



Scientific Committee on Consumer Safety

SCCS

OPINION ON

Platinum (nano), Colloidal Platinum (nano) and Acetyl tetrapeptide-17 Colloidal Platinum (nano)



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

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

The SCCS members:

Dr U. Bernauer (Chairperson)
Dr L. Bodin
Prof. Q. Chaudhry
Prof. P.J. Coenraads
Prof. M. Dusinska
Dr E. Gaffet
Prof. E. Panteri
Dr C. Rousselle
Dr M. Stepnik
Dr S. Wijnhoven (Rapporteur)

The SCHEER members

Dr W.H. de Jong

External experts

Dr N. von Goetz

All Declarations of Working Group members are available on the following webpage:

[Register of Commission expert groups and other similar entities](#)

This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 16 April until 14 June 2021). There were no comments received.

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 A (Platinum and Colloidal Platinum) and B (Acetyl tetrapeptide-17 (and) Colloidal Platinum) 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?*

Having considered all the information provided by the Notifiers, and that obtained from other sources, the SCCS is of the opinion that it is not possible to carry out a safety assessment of any of the notified platinum nanomaterials due to limited or missing essential information. The limited amount of data provided by the Notifiers also does not correspond to the requirements and data standards as indicated in the SCCS Guidance (SCCS 1611/19), and the SCCS Memorandum (SCCS/1524/13).

To enable safety assessment by the SCCS, the Notifiers need to provide the necessary information, a summary of which is provided in Annex I.

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

In the absence of sufficient data to allow safety assessment, the SCCS has considered the different aspects of platinum nanomaterials 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 platinum, colloidal platinum, and acetyl tetrapeptide-17 colloidal platinum (nano) in cosmetic products can pose a risk to the consumer. The SCCS will be ready to assess any evidence provided to support safe use of the materials in cosmetic products.

Keywords: SCCS, scientific opinion, Platinum, Colloidal Platinum, Acetyl tetrapeptide-17 Colloidal Platinum, nano, CAS No 7440-06-4, EC No. 231-116-1, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Platinum (nano), Colloidal Platinum (nano) and Acetyl tetrapeptide-17 Colloidal Platinum (nano), final version of 24-25 June 2021, SCCS/1630/21

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.).

Scientific Committee members

Ulrike Bernauer, Laurent Bodin, Qasim Chaudhry, Pieter Jan Coenraads, Maria Dusinska, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Berit Granum, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara Vanhaecke, Susan Wijnhoven

Contact

European Commission
Health and Food Safety
Directorate C: Public Health
Unit C2: Health information and integration in all policies
L-2920 Luxembourg
SANTE-SCCS@ec.europa.eu

© European Union, 2022

PDF ISSN 1831-4767 ISBN 978-92-76-54760-0 doi:10.2875/56282 EW-AQ-22-011-EN-N

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

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

TABLE OF CONTENTS

ACKNOWLEDGMENTS	2
1. ABSTRACT	3
2. MANDATE FROM THE EUROPEAN COMMISSION	6
3. OPINION.....	8
3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS	8
3.1.1 Chemical identity	8
3.1.2 Physical form	10
3.1.3 Molecular weight	11
3.1.4 Purity, composition and substance codes.....	11
3.1.5 Impurities / accompanying contaminants	14
3.1.6 Solubility	14
3.1.7 Partition coefficient (Log P _{ow})	15
3.1.8 Additional physical and chemical specifications.....	15
3.1.9 Particle size	16
3.1.10 Microscopy	16
3.1.11 Crystal structure.....	17
3.1.12 UV absorption	18
3.1.13 Surface characteristics.....	18
3.1.14 Droplet size in formulations.....	19
3.1.15 Homogeneity and stability.....	19
3.1.16 Other parameters of characterisation.....	19
3.1.17 Summary on supplementary physicochemical characterisation ...	20
3.2 TOXICOKINETICS	20
3.2.1 Dermal / percutaneous absorption.....	20
3.2.2 Other studies on toxicokinetics	24
3.3 EXPOSURE ASSESSMENT	24
3.3.1 Function and uses.....	24
3.4 TOXICOLOGICAL EVALUATION.....	25
3.4.1 Acute toxicity	25
3.4.2 Irritation and corrosivity	26
3.4.3 Skin sensitisation.....	26
3.4.4 Repeated dose toxicity	27
3.4.5 Mutagenicity/genotoxicity	27
3.4.6 Carcinogenicity.....	32
3.4.7 Reproductive toxicity	33
3.4.8 Photo-induced toxicity	33
3.4.9 Human data.....	33
3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)	34
3.6 DISCUSSION.....	34
4. CONCLUSION	35
5. MINORITY OPINION.....	35
6. REFERENCES	36
7. ANNEX I	38
8. ANNEX II	42

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.

The Commission services received under Article 16 of the Cosmetics Regulation via the Cosmetic Product Notification Portal (CPNP):

- 18 notifications for cosmetic products containing Platinum (10 notifications) and Colloidal Platinum (8 notifications) with CAS No 7440-06-4 and EC No. 231-116-1 in nano form. Platinum is reported in CosIng database with the following functions: antimicrobial, antioxidant and skin conditioning, whereas Colloidal Platinum is reported with antimicrobial, antioxidant and deodorant function. In both cases, there is no reference to the nano forms and they are not regulated under the Cosmetic Regulation (EC) No 1223/2009. According to the notifications submitted, both ingredients (Platinum and Colloidal Platinum) are used in nano form in leave-on and rinse-off skin cosmetic products with different concentrations and specifications as reported in the attached list.
- 13 notifications for cosmetic products containing Acetyl tetrapeptide-17 Colloidal Platinum [CAS and EC No. not available] in nano form, as reported in the attached list. Acetyl tetrapeptide-17 Colloidal Platinum (nano) is not reported in the CosIng database. According to the notifications submitted, this ingredient is used in leave-on and rinse-off skin care cosmetic products, with different concentrations and specifications as reported in the attached list.

