Scientific Committee on Consumer Safety

SCCS

OPINION ON

Copper (nano) and Colloidal Copper (nano)

The SCCS adopted this document
by written procedure on 5 March 2021

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of a minimum eight weeks after its initial publication (from 9 November 2020 until 4 January 2021). Comments received during this time were related to the general toxicity and risk assessment of copper. As the comments were not related to the nanoform of copper, the comments did not result in a change of the Opinion.

1. ABSTRACT

In total, 9 manufacturers notified the European Commision about 36 different products containing nanosized copper (nanoCu). For every copper dispersion, four different files were presented: a notification file, a safety file, a specification file and a file on the toxicity profile. After initial evaluation and a request for additional information, two manufacturers withdrew their products (n=7) from the notification. Therefore, these 7 products were excluded from evaluation, resulting in the final evaluation of 29 nanosized copper products produced by 7 manufacturers.

For three different copper nanomaterials, notification files were provided. These were Copper Water nano-Tech 100 ppm, Copper Water nano-Tech 50 ppm, and ECO COPPER NANO-COLOID.

For all three copper nanomaterials, the notified files were insufficient regarding their characterisation and for conducting a safety evaluation. All copper nanomaterials claimed to have an antimicrobial activity. Most files contained declarations of safety without any supporting data or documentation.

Based on the provided information, it is not possible to perform a safety assessment on these copper nanomaterials.

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 Copper and Colloidal Copper safe when used in leave-on and rinse-off dermal and oral cosmetic products including: skin, nail and cuticle, hair and scalp and oral hygiene products, at a maximum concentration of 1 % (for both Copper and Colloidal Copper) and with specifications as 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 Copper and Colloidal Copper due to the limited or missing essential information.

Much of the information provided on toxicity relates to copper as such, and in the absence of full study reports, it is not possible to determine the relevance of the data for nano-forms of copper under the current evaluation.

Adequate information on various aspects both for the nanomaterial characterisation and the toxicological evaluation including experiment performance and experimental data obtained need to be provided (as summarised in Annex I).

2. Does the SCCS have any further scientific concerns with regard to the use of Copper and Colloidal Copper in nano form in cosmetic products?

The information provided by the Notifiers, and obtained from scientific literature, suggests possible systemic uptake of Cu nanoparticle (and/or ionic Cu), which may lead to accumulation in certain organs - notably the liver and spleen. In addition, the available literature data indicate potential mutagenic/genotoxic and immunotoxic/nephrotoxic effects of copper nanomaterials. These indications raise an alert that warrants further safety evaluation of copper nanomaterials used as cosmetic ingredients. The SCCS concerns for consumer safety in this regard are detailed in Annex II.
Annex I provides an overview of the information provided by the Notifiers compared to the data requirements as given in the SCCS checklists for Applicants submitting dossiers on Cosmetic Ingredients to be evaluated by the SCCS (SCCS/1588/17).

Keywords: SCCS, scientific opinion, Copper (nano), Colloidal Copper (nano), CAS No 7440-50-8, EC No. 231-159-6, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Copper (nano) and Colloidal Copper (nano), preliminary version of 27-28 October 2020, final version of 5 March 2021, SCCS/162/2020

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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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https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Article 2(1)(k) of Regulation (EC) No 1223/2009 (Cosmetics Regulation) establishes that "nanomaterial" means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.
That definition covers only materials in the nano-scale that are intentionally made and are insoluble/partially-soluble or biopersistent (e.g. metals, metal oxides, carbon materials, etc.). 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, the Commission shall refer it to the Scientific Committee on Consumer Safety (SCCS) for a full risk assessment.
The Commission services received 35 notifications under Article 16 of the Cosmetics Regulation via the Cosmetic Product Notification Portal (CPNP) for cosmetic products containing Copper (30 notifications) and Colloidal Copper (5 notifications) with CAS No 7440-50-8, EC and No. 231-159-6 in nano form, as reported in the attached list. Only Copper is reported in the CosIng database as a colorant (CI 77400) without any reference to the nano form and it is regulated according to entry 132 of Annex IV (IV/132) of the Cosmetic Regulation (EC) No 1223/2009.
According to the available notifications, both ingredients are used in nano uncoated form with an anti-microbial function, in both leave-on and rinse-off dermal and oral cosmetic products. The notifications concern skin, nail and cuticle, hair and scalp products, as well as oral hygiene products, with maximum reported concentration being 1% (for both Copper and Colloidal Copper). The notifications and respective specifications are reported in the attached list.
The Commission has concerns on the use of Copper and Colloidal Copper in nano form because of the potential for nanoparticles to be absorbed dermally or across a mucous membrane and to enter cells. Therefore, we request the SCCS to carry out a safety assessment of the nano form of Copper and Colloidal Copper 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 Copper and Colloidal Copper safe when used in leave-on and rinse-off dermal and oral cosmetic products including: skin, nail and cuticle, hair and scalp and oral hygiene products, at a maximum concentration of 1 % (for both Copper and Colloidal Copper) and with specifications as 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 Copper and Colloidal Copper in nano form in cosmetic products?

3. OPINION

Preamble

In total, 9 manufacturers sent notifications to the European Commission on 36 different products containing nanosized copper (nanoCu). For every copper dispersion, four different files were presented: a notification file, a safety file, a specification file and a file on the toxicity profile. After initial evaluation and a request for additional information, two manufacturers withdrew their products (n=7) from the notification. Therefore, these 7 products were excluded from evaluation resulting in the final evaluation of 29 nanosized copper products of 7 manufacturers.

Notification files were presented for three different copper nanomaterials:Copper Water nano-Tech 100 ppm, Copper Water nano-Tech 50 ppm and ECO COPPER NANO-COLOID. All copper nanomaterials were claimed to have an antimicrobial activity. A number of additional files were presented at the request of the SCCS.

In addition to the information provided by the Applicants, a Call for Data on copper nanomaterials was published by the Commission from 11 June 2019 to 10 November 2019. Information received as a result of this Call has also been considered by the SCCS. Furthermore, information from the literature search performed by the Commission was also considered by the SCCS. However, it was not possible to relate toxicological data from this information with the type of materials considered in this assessment. As such, the SCCS has considered that the information available at present is insufficient to allow drawing conclusions on the safety of copper nanomaterials included in this Opinion.

A commenting period on the preliminary Opinion on Copper (nano) and Colloidal Copper (nano) was open on the website of the non-food scientific committees from 9 November 2020 to 04 January 2021. Comments received in the commenting period were related to the general toxicity and risk assessment of copper. As the comments were not related to the nanoform of copper, the comments did not result in a change of the Opinion.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI (International Nomenclature Cosmetic Ingredients) name: CI 77400
IUPAC: copper

SCCS comment

CI 77400 as indicated by Notifier is indicated in INCI as copper powder, bronze powder used as colorant.

Ref.: 1

3.1.1.2 Chemical names

Copper (colloidal)

SCCS comment

The term colloidal copper implies that particles are in aqueous suspension. However, some part of the copper content may also be in the form of ionic copper depending on the solubility of the copper nanoparticles.

3.1.1.3 Trade names and abbreviations

In the following Tables, the trade names of colloidal copper dispersions from different manufacturers are described.

Table 1: Trade names of the various notified colloidal copper dispersions (coded by the SCCS)

Code manufacturer	**Code number SCCS**	**Cosmetic product name**	**CPNP code**	**Product use**
A	1	Copper water	1000980	Dermal, leave on
B	2	Vertere Colloidal Nano Copper 50 ppm	1002550	Dermal, leave on
B	3	Quantum Gold Golden Ratio Formula Serum	1002551	Dermal, leave on
B	4	Quantum Gold Golden Ratio Formula Cream	1002552	Dermal, leave on
B	5	Quantum Nano Gold Micellar Water	1002553	Dermal, leave on
B	6	Quantum Silver Action Cleansing Gel	1002554	Dermal, rinse off
B	7	Quantum Platinum Enlightenment Cream	1002555	Dermal, leave on
B	8	Quantum Gold New Age Cream	1002556	Dermal, leave on
B	9	Quantum Gold New Age Serum	1002557	Dermal, leave on
B	10	Quantum Gold Ultimate Revival Hair Serum	1002558	Dermal, leave on
B	11	Quantum Gold Golden Ratio Cellural Eye	1002559	Dermal, leave on
B	12	Quantum Silver Peel Solution	1002560	Dermal, rinse off

B	13	Quantum Gold Algae Mask	1002561	Dermal, leave on
C	14	C Nano Active Krem do twarzy redukujący przebarwienia ANTI-OX C Nano Active Face Enlightenment Cream ANTI-OX	1002906	Dermal, leave on
D	15	Maseczka oczyszczająco-kojąca, do skóry ze skłonnością do zmian trądzikowych Cleansing-soothing mask for skin with acne changes	1002940	Dermal, rinse off
D	16	Maseczka pielęgnująco-oczyszczając Caring and cleansing mask	1002941	Dermal, rinse off
D	17	Maseczka odżywczo-relaksująca Nourishing and relaxing mask	1002942	Dermal, rinse off
D	18	Maseczka nawilżająco-liftingująca Moisturizing and lifting mask	1002943	Dermal, rinse off
D	19	Maseczka odświeżająco-detoksykacyjna Refreshing and detoxifying mask	1002944	Dermal, rinse off
E	20	Podopharm Professional Tinktura na grzybicę paznokci Podopharm Tincture for onychomycosis	1002989	Dermal, leave on
F	21	ENILOME INTIMA Pianka do higieny intymnej	1003013	Dermal, rinse off

		ENILOME INTIMA Intimate hygiene foam		
F	22	SELECTA INTIMA Dermokosmetyk do higieny intymnej SELECTA INTIMA Intimate hygiene dermocosmetic	1003014	Dermal, rinse off
D	23	Maseczka odświeżająco detoksykacyjna Refreshing and detoxifying mask	1003061	Dermal, rinse off
D	24	Maseczka nawilżająco – liftingująca Moisturizing and lifting mask	1003062	Dermal, rinse off
D	25	Maseczka odżywczo relaksująca Nourishing and relaxing mask	1003063	Dermal, rinse off
D	26	Maseczka pielęgnująco oczyszczająca Caring and cleansing mask	1003064	Dermal, rinse off
D	27	Maseczka oczyszczająco-kojąca, do skóry ze skłonnością do zmian trądzikowych Cleansing-soothing mask for skin with acne changes	1003065	Dermal, rinse off
G	28	Nano Koloid Eco Miedz (ECO COPPER NANO-COLLOID)	1003371	Dermal, leave on
G	29	Nano Koloid Eco Miedz (ECO COPPER NANO-COLLOID)	1003373	Dermal, leave on

Table 2: Composition of the various colloidal copper dispersions (as identified by CPNP code)

Code product manu-facturer	Code number	CPNP code	Cu NP size, nm (%)	Concentration	Copper identification	References
A	1	1000980	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1000980 SpecCu100-Eng 1000980_35406_spec_file 2013-6-28-14-18-1
B	2	1002550	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002550_100234_spec_file 2016-6-26-9-58-11
B	3	1002551	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002551_100234_spec_file 2016-6-26-9-58-11
B	4	1002552	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002552_100234_spec_file 2016-6-26-9-58-11
B	5	1002553	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002553_100234_spec_file 2016-6-26-9-58-11
B	6	1002554	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002554_100234_spec_file 2016-6-26-9-58-11
B	7	1002555	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002555_100234_spec_file 2016-6-26-9-58-11
B	8	1002556	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002556_100234_spec_file 2016-6-26-9-58-11
B	9	1002557	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002557_100234_spec_file 2016-6-26-9-58-11
B	10	1002558	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002558_100234_spec_file 2016-6-26-9-58-11
B	11	1002559	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002560_100234_spec_file 2016-6-26-9-

						58-11
B	12	1002560	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002561_100234_spec_file 2016-6-26-9-58-11
B	13	1002561	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002550_100234_spec_file 2016-6-26-9-58-11
C	14	1002906	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002906_69005_spec_file 2017-5-10-9-43-6
D	15	1002940	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002940 - 112784 spec file 2017-5-31-7-37-34
D	16	1002941	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002941 - 112784 spec file 2017-5-31-7-37-34
D	17	1002942	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002942 - 112784 spec file 2017-5-31-7-37-34
D	18	1002943	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002943 - 112784 spec file 2017-5-31-7-37-34
D	19	1002944	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1002944 - 112784 spec file 2017-5-31-7-37-34
E	20	1002989	2-5 (70-75%) 5-100 (25-30%)	50 ppm	Axonnite Copper nano-Tech	1002989 - 72148 spec file 2017-8-1-8-33-14
F	21	1003013	2-5 (70-75%) 5-100 (25-30%) 3-5 (80-85%) 5-100 (15-20%)	50 ppm 100 nm	Axonnite Copper nano-Tech Copper Water nano-TECH	1003013 - 104652 spec file 2017-8-23-13-2-30 1003013-104652_exposure_file 2017-8-23-13-2-30
F	22	1003014	2-5 (70-75%) 5-100 (25-30%) 3-5 (80-85%) 5-100 (15-	50 ppm	Axonnite Copper nano-Tech Copper Water	1003014 - 104652 spec file 2017-8-23-13-2-30

			20%)	100 nm	nano-TECH	1003013-104652_exposure_file 2017-8-23-13-2-30
D	23	1003061	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1003061 - 112784 spec file 2017-5-31-7-37-34
D	24	1003062	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1003062 - 112784 spec file 2017-5-31-7-37-34
D	25	1003063	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1003063 - 112784 spec file 2017-5-31-7-37-34
D	26	1003064	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1003064 - 112784 spec file 2017-5-31-7-37-34
D	27	1003065	3-5 (80-85%) 5-100 (15-20%)	100 ppm	Copper Water nano-TECH	1003065 - 112784 spec file 2017-5-31-7-37-34
G	28	1003371*	No data	No data	ECO COPPER NANO-COLOID	1003371 - 86357 spec file 2018-10-3-11-26-3
G	29	1003373*	No data	No data	ECO COPPER NANO-COLOID	1003373 - 86357 spec file 2018-10-3-11-26-3

* Notification 1003371 indicates presence of the element silverin stead of copper. Both Notification 1003371 and 1003373 indicate under heading 'other information' that the product 'does not cause allergies except for specific allergy to silver'.

SCCS comment

Copper Water nano-Tech 100 ppm concentration and copper water in axonnite technology 50 ppm

In total, 7 manufacturers notified the Commission about 29 different products containing nanosized copper (nanoCu). For every copper dispersion, four different files were presented: a notification file, a safety file, a specification file and a file on the toxicity profile.

For 2 products (product 21 and 22 of manufacturer F), an exposure file was sent as notification. However, these "exposure" files contained information on the identification of the Cu NPs as being Copper Water Nano-Tech 100 ppm. For these two products, two different identification files for nano-copper were provided, one identifying it as NanoTech 50 ppm and the other as NanoTech 100 ppm.

Three different Cu nanomaterials were used in the various products for which notification was submitted.

In the files presented, Ag is indicated:
Copper Water nano-TECH 100 ppm. Characteristics "Content of Ag",
Spec file 2013-6-28-14-18-1,
Spec file 2016-6-26-9-58-11,
Spec file 2017-5-10-9-43-6,
Spec file 2017-5-31-7-37-34,

All Spec files are including the same pdf file "Spec.Cu100-Eng" related to Copper Water nano-TECH 100 ppm.

Axonnite Copper nano-TECH, Copper Water in Axonnite Technology, 50 ppm:
Characteristics "Content of Ag".
Spec file 2017-8-1-8-33-14,
Spec file 2017-8-23-13-2-30.

ECO COPPER NANO-COLOID
For #28 and #29 (ECO COPPER NANO-COLOID) additional information has already been requested by the EC. Both notification files contain the same information with the exception that in notification 1003371, the element silver is reported and in the notification file 1003373, the element silver is changed into the element copper.

Ref.: 2, 3. See also file numbers in Table 2.

3.1.1.4 CAS / EC number

CAS: 77440-50-8
EC Number: 231-159-6

Copper water in Axonnite Technology
INCI name: water/aqua
CAS number: 7732-18-5

SCCS comment
Cu CAS and EC number missing in notification 1002906, 1002940, 1002941, 1002942, 1002943, 1002944, 1002989. Products C14, D15-19, and E20 (Table 2).

Ref.: 2

3.1.1.5 Structural formula

Cu

3.1.1.6 Empirical formula

Cu

3.1.2 Physical form

The copper nanomaterials are presented as dispersions in water of copper nanoparticles. For the various products (1-29) of the different manufacturers (A-G) 3 different Cu nanoformulations were used as presented in Tables 3 and 4.

Table 3. Specifications of copper nanomaterials in notified products

Manufacturer	Product	Specification file number	Specification
A	1	2013-6-28-14-18-1	Copper Water nano-TECH 100 ppm
B	2-3-4-5-6-7-8-9-10-11-12-13	2016-6-26-9-58-11	Copper Water nano-TECH 100 ppm
C	14	2017-5-10-9-43-6	Copper Water nano-TECH 100 ppm
D	15-16-17-18-19-23-24-25-26-27	2017-5-31-7-37-34	Copper Water nano-TECH 100 ppm
E	20	2017-8-1-8-33-14	Copper Water nano-TECH 50 ppm
F	21-22	2017-8-23-13-2-30	Copper Water nano-TECH 50 ppm
G	28-29	2018-10-3-11-26-3	ECO COPPER NANO-COLOID

Table 4. Physical form, shape and agglomeration/aggregation state.

Copper nanomaterial	Physical form, shape and agglomeration/aggregation state
Copper Water nano-TECH 100 ppm	Dispersion, crystalline, dispersed free particles
Copper Water nano-TECH 50 ppm	Dispersion, crystalline, dispersed free particles
ECO COPPER NANO-COLOID	Solution, crystalline, dispersed free particles

Ref.: 2, 3, 4, 5, 6, 7, 8.

3.1.3 Molecular weight

63.55

3.1.4 Purity, composition and substance codes

Products are described as water dispersion (colloid) of copper nanoparticles:

Products 1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-23-24-25-26-27 composition: copper 100 ppm ± 10%.

Products 20-21-22 composition: copper 50 ppm ± 10%.

Product 28-29 (ECO COPPER NANO-COLOID): The product contains no ingredients listed in Annex I to the Regulation. According to the notified recipes and ingredient information and technological description, the following was stated: Formaldehyde, mercury, lead, cadmium, arsenic, antimony, 1.4 dioxan, phthalates, secondary amines and nitrosamines are below allowed level (levels indicated in Notification_1003371_86357_need_info_safety_file EC_2019-3-24-20-19-8 English).

SCCS comment
Information was not provided for one of the copper nanomaterials (present in product 28-29).

For product 21 and 22, presented files indicate that it is composed of NanoTech 50 ppm whereas other files indicate that it is composed of NanoTech 100 ppm.

Product 21: Notification 1003013 (NanoTech 50 ppm), Spec file 2017-8-23-13-2-30 (NanoTech 50 ppm), Safety file 2017-8-23-13-2-30 whereas the file 1003013-104652_exposure_file 2017-8-23-13-2-30 refers to the Spec.Cu 100-Eng file on NanoTech 100 ppm.
Product 22: Notification 1003014 (NanoTech 50 ppm), Spec file 2017-8-23-13-2-30 (NanoTech 50 ppm), Safety file 2017-8-23-13-2-30 whereas the file 1003013-104652_exposure_file 2017-8-23-13-2-30 refers to the Spec.Cu 100-Eng file on NanoTech 100 ppm.

Information was not provided on the methodology used for determination of the copper content for the other copper nanomaterials.

For NanoTech 100 ppm (Spec.Cu 100-Eng), "metallic nanomolucular copper Cu4N" and for NanoTech 50 ppm (Spec file 2017-8-23-13-2-30), "metallic nanomolucular silver Cu4N (chemical analysis 928/05) is indicated. Cu4N does not exist.

NOTE Ag4N is a quality indication for Ag.

In several places in various files, 'silver' is presented instead of 'copper'.

A detailed analytical report on determination of copper levels should be provided.

Ref.: 2, 3, 4.

3.1.5 Impurities / accompanying contaminants

Table 5: Impurities/contaminants

Copper nanomaterial	Impurities/contaminants
Copper Water nano-TECH 100 ppm	Copper Water nano-TECH does not contain any of 36 listed allergens, is not mentioned in the Annex to Minister of Health Regulation as a forbidden substance and does not contain any substance of CMR group.
Copper Water nano-TECH 50 ppm	Copper Water nano-TECH does not contain any of 36 listed allergens, is not mentioned in the Annex to Minister of Health Regulation as a forbidden substance and does not contain any substance of CMR group.
ECO COPPER NANO-COLOID	-

SCCS comment

Information was not provided on the presence of impurities/contaminants with the exception for the statements on the absence of allergens and forbidden substances as presented in Table 5. A detailed analytical report on purity of the material and any impurities/contaminants should be provided.

Ref.: 2, 3, 4.

3.1.6 Solubility

Table 6: Solubility

Copper nanomaterial	Solubility
Copper Water nano-TECH 100 ppm	<0.01 mg/L (MSDS unlimited)
Copper Water nano-TECH 50 ppm	<0.01 mg/L
ECO COPPER NANO-COLOID	It dissolves

SCCS comment
Information was not provided on the solubility for the copper nanomaterials or for how solubility was determined. The statement 'It dissolves' is not acceptable without supporting data. A detailed report on the methodology used for determination of copper solubility should be provided.

Ref.: 2, 3, 4, 5, 6.

3.1.7 Partition coefficient (Log P_{ow})

Table 7. Partition coefficient (Log P_{ow})

Copper nanomaterial	Partition coefficient (Log P_{ow})
Copper Water nano-TECH 100 ppm	Not applicable
Copper Water nano-TECH 50 ppm	Not applicable
ECO COPPER NANO-COLOID	Not applicable

Ref.: 2, 3, 4, 5, 6, 7, 8.

3.1.8 Additional physical and chemical specifications

Table 8: Physical characteristics of copper nanomaterials

Product/Parameter	Copper Water nano-TECH 100 ppm	Copper Water nano-TECH 50 ppm			ECO COPPER NANO-COLOID
Melting point	/	/	≠	≠	/
Boiling point	Approx 100°C				Ca 100°C
Flash point	/	/	≠	≠	/-
Vapour pressure	/	/	≠	≠	/-
Density	0.990 – 1.010	0.990 – 1.010			-
Viscosity	1000x10^{-6} Pa	1000x10^{-6} Pa			-

	x s	x s			
pKa					
Refractive index					
pH	6.5+/-0.5 (5-6.5) (5.0-7.5)	6.5+/-0.5 (6.0+/-1) (5.0-7.5)			5.0 7.0
SSA (BET)	1 m^2 /g	1 m^2 /g			-
VSSA	1 m^2/cm^3	1 m^2/cm^3			-

SCCS comment
Limited information was provided. Boiling point of 'approximately 100°C' is the boiling point of the vehicle water and not of copper.

3.1.9 Particle size

Table 9. Particle size

Copper nanomaterial	Particle size
Copper Water nano-TECH 100 ppm	3-5 nm (80%-85%), 5-100 nm (15%-20%) Notification [Min – Max: 1 – 100]
Copper Water nano-TECH 50 ppm	2-5 nm (70%-75%), 5-100 nm (25-30%) Notification [Min – Max: 1 – 100]
ECO COPPER NANO-COLOID	-

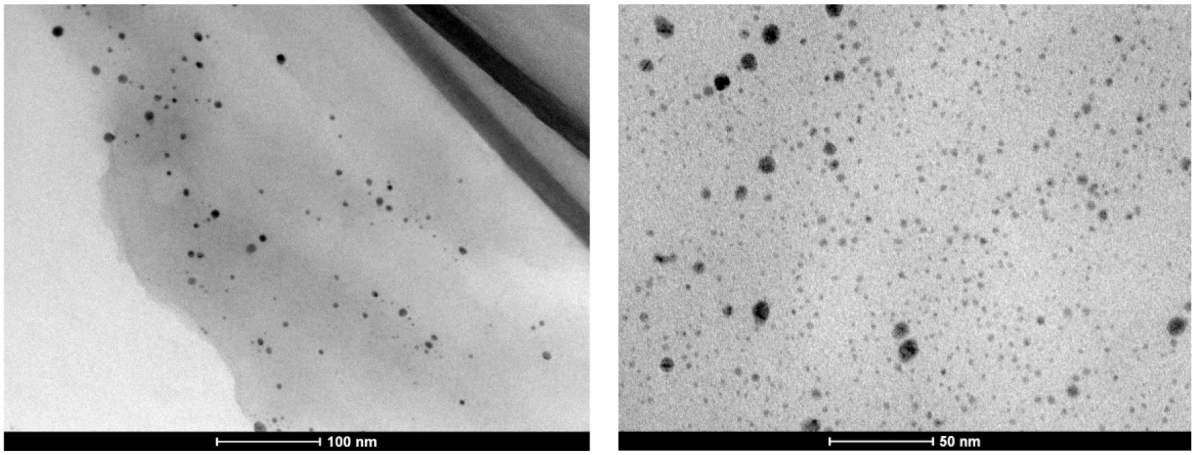
SCCS comment
Information on how particle size measurements were performed was not provided. This information should be provided to ascertain the validity of the data reported in Table 9.

3.1.10 Microscopy

Table 10. Electron microscopy

Copper nanomaterial	Electron microscopy
Copper Water nano-TECH 100 ppm	No pictures were provided
Copper Water nano-TECH 50 ppm	No pictures were provided
ECO COPPER NANO-COLOID	Pictures were provided

Figure ECO COPPER NANO-COLOID nanomaterial.



Rys. 2.2.1. Obrazy TEM w jasnym polu cząstek srebra

Translation title: Fig. 2.2.1. TEM images in a bright field of silver particles

SCCS comment
Limited information was provided. Only for the ECO COPPER NANO-COLOID nanomaterial TEM were pictures provided, but in the photo captions they were said to represent silver nanoparticles.

3.1.11 Crystal structure

Table 11. Crystal structure

Copper nanomaterial	Crystal structure
Copper Water nano-TECH 100 ppm	Crystalline
Copper Water nano-TECH 50 ppm	Crystalline
ECO COPPER NANO-COLOID	-

SCCS comment
Limited information was provided. A description of the crystalline shape should be provided.

3.1.12 UV absorption

SCCS comment
Information was not provided. Information on the UV absorption and the methodology used should be provided.

3.1.13 Surface characteristics

SCCS comment
Information was not provided. Information on the surface characteristics and the methodology used should be provided.

3.1.14 Droplet size in formulations

SCCS comment
Information was not provided. However, this information is not considered relevant as the formulations are intended for dermal applications only. Information would be needed if formulations were intended to be used as spray applications.

3.1.15 Homogeneity and stability

Table 12. Homogeneity and stability

Copper nanomaterial	Homogeneity and stability
Copper Water nano-TECH 100 ppm	Shelf life 24 months
Copper Water nano-TECH 50 ppm	Shelf life 30 months
ECO COPPER NANO-COLOID	-

SCCS comment
Limited information was provided for Copper Water Nanotech-100 and Copper Water Nanotech-50 for their shelf life. A detailed report on the methodology used for determination of the shelf life should be provided.

3.1.16 Other parameters of characterisation

SCCS comment
Information was not provided.

3.1.17 Summary on supplementary physicochemical characterisation

Table 13. Physical characteristics of copper nanomaterials.

Product/Parameter	Copper Water nano-TECH 100 ppm	Copper Water nano-TECH 50 ppm	ECO COPPER NANO-COLOID
Concentration	100 ppm ± 10% (0.01%)	50 ppm ± 10% (0.005%)	25 ppm (0.0025%)
Size	3-5 nm (80%-85%) 5-100 nm (15%-20%)	2-5 nm (70%-75%) 5-100 nm (25-30%)	<5, or 5-10 nm
Surface area	-	-	-
Surface charge	-	-	-
pH	6.5 ± 0.5	6.5 ± 0.5	5.0 – 7.0

	(5.0-7.5) PN-EN-ISO 3696.	(5.0-7.5) PN-EN-ISO 3696.	
Solubility	<0.01 mg/L	<0.01 mg/L	It dissolves
Shelf life	24 months	30 months	-
Form	-	-	-
Colour	-	-	Colorless

SCCS comment on physicochemical characterisation
Information provided on characterisation was limited and missing on various parameters. These include surface area, surface charge, solubility, and crystalline shape for all 3 copper nanomaterials (Copper Water nano-TECH 100 ppm, Copper Water nano-TECH 50 ppm, and the ECO COPPER NANO-COLOID nanomaterial).

The information on the characterisation of the three copper nanomaterials was limited and patchy. Not all parameters were provided for the three formulations. For the ECO COPPER NANO-COLOID nanomaterial (Manufacturer G product #28 and #29) no information was provided at all.

Information was not provided on the presence of impurities/contaminants. A detailed analytical report on purity of the material and any impurities/contaminants should be provided.

3.2 FUNCTION AND USES

The following information was provided by the Notifiers as presented in Table 14.

Table 14. Product names, product type and use concentration of the various colloidal copper dispersions

Code manufacturer	**Code number SCCS**	**Name of colloidal copper dispersion/cosmetic product name**	**CPNP code**	**Product type**	**Use concentration**
A	1	Copper water	1000980	Dermal, leave on antibacterial	1% w/w
B	2	Vertere Colloidal Nano Copper 50 ppm	1002550	Dermal, leave on antibacterial	0.005% w/w
B	3	Quantum Gold Golden Ratio Formula Serum	1002551	Dermal, leave on antibacterial	0.0055% w/w
B	4	Quantum Gold Golden Ratio Formula Cream	1002552	Dermal, leave on antibacterial	0.0055% w/w
B	5	Quantum Nano Gold Micellar Water	1002553	Dermal, leave on	0.0008% w/w

				antibacterial	
B	6	Quantum Silver Action Cleansing Gel	1002554	Dermal, rinse off antibacterial	0.008% w/w
B	7	Quantum Platinum Enlightenment Cream	1002555	Dermal, leave on antibacterial	0.0034% w/w
B	8	Quantum Gold New Age Cream	1002556	Dermal, leave on antibacterial	0.0034% w/w
B	9	Quantum Gold New Age Serum	1002557	Dermal, leave on antibacterial	0.0034% w/w
B	10	Quantum Gold Ultimate Revival Hair Serum	1002558	Dermal, leave on antibacterial	0.0034% w/w
B	11	Quantum Gold Golden Ratio Cellural Eye	1002559	Dermal, leave on antibacterial	0.0055% w/w
B	12	Quantum Silver Peel Solution	1002560	Dermal, rinse off antibacterial	0.0008% w/w
B	13	Quantum Gold Algae Mask	1002561	Dermal, leave on antibacterial	0.0034% w/w
C	14	C Nano Active Krem do twarzy redukujący przebarwienia ANTI-OX C Nano Active Face Enlightenment Cream ANTI-OX	1002906	Dermal, leave on antibacterial	0.0005% w/w
D	15	Maseczka oczyszczająco-kojąca, do skóry ze skłonnością do zmian trądzikowych Cleansing-soothing mask for skin with acne changes	1002940	Dermal, rinse off antibacterial	0.0002% w/w
D	16	Maseczka pielęgnująco-oczyszczając Caring and cleansing	1002941	Dermal, rinse off antibacterial	0.0002% w/w

		mask			
D	17	Maseczka odżywczo-relaksująca Nourishing and relaxing mask	1002942	Dermal, rinse off antibacterial	0.0002% w/w
D	18	Maseczka nawilżająco-liftingująca Moisturizing and lifting mask	1002943	Dermal, rinse off antibacterial	0.0002% w/w
D	19	Maseczka odświeżająco-detoksykacyjna Refreshing and detoxifying mask	1002944	Dermal, rinse off antibacterial	0.0002% w/w
E	20	Podopharm Professional Tinktura na grzybicę paznokci Podopharm Tincture for onychomycosis	1002989	Dermal, leave on antibacterial	0.0000003% w/w
F	21	ENILOME INTIMA Pianka do higieny intymnej ENILOME INTIMA Intimate hygiene foam	1003013	Dermal, rinse off antibacterial	0.00001% w/w
F	22	SELECTA INTIMA Dermokosmetyk do higieny intymnej SELECTA INTIMA Intimate hygiene dermocosmetic	1003014	Dermal, rinse off antibacterial	0.0001% w/w
D	23	Maseczka odświeżająco – detoksykacyjna Refreshing and detoxifying mask	1003061	Dermal, rinse off antibacterial	0.0005% w/w
D	24	Maseczka nawilżająco – liftingująca Moisturizing and lifting mask	1003062	Dermal, rinse off antibacterial	0.0005% w/w
D	25	Maseczka odżywczo – relaksująca Nourishing and relaxing mask	1003063	Dermal, rinse off antibacterial	0.0005% w/w
D	26	Maseczka pielęgnująco – oczyszczająca Caring and cleansing	1003064	Dermal, rinse off	0.0005% w/w

		mask		antibacterial	
D	27	Maseczka oczyszczająco-kojąca, do skóry ze skłonnością do zmian trądzikowych Cleansing-soothing mask for skin with acne changes	1003065	Dermal, rinse off antibacterial	0.0005% w/w
G	28	Nano Koloid Eco Miedz (ECO COPPER NANO-COLLOID)	1003371	Dermal, leave on, antimicrobial	0.005% w/w
G	29	Nano Koloid Eco Miedz (ECO COPPER NANO-COLLOID)	1003373	Dermal, leave on, antimicrobial	0.005% w/w

The products are used on the skin either as 'leave on' or 'rinse off' products.

In summary:

Dermal leave-on products: A1, B2-5, B7-11, B13, C14, E20, and G28-29.
Dermal rinse-off products: B6, B12, D15-19, F21-22, and D27-31.

According to the additional information provided by the Notifiers, the 'use concentration' is the concentration of the nanoparticles in the final cosmetic product.

3.3 TOXICOLOGICAL EVALUATION

Table 15. Toxicological evaluation

Copper nanomaterial	General toxicological information
Copper Water nano-TECH 100 ppm	The safety assessor can estimate that, given the present level of knowledge, the product does not show any foreseeable risk to human health under conditions of normal use. SCCS NOTE: based on statement for NanoTech 50 ppm also used for NanoTech 100 ppm. Notification_1002940_112784_safety_file_2017-5-31-7-37-34 (polish and English (translated)), and Notification_1002550_100234_tox_profile_2016-6-26-9-58-11 (Polish and English (translated)). Product not classified as a component dangerous to health and environment according to the Minister of Health Regulation of September 2, 2003 on the criteria and ways of classification of chemical components and substances (Dz. U. Nr 171, poz. 1666). Notification1002940_112784_spec_file 2017-5-31-7-37-34 It does include dangerous components for heath and

	environments. Notification_1003051_104700_safety_file_2017-11-3-8-14-4 consists of a Material Safety Data Sheet (MSDS) for Copper Water nano-Tech 100 ppm.
Copper Water nano-TECH 50 ppm	The safety assessor can estimate that, given the present level of knowledge, the product does not show any foreseeable risk to human health under conditions of normal use. Notification_1002989_72148_tox_profile_2017-8-29-8-46-56 [equal to Notification_1002940_112784_safety_file_2017-5-31-7-37-34 (Polish and English (translated)), and Notification_1002550_100234_tox_profile_2016-6-26-9-58-11 (Polish and English (translated)). Product not classified as a component dangerous to health and environment according to the Minister of Health Regulation of September 2, 2003 on the criteria and ways of classification of chemical components and substances (Dz. U. Nr 171, poz. 1666). Notification_1003013_104652_spec_file_2017-8-23-13-2-30.
ECO COPPER NANO-COLOID	Hazard labels H317 (skin allergic reaction), H319 (eye irritation). All components of the product (see Part A. 1. Qualitative and quantitative composition of the cosmetic product) have been tested for their suitability for use as ingredients in cosmetics, or they are known as such ingredients (see, inter alia, Datenbank Cosmetics-CosIng). Therefore, general studies for their usefulness can be waived, as stated in The SCCS'S Notes of Guidance for The Testing of Cosmetic Substances and Their Safety Evaluation 8th Revision. Taking into account the above data and the use of the product in accordance with intended use, no significant risk has been proven. It should be emphasized that in case of hypersensitivity to any component of the product (e.g. Copper), adverse reactions cannot be excluded. However, it should be emphasized that individual substances are contained in the finished product at concentrations that do not cause allergic reactions, what was confirmed by a dermatological test. Ref.: Polish and English translation of the CPNP Notification no. 1003371 file (86357_need_info_safety_file_EC_2019-3-24-20-19-8-20)

Additional information provided on request by the SCCS

Document: Pawlowska *et al.* (2020)
Colloidal Copper (nano)/copper (nano), presenting an overview on toxicity of Cu (in ionic form) and Cu NPs.

Reference 9.

Document: Background document for development of WHO Guidelines for Drinking-water Quality (WHO, 2004)
This WHO document contains information on the levels of copper considered safe in drinking water. These include various forms of Cu including Copper(II) acetate monohydrate [$Cu(C_2H_3O_2)_2{\cdot}H_2O$] CAS No. 6046-93-1, Copper(II) chloride [$CuCl_2$] CAS No. 7447-39-4, Copper(II) nitrate trihydrate [$Cu(NO_3)_2{\cdot}3H_2O$] CAS No. 10031-43-3, Copper(II) oxide [CuO]

1317-38-0, Copper(II) sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$] CAS No. 7758-99. For Cu, the guideline value was established at 2 mg/L drinking water that should permit consumption of 2 or 3 litres of water per day, use of a nutritional supplement and copper from foods without exceeding the tolerable upper intake level of 10 mg/day (IOM, 2001) or eliciting an adverse gastrointestinal response.

References 10., 11.

SCCS comment

Copper Water NanoTech 100 ppm
The safety file (Notification_1002940_112784_safety_file_2017-5-31-7-37-34) presented for Copper Water nano-Tech 100 ppm is for NanoTech 50 ppm.
File Notification_1003051_104700_safety_file_ 2017-11-3-8-14-4 consists of a material safety data sheet (MSDS) for Copper Water nano-Tech 100 ppm without references.

Copper Water NanoTech 50 ppm
The files notified as 'safety' or 'toxprofile' files did not contain information on toxicological endpoints for the Copper Water nano-TECH 50 ppm product.
The safety file (Notification_1002940_112784_safety_file_2017-5-31-7-37-34) notified for Copper Water nano-Tech 100 ppm is for Copper Water nano-Tech 50 ppm.

Copper Water nano-TECH 50 ppm and Copper Water nano-TECH 100 ppm
References to or studies referring to the presented data/information were not presented. For both NanoTech 50 ppm and NanoTech 100 ppm the same general statement on safety was provided while data or references were not presented. Additional information asked for by the SCCS was provided by the manufacturer. However, additional data for these Cu-NPs were not provided. Some results on a genotoxicity test was provided.

ECO COPPER NANO-COLOID
Information on toxicity of copper is included in the file indicated as 'copper complexed'. Information on the toxicity tests on the nanoformulation is not provided.
Notification_1003371_86357_need_info_safety_file_EC_ 2019-3-24-20-19-8 (English and polish).
Notification_1003371_86357_tox_profile_file 2018-10-3-11-26-3 and Notification_1003373_86357_tox_profile_file 2018-10-3-11-26-3 files contained a publication on human exposure studies on commercial silver nanoparticles (Munger *et al.* 2014, Ref 11).
Literature is cited without providing the full references. Some literature is cited, indicating that more research is needed on skin penetration of nanoparticles. Only for the human skin irritation study was information provided on the study protocol and the study results.

Pawlowska *et al.* (2020)
The conclusions drawn by the authors in the document were not supported by data or any reference to already published work. Data on toxicity and skin penetration of Cu nanoparticles were not provided.

WHO (2004) Copper in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality.(WHO/SDE/WSH/03.04/88). WHO, Geneva, Switzerland. 2004.
The WHO document deals with copper materials in various forms, but does not address copper nanoparticles. There was no information presented in the document on Cu-NP toxicity. Skin toxicity was also not addressed in this document. Therefore, the provided

information has not been considered relevant by the SCCS for the evaluation of the products that were notified to the Commission.

SCCS remarks on toxicological information provided
For all three copper nanomaterials the information on toxicity in the notified information is very limited or absent altogether. Where information is provided, supporting experimental data/documentation and/or references to studies are lacking. In addition, in many cases information is not specific for the nanomaterials as presented in the notifications but based on literature on general copper toxicity. The request for additional data did not result in the submission of any data for the notified copper nanomaterials, except some results on genotoxicity testing for one material.

3.3.1 Acute toxicity

Table 16. Information on acute toxicity of copper (nano) and colloidal copper (nano) as provided by the Notifiers.

Copper nanomaterial	Acute toxicity
Copper Water nano-TECH 100 ppm	Lowest published toxic dose administered orally to humans is 0.01 mg/kg. Lowest published lethal dose administered subcutaneously to rabbits is 375 mg/kg. Lowest published toxic dose administered orally to rabbits is 3 g/kg/60 days. Notification_1002940_112784_ safety file_2017-5-31-7-37-34 (describes NanoTech 50 ppm) (Polish and English (translated)).
Copper Water nano-TECH 50 ppm	Lowest published toxic dose administered orally to humans is 0.01 mg/kg. " Lowest published lethal dose administered subcutaneously to rabbits is 375 mg/kg. Lowest published toxic dose administered orally to rabbits is 3 g/kg/60 days. Notification_1002989_72148_tox_profile_2017-8-29-8-46-56 (Polish and Englis (translated)), and NanoTech 100 file: Notification_1002940_112784_ safety file: 2017-5-31-7-37-34 (Polish and English (translated) describes NanoTech 50 ppm)
ECO COPPER NANO-COLOID	-

SCCS comment
Although some statements were given on the oral toxicity of colloidal copper, original study reports were not provided. Information on material characterisation for the materials used in the studies was not presented. It is not clear whether (and if so in which respect) the material used in the acute oral toxicity studies corresponds to the materials in this notification. Proper assessment of the acute toxicity relevant to the materials under consideration should be provided (see SCCS/1611/19).

Based on the information provided by the Notifiers, a conclusion cannot be drawn on acute toxicity of colloidal copper formulations as used in the notified products.

3.3.2 Irritation and corrosivity

Table 17. Information on irritation and corrosivity of copper (nano) and colloidal copper (nano) as provided by the Notifiers

Copper nanomaterial	Irritation and corrosivity
Copper Water nano-TECH 100 ppm	It does not exert specificity irritating and sensitizing. Irritation of eye can evoke at longest contact. Notification_1003051_104700_safety_file_2017-11-3-8-14-4 (MSDS for Copper Water nano-Tech 100 ppm).
Copper Water nano-TECH 50 ppm	Dermatological tests were also carried out showing no irritant or sensitizing properties. Notification_1002989_72148_safety_file_2017-7-21-9-56-51 (Polish and English (translated)). The results of dermatological tests carried out with the semi-open contact test confirmed that the preparation with a concentration of 25 ppm copper - does not show irritating and sensitizing properties. Notification_1002940_112784_safety file_ 2017-5-31-7-37-34 (Polish and English (translated)).
ECO COPPER NANO-COLOID	H319 causes serious eye irritation Notification_1003371_86357_need_info_safety_file_EC_2019-3-24-20-19-8-20 Polish and English translation.

SCCS comment
The safety file (Notification_1002940_112784_safety_file_2017-5-31-7-37-34) presented for Copper Water nano-Tech 100 ppm is for Copper Water nano-Tech 50 ppm. The information on skin and eye irritating properties of copper (nano) and colloidal copper (nano) should be supported by data (study reports). Furthermore, it is not clear whether the test results were obtained with the materials under consideration. There are indications of serious eye irritation, and therefore, a potential for skin and eye irritation cannot be excluded on the basis of the limited information provided.

3.3.3 Skin sensitisation

Table 18. Information on skin sensitizing properties of copper (nano) and colloidal copper (nano) as provided by the Notifiers.

Copper nanomaterial	Skin sensitisation
Copper Water nano-TECH 100 ppm	The results of dermatological tests carried out with the semi-open contact test confirmed that the preparation with a concentration of 25 ppm copper - does not show irritating and sensitizing properties. Notification_1002940_112784_safety file_ 2017-5-31-7-37-34 (Polish and English (translated)).

	It does not exert specificity irritating and sensitizing. Notification_1003051_104700_safety_file_2017-11-3-8-14-4 (MSDS for Copper Water nano-Tech 100 ppm).
Copper Water nano-TECH 50 ppm	The results of dermatological tests carried out with the semi-open contact test confirmed that the preparation with a concentration of 25 ppm copper - does not show irritating and sensitizing properties. Notification_1002940_112784_safety file_ 2017-5-31-7-37-34 (Polish and English (translated)). Dermatological tests were also carried out showing no irritant or sensitizing properties. Notification_1002989_72148_safety_file_2017-7-21-9-56-51 (Polish and English (translated)).
ECO COPPER NANO-COLOID	H317 may cause an allergic skin reaction Dermatological allergy tests came negative (does not cause allergies except for specific allergy to silver) Notification_1003371 and Notification_ 1003373.

SCCS comment
The safety file (Notification_1002940_112784_safety_file_2017-5-31-7-37-34) presented for Copper Water nano-Tech 100 ppm is for Copper Water nano-Tech 50 ppm.
Statements made by the Notifiers have not been supported by data. Study data on skin sensitisation have not been provided. Adequate information on skin sensitisation on relevant materials should be part of the information on a cosmetic ingredient (see SCCS/1611/19). Information on material characterisation for the materials used in the dermatological tests has not been presented.
For ECO copper nano-colloid a statement of possible allergy to silver was included that does not belong in a copper file notification. A conclusion cannot be drawn with respect to skin sensitisation based on the information provided, apart from the indication that ECO copper nano-colloid might cause allergic reactions.

3.3.4 Dermal/percutaneous absorption

SCCS comment
Information on studies on skin penetration for the three copper nanomaterials was not provided in the initial notification.

Additional information provided on request by the SCCS

Document: Nanocopper Absorption (Warsaw, September 20, 2016)

Additional information was provided at the request of the SCCS for Axonnite nanoparticles composed of demineralised water and copper (nano) with a concentration of 50 ppm manufactured by NANO-TECH.

The experiment was carried out in three independent trials using pig's skin. Pig ears, collected from the butcher within 3 hours after slaughter, were washed under running water, dried and cut the bristles with an electric shaver to a length of 1 mm. Using a scalpel the skin was carefully separated from cartilage. Then equal skin fragments of 0.64 mm thick were cut using a dermatome (Padgett Electro Dermatome model B; Integra Lifesciences

Padgett Instruments, Plainsboro, NJ, USA). Skin disks with a diameter of 25mm were cut from the obtained thin layers of skin and placed in Franz's chamber. Before the permeation tests, the membranes were placed in the chambers in demineralized water for 24 hours to get stabilized. Permeation testing was conducted using the finished Axonnite Copper product with known content of nano-copper. After starting the study, 1mL of solution was taken for further analysis from the acceptor chambers at defined intervals (after 2h, 4h, 6h, 8h, 10 and 24h). The experiments were carried out in an air-conditioned room at 20-22°C for 24 hours. The samples were analysed on a RIGAKU NEX DE spectrometer at ANITEPO Sp. z o. Results of the analysis did not reveal any presence of copper elements in the tested samples after 2h, 4h, 6h, 8h, 10 and 24h. The results of the experiment indicate that after 2h, 4h, 6h, 8h, 10 and 24h the amount of nano-copper in the acceptor chamber has not changed in relation to the background, which was pure water.

Reference 12.

SCCS comment
One experiment with three trials was reported, which is not in agreement with the SCCS guidelines for skin-penetration studies. The data of the test outcome were not appropriately presented, therefore the conclusions of the report are not appropriate for the SCCS evaluation.

A dermal/percutaneous absorption study with relevant copper nanoparticle dispersions, along with proper material characterisation, carried out according to the SCCS Guidance on nanomaterials (SCCS/1611/19), should be provided to demonstrate whether or not there is evidence for systemic absorption of copper nanoparticles via the skin. The dermal penetration data required should include *in vitro* studies carried out using the copper nanoparticle dispersions presented in the notifications, and under current evaluation, and/or other relevant studies that may be available in published literature.

3.3.5 Repeated dose toxicity

Table 19. Information on repeated dose toxicity of copper (nano) and colloidal copper (nano) provided by the Notifiers

Copper nanomaterial	Repeated dose toxicity
Copper Water nano-TECH 100 ppm	-
Copper Water nano-TECH 50 ppm	NanoTech 100 file: 1002940 safety file: 2017-5-31-7-37-34 Lowest toxic dose oral rabbit 60 days study: 3 g/kg
ECO COPPER NANO-COLOID	-

SCCS comment
The information provided on Copper Water nano-TECH 50 ppm is not supported by experimental data. As such, it is not clear whether the information is relevant for the particular material under current evaluation.
The Notifiers should consider the likelihood of systemic exposure under intended and foreseeable uses. Information on repeated dose toxicity is necessary for the risk evaluation where the product use could lead to internal exposure of the consumer to copper (nano) and colloidal copper (nano). In case of significant uptake, information on certain chronic toxicity endpoints would also be needed.

3.3.6 Mutagenicity/genotoxicity

Table 20. Information provided by the Notifiers on genotoxicity of copper (nano) and colloidal copper (nano).

Copper nanomaterial	Mutagenicity/genotoxicity
Copper Water nano-TECH 100 ppm	Not cytotoxic and genotoxic in micronucleus test
Copper Water nano-TECH 50 ppm	-
ECO COPPER NANO-COLOID	Information on toxicity of copper is included in the file indicated as "copper complexed". Notification_1003371_86357_need_info_safety_file_EC_ 2019-3-24-20-19-8 (English and polish). A review on genotoxicity of cobalt-, nickel-, and copper nanoparticles is cited but no data or reference is presented.

Additional information provided on request by the SCCS

Both, a cytotoxicity test *in vitro* (agar diffusion) and a genotoxicity test (micronucleus test) have been performed according to protocols as described in PN-EN ISO 10993-5, PN-EN ISO 10993-3 and PN-EN ISO 10993-12.

No.	Parameter	Test method	Requirement	Result
1.*	Cytotoxicity in vitro	agar diffusion acc. to PN-EN ISO 10993-5:2009	-	cytotoxicity grade – "0" interpretation – none cytotoxicity final result – **sample non cytotoxic**
2.	Genotoxicity	micronucleus test acc. to PN-EN ISO 10993-3:2014 PN-EN ISO 10993-12:2012	-	**sample non genotoxic**

** method within the scope of accreditation no. AB 774*

Cytotoxicity:
The cytotoxicity test was performed as an agar diffusion test according to PN-EN ISO 10993-5:2009, using NCTC clone 929 ATCC and a negative, positive, intact cells and neutral carrier as controls with a concentration of 50 ppm. Regarding cell response, there was no detectable zone around or under the specimen.

Micronucleus test:
The genotoxicity test was performed according to PN-EN ISO 10993-3:2014 and PN-EN ISO 10993-12:2012. A micronucleus genotoxicity test was performed using the mouse fibroblast cells NCTC clone 929 ATCC. Positive controls were for the short-term test (37 ± 1°C for 6 hours) without metabolic activation methyl methanesulfonate and with metabolic activation cyclophosphamide, and for the long term test (37 ± 1°C for 30 hours) without metabolic activation colchicine. The results are expressed as % binucleated cells with micronuclei in the population of binucleated cells (Table 21). The results are the mean of two independent extractions (±SD). Statistical significance was evaluated using t-Student test, with σ value of 0.05. In all tests, statistically significant increase in the numbers of binucleated cells with micronuclei in population of binucleated cells was not observed; which means that the tested sample is non genotoxic.

Table 21. The results of the micronucleus test *in vitro* (given as % of binucleated cells with micronuclei in population of binucleated cells):

Test without metabolic activation, short-term		
Cell culture control	**Positive control**	**Tested sample**
3,19% ± 0,48%	36,67% ± 1,57% (YES)	3,68% ± 1,02% (NO)

Test without metabolic activation, long-term		
Cell culture control	**Positive control**	**Tested sample**
2,92% ± 1,18%	67,80% ± 7,22% (YES)	2,88% ± 1,45%* (NO)

** significant inhibition of cellular division was observed.*

Test with metabolic activation S9, short-term		
Cell culture control	**Positive control**	**Tested sample**
2,27% ± 0,99%	14,38% ± 1,45% (YES)	1,97% ± 0,23% (NO)

The conclusion from the study is that within the tested scope, the sample is not cytotoxic nor genotoxic.

Reference 13.

SCCS comment

Information on mutagenicity/genotoxicity was not provided in the initial notifications. The information was later provided in the form of an additional study on cytotoxicity and genotoxicity. However, the provided information is neither acceptable, nor sufficient. The SCCS considers that results of the whole study (both cytotoxicity and genotoxicity) are not reliable for the following reasons:

With respect to cytotoxicity study:

- The original study data was not provided.
- 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 the concentration of 100 ppm was tested while on the 2nd page (paragraph 6) it is stated that the concentration of 50 ppm was tested. From the information available in published literature, it is known that the EC50 for copper nanoparticles may vary and can be below 100 µg/mL, depending on cell types and particle sizes (Hanagata *et al.*, 2011, He *et al.*, 2020, Semisch *et al.*, 2014).
- No information on control substances used was given, neither positive nor negative.
- No data were provided on stability of the copper nanoparticle suspension and how it was applied on the agar.
- The information on possible interference in the test by Cu nanoparticles was not provided.
- No information was provided on the number of replicates.

- The agar diffusion test used is not considered suitable to determine cytotoxic properties of copper 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 substances.
- More specifically for nanomaterials, ISO 19007 describes an *in-vitro* MTS assay for measuring cytotoxic effects of nanomaterials. Other quantitative assessments might also be used (such as the NRU cytotoxicity test; the colony formation cytotoxicity test; the MTT cytotoxicity test and the XTT cytotoxicity test) 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 efficacy. The cytotoxicity test should be carried out at different concentrations to enable calculation of EC50 to compare the relative toxicity of the various colloidal copper dispersions in nano form.

Ref.: 14-16

With respect to genotoxicity study:

- It is not clear to the SCCS why an ISO guideline for testing medical devices was followed, as cosmetic ingredients should be tested using OECD test guidelines or EU methods (See SCCS/1611/19). Also within ISO 10993-3:2014, a clear reference is made to the OECD TG 487 for performing a micronucleus test (but modified for medical devices).
- L929 fibroblasts are not suggested in OECD TG 487: the choice of the cell line was not justified
- No information was provided on positive control substances (concentrations, vehicles, etc.)
- Cytokinesis blockage by Cytochalasin B was not reported. For the evaluation of genotoxicity of nanomaterials using the micronucleus test, it is critical to add the cytokinesis blocking agent some time after the exposure to the nanomaterials as the blocking agent may also block the uptake of the nanoparticles.
- No data have been provided which are necessary to demonstrate that the cells in culture had divided, so that a substantial proportion of the cells scored had undergone division during or following treatment with the test chemical. 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.
- Information on the uptake of the particles by the cells used in the assay was not provided

The SCCS is of the opinion that mutagenicity/genotoxicity data on copper nanoparticles provided by the Notifiers are not sufficient. Only results on chromosomal aberrations for one material have been provided and these are not acceptable. According to the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19) results on gene mutation in mammalian cells are additionally required.
The provided studies were not performed or reported according to GLP system.
Mutagenicity/genotoxicity data from *in vitro* studies would be required for all colloidal copper dispersions as part of the base set requirements for assessment of cosmetic products (including proper material characterisation). Any existing data should also be provided.

3.3.7 Carcinogenicity

SCCS comment
Information on carcinogenicity was not provided. 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 systemic availability via the relevant uptake route(s) or on genotoxicity to allow discounting the need for information on carcinogenicity.

3.3.8 Reproductive toxicity

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

SCCS comment
Information was not provided. Information on photo-induced toxicity should be provided for those products intended to be used on skin that is exposed to sunlight (SCCS/1611/19).

3.3.10 Human data

Table 22. Information on studies in humans provided by the Notifiers.

Copper nanomaterial	
Copper Water nano-TECH 100 ppm	-
Copper Water nano-TECH 50 ppm	-
ECO COPPER NANO-COLOID	In the group of 40 patients, including 40 with a history of allergy, no positive readings were found, which indicates that the product does not show irritating and sensitizing properties. Notification_1003371_863557_other file_2018-10-17-14-19-59 Polish and English translation.

ECO COPPER NANO-COLOID
Dermatological study performed as Contact Extended Semi-Open Test.
For the study, 40 people (38 women and 2 men) with a positive allergy history were selected, including 25 with atopic history, 9 with documented contact allergy, 16 with undocumented allergy (from an interview), and 40 showing hypersensitivity to cosmetics, household chemistry and washing products.

In this group, none of the people had documented hypersensitivity, nor did they report any adverse reactions to individual components of the tested product in the interview.
The skin at the application site (arms on the right side and back) was normal, with no lesions.

The test article was applied as a solution at the concentration of 25 ppm on paper discs (Whatmann 3), which were fixed with a porous hypoallergenic (surgical) patch on the shoulders on the right side or on the back. The samples were taken off after 48 hours. The first reading immediately after taking off the samples, the next one (in doubtful cases) 72 hours after the test was initiated. Assessment of reactions was made using a scale that is consistent with the generally accepted scale in dermatological studies.

In the group of 40 patients, including 40 with a history of allergy, no positive readings were found, which indicates that the product does not show irritating and sensitizing properties.

Ref 17.

SCCS comment
ECO COPPER NANO-COLOID: data from the study should be presented in detail. From the limited information it can be deduced that this test cannot inform about a sensitisation potential.
Copper Water nano-TECH 100 ppm and Copper Water nano-TECH 50 ppm: information on human studies was not provided.

3.3.11 Special investigations

Information on special investigations was not provided.

3.4 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

SCCS comment

Based on the notified and submitted information, it is not possible to perform a safety evaluation for any of the 3 copper dispersions discussed in this Opinion.

3.5 DISCUSSION

The information provided by the Notifiers is insufficient to perform a safety evaluation and risk assessment of the three notified nanomaterials composed of Cu-NPs.
In addition to the information provided by the Notifiers, a Call for Data was made by the European Commission. Information received as a result of this Call has also been considered by the SCCS. Furthermore, information from literature search performed by the Commission was also considered by the SCCS. However, it was not possible to relate toxicological data from this information with the type of materials considered in this assessment. As such, the SCCS has considered that the information available at present is insufficient to allow drawing conclusions on the safety of colloidal copper materials included in this Opinion.

For a proper safety evaluation the following information/data should be provided:

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

In addition, information on possible risks of copper nanomaterials was evaluated based on public literature. This information is provided in ANNEX II.

4. CONCLUSION

1. In view of the above, and taking into account the scientific data provided, does the SCCS consider the nanomaterials Copper and Colloidal Copper safe when used in leave-on and rinse-off dermal and oral cosmetic products including: skin, nail and cuticle, hair and scalp and oral hygiene products, at a maximum concentration of 1 % (for both Copper and Colloidal Copper) and with specifications as 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 Copper and Colloidal Copper due to the limited or missing essential information.

Much of the information provided on toxicity relates to copper as such, and in the absence of full study reports, it is not possible to determine the relevance of the data for nano-forms of copper under the current evaluation.

Adequate information on various aspects both for the nanomaterial characterisation and the toxicological evaluation including experiment performance and experimental data obtained need to be provided (as summarised in Annex I).

2. Does the SCCS have any further scientific concerns with regard to the use of Copper and Colloidal Copper in nano form in cosmetic products?

The information provided by the Notifiers, and obtained from scientific literature, suggests possible systemic uptake of Cu nanoparticle (and/or ionic Cu), which may lead to accumulation in certain organs - notably the liver and spleen. In addition, the available literature data indicate potential mutagenic/genotoxic and immunotoxic/nephrotoxic effects of copper nanomaterials. These indications raise an alert that warrants further safety evaluation of copper nanomaterials used as cosmetic ingredients. The SCCS concerns for consumer safety in this regard are detailed in Annex II.
Annex I provides an overview of the information provided by the Notifiers compared to the data requirements as given in the SCCS checklists for Applicants submitting dossiers on Cosmetic Ingredients to be evaluated by the SCCS (SCCS/1588/17).

5. MINORITY OPINION

None.

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ANNEX I

Overview of the information provided by the Notifiers compared to the data requirements as given in the SCCS checklists for Applicants submitting dossiers on Cosmetic Ingredients (SCCS/1588/17) and/or nanomaterials (SCCS/1611/19) to be evaluated by the SCCS

Table 1. Summary of material characterisation data on copper (nano) and colloidal copper as provided by Notifiers in this Opinion.

Information required[a]	**Copper Water Nano-TECH 100**	**Copper Water Nano-TECH 50**	**ECO COPPER NANO-COLOID**
Chemical identity	Y	Y	Y
Chemical composition	Y	Y	Y
Particle size[b]	Y	Y	Y
Morphology	N	N	N
Surface characteristics	N	N	N
Solubility	Y	Y	N
Surface area	Y	Y	N
Catalytic activity	N	N	N
Concentration[c]	Y	Y	Y
Dustiness[c]	N	N	N
Density and pour density[d]	N	N	N
Redox potential	N	N	N
pH[e]	Y	Y	Y
Viscosity[f]	N	N	N
Stability	Y	Y	N
UV absorption	N	N	N
Other	N	N	N

Y = yes, N = no, Y/N = partly

a) For details on the parameters see Table 2 of SCCS/1611/19;
b) For any spray products, size distribution of the droplets as well as of the dried residual particles should be provided;
c) For dry powder products only;
d) For granular materials only;
e) For aqueous solutions;
f) For liquid dispersions.

The Y/N in the abovementioned Table is referring to the **availability of the data** in the submitted/notified files, not the **quality** of the submitted/notified data.

Table 2. Summary of information on toxicological information on copper (nano) and colloidal copper as provided by Notifiers in this Opinion.

Information required[a]	**Copper Water Nano-TECH 100**	**Copper Water Nano-TECH 50**	**ECO COPPER NANO-COLOID**
Likelihood and extent of internal exposure via skin, lung or oral route considering use type	N	N	N
Dermal absorption for dermally-applied products	N	N	N
Biokinetic behaviour, aggregation/agglomeration considered during tests?	N	N	N
Acute toxicity	Y	Y	N
Irritation and corrosivity	Y	Y	Y
Skin sensitisation	Y	Y	Y
Mutagenic/genotoxicity[a]	Y	Y	N
Repeated dose toxicity	N	Y	N
Phototoxicity – for products intended for use in sunlight exposed skin	N	N	N
Human data	N	N	Y
Reproductive toxicity[b]	N	N	N
Carcinogenicity[c]	N	N	N
Other relevant information	N	N	N

Y = yes, N = no, Y/N = partly

a) The Ames test is not considered appropriate for nanomaterial mutagenicity assessment. The following scheme based on *in vitro* assays is proposed (SCCS/1611/19)
   1) Mammalian cell chromosome aberration/clastogenicity – determined either by *in vitro* chromosome aberration test or micronucleus test. The micronucleus test can be performed by the mononucleate or cytokinesis blocked protocols. In the cytokinesis blocked micronucleus assay, co-exposure to both cytochalasin B and the test nanomaterial for the duration of the experiment is not considered acceptable.
   2) An *in vitro* mammalian cell gene mutation test (e.g. hprt, tk or xprt tests). Other indicator tests, such as cell transformation assays or toxicogenomic approaches may also be useful for identification of genotoxic as well as non-genotoxic carcinogen nanomaterials.
   3) *In vitro* genotoxicity studies should be accompanied by an assessment of cellular and nucluclear uptake to demonstrate target exposure to enable a complete evaluation of data-outputs.

b) Where points a and 2 indicate significant systemic uptake.

c) Where points 1 and 2 indicate significant uptake and/or bioaccumulation

The Y/N in the abovementioned Table is referring to the **availability of the data** in the submitted/notified files, not the **quality** of the submitted/notified data.

Table 3. Comparison of exposure information for copper (nano) and colloidal copper in this notification with requirements as given in the SCCS checklists for Applicants submitting dossiers on Cosmetic Ingredients and/or nanomaterials to be evaluated by the SCCS (SCCS/1588/17 and SCCS/1611/19)

Information required[a]	**Copper Water Nano-TECH 100**	**Copper Water Nano-TECH 50**	**ECO COPPER NANO-COLOID**
Category of cosmetic products in which the ingredient is intended for use	Y	Y	Y
Category of the ingredient in the finished cosmetic product	Y	Y	Y
Quantity of the product used in each application	Y	Y	Y
Frequency of use	N	N	N
Total area of skin contact	N	N	N
Duration of exposure	N	N	N
Foreseeable misuse which may increase exposure	N	N	N
Consumer target groups (e.g. children, people with sensitive, damaged or comprised skin) where specifically required	N	N	N
Quantity likely to enter the body (fraction absorbed) for each target group	N	N	N
Estimated dermal exposure based on the intended use of the product	N	N	N
Estimated oral exposure based on the intended use of the product	N	N	N
Estimated inhalation exposure based on the intended use of the product	N	N	N
Exposure calculation for each target group	N	N	N
Other relevant information	N	N	N

Y = yes, N = no, Y/N = partly

a) In the absence of information, the SCCS may use default values for some of the parameters (SCCS Notes of Guidance SCCS/1602/18).

ANNEX II

Safety concerns for Cu-nanoparticles used as cosmetic ingredient based on public information

As the information and data sent in the notification to the European Commission were insufficient to perform a safety assessment, additional information on copper nanoparticles was obtained from the literature. From the evaluation and other relevant information from published literature, the SCCS has concluded that there is a basis for concern that the use of colloidal copper (nano) in cosmetic products can pose a risk to the consumer because of the following considerations:

Physicochemical aspects

1. Colloidal copper (nano) is comprised of primary particles that are in the nano-scale. The particle sizes are reported to range from 3 nm to 100 nm (Table 2.).
2. Besides Cu (nano) particles (Cu-NP) also Cu ions may be present in Cu nanoformulations, as potential transformation between particulate and ionic forms of copper is likely. Indeed micronized copper (with a hydrodynamic size median of 124 nm in mass metrics and 34 nm in number metrics) shows a slow dissolution in a slightly acidic environment (Pantano *et al.*, 2018 Supplementary Information). In addition, in a study by De Jong *et al.* (2019) shrinking of Cu-NP formulations (CuO and $Cu_2CO_3(OH)_2$) was observed under intestinal conditions indicative for dissolution. These data would suggest that it is relevant to consider the possible toxicological effects of both the Cu ions and the Cu-NPs especially after oral exposure. The dissolution of copper nanoformulations is slow for short time periods even at gastric low pH levels (Lei *et al.* 2008, De Jong *et al.* 2019). At more neutral pH dissolution is almost absent reaching approximately 2% after 20 hours and approximately 8% after 80 hours (Pantano *et al.* 2018).

Toxicological aspects

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

Genotoxicity/mutagenicity

Sadiq *et al.* (2015) reported on the mutagenicity of Cu-NPs. Cu-NPs were found to be mutagenic in the TA98 and TA100 Salmonella typhimurium strains. In the micronucleus test there was an increase in binucleated cells with micronuclei, while in the comet assay DNA strand breaks and oxidative DNA damage were identified (Sadiq *et al.* 2015). Cu-NPs also induced DNA strand breaks and oxidative DNA damage in monkey kidney CHS-20 cells (Sadiq, 2015). In addition, Cu-NPs caused DNA damage in human cell lines in a number of *in vitro* studies (Alarifi *et al.*, 2013; Di Bucchianico *et al.*, 2013; Karlsson *et al.*, 2008; Semisch *et al.*, 2014). For CuO NPs genotoxicity was reported by induction of DNA strand breaks in Hela S3 cells while the increase in micronuclei in A549 lung cells was not significantly present (Semisch *et al.*, 2014). In a study by Alarifi *et al.* (2013) CuO NPs were observed to be cytotoxic by LDH (membrane leakage) assay and MTT (viability) assay, and induced DNA breakage (comet assay) in the human skin epidermal cell line HaCaT (Alarifi *et al.*, 2013).

Ames test	Any proof of cellular internalization?
Cu: nanoparticles were found mutagenic in *S. typhimurium* TA98 and TA100 strains with and without metabolic activation (Sadiq *et al.* 2015)	no
CuO: nanoparticles showed marginal mutagenicity in *S. typhimurium* TA97a and TA100 strains (Pan *et al.*, 2010).	no
Micronucleus test	
Cu: nanoparticles induced a significant increase in micronuclei frequencies in monkey kidney CHS-20 cell line (Sadiq, 2015).	no
CuO: nanoparticles increased frequency of micronuclei in the mouse neuroblastoma cell line Neuro-2A (Perreault *et al.*, 2012), and in RAW 264.7 mouse macrophages and human PBL (Di Bucchianico *et al.*, 2013). In the latter study significant dose–effect relationships were observed for the different CuO NPs shapes. CuO NPs induced a significant increase in micronucleated erythrocytes in peripheral blood after i.p. injection in mice (Song *et al.*, 2012).	Perreault – no Di Bucchianico – no Song – Cu concentration after i.p. injection in serum increased immediately after injection and reached a maximum level after 1h, and control levels after 24 h; no proof of NPs in bone marrow
Comet assay	
Cu: nanoparticles induced DNA strand breaks and oxidative DNA damage in monkey kidney CHS-20 cells (Sadiq *et al.*, 2015).	Sadiq – no
CuO: nanoparticles caused DNA damage in human cell lines in a number of *in vitro* studies (Alarifi *et al.*, 2013; Di Bucchianico *et al.*, 2013; Karlsson *et al.*, 2008; Semisch *et al.*, 2014).	Alarifi – yes, TEM showed NPs to be distributed inside HaCaT cells mostly in cytoplasm, but also in nucleus (comment: TEM image in the paper is of exceptionally poor quality) Di Bucchianico – no Karlsson 2008 – no Semisch – yes, CuO NPs or $CuCl_2$ provoked a concentration-dependent Cu accumulation in the cytoplasmic fraction (ICP-MS)

Available data indicate the potential mutagenic/genotoxic effects of copper nanomaterials if there is an internal exposure. Production of reactive oxygen species (ROS) seems to be the major factor for the genotoxic potential of copper and copper oxide nanoparticles that partially release Cu ions.

General toxicity:

In vitro

In *in vitro* studies Cu-NP toxicity was demonstrated. In a study by Prabhu *et al.* (2010), dorsal root ganglion neurons of rat origin were exposed to copper nanoparticles of increasing concentrations (10–100 µM) and sizes (40, 60 and 80 nm) for 24 h. LDH (cell membrane damage) and MTS (cell viability) assays revealed that exposure to copper nanoparticles had a low but significant toxic effect with all the sizes tested when compared to unexposed control cultures. Further analysis of the results showed that copper nanoparticles of smaller size and higher concentration exerted the maximum toxic effects, and that intracellular deposition of copper was present (Prabhu *et al.* 2010). Overall the EC50 for copper NPs may vary and can be below 100 µg/mL, depending on cell types and particle sizes (Hanagata *et al.*, 2011, He *et al.*, 2020, Semisch *et al.*, 2014).

In vivo

For acute effects the LD50 values of nano-copper (23.5 nm) and ionic copper (0.072 nm) particles are 413 and 110 mg/kg body weight, respectively, and can both be considered as moderately toxic (Chen *et al.* 2006). Comparably, the LD50 value of micro-copper (17 µm) is above 5000 mg/kg, considered to be non-toxic (Chen *et al.* 2006). More recent data indicated LD50 values for Cu ions, 30 nm, 50 nm, 80 nm, and 1 µm copper particles were 359.6, 1022, 1750, 2075, and>5000 mg/kg, respectively (Tang *et al.* 2018).

For Cu NPs, *in vivo* liver and kidney toxicity was reported in mice and rats after short term (1-5 days) oral administration (Chen *et al.* 2006; Lei *et al.* 2008; Sarkar *et al.* 2011; Liao and Liu 2012, Tang *et al.* 2018), which was also observed in subchronic studies up to 4 weeks exposure (Cholewińska *et al.* 2018, Xu *et al.* 2018). Also spleen toxicity accompanied by immunotoxicity was observed for Cu-NPs after 28 days intragastric administration to rats (Zhou *et al.* 2019). For toxic effects on the kidney, the toxicity might be attributed to the relatively high chemical reactivity of small size (23.5 nm) Cu NPs compared to big size (17 micron) particles, suggesting that both the toxicity of the particles and the resulting ions should be explored (Meng *et al.*, 2007).

No information on long-term repeated dose toxicity of Cu-NPs is available.

In conclusion, oral exposure to Cu NPs may induce systemic toxicity depending on the dose, and part of that toxicity maybe due to release of Cu ions.

Non-nano copper

The oral toxicity of Cu ions is well established with an acceptable drinking water standard of 1.3 – 2.0 mg/L (IOM 2001, WHO 2004, ATSDR 2004).

For copper in non-nano form, EFSA evaluated the risk for the pesticide active substance copper (EFSA 2018). Five variants of copper were considered with as active substances copper (I) and copper (II) ions. Based on animal data (90 d rat study) a NOAEL of 16 mg Cu/kg bw per day was derived.

Dermal toxicity

For Cu-NPs a role is considered in wound healing (Gopal *et al.* 2014, Alizadeh *et al.* 2019). Recently, in contrast to the observed cell toxicity as indicated above, it was reported that 40 nm Cu-NPs and 80 nm Cu-NPs were not toxic to cultured fibroblast, endothelial, and keratinocyte cells, and also that 80 nm Cu-NPs enhanced endothelial cell migration and proliferation (Alizadeh *et al.* 2019). Extensive assessment of *in vivo* wound healing demonstrated that treatment with the 80 nm Cu-NPs accelerated wound healing over a shorter time via formation of granulation tissue and higher new blood vessels. Importantly, serum biochemical analysis confirmed that 40 nm Cu-NPs (10 µM) and 80 nm Cu-NPs (1 µM) did not show any accumulation in the liver during wound healing. In addition, Cu-NPs added to scaffolds for skin tissue engineering showed no negative effect on cell adhesion and proliferation when Cu-NPs were present at concentrations of 0.01%, 0.02%, and 0.03%

(Kumari *et al.* 2019). Unfortunately, the type of cell used for these studies was not reported.
For plant extract mediated synthesis of Cu-NPs a high level of photocatalytic activity was reported (Nazar *et al.* 2018).
CuO NPs were observed to be more toxic than micro-sized particles in human skin organ cultures while skin penetration was not observed (Cohen *et al.* 2013). It was suggested that CuO NPs adhered to the skin, reacted with the local acidic environment generating locally toxic ionic Cu.

Non-nano copper
Dermal toxicity of Cu itself (non-nano form) seems limited. In the 2002 update of the "Toxicological Profile on Copper" of 2002, only limited effects are indicated for dermal exposure (ATSDR 2004). No studies were found for systemic effects after dermal exposure. Eye irritation has been reported by factory workers exposed to copper dust, whereas no studies in animals were noted. Skin effects were reported as pruritic dermatitis and allergic contact dermatitis.

Skin penetration
Cellular and transdermal permeation of Cu-NPs was demonstrated to be shape dependent when evaluated for their transdermal drug delivery potential and having antimicrobial activity (Murugan *et al.* 2016). Spheric Cu-NPs showing the highest perfusion per unit area after 12 hours of incubation using excised BALB/c mice dermal tissue in which the. Cu-NP spheric nanoparticle flux was higher for the 5 nm spheres than for the 90 nm spheres.

Non-nano copper
Cu skin penetration was studied in humans under occlusive and semi-occlusive conditions (Hostynek *et al.* 2006). There was a decreased Cu content from the superficial to the deeper layers of the stratum corneum as demonstrated by tape stripping of the exposure sites. Under occlusive conditions from the tenth tape strip, Cu content was similar to background levels, while under semi-occlusion with breathable tape Cu levels were still increased above the background at the twentieth strip reaching the epidermal layer. These results indicate that Cu, when applied as powder, can penetrate the stratum corneum of the human skin (Hostynek *et al.* 2006).

Exposure aspects
When nano form of copper is used in cosmetic products, it will present a potential for consumer exposure to nanoparticles of copper. The extent of such exposure would be dependent on the concentration of nano copper used in a product and the nature and frequency of the product use.
There is a general exposure to non-nano forms of copper via food and food contaminants (EFSA 2008, 2018, WHO 1998). However, in the WHO report of 1998 it considered at that moment that there is greater risk of health effects from deficiency of copper intake than from excess of copper intake (WHO, 1998). Also, there is the natural homeostatic mechanisms that regulate copper in the body.

Conclusions
With a collective consideration of the physicochemical, toxicological and exposure aspects noted above, the SCCS is of the view that there is a basis for concern that the use of colloidal copper (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.

Appendix

Scoring system used by Brand *et al.* 2019

Recently, a scoring system has been proposed by Brand *et al.* (2019) that combines consideration of the key aspects of nanomaterials that can trigger a 'signal' for risk and expert judgment to assign a score for prioritisation on the basis of risk potential for human health. Table-1 below has been adapted from Brand *et al.* (2019) and has been used here to analyse Cu-NPs for further action regarding regulatory action and/or safety assessment.

Table 1. Scoring system with key questions to assess a selected signal for prioritisation on risk potential for human health.

Descriptor	Question	Answer[a] *(score)*		
		Yes *(3)*	No *(0)*	? *(1)*
Physico-chemical properties[b] *(max 12 pts)*	Indication of low or no dissolution or degradation rate in physiologically relevant media?	X		
	Indication of reactivity? E.g. due to surface area, type of chemical, surface treatment.			X
	Indication of release of toxic ions or molecules?	X		
	Indication that the nanomaterial is persistent and rigid, e.g. a High Aspect Ratio Nanoparticle (HARN)[c] ?		X	
Hazard *(max 12 pts)*	Is the chemical itself a substance of very high concern, relating to human health hazard[d] ?		X	
	Indication of mutagenicity/carcinogenicity (of the material)?	X		
	Indication of immunotoxicity (of the material)?	X		
	Indication of other toxicity (of the material)?	X		
Kinetics *(max 12 pts)*	Indication of absorption?	X		
	Indication of distribution to brain or reproductive organs?	X		
	Indication of accumulation in any tissue?	X		
	Indication of change in kinetic profile compared to non-nano situation?	X		
Exposure [e] *(max 12 pts)*	Products used or likely to be used much or in many products and/or by wide population?	X		
	Is exposure of sensitive subgroups anticipated? (e.g. babies or elderly people)	X		
	Is exposure likely to occur frequently (more than a few incidental times)?	X		
	Is there potential for nanomaterial exposure likely, based on the product use description?	X		
	Total marks	13	2	1
		x 3	x 0	x 1
	Sub-score	39	0	1
	Total score[1]		**40**	

[1] [a] An indication for a specific physicochemical property, hazard, (toxico)kinetic behaviour or exposure is sufficient to attribute the maximum score of 3. Unknown (=?) can also be interpreted as 'maybe', in case the indications are weak.
[b] Take into account that outer layers may not be stable and therefore consider changes in surface properties.
[c] HARN = a material that has a diameter <100 nm and a length many times greater than its diameter (aspect ratio greater than 3 or 5:1), as defined by ECHA (2017) [11].
[d] In the sense of Article 57 of Regulation EU 1907/2006 with respect to human health-related endpoints.
[e] Restricted to exposure of consumers.

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