence of exposure to a non-cytotoxic dose of UVA light using the 3T3 BALB/c cell line Clone A31).
The Test Item was prepared in three different pH mediums: one acidic pH medium (0.56), one basic pH medium (12) and one neutral pH medium (7.4).
Molar extinction/absorption coefficient (ε) has been calculated for all maximum absorption of the Test Item with the following formula: Molar extinction/absorption coefficient (ε) has been calculated for all maximum absorption of the Test Item with the following formula: $\varepsilon = A / (Ci \times d)$,

whereby ε= the molar extinction coefficient A= absorbance, Ci= the molar concentration (mol/L), d= absorption path length (cm).

The peaks and valleys in the UV radiation spectrum from wavelengths 190 to 400 nm from each treatment were recorded by a spectrophotometer.

Table 18: Values from Reference control

λ	A	Ci	d	ε	log ε
350.5	0.938	0.0003	1	3126.67	3.50
313.5	0.410	0.0003	1	1366.67	3.14
258	1.243	0.0003	1	4143.33	3.62
233.8	0.950	0.0003	1	3166.67	3.50

Table 19: Values from Test Item

20/004	λ	A	Ci	d	ε
Test item 10% basic pH	239.5	1.481	1.53E-08	1	96989482
Test Item 10% neutral pH	NO	Sample did not show any peak or valley	1.53E-08	1	<1000
Test Item 10% acid pH	235	1.485	1.53E-08	1	97251439

According to OECD TG 432, due to the fact that molar extinction coefficient (ε) is not greater than 1000 $M^{-1}\cdot cm^{-1}$ in neutral pH of Test Item and the absorbance obtained was not between 400 and 315 nm, the Test Item is unlikely to be photoreactive and the OECD TG 432 *"In Vitro 3T3 NRU Phototoxicity Test"* was not necessary to be performed.

SCCS comment
The study report did not specify the concentration of the gold-hyaluronic acid particles in the test article. The media to obtain an acidic, neutral or basic test solution are not specified. The SCCS agrees that the test does not indicate a phototoxic potential.

Ref.: 29

General SCCS comments on the provided photo-induced toxicity test results
Phototoxicity test results were submitted for A(G-10), A(CG-3) and B(SMG-2).
Regarding A(G-10), it is as yet not certain whether the test system (3T3 NRU) is suitable for testing nanoparticles.
For A(CG-3) the *in vivo* tests points to absence of phototoxicity.
Nanogold particles in the range of 3 - 6 nm can exhibit photocatalytic activities. According to the notifications related to A(G-10) and A(CG-3), the tested nano materials are larger than 6 nm.
As noted in the SCCS Guidance, UV-VIS spectra of the compound along with Molar Extinction Coefficient (MEC) determined according to a harmonized procedure should be provided. There is no need to perform phototoxicity testing of compounds with a MEC below 1000 L mol−1 cm−1. Also, *in vitro* phototoxicity testing is not needed when the test material only absorbs at wavelengths lower than 313 nm and if there is insufficient absorption at longer wavelengths.

3.3.9.2 Phototoxicity/Photomutagenicity/photoclastogenicity

3.3.10 Human data

SCCS comment
Human data were not provided, except the HRIPT studies (see 3.3.3 Skin sensitization).

3.3.11 Special investigations

A(G-10)

Guideline:	OECD 129 (2010)
Cells:	mouse fibroblast cell line, Balb/c 3T3, clone A31, from the American Type Culture Collection (ATCC cell line No. CCL-163)
Material:	A(G-10)
Solvent:	$DMEM_0$
Batch:	161017
Purity / Composition:	66.6 ± 1.4 mg/kg
Concentrations:	Two experiments: 0, 588.63, 1265.55, 2720.93, 5850, 12577.51, 27041.64, 58139.53 and 125000 µg/mL
GLP compliance:	Yes
Study Period:	November– December 2017

The assay evaluates the cytotoxicity of the test item applied to mouse fibroblasts (Balb/c 3T3, clone A 31). Cytotoxicity is measured as the inhibition of the capacity to take up the vital dye, Neutral Red (NR). NR readily penetrates cell membranes by non-diffusion and accumulates in the cell lysosomes. Damage to the lysosomal membrane leads to irreversible lysosome fragility. Damage to lysosomes by a test item results in a decrease in the uptake and accumulation of NR, allowing the quantification by spectrophotometry of viable, damaged or dead cells. The positive control was the Sodium Lauryl Sulfate.

Results
Preliminary test
The preliminary test was performed to determine the relevant concentration range at which cytotoxicity is obtained. Using a treatment volume of 50% (50 µL in 50 µL of culture medium), the concentrations tested in this preliminary test were: 0.01, 0.1, 1, 10, 100, 1000, 10000 and 100000 µg/mL in DMEM.

The following results were obtained after 48 hours incubation: no decrease in cell viability (decrease in NRU) was noted at any concentrations and therefore no IC_{50} was estimated.

These results were taken into account to select a more appropriate test item concentration range for the main tests.

Main tests
Two independent experiments were performed. In both experiments, the following concentrations were used: 588.63, 1265.55, 2720.93, 5850, 12577.51, 27041.64, 58139.53 and 125000 µg/mL.

The following results were obtained after 48 hours incubation: no decrease in cell viability (decrease in NRU) was noted at any concentrations, therefore no IC50 and no LD50 was estimated.

Conclusion
Under the experimental conditions of this study and after treatment of cells for 48 hours, the test item, ALM70c, is not considered cytotoxic in this *in vitro* test system. The mean IC50 and the corresponding LD_{50} for rats could therefore not be determined.

Ref.: 30

SCCS comment
The SCCS assumes that A(G-10) (Batch 161017) is a mixture containing colloidal gold (CAS number 7440-57-5) nanoparticles in suspension at 66.6 mg/kg (±1.4 mg/kg) in water

(determined by ICP-MS method) with traces of plant extracts (Hubertia ambavilla). It can be calculated that the highest concentration tested of 125000 µg/mL (prepared from nanogold stock solution) corresponded to the final concentration of gold nanoparticles of 8.325 µg/mL. The highest vehicle concentration used for cell exposure corresponded to 12.5% v/v. The laboratory performing the cytotoxicity test should provide a confirmation that this relatively high concentration did not influence the normal growth of the cells after 48 h.

B(SMG-2)

Protocol:	DB-ALM Protocol no. 3: The FRAME Modified Neutral Red Uptake Cytotoxicity Test; DB-ALM Protocol no. 17: MTT Assay
Cells:	Human hepatocarcinoma HepG2; mouse fibroblast Balb/c 3T3 Clone A31, human colorectal carcinoma CaCo-2; human lung carcinoma A549
Material:	B(SMG-2)-GH53 liquid
Particle size:	20±5 nm
Solvent:	MEM or DMEM culture medium
Batch:	GH-53
Purity / Composition:	H_2O + sodium citrate + Gold particles (12 nm) + Hyaluronic acid (5-10 kDa)
Concentrations:	Three experiments: 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125 % v/v
Positive control:	SLS
GLP compliance:	Yes
Study Period:	May – August 2020

The aim of this study was to evaluate the basal 24-hour *in vitro* cytotoxicity of the Test Item B(SMG-2) measuring the viability of HepG2 hepatocarcinoma, Balb/c 3T3 Clon A31 mouse fibroblast, CaCo-2 human colorectal carcinoma and A549 human lung carcinoma cell lines by MTT and Neutral Red (NR) uptake. Additionally, a qualitative evaluation of the morphological changes of the cells after exposure was performed.

Results

Tests consisted in the cell exposure of eight Test Item concentrations ranging from 10% to 0.078% by 2-fold serial dilution during 23.03 to 23.35 hours at 36.8 ±. 0.2°C with 4.75 ± 0.25% CO_2 in humidified atmosphere. Once the period incubation ended, the percentage of Test Item cell viability was obtained ranging from 82.79-102.45% and 70.17-132.38% for NR and MTT, respectively. A qualitative evaluation of the morphological changes determined no cytotoxicity (grade < 2) for all Test Item concentrations in the different cell lines.

Conclusion

Under the test conditions described above, and taking into account that any concentrations of the Test Item showed no major morphological changes (grade< 2) nor any reduction in cell viability of more than 30%, the Test Item (B(SMG-2) can be considered as non-cytotoxic up to 10% on 3T3, HepG2, CaCo-2 and A549 cell lines.

Ref.: 31

The SCCS comment

Based on the study report, the exact concentration of the gold nanoparticles used for cell exposure cannot be ascertained. According to the SCCS calculation (assuming the concentration of 0.005-0.01% B(SMG-2) in the test suspension (D-Safety Report), the final maximum concentration of gold nanoparticles used for cell exposure would be 0.001% (10 µg/mL).

The Test Item B(SMG-2) can be considered as non-cytotoxic up to 10% on 3T3, HepG2, CaCo-2 and A549 cell lines after 24 h exposure. However, 24 hours might not be enough to assess influence of the test item on more subtle functions of the cells.

The SCCS notes a fairly large difference in response of the four cell lines to SLS used as a positive control (IC_{50} in MTT test ranging from 0.00058%-0.041% and in NR test ranging from 0.00087% to 0.055%).

3.4 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

Based on the notified and subsequently provided information, it is not possible to perform a safety evaluation for any of the materials under categories of gold (nano), colloidal gold (nano) and surface modified gold (nano) materials discussed in this Opinion.

3.5 DISCUSSION

The information provided by the Notifiers through CPNP on the materials considered in this Opinion was assessed by the SCCS, and further clarifications were asked where appropriate. Additionally, a call for information was made and a literature search performed by the Commission to obtain further information from other sources. In developing this Opinion, the SCCS has taken into account the responses received from the Notifiers, the information received from the Commission's call for information, and the results of the literature search. Having considered all the available information, the SCCS is of the view that the information available at present is insufficient to allow drawing conclusions on the safety of Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) materials included in this Opinion.

For a proper safety evaluation the following information/data relevant to each type of materials should be provided:

- Data on the Gold (nano), Colloidal Gold (nano), Surface Modified Gold (nano) materials as notified regarding characterisation, and the methodology used, for impurities/contaminants, particle size, crystallinity and crystal form, solubility, surface characteristics, UV absorption and microscopy.
- Data on systemic uptake of the Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) nanomaterials as notified via the relevant uptake route(s).
- Data on the Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) materials as notified regarding toxicity and the methodology used for acute toxicity, irritation/sensitisation, and mutagenicity/genotoxicity. This should be supplemented with reproductive toxicity and carcinogenicity if significant systemic exposure is indicated.

In the absence of sufficient data to allow safety assessment, the SCCS has considered the different aspects of Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) materials that could raise a concern over consumer safety. As detailed in Annex II, the SCCS has concluded that there is a basis for concern that the use of Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) materials in cosmetic products can pose a risk to the consumer.

4. CONCLUSION

1. In view of the above, and taking into account the scientific data provided, does the SCCS consider the nanomaterials A, B and C are safe when used in leave-on cosmetic products according to the maximum concentrations and specifications reported in the attached list, taking into account reasonably foreseeable exposure conditions?

The SCCS has considered all the information provided by the Notifiers and is of the opinion that it is not possible to carry out safety assessment of the nanomaterials (Gold, Colloidal Gold and Surface Modified Gold) due to limited or missing essential information. Much of the information provided on toxicity relates to gold as such, and it is not possible to determine the relevance of the data for nano-forms of any of the materials under the current evaluation due to the absence of full study reports.

Detailed data and information need to be provided on physicochemical characterisation and toxicological evaluation, along with experiment performance to allow safety assessment of the nanomaterials.

In regard to surface modified gold, all notifications relating to Acetyl heptapeptide-9 Colloidal gold (nano) were withdrawn by the Notifiers and therefore only Gold Thioethylamino Hyaluronic Acid has been considered in this Opinion.

2. Does the SCCS have any further scientific concerns with regard to the use of materials A, B and C in nano form in cosmetic products?

The information obtained from scientific literature suggests possible systemic uptake of gold nanoparticles which may lead to accumulation in certain organs - notably the liver and spleen. In addition, the available data from literature indicate potential mutagenic/genotoxic effects of gold nanomaterials. These indications raise an alert that warrants further safety evaluation of gold nanomaterials when used as cosmetic ingredients. In the absence of sufficient data to allow safety assessment, the SCCS has considered these aspects and has concluded that there is a basis for concern that the use of Gold (nano), Colloidal Gold (nano) and Surface Modified Gold (nano) materials in cosmetic products can pose a risk to the consumer. The SCCS concerns for consumer safety in this regard are detailed in Annex II.

The SCCS would be ready to assess any evidence provided to support the safe use of this materials in cosmetic products.

5. MINORITY OPINION

None.

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7. ANNEX I

Table 1: Trade names of the various Gold (nano), Colloid Gold (nano) and Surface Modified Gold substances, * notification withdrawn

Materials (266 Notifications)	**Name**	**Notifications**	**Function and uses**	**Leave on / Rinse off : % w/w**
		A. Gold (nano) and Colloidal Gold (nano) 237 notifications 68 Notifications Gold (Nano) and 169 Colloidal Gold (Nano)		
G – 1 40 notifications	Opis Gold Water	1003569, 1003475, 1003474, 1003473, 1003472, 1003471, 1003470, 1003469, 1003468, 1003411, 1003223, 1003138, 1003135, 1003134, 1003133, 1002947, 1002887, 1002658, 1002652, 1002651, 1002650, 1002649, 1002648, 1002589, 1002588, 1002587, 1002533, 1002532, 1002531, 1002530, 1002529, 1002528, 1002527, 1002526, 1002525, 1002524, 1002523, 1002522, 1002521, 1000420	eye contour products face care products other than face mask face mask hair conditioner hand care products make up remover products other hair and scalp care and cleansing other skin care products other skin products scalp and hair roots care products	Leave on : 0.0001 to 0.0139 Rinse off : 0.0001
G-2 15 notitifications	aXonnite Gold	1003293, 1003255, 1003254, 1003241, 1003236, 1003235, 1003234, 1003233, 1003231, 1003230, 1003229, 1003228, 1003226, 1003091, 1003090, 1009091*	Body care products eye contour products face care products other than face mask face mask	Leave on : 0.00001 to 0.00025 Rinse off : 0.00001
G-3 1 notification	Axonnite Gold nano-TECH	1003253	eye contour products	Leave on : 10
G-4 3 notifications	PSQ-Au	1003033, 1002008, 1002006	other face make - up products other lip m ake - up products	Leave on : 0.000020
G-5 1 notification	Nanozloto	1001523	face care products other than face mask	Leave on : 0.09
G-6 1 notification	Nano gold partical	1001422	face mask	Rinse off : 0.1
G-7 1 notification	Goldex ZŁOTO NANOKOLIDALNE (H2O Au) NIECHEMICZNE	1001412	Body care products	Leave on : 0.005
G-8 2 notifications	Water&Cellulose Gum&SodiumCarbonate&Gold&Silver	1003408, 1003413	face mask	Leave on : 0.005
G-9 3 notifications	//	1002916, 1002928, 1002933	Body care products	Leave on : 0.0018 to 0.0027
G-10 1 notification	ALM70c, Au@TSK1	1003539	Body care products	Rinse off : 6
CG-1 10 notifications	Złota Woda nano-TECH	1000984, 1000986, 1000987, 1000988, 1000989, 1000990, 1000991, 1000992, 1001061, 1001090	Body care products eye contour products face care products other than face mask	Leave on : 0.00015 to 4 Rinse off : 0.005

			face mask make up remover products	
CG-2 14 notifications	Gold Water nano-TECH	1001180, 1001181, 1001182, 1001183*, 1001184, 1001185, 1001186, 1001187, 1001188, 1001189, 1001190, 1001191, 1001192 ,1001193	Bath / shower product Body care products Chemical exfoliation products eye contour products face care products other than face mask make up remover products other skin care products	Leave on : 1 Rinse off : 1
CG-3 9 notifications	GWE – 1000	1001908, 1001909, 1001910, 1001946, 1001947, 1001948, 1002306, 1002795 1000999	Body care products scalp and hair roots care products	Leave on : 0.0004 to 0.2 Rinse off : 0.0012 to 0.1
CG-4* 19 notifications	Golden Gollagenine (PF)	1002371*, 1002372*, 1002373*, 1002374*, 1002375*, 1002376*, 1002377*, 1002378* 1002379*, 1002383*, 1002388*, 1002389*, 1002390*, 1002391*, 1002392*, 1002393*, 1002394*, 1002395*, 1002396*	Body care products eye contour products face care products other than face mask face mask make up remover products	Leave on : 0.0000006
CG-5 98 notifications	Gold Colloid Metalor	1002599, 1002600, 1002601, 1002602, 1002603, 1002604, 1002605, 1002606, 1002607, 1002608, 1002609, 1002610, 1002611, 1002612, 1002613, 1002614, 1002615, 1002616, 1002617, 1002618, 1002619, 1002620, 1002621, 1002622, 1002623, 1002624, 1002625, 1002626, 1002627, 1002628, 1002629, 1002630, 1002631, 1002632, 1002633, 1002634, 1002635, 1002636, 1002637, 1002638, 1002639, 1002640, 1002641, 1002642, 1002807, 1002808, 1002810, 1002811, 1002812, 1002813, 1002814, 1002815, 1002816, 1002817, 1002818, 1002819, 1002820, 1002821, 1002822, 1002823, 1002824, 1002825, 1002826, 1002828, 1003094*, 1003313, 1003314, 1003315 1003316, 1003317, 1003318, 1003319, 1003320, 1003321, 1003322, 1003323, 1003324, 1003325, 1003326, 1003327, 1003328, 1003329, 1003330, 1003331, 1003332, 1003333, 1003334, 1003335, 1003336, 1003337, 1003338, 1003339, 1003549, 1003550 1003551, 1003552, 1003553, 1003555	Body care products eye contour products face care products other than face mask face mask	Leave on : 0.00000165 to 0.00055 Rinse off : 0.001
CG-6 12 notifications	Lipobelle Gold	1002288, 1002950, 1002951*, 1002952*, 1002953, 1002954, 1003015, 1003055, 1003056, 1003057, 1003058, 1003059	eye contour products face care products other than face mask face mask	Leave on : 0.00055 to 0.005 Rinse off : 0.002 to 0.005
CG-7* 3 notifications	Colloid Gold – P	1003478*, 1003479*, 1003577*	eye contour products face mask other skin cleansing products	Leave on : 0.05 Rinse off : 0.000003
CG-8 1 notification	Colloid PMG-PG	1001542	face care products other than face mask	Leave on : 0.00005
CG-9 1 notification	spec file as Silver	1003372	other skin care products	Leave on : 0.005
CG-10 1 notification	PurestColloids-MesoGold	1001196	Body care products	Leave on : 0.000001

CG-11 1 notification		1002564	other skin care products	Leave on : 0.001
B. Gold Thioethylamino Hyaluronic Acid (nano) 11 notifications				
SMG-2 9 notifications	Endor -GH	1000831, 1002147, 1002148, 1002149, 1002150, 1002166, 1002167, 1002168, 1002169	eye contour products face care products other than face mask	Leave on : 3 to 4
SMG-3 2 notifications	Hyalgen	1002910, 1002911	face care products other than face mask	Leave on : 0.000225
C*. Acetyl heptapeptide-9 Colloidal gold (nano) 18 Notifications				
SMG-1* (18 notifications)	Golden Collagenine	1000600*, 1000720*, 1000721*, 1000722*, 1000740*, 1000741*, 1000742*, 1000743*, 1000744*, 1000745*, 1000748*, 1000749*, 1000750*, 1000753*, 1000754*, 1000755*, 1000756*, 1000757*	Body care products face care products other than face mask face mask	Leave on : 0.0000006 to 0.000012

8. Annex II

Safety concerns for Gold-nanomaterials used as cosmetic ingredient based on public information

In this Opinion, the SCCS has evaluated the safety of gold nanomaterials when used in cosmetics. From this evaluation, and other relevant information from published literature, the SCCS has concluded that there is a basis for concern that the use of gold, colloidal gold and surface modified gold (nano) in cosmetic products can pose a risk to the consumer because of the following considerations:

Physicochemical aspects:
Gold, Colloidal Gold and Surface Modified Gold are comprised of primary particles that are in the nano-scale. For most of the materials, the particle sizes are reported to range from 1 nm to 100 nm (Table 2). For some materials, nanoparticles have been reported in the size range between 2-5 nm.

The solubility for Gold, Colloidal Gold and Surface Modified Gold has been reported to be below 0.01 mg/L, indicating that these materials are practically insoluble.

The gold nanoparticles are reported to be in different shapes, such as nanospheres, nanotriangles, nanoprisms and nanorods. Other shapes that have also been reported in the literature include tetrahedral, sub-octahedral, octahedral, decahedral, icosahedral, multiple twined and irregular shapes (Schaeublin *et al.*, 2012; Tian *et al.*, 2015; Khan *et al*., 2014; Adewale *et al*., 2019).

Gold nanoparticles in the size ranging from 1 to 6 nm have been found to exhibit catalytic activity (Valden *et al.*, 1998; Cunningham *et al.*, 1998; Nafiu *et al.*, 2020).

Toxicological aspects:
The chemical and particulate nature of gold nanoparticles (AuNPs) and colloidal gold (nano) suggests a potential for toxicological hazard, as detailed below:

General Toxicity
In vitro
Spherical (Shukla *et al.,* 2005; Kahn *et al.*, 2007; Connor *et al.*, 2005; Gu *et al.*, 2009); Villiers *et al.*, 2010) and rod shaped gold particles (AuNPs) (Alkilany *et al.*, 2009) tested in a number of different cells showed no or only negligible cytotoxicity. Carnovale *et al.* (2019) found no cytotoxicity when cells were treated by cetyltrimethylammonium bromide (CTAB)-stabilized rod- and cube-shaped gold nanoparticles (5 nm size), whereas toxicity was observed in the case of CTAB-stabilized spherical and prismatic gold nanoparticles.

Schaeublin *et al*. (2011) evaluated differently charged AuNPs (1.5 nm size, positive, neutral and negative charge) and found that charged, but not neutral particles, caused significant mitochondrial stress as indicated by a decreased mitochondrial membrane potential and decreased intracellular Ca^{2+} levels.

There are several studies indicating that AuNPs (1 – 200 nm size) can be toxic when used in biological systems in a certain range of concentrations (Jia *et al.*, 2017). Under *in vitro* conditions, AuNPs can induce production of reactive oxygen species (ROS) after entering the cells, and oxidative stress-related cytotoxicity, such as DNA damage, cell death (apoptosis and necrosis) and cell cycle arrest.

It has been shown that AuNPs with a similar size (14.8±3.2 nm and 15.7±2.6 nm) and shape, but different surface charges, may elicit different cellular responses, i.e., the pathways of internalization, cell activation and inflammation in immune cells (Srijampa *et al*., 2019).

In vivo

In *in vivo* animal (rat, mouse) studies using either the intravenous (i.v.) or intraperitoneal (i.p.) administration route, several observations were made pointing to toxic effects of different forms of gold-NPs when systemically available:

- acute inflammation with neutrophils influx in the mouse liver (Cho *et al.*, 2009)
- activation of hepatic CYP1A1 and CYP2B enzymes (Cho *et al.*, 2010)
- increase in lipid peroxidation and protein carbonylation (Lopez-Chavez *et al.* (2018)
- effects (not further specified) on white blood cells and liver enzymes (Zhang *et al.*, 2011)
- Kidney effects (tubular alterations and histological alterations in cortex and proximal tubules) (Abdelhalim and Mady, 2011; Abdelhalim and Jarrar, 2011)
- Liver effects (e.g. alterations in hepatocytes, Kupffer cell hyperplasia or inflammatory cell infiltration) (Abdelhalim and Mady, 2011; Abdelhalim and Jarrar, 2011)

Non nano form

Data on the oral toxicity of elemental gold is limited. The acute toxicity of elemental gold seems to be low, as rats were unaffected by a single dose of 2000 mg nanoparticles/kg of body weight. Information on repeated dose toxicity is also very limited. Skin rashes have been reported in humans following the ingestion of liquors containing gold flakes (Russell *et al.*, 1996, 1997).

The release of gold from dental fillings, leading to elevated gold concentrations in the plasma and urine, has also been reported (Ahnlide *et al.*, 2002; Becker *et al.*, 2003; Drasch *et al.*, 2000; Komaromy-Hiller *et al.*, 2000).

Genotoxicity

Spherical 12 nm gold nanoparticles (uncoated or coated with hyaluronic acid (HA)) in comparison to the gold salt, $HAuCl_4$ *$3H_2O$ were comparatively investigated for *in vitro* cytotoxicity (MTT assay), genotoxicity (Comet assay) and cellular uptake (TEM) by using BALB/c 3T3 cells (DiGuglielmo *et al.*, 2012). It was demonstrated that nanoparticles were internalised by an endo-lysosomal pathway. Coating reduced cytotoxicity as well as internalisation into cells. DNA damage was observed and it was concluded that this was most probably due to an indirect mechanism (oxidative stress).

Wang *et al.* (2010) studied size-dependent endocytosis of gold nanoparticles and found that the amounts of cellular uptake decreased with the increase of particle size. On the other hand, Vales *et al.* (2020) demonstrated that uptake and cytotoxicity of gold NPs are clearly enhanced by positive surface charge.

The reactivity of gold NPs might lead to interferences with several *in vitro* assays. For example, 14-nm citrate-stabilized AuNPs (negative charge) interfered with the alkaline Comet assay during critical steps where cell membranes are lysed, and the intracellular NPs have the potential to directly interact with the DNA (George *et al.*, 2017).

Most *in vitro* studies on the genotoxicity of AuNPs have reported positive results with both the comet assay and micronucleus assay, but several studies have also reported negative responses (Hadrup *et al.*, 2015; Wang *et al.*, 2020; Vales *et al*., 2020). Xia *et al. (*2017) reported no DNA damage by the Comet assay after 20 and 50 nm AuNPs in HepG2, whereas 5 nm AuNPs induced a dose-dependent increment in DNA damage after 24-h exposure. Furthermore, 5 nm AuNPs induced cell cycle arrest in G1 phase in response to DNA damage and promoted the production of reactive oxygen species (ROS). Vales *et al.* (2020) exposed BEAS2B cells with two core (5 nm and 20 nm) and three functionalized gold nanoparticles and found that DNA damage was induced by 20-nm ammonium and PEGylated gold nanoparticles, while micronucleus induction was increased by 5-nm ammonium and 20-nm PEGylated gold nanoparticles.

Studies on *in vivo* genotoxicity testing provide some evidence on potential harmful effects (Xia *et al.*, 2017; Wang *et al*., 2020). In the standard *in vivo* micronucleus test, no obvious increase in the frequency of micronucleus formation was found in mice after 4 day exposure of AuNPs (Xia *et al.,* 2017). However, when the exposure period was extended to 14 days, 5 nm AuNPs presented significant clastogenic damage, with a dose-dependent increase of micronuclei frequencies.

The findings suggest that different factors may play critical roles in determining the genotoxic potential of AuNPs, e.g. particle size and surface coating; concentrations of AuNPs; cell models; experimental conditions (medium, serum) and test procedures; genotoxicity end points; and durations of exposure. Both negative and positive results were obtained with different genotoxicity endpoints and various gold nanoparticles. Thus, genotoxic potential of AuNPs cannot be excluded a prori, therefore it should be considered individually for each new nanomaterial assessed.

Immunotoxicity

Almeida et al. (Almeida *et al.*, 2013) concluded that immune cell populations carry AuNPs and migrate through the spleen rather than the particles migrating through the tissue by cell–cell transfer. An immunomodulatory effect of nano-particles, including those of gold, on skin allergy has been postulated (Jatana *et al.*, 2017). Some gold nanoparticles attenuate an allergic response of the skin in sensitised animals. It is as yet unclear whether this may represent a beneficial effect. A sensitisation study in mice suggests that gold nanoparticles are unlikely to cause sensitisation (Roach *et al.*, 2020).

Malaczewka (2015) has reported that the effect of gold nanocolloid administered orally on the peripheral blood leukocytes in mice was limited to the increased activity of phagocytes and changes in percentages of lymphocyte populations. Enhanced activity of granulocytes and monocytes was a transient phenomenon, noticed only after a short time of nanogold administration, which seems indicative of the adaptability of the organism to the presence of nanoparticles. However, the phenotypic changes among lymphocytes did not occur until 28 days of the administration of nanoparticles, which in turn might indicate exhaustion of compensatory mechanisms and certain immune dysregulation due to long-lasting contact with nanoparticles.

Non nano form

According to Hadrup et al. (2015), gold released from dental restorations has been reported to increase the risk of developing gold hypersensitivity.

Exposure aspects:

Frequency of use

Humans are exposed to gold from various sources; as a food coloring agent, use in dental fillings and inert carriers for medical purposes. Non-oral sources include jewelry and during the manufacturing of gold containing products (Brune *et al.*, 1980; Hamilton and de Gannes, 2011; Hewitt, 1988; Rapson, 1985). Oral sources include food, dental fillings, tobacco and pharmaceuticals (Ahnlide *et al.*, 2002; Iyengar et al., 2000; Krachler *et al.*, 2000; Nada *et al.*, 1999; Wittsiepe *et al.*, 2003; Ysart *et al.,* 1999). The human intake of dietary gold has been reported to be 10– 14 ng/kg bw/day for small children (Wittsiepe *et al.,* 2003; Ysart *et al.*, 1999) and 10 ng/kg bw/day for people consuming a typical American diet (Iyengar *et al*., 2000). Gold complexes have also been used as antirheumatic pharmaceuticals. These complexes, e.g., sodium aurothiomalate and auranofin, can be converted to other gold complexes in the mammalian body.

Bioavailability/Toxicokinetics (uptake and tissue distribution)

The uptake, distribution and toxicity of gold nanoparticles depend greatly on the size (Wang *et al*., 2010; 2015) shape (Carnovale *et al.* 2019), interaction between the particle surface

and the surrounding biological media (Alkilany & Murphy, 2010; Mahmoudi *et al.,* 2011), e.g. capping agents and protein corona (Carnovale *et al.,* 2019).

Non nano form

According to a review by Hadrup *et al.* (2015), gold could be detected in organs, such as the liver, heart, kidneys and lungs. The main excretion route of absorbed gold is through urine.

The release of gold from dental fillings, leading to elevated gold concentrations in the plasma and urine, has also been reported [Ahnlide *et al.* (2002); Becker *et al.* (2003); Drasch *et al.* (2000); Komaromy-Hiller *et al.* (2000)].

Studies with different age groups indicate that gold is not accumulated to a large extent in humans because older individuals do not have higher body burdens of gold than younger individuals (Masiak *et al.*, 1981; Parr and Taylor, 1963). Nevertheless, in subjects aged below 60 yr a higher Au level was demonstrated in the serum than in the subjects aged above 60 yr (Masiak *et al.*, 1981).

Regarding distribution in humans, gold has been found in a range of tissues, including the blood, liver, lung, kidney, heart, spleen, brain, bladder and endometrium. In human milk, gold has been reported in the range of 0.1–2.1 µg/L (Krachler *et al*., 2000; Prohaska *et al*., 2000).

Information available in open literature indicates that gold NPs can be taken up by the oral, dermal route and inhalation route. The latter route is not considered because the inhalation route was not indicated in the notifications for cosmetic applications.

Dermal route:

AuNPs penetration in the stratum corneum layer has been reported (Graf *et al.*, 2009); Labala *et al.*, 2015; Labouta *et al.*, 2013b; Liu *et al.*, 2012), up to the epidermis layer (Hao *et al.*, 2017; Raju *et al.*, 2018), and deeper skin penetration in the dermis/ hypodermis layer (Chen *et al.*, 2017; Fernandes *et al.*, 2014, 2015; Goldstein *et al.*, 2014; Hsiao *et al.*, 2019; Huang *et al.*, 2010, Labouta *et al.*, 2011; Larese Filon *et al.*, 2011; Mahmoud *et al.*, 2017, 2018).

Various parameters have been reported to influence the observed skin penetration:

- surface modification (Bessar *et al.*, 2016; Chen *et al.*, 2017; Fernandez *et al.*, 2014, 2015; Hao *et al.*, 2017; Labala *et al.*, 2015; Labouta *et al.*, 2013a; Mahmoud *et al.*, 2016),
- surface charge (Chen *et al.*, 2017); Fernandes *et al.*, 2014, 2015; Hao *et al.,* 2017, Labala *et al.*, 2015; Lee *et al.*, 2013; Mahmoud *et al.*, 2017)
- hydrophobic/ hydrophilic character (Labouta *et al.*, 2011, 2013b; Gupta *et al.,* 2017; Mahmoud *et al.*, 2017, 2018; Sonavane *et al.*, 2008); Xiong *et al.*, 2016)
- Au nanoparticles shape (Fernandes *et al.*, 2014, 2015; Hsiao *et al.*, 2019)
- aggregation / agglomeration state (El-Sayed *et al.*, 2016; Labouta *et al.*, 2011, 2012; Mahmoud *et al.*, 2016)
- Au nanoparticle size (Gupta *et al.*, 2017; Huang *et al.*, 2010; Raju *et al.*, 2018; Sonavane *et al.*, 2008, Hsiao *et al.*, 2019).

Based on experiments performed using the Franz diffusion cell method with intact and damaged human skin, Larese Filon *et al*. (2011) have reported twenty-four hours gold flux permeation was 7.8 ± 2.0 ng cm^{-2} h^{-1} and 7.1 ± 2.5 ng cm^{-2} h^{-1} in intact and damaged skin, respectively, with a lag time less than 1 hour.

Oral route:

Hillyer and Albrecht (2001) have published a study on four sizes of gold nanoparticles (4, 10, 28 and 58 nm) administered to mice for 7 days. The concentration of each particle size was 200 mg/L of drinking water, estimated to be equivalent to 36 mg/kg bw/day. The investigators

found that the smaller particles (4 and 10 nm) crossed the gastrointestinal membrane more readily than the larger particles (28 and 58 nm) and that uptake occurred in the small resulting in the distribution of gold to the blood, brain, lungs, heart, kidneys, spleen, liver, small intestine and stomach.

Zhang *et al.* (2010) reported that the administration of 2.2 mg/kg bw/day gold nanoparticles (13.5 nm) by oral gavage for 14 days to mice resulted in gold nanoparticles occurring in the blood and in bone marrow cells.

Schleh *et al*. (2012) investigated radiolabelled gold nanoparticles with sizes ranging from 1.4 to 200 nm that were either stabilized with mono-sulfonated triphenylphosphine or citrate. The nanoparticles were administered by oral gavage at doses in the range of 4–108 mg/kg bw. After 24 h, the absorption of gold was reported to be in the range of 0.02–0.4% of the administered gold.

Alalaiwe *et al.* (2017) have investigated the influence of PEG coating on the oral bioavailability of gold nanoparticles (5 nm) in rats. Blood concentrations following oral administration were inversely related to PEG size, and the AUC (Area Under the Curve) in blood was significantly greater for the 1 kDa PEG-coated AuNPs than particles coated with 2 or 5 kDa PEG. Bioavailabilities of all of the particles were below 0.1%. Concentrations in liver, spleen and kidney were similar after the intravenous doses, but kidney showed the highest concentrations after an oral dose

Distribution

Systemically available gold can be distributed to a variety of tissues and even cross the blood brain (Sonavane *et al.*, 2008), the blood testis or the placental (Lin *et al*., 2015)-barrier. The distribution is influenced by NP size and surface properties (De Jong *et al.*, 2008). Available information also indicates, that gold may stay over considerable periods of time in certain tissues.

Schleh *et al.* (2012) report that in their study mentioned above (gold nanoparticles with sizes ranging from 1.4 to 200 nm, administrated by oral gavage), gold was found in the liver, kidneys, blood, lungs, heart, brain and spleen. In addition, Schleh *et al.* found approximately 0.05% of the administered gold in 24 h urine, suggesting this as a route of elimination however it is unclear whether the absorbed amounts of gold were of particulate nature.

Lin *et al.* (2015) in their literature overview report that after intraesophageal instillation of negatively (1.4–200 nm) or positively (2.8 nm) charged AuNPs to rats, AuNPs were able to cross the gastrointestinal barrier, but the absorption was incomplete within 24 h and absorption efficiency was low and size-dependent, ranging from 0.37% for small sizes (1.4–2.8 nm) to 0.01% for large size (200 nm). Negatively charged 2.8 nm AuNPs had a higher absorption than (0.37% vs 0.14%) positive 2.8 nm particles.

Conclusion:

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

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