The Commission has concerns about the use of Platinum - Colloidal Platinum and Acetyl tetrapeptide-17 Colloidal Platinum 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 Platinum - Colloidal Platinum and Acetyl tetrapeptide-17 Colloidal Platinum 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:*
- a) *does the SCCS consider the nanomaterials Platinum and Colloidal Platinum safe when used in leave-on and rinse-off skin cosmetic products according to the maximum concentrations and specifications reported in the attached list, taking into account reasonably foreseeable exposure conditions?*
 - b) *does the SCCS consider the nanomaterial Acetyl tetrapeptide-17 Colloidal Platinum 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*
- a) *Platinum and Colloidal Platinum in nano form in cosmetic products?*
 - b) *Acetyl tetrapeptide-17 Colloidal Platinum in nano form in cosmetic products?*

3. OPINION

Preamble

In total, 8 manufacturers notified 31 different products containing nanosized platinum i.e. nano platinum, colloidal platinum and acetyl tetrapeptide-17 colloidal platinum (nano). For every platinum dispersion, four different files were presented; a notification file, a safety file, a specification file and a file on the toxicity profile. For two platinum dispersions, additional files have been submitted. After initial evaluation and a request for additional information, one manufacturer withdrew two different platinum dispersions (in total 19 notifications) from the notification portal. Therefore, these products were excluded from evaluation, resulting in the final evaluation of 12 nanosized platinum products from 7 manufacturers.

For four different platinum nanomaterials, notification files were presented, being aXonnite Platinum, APS-WM100, Platinum MatrixEM (PF) and one dispersion without a name. A number of additional files were provided at the request of the SCCS.

In addition to the information provided by the Notifiers, a Call for Data on platinum nanomaterials was published by DG Health and Food Safety from 11 June 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 types of materials considered in this assessment.

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 (according to the notification file):

P1: Aqua, Platinum
P2: Aqua and Platinum
P3: Colloidal Platinum
P4: Platinum, acetyl-L-cystein-S-yl-L-lysinyL-L-phenylalaninyl-L-arginine;
Acetyl tetrapeptide-17 (and) Colloidal platinum

3.1.1.2 Chemical names

Metallic nanoparticle platinum
Colloidal platinum
Acetyl tetrapeptide-17 (and) Colloidal platinum

3.1.1.3 Trade names and abbreviations

aXonnite platinum
APS-WM100
Platinum matrixEM (PF)

Opinion on Platinum (nano)

In the following Table, the trade names of platinum (nano), colloidal platinum (nano) and acetyl tetrapeptide-17 (and) colloidal platinum (nano) from different manufacturers are described.

Table 1: Trade names of the various platinum dispersions and corresponding CPNP codes (manufacturers' names have been coded by the SCCS)

Nr	Name of the platinum dispersion	Manufacturer	CPNP code(s)	References
P1 (Platinum)	aXonnite platinum	A B C D	1002908 1003132 1002945 1002946 1003541 1002990 1002991 1003464 1003465	1-4 5-8 9-12 13-16 17-20 21-24 25-28 29-32 33-36
P2 (Platinum)	APS-WM100	E	1003414	37-40
P3 (Colloidal platinum)	/	F	1003374	41-45
P4 (Acetyl tetrapeptide-17 (and) colloidal platinum)	Platinum matrixEM (PF) NP0003PF	G	1001866	46-50

In the original submission, seven different Notifiers have submitted data on four different nano platinum dispersions (See Table 1). For every nano platinum dispersion, at least four different files have been submitted; a notification file, a safety file, a specification file and a file on the toxicity profile. For some notifications, additional files have been submitted.

SCCS comment

The SCCS noted that there is a great deal of overlap in the files submitted. For P1, there is one specification file submitted for all the dispersions - although the files sometimes have different names. The safety and toxicity files are identical for all CPNP notifications.

For P2, there is one file submitted, which is used as specification, safety and toxicity file (reference 38, 39 and 40 are the same). For P3, no relevant documents have been submitted as the specification and toxicity files are on nano silver (not platinum), and the safety file is not accessible because it is locked by a password. This password was not provided by the Notifier, even when requested by the SCCS. One additional file has been provided for P3, named "other file", with information relating to nano copper instead of platinum.

For P4, one of the two Notifiers has withdrawn his notification during the reviewing process.

Opinion on Platinum (nano)

3.1.1.4 CAS / EC number

CAS number

P1, P2, P3: 7440-06-4

P4: Not available

EC number

P1, P2, P3: 231-116-1

P4: Not available

SCCS comment

According to the Notifier, no CAS number for the peptide is available for P4, instead, the CAS number for platinum is given in the exposure file (Notification_1001866_42328_exposure_file_).

Ref. 50

3.1.1.5 Structural formula

Platinum: Pt

3.1.1.6 Empirical formula

Platinum: Pt

3.1.2 Physical form

Table 2: Physical form, shape and agglomeration/ aggregation state

Platinum nanomaterial	Physical form, shape and agglomeration/ aggregation state
P1: aXonnite platinum	Dispersion, Spherical/ Crystalline, Dispersed free particles
P2: APS-WM100	Solution, Irregular, Dispersed free particles
P3:/	Solution, Crystalline, Dispersed free particles
P4: Platinum matrixEM (PF)	Dispersion, Crystalline, Dispersed free particles

Ref. 1, 5, 9, 13, 17, 21, 25, 29, 33, 27, 41, 46

According to the notification files, Platinum nano is described as either a clear colourless water dispersion of platinum nanoparticles (P1 and P4) or a solution (P2 and P3) as presented in Table 2.

For P4, according to the Notifier, the "nanomaterial preparation is in the form of a dark brown solution".

Ref. 47, 49, 50

The following product specifications of the various forms of Platinum nano have been reported.

Table 3: Product specifications as provided by the Notifiers

Dispersions	Concentration	Particle size (% of the total amount)	Specific surface area	Surface charge (Zeta potential)	REFs
P1	50 ppm/ 100 ppm ±10% (50/ 100 mg/kg)	2-5 nm (70-75%) and 5-100 nm (25-30%)	1 m ² /g (BET) 1 m ² /cm ³ (VSSA)		1,3,5,7,9,11 13,15,17,19, 21,23,25,27, 29,31,33,35
P2	0.1% w/w	19-23.6 nm			37,38,39,40
P3		3-12 nm	1 m ² /g (BET) 1 m ² /cm ³ (VSSA)		41
P4	7.8 × 10 ⁻⁸ M or 4.7 × 10 ¹⁵ nanoparticles/L	1.5-6.5 nm (medium diameter 3.6 ± 1.2 nm)	38.5 nm ²	> 20 mV (measured Z potential was 32.9 A ±8.3 mV peak 1: -30.4 mV, peak 2: -53.4 mV)	46,47,49,50

SCCS comment

Platinum dispersions are differently described by the different Notifiers, either as a dispersion or as a solution. Solution is not the correct term for P4, as the nanoparticles are dispersed, free particles.

The specific surface area and VSSA of P1 and P3 is far too low for such small sized nanoparticles and should be corrected.

The specific surface area for P4 should also be corrected for the metric used.

3.1.3 Molecular weight

Platinum, 195.08 g/mol

Acetyl tetrapeptide-17 (and) Colloidal platinum, 735.859 g/mol (calculated from molecular weights).

3.1.4 Purity, composition and substance codes

Products are described as colourless clear water dispersion (colloid) of platinum nanoparticles (P1-P3) or a dark-brown solution (P4):

Composition of P1-P3:

P1: Pt content is 50 ppm / 100 ppm ± 10% (50/100 mg/kg)

P2: 0.1 % w/w

P3: not given

Ref. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35

Ref. 37, 38, 39, 40

Opinion on Platinum (nano)

Composition of P4 (pre-formulation solution):

>99% Aqua (water) used as solvent

0.4-0.6% Phenoxyethanol, used as a preservative

0.004-0.006% Acetyl tetrapeptide-17 (and) Colloidal platinum

Table 4: Identification of raw materials (preformulation solution P4)

Platinum MatrixEM (PF)	Infinitec Activos SL	0.1	Demineralized Water (Aqua)	1	0.0995
			Phenoxyethanol	11	0.00045
			Acetyl Tetrapeptide-17, Colloidal Platinum	30	0.00005

Ref. 50

According to the Notifier of P4:

Acetyl tetrapeptide-17 purity and identity was analysed by HPLC. Column C18 4,6X150mm 5µm, Waters; Waters 996 photodiode array detector equipped with the Waters 2695 separation module and the Millenium Software. UV detection at 220nm, and linear gradients from 5 to 100% of CH₃CN (+0.036% TFA) into water (+0.045% TFA) were run at 1.0 mL/min flow rate. Acetyl tetrapeptide-17 elution time is 3.5 min.

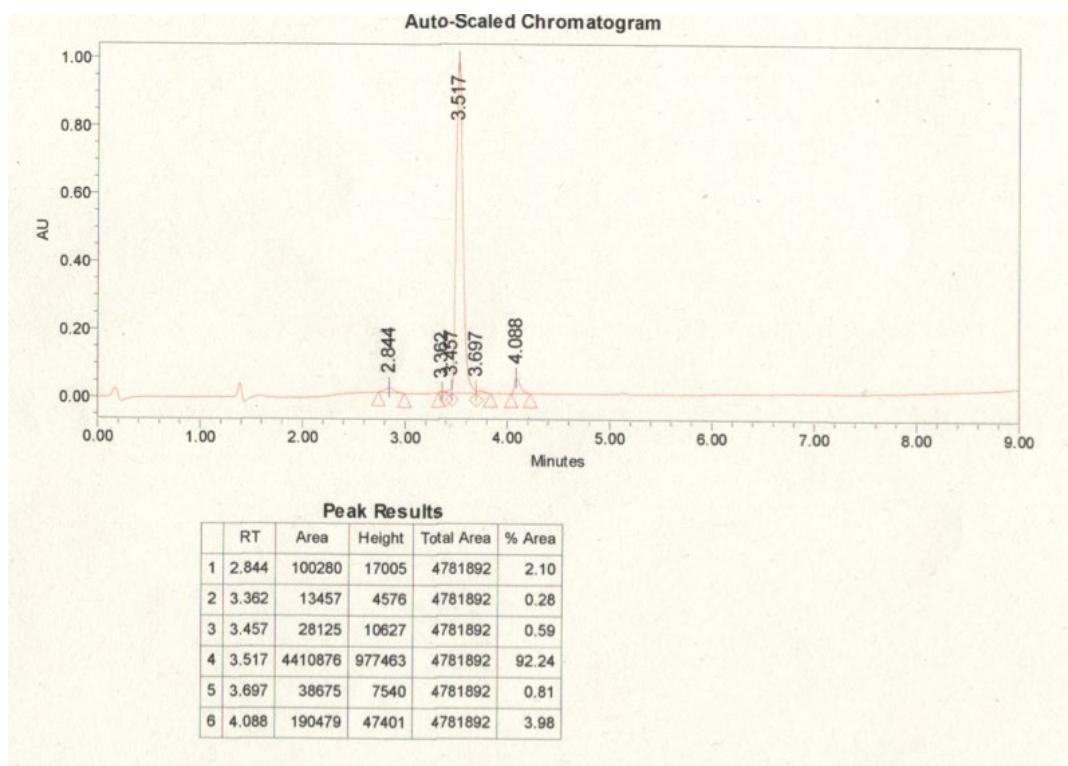


Figure 1. HPLC chromatogram of Acetyl tetrapeptide-17 (pick 3.5 min).

Colloidal platinum core was analyzed by High Resolution-TEM (High Resolution Transmission Electron Microscopy). According to the Notifier (P4), this technique allows observing crystalline layers of the platinum core. The distance between two layers is characteristic of each material, and therefore the technique allows confirming that nature of the core is platinum. TEM was chosen for being the more appropriate to this kind of nanoparticles, it

Opinion on Platinum (nano)

allows to observe a direct image of the particle without interpretation required by other structural techniques and is therefore very conclusive.

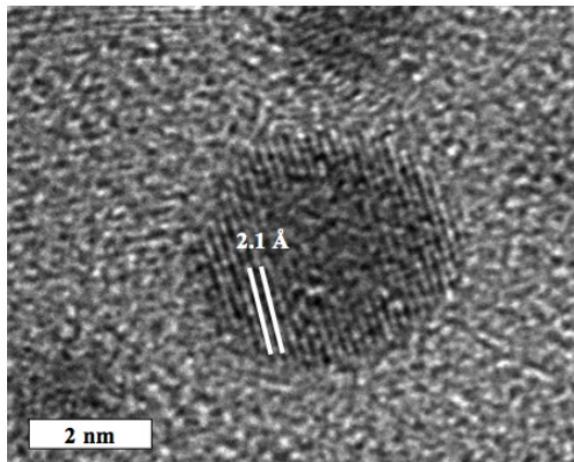


Figure 2: High Resolution TEM Image of Platinum MatrixEM nanoparticles, showing interlayer's distance of Interplanar distance = 2.10 Å, which corresponds to (111) plane

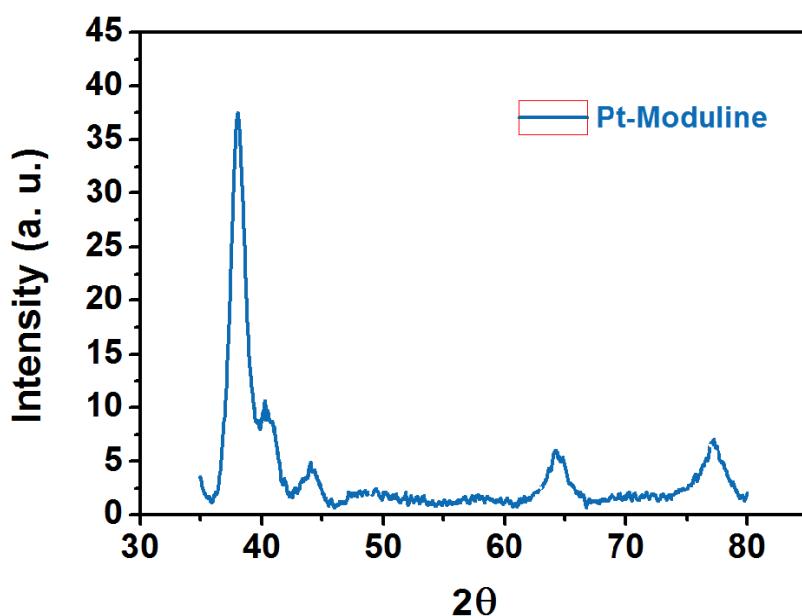


Figure 3: X ray diffraction analysis (XRD) of Platinum MatrixEM nanoparticles

According to the Notifier of P4, the measured diffraction peaks were attributed to (111), (200), (220), and (311) planes by using approximately the lattice parameter for platinum, which means that face-centered crystalline nanoparticles were obtained (see Figure). Colloidal platinum formation is responsible for the dark brown characteristic colour of the solution obtained as manufactured product.

Ref 47, 49, 50

SCCS comment

In Figure 3, which was submitted by the Notifier, an extra X-ray diffraction peak is observed on the right side of the (111) XRD peak to the Pt structure which is not in line with the face centered cubic (FCC) structure of platinum. Information has not been provided to explain the origin of such an extra XRD peak.

The SCCS has also noted that the particle surface of material P4 is coated with citrate. Since this material is also surface-modified with a peptide (acetyl tetrapeptide-17), it should be

Opinion on Platinum (nano)

explained whether citrate coating was applied to colloidal Pt particles before or after surface-modification with the peptide.

3.1.5 Impurities / accompanying contaminants

P1:

According to the Notifier, the nonionic Silver Colloid preparation does not contain any of the 36 allergens listed, is not mentioned in the Annex to Minister of Health Regulation as a forbidden substance and does not contain any substance of the CMR group.

Ref. 3, 7, 11, 15, 19, 23, 27, 31, 35

Information on impurities of P2 and P3 has not been provided.

P4:

According to the Notifier, coating agent/surface moiety: sodium citrate. The presence of citrate is required for adequate solubility of the nanoparticle. Otherwise non soluble particle would be obtained, and visible precipitation will occur.

No doping material included

No encapsulating materials included

No processing chemicals included

No dispersing agents included

No other additives or formulants, e.g. stabilizer used than described.

Ref. 47, 49, 50

SCCS comment

In the P1 notification, a non-ionic silver colloid preparation is mentioned, while this file is on non-colloidal platinum. Furthermore, information was not provided on the presence of impurities/contaminants, with the exception of the statements on the absence of allergens and forbidden substances as presented above. A detailed analytical report on purity of the material and any impurities/contaminants should be provided.

Information on impurities of P2 and P3 has not been given and this information should be provided.

3.1.6 Solubility

Table 5: Solubility as described in the notification files

Platinum nanomaterial	Solubility/dissolution (in relevant solvents): Aqueous media (mg/L)
P1: aXonnite Platinum	Below 0.01
P2: APS-WM100	Below 0.01
P3: /	Below 0.01
P4: Platinum matrixEM (PF)	Below 0.01

According to one of the Notifiers (P4), acetyl tetrapeptide-17 (and) colloidal platinum (P4) is soluble in water and water-glycerine (50%). It is not soluble in ethanol, toluene or chloroform. Solubility is readily confirmed by absence of precipitate.

Opinion on Platinum (nano)

Solubility: instability of the system would result in visible precipitation, which is not observed in retain samples beyond expire date, nor stability studies during 3 months at 40°C.

Ref. 47, 49, 50

SCCS comment

The term 'solubility' has been wrongly used to describe what is essentially 'dispersibility' and/or stability of the dispersion. Specifically, it is not correct to assume that the 'absence of precipitate' indicates solubility of nanoparticles.

The SCCS is of the opinion that platinum and Pt-tetrapeptide nanoparticles are practically insoluble, and persistent. This is confirmed by the notification files of all notified Pt dispersions (platinum, colloidal and surface modified colloidal platinum), which indicate a solubility of < 0.01 mg/L. No additional information (apart from the <0.01 value in the notification file) is given for the other Pt dispersions and this information should be provided. A detailed report on the methodology used for determination of platinum solubility should also be provided.

3.1.7 Partition coefficient (Log P_{ow})

No information on partitioning between aqueous and organic phases is available.

Ref. 47, 49, 50

3.1.8 Additional physical and chemical specifications

Table 6: Additional physical and chemical specifications provided by the Notifiers

Specification	P1	P2	P3	P4	Comments column P4
	Ref 3,7,11,15,19,23, 27,31,35			Ref 47,49,50	
Melting point	-	-	-	-	
Boiling point	-	-	-	-	
Density	0.990 - 1.010 g/m ³ 1.00 kg/L (20 C)			21.5 kg/L	Determined by a pycnometer Precision level of density in column I is too high
Viscosity	1000 x 10 ⁻⁶ Pa x s			<500 cps (5/50, 25 C)	Brookfield digital viscosimeter MODEL DV-I+
pH	6.5 ±1			3.00-7.00	Provided by standard laboratory pH meter
Conductivity	2 - 50 µS				Tested by conductometer
Turbidity/Opacity	max. 8 NTU				

Opinion on Platinum (nano)

Colour	Colourless to pale pink			Dark brown	
--------	-------------------------	--	--	------------	--

SCCS comment

Physicochemical characterisation should be provided for all the different platinum nano dispersions considering recommendations in the SCCS Guidance on nanomaterials (SCCS/1611/19). The provided density of dispersion P1 corresponds to the density of water and not of platinum.

3.1.9 Particle size

The average particle size of the products is:

P1: 2-5 nm (70-75%) and 5-100 nm (25-30%)

P2: 19-23.6 nm (lowest cut off level 10 nm)

P3: 3-12 nm

P4: 1.5-6.5 nm (medium diameter 3.6 ± 1.2 nm)

According to the Notifier, the TEM measurement of particle size (P4):

Number of Platinum (Pt) atoms in one nanoparticle can be calculated from particle size diameter (D) determined by TEM (15.3 nm) using the formula,

$$N = \frac{\pi \rho D^3}{6 M} N_A$$

where N is the number of atoms, r is the density of platinum (21.5 Kg/L), M is the molecular weight of Pt 195.08 g/mol, and NA is Avogadro number 6.0221E+23 (1/mol).

From this calculation, the number of atoms of Pt in a nanoparticle is 289888. Therefore, the nanoparticle molecular weight is 1485 Pt atoms/ nanoparticles.

Concentration of Pt(0) $1.2 \times 10^{-4} M$

Therefore, concentration of nanoparticles is $7.8 \times 10^{-8} M$ or 4.7×10^{15} nanoparticles/L.

Number of atoms (N) of Pt at the surface can be calculated with the formula,

$N_{\text{Surf}} = 4\pi R^2 / (a^2/2)$, where a is a net parameter (distance between atoms in a unit cell).

Calculated Number of atoms of Pt at the surface is 502 to which 252 citrate molecules may be linked.

Ref. 47, 49, 50

SCCS comment

The SCCS has noted that the particle size distribution for different platinum materials range from 2 to 100 nm for P1; 19 to 23.6 nm for P2; 3 to 12 nm for P3; and 1.5 to 6.5 nm for P4. The median particle diameter is only reported for P4 as 3.6 ± 1.2 nm, and should also be provided for the other materials.

3.1.10 Microscopy

According to the Notifier of P4, the use of more than one method (one being electron microscopy based imaging) for determination of size parameters has been recommended by EFSA (2011) and OECD (2010). For platinum nanoparticles, TEM was the method chosen, provided that it measures the real particle size. Compared to other techniques, such as Dynamic Light Scattering (DLS), which measures the Hydrodynamic Ratio and is including not only the particle but also the associated environment (i.e. water, more pH depending and functionalization) providing higher levels of particle. Therefore, in the present case the more

Opinion on Platinum (nano)

scientifically reliable data are only provided by TEM. Additionally, High Resolution TEM provided complementary information (see 3.1.).

TEM measures of Platinum MatrixEM (P4) nanoparticles exhibited:
 Medium diameter 3.6 ± 1.2 nm
 Size Range 1.5- 6.5 nm

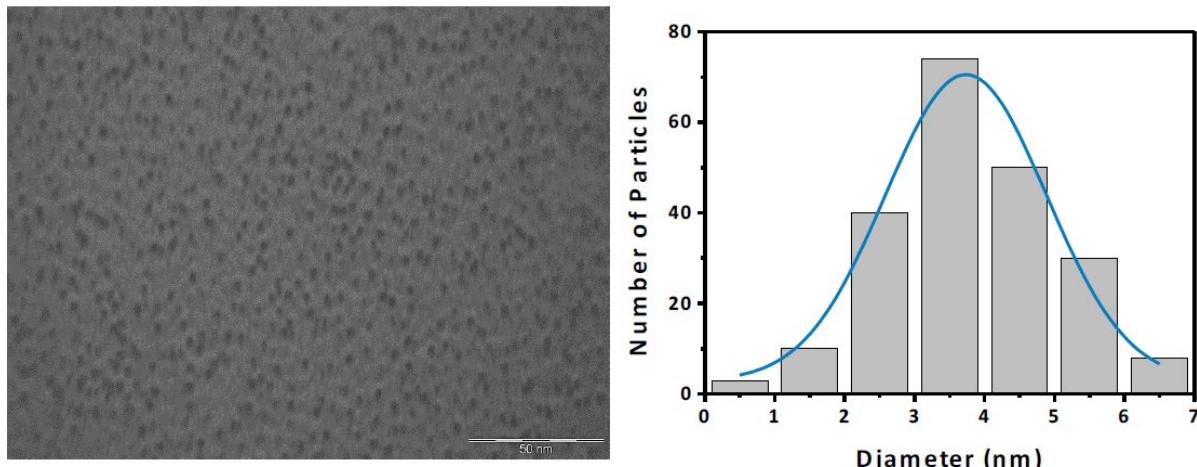


Figure 4. TEM images (JEOL 1010, 100 KeV) and measured distribution of particle size.

Ref 47, 49, 50

SCCS comment

The SCCS recommends the use of two different methods for size determination, including one electron microscopy method (SCCS/1611/19).

For P3, TEM images are provided for silver particles, and have thus not been considered in this Opinion.

3.1.11 Crystal structure

Table 7: Crystal structure

Platinum nanomaterial	Morphology and Crystal structure
P1: aXonnite Platinum	Spherical, Crystalline
P2: APS-WM100	Irregular
P3: /	Crystalline
P4: Platinum matrixEM (PF)	Crystalline

SCCS comment

The provided information is not sufficient. Further details of the crystalline shape and aspect ratio of the particles should be provided.

3.1.12 UV absorption

Information on UV absorption has not been provided.

SCCS comment

Information on the UV absorption and the methodology used should be provided.

3.1.13 Surface characteristics

Information on BET (Brunauer Emmet and Teller method) for the specific surface area measurement is provided for platinum dispersion P1 and P3 and is $1 \text{ m}^2/\text{g}$. Also volume specific surface area (VSSA) is given for P1 and P3 and is $1 \text{ m}^2/\text{cm}^3$.

Ref. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35

For P4, more information is provided on analysis of the surface characteristics.
According to the Notifier:

Analytical methods used

Information on surface charge with Z-Potential:

Measured Z potential was $32.9 \pm 8.3 \text{ mV}$. Peak 1: -30.4 mV, Peak 2 -53.4 mV.

Negative Z potential means that the particle is surrounded by negative charges, in this case due to the presence of sodium citrate used as coating agent. The higher the Z potential, the more stable the colloid is, for values $> 20 \text{ mV}$, in absolute values, the colloid is considered stable, like in the present case.

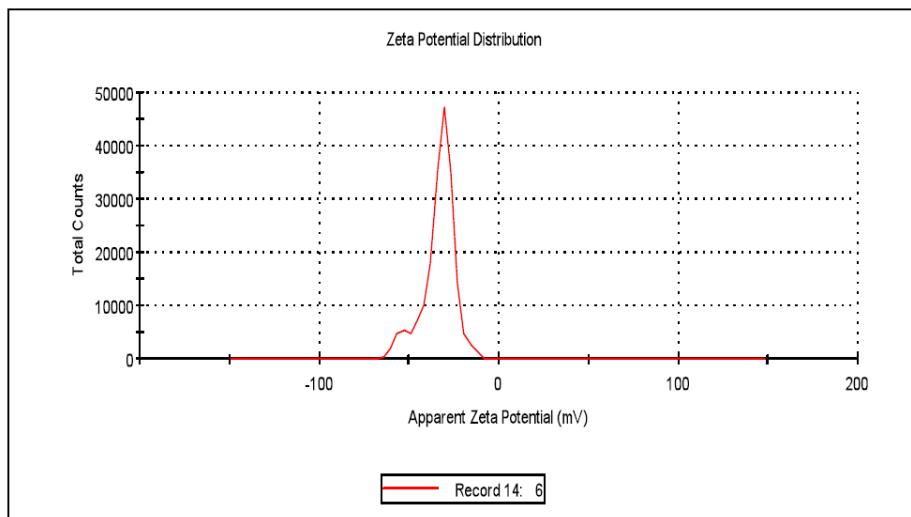


Figure 5. Z Potential Distribution of Platinum Matrix EM

Surface area of P4 is 38.5 nm^2 , calculated from the diameter determined by TEM (medium value 3.5 nm) and surface area of spheres ($4\pi r^2$).

Ref. 47, 49, 50

SCCS comment

There is a minus (negative) sign missing in measured Z potential. The value given for surface area is quoted per particle. This should be given as m^2/g . Also, the value of the VSSA given is incorrect and needs correcting.

As mentioned above in the SCCS comment on page 11, the specific surface area and VSSA of P1 and P3 is far too low for such small sized nanoparticles and should be corrected.

3.1.14 Droplet size in formulations

Information on droplet size in formulations has not been provided

SCCS comment

Inhalation exposure is not expected during the use of the submitted products, so information on droplet size is not relevant.

3.1.15 Homogeneity and stability

According to the Notifier, the shelf life of P1 is 24 months.

No information has been provided on homogeneity and stability for P2 and P3.

According to Notifier of P4: Z potential gives indication of stability ($> 20\text{mV}$). Instability of the system would result in visible precipitation, which is not observed in retained samples beyond expire date, nor stability studies during 3 months at 40°C .

SCCS comment

The provided information is incomplete. Studies on stability of the dispersions should be provided.

3.1.16 Other parameters of characterisation

The Notifier of P4 has provided the following additional information:

Catalytic activity

Nanomaterial core chemical reactivity.

Core material is functionalized with peptidic compound and coated with citrate, which does not provide reactive available points at the surface and confers sterical hindrance, which makes difficult the access to the surface of the core material. Adequate citrate coating is demonstrated with solubility, inefficient coating would yield to visible nanoparticle precipitation, and by UV-VIS absorption maximum (agglomeration of particles would result in peak broadening).

Nanomaterial surface chemical reactivity. The expected reactivity of the conjugated compound at the surface of the nanoparticle is that of any peptidic compound. Nanomaterial photocatalysis and radical formation is not expected due to the chemical nature of the components, there is no presence of photoactive functional groups.

Redox potential

Nanomaterial is chemically colloidal platinum, as demonstrated by HR-TEM, and the redox state of this is 0, therefore no redox potential can be measured.

SCCS comment

Information on surface reactivity is provided only for P4, but this information is incomplete. Complete information should be provided for all materials.

Platinum particles (especially nanoparticles) are known for their surface reactive/catalytic activity. Therefore, data on the catalytic activity for all the notified platinum nanomaterials should be provided.

Opinion on Platinum (nano)

3.1.17 Summary on supplementary physicochemical characterisation

Table 8: Physical characteristics of platinum nanomaterials

Product Parameter	P1	P2	P3	P4
Concentration	50 ppm ±10% (50 mg/kg) 100 ppm ±10% (100 mg/kg)	0.1% w/w	/	7.8x10 ⁻⁸ M or 4.7x10 ¹⁵ nanoparticles/L
Size	2-5 nm (70-75%) 5-100 nm (25-30%)	3-12 nm (from notifications)	19-23.6 nm	1.5-6.5 nm (medium diameter 3.6 ± 1.2 nm)
Surface area	1 m ² /g (BET) 1 m ² /cm ³ (VSSA)		1 m ² /g (BET) 1 m ² /cm ³ (VSSA)	38.5 nm ²
Surface charge	/	/	/	> 20 mV (measured Z potential was 32.9 ±8.3 mV peak 1: -30.4 mV, peak 2: -53.4 mV)
pH	6.5 +/- 1	/	/	3-7
Solubility	< 0.01 mg/L	< 0.01 mg/L	< 0.01 mg/L	< 0.01 mg/L
Shelf life	24 months	/	/	40 months
Form				
Colour	Colourless			Dark brown

SCCS comment

See SCCS comments above.

3.2 TOXICOKINETICS**3.2.1 Dermal / percutaneous absorption**

In the first submission/ notification round, information on the dermal absorption has not been provided for P1, P2 nor P3. However, the Notifier of aXonnite Platinum (P1) provided an additional document on the dermal absorption in response to the Call for data by the European Commission's Directorate General for Health and Food Safety (from 11 June 2019 to 10 November 2019).

Opinion on Platinum (nano)

P1: Notifier's text

Purpose of the test

According to the Notifier (from an English translation of the original Polish text), the purpose of the dermal penetration study was to assess the penetration ability of nano-platinum from aXonnite Platinum, through undamaged pig skin under *in vitro* conditions.

Physico-chemical characterization of aXonnite Platinum produced by Nano-Tech

Unlike ionic forms, aXonnite nanoparticles are obtained by physical methods, therefore they are characterized by very high chemical purity.

- demineralized water, platinum (nano) with nanoparticles at the concentration of 50 ppm.

Research methodology

Permeation tests were performed on membranes prepared from intact swine skin. Pig ears, collected from the butcher within 3 hours after slaughter, were washed under running water, dried and the bristles was cut with an electric shaver to a length of 1 mm. The skin was carefully separated from the cartilage with a scalpel. Then skin fragments of 0.64 mm in thickness were cut with a dermatome (Padgett Electro Dermatome model B; Integra Lifesciences Padgett Instrument Plainsboro, NJ, USA). From the thin skin layers, circles with a diameter of 25 mm were cut and placed in Franz's chamber.

Before the start of the permeation study, the skin membranes placed in the chambers were subject to a 24-hour equilibration in demineralized water. Permeation study was conducted using the final product aXonnite Platinum with a determined nano-platinum content. After starting the test, 1 mL of the solution from the acceptor compartment was taken for further analysis at specified time intervals (after 2, 4, 6, 8, 10 and 24 hours). The experiments were carried out in an air-conditioned room at temperature of 20-22°C for 24h. The samples were analyzed on a RIGAKU NEX DE spectrometer in the facility of ANITEPO Sp. z.o.o.

Results

Date of the study: August 2016

The experiment was carried out in three independent repeats. As a receiving system demineralized water was applied. The limit of quantification was established as for pure water. The analysis did not reveal any presence of platinum in the samples tested after 2, 4, 6, 8, 10 and 24 hours.

Conclusions

The results of the conducted experiment indicated that the amount of nano-platinum in the acceptor chamber did not change after 2, 4, 6, 8, 10 and 24h relative to the background value, i.e. pure water. This indicates the lack of penetration capacity of platinum nanoparticles applied in the form of aXonnite Platinum.

Ref. 58

SCCS comment on P1

The study report on dermal absorption for the aXonnite platinum is not complete, as no experimental data have been provided. Only statements are provided in the form of results and conclusions, which are not sufficient. These experimental data should be provided, including the analyses used.

The analytical method should be sensitive enough to detect platinum particles. The SCCS is of the opinion that spectrophotometric analysis is not sensitive enough.

The receiving system (demineralized water) might not be appropriate as the nanomaterial is not soluble in water. According to the SCCS NoG 10th revision (SCCS/1602/18): Adequate detection of substances that are poorly soluble in water is important in the receptor fluid of an *in vitro* dermal absorption study to ascertain that the dermal absorption concerns the

Opinion on Platinum (nano)

active substance and not the impurities. However, this does not make sense when water is chosen as the receptor fluid in the study.

P4: Notifier's text

According to the Notifier, Platinum MatrixEM (P4) absorption was studied on skin biopsies (OECD 428) determining platinum particles by ICP-MS (Induced-Coupled Plasma-Mass Spectrometry). This technique has the adequate sensitivity to measure platinum concentration in the ppm range. 10% of Platinum MatrixEM (versus recommended dose of use 0.3%) was tested and compared to untreated control.

Experimental data exhibited concentration of platinum at the more external epidermic layers of skin biopsies (0.33 ppm; untreated control 0.01 ppm), at the level of the test solution. Concentration at the most internal layers of skin was no significantly different than untreated control (0.01 ppm at dermis, PBS collection solution an intreated control). Therefore, no significant detection of platinum occurred in the most internal layers of skin corresponding to the dermis neither at the collection solution used, therefore it can be concluded that no systemic absorption is expected for Platinum MatrixEM nanoparticles.

Methodology

Experimental procedure

Franz cells were used to carry out the assay with pig skin. Skin was washed with PBS and fat layer removed before the study. The test item was inoculated onto the skin and incubated during 24 hours at 32°C.

At the end of incubation procedure, the skin was frozen at -80°C and then cut using a Cryostat for further Pt analysis.

The test consisted on three phases:

1. Skin incubation with test item or PBS (negative control) in Franz cells

The fat was removed from the skin to roughly 0.5 mm and washed with PBS.

The test item was 10% Platinum Matrix EM. The assay was carried out in Franz cells during 24 hours at 32°C with constant shaking. Once incubated, receptor fluid was removed and the skin was stored at -80 ° C until the tissue cutting process.

2. Slicing of tissue using a Cryostat

The slicing of the skin was performed with a Cryostat at 20um thick at -20°C.

The tissue sections were placed into vials until quantification. A skin sample without treatment (only PBS) was used as a negative control.

3. Quantification of Platinum by induction coupled plasma mass spectroscopy

The quantification was performed by induction coupled plasma mass spectroscopy (ICP-MS) technique in standard conditions in a Perkin Elmer ELAN 6000. Five calibrated standards were prepared with a solution of 200 ppm of thiourea in 1% HCl and 4% HNO₃, and Pt pattern of 1g/L NIST traceable.

The samples were digested using 1/10 and 1/15 dilutions.

Results

The following table shows the results of the Platinum levels detected in all skin samples. Results indicate that Pt remains at the skin epidermis surface and that Pt level in dermis is similar to that in untreated control skin. No any Pt was detected in the receptor PBS.

Opinion on Platinum (nano)

Table 9: Platinum levels detected in all skin samples

	Pt (ug/g)
Standard Solution 10% Pt	0,30 ± 0,008
Control skin (untreated)	0,01
Pt treated skin epidermis	0,33
Pt treated Dermis	0,01
PBS receptor	0,01

Conclusion

From experimental results we conclude that 10%- Platinum Matrix EM does not penetrate into the dermis.

Ref. 47-49

SCCS comment on P4

Although OECD TG 428 was followed, it is not possible to interpret the results without further clarification, because the way the data was presented is very confusing. For example:

1. The product name is '10% Platinum Matrix Em (PF)' (which does not indicate concentration)
2. The concentration of (presumably of Pt in the test substance) is indicated as 2.2 µg/mL (i.e. 0.0022 µg/µL).
3. The volume applied for the test was 25 µL (which makes it equal to 0.0022 x 25=0.055 µg of Pt applied)

However when Pt is analysed using ICP-MS, after solubilising the samples:

- a. The standard solution comes out as 0.30 ± 0.008 (indicated as µg/g)
- b. In Pt-treated skin epidermis = 0.33 (indicated as µg/g)

The weight of each skin sample analysed (indicated as '/g' here) was unclear, as was the proportion of the applied amount of Pt that was recovered in each analysed skin section. In addition, from the Notifier's provided interpretation, it is possible that the stratum corneum may have been mistaken for epidermis.

In an additional letter to the Notifier, the SCCS requested additional information for further clarification of the test results, e.g. on the exact amount of platinum dispersion that was applied to the skin and on the recovery rate. No additional information or clarification was provided in response to this request.

In the absence of further clarification, the SCCS has concluded that the provided data show skin penetration of platinum P4 particles.

Opinion on Platinum (nano)

3.2.2 Other studies on toxicokinetics

According to the Notifier of P4, no additional toxicokinetic studies were relevant as no dermal penetration has been demonstrated.

SCCS comment

The SCCS is of the opinion that the absence of dermal absorption of platinum P4 dispersion has not been demonstrated. Notwithstanding the ambiguities in the data, it points to dermal penetration of Pt nanoparticles.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

The following information was provided by the Notifiers as presented in Table 10.

Table 10. Product names, product types and use concentrations of the various notified platinum nanomaterials

Code number Pt	Description of platinum product	CPNP notification	Product type	Cosmetic products the platinum (nano) is used in	Use concentration (w/w %)
P1	Platinum	1002908	Dermal, leave on	Face care products other than face mask	0.0005
P1	Platinum	1002945	Dermal, rinse off	Face mask	0.0001
P1	Platinum	1002946	Dermal, rinse off	Face mask	0.0001
P1	Platinum	1002990	Dermal, leave on	Face care products other than face mask	0.00004
P1	Platinum	1002991	Dermal, leave on	Face care products other than face mask	0.00002
P1	Platinum	1003132	Dermal, leave on	Face care products other than face mask	0.00002
P1	Platinum	1003464	Dermal, leave on	Face care products other than face mask	0.00002
P1	Platinum	1003465	Dermal, leave on	Face care products other than face mask	0.00004

Opinion on Platinum (nano)

P1	Platinum	1003541	Dermal, leave on	Face care products other than face mask	0.00001
P2	Platinum	1003414	Dermal, leave on	Face mask	0.1
P3	Colloidal platinum	1003374	Dermal, leave on	Other skin care products	0.002
P4	Acetyl tetrapeptide-17 (and) colloidal platinum	1001866	Dermal, leave on	Face mask	0.00005*

In response to the SCCS query, the Notifiers confirmed that the 'use concentration' is the concentration of the nanomaterials in the final cosmetic product. Considering this information, the platinum nanomaterials are used in a variety of products with the highest concentration in face products at 0.1%, and for other skin care products at 0.002%.

3.4 TOXICOLOGICAL EVALUATION

3.4.1 Acute toxicity

In the first round of data submission, no information on acute toxicity of either of the platinum dispersions has been provided. However, in response to the Call for data by the European Commission's Directorate General for Health and Food Safety (from 11 June 2019 to 10 November 2019), the Notifier of aXonnite Platinum (P1) provided an additional document with a statement on the oral as well as the dermal acute toxicity of P1.

3.4.1.1 Acute oral toxicity

P1: Lowest oral toxicity dose is 9100 mg/kg

Ref.59

3.4.1.2 Acute dermal toxicity

P1: Lowest toxicity of dermal implants is 23 g/kg

Ref.59

3.4.1.3 Acute inhalation toxicity

P1: Respiratory: the product does not cause respiratory disorders.

Ref. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36

General SCCS comment on acute toxicity

The provided information only consists of statements on toxicity, and detailed information on acute toxicity has not been provided for any of the platinum dispersions. Full study reports

Opinion on Platinum (nano)

need to be provided for assessment of the quality and relevance of the studies for use in safety assessment.

3.4.2 Irritation and corrosivity

P1:

Skin contamination: the product does not cause skin irritation.

Eye contamination: the product does not cause eye irritation.

Irritating effect.

Does not irritate eyes.

Does not induce skin irritation (rabbit).

Ref. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36

For P2 and P3, no data on irritation and corrosivity have been provided

P4:

The product as manufactured was non irritant according to HRIPT (see 3.4.9) and non ocular irritant in HET-CAM, where product pure as manufactured scored 0.

Ref. 50

SCCS comment

Although some statements were provided on irritation and corrosivity of aXonnite platinum (P1), original study reports were not provided. Information on material characterisation for the materials used in the studies was also not provided. The information provided on aXonnite platinum (P1) is not supported by experimental data.

Information on irritation and corrosivity has not been provided for P2 and P3. This information should be provided.

The HET-CAM study mentioned in the dossier has not been reported in any detail in the Annexes of the dossier (on P4). In addition, a HET-CAM test can be used for screening strong irritants, and as supportive information when used together with another test. The HET-CAM test is not an officially accepted OECD test guideline and it is unclear whether it can be used for nanomaterials. Data of a HET-CAM test alone are not sufficient to draw conclusions on eye-irritation.

3.4.3 Skin sensitisation

P1: Does not induce skin sensitization (guinea pig, OECD 406).

Ref. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36

For P2, information on skin sensitization has not been provided

P3: Dermatological allergy tests came negative (does not cause allergies except for a specific allergy to silver)

Ref. 41

P4: The product as manufactured was tested under occlusion with HRIPT (Human Repeated Insult Patch Test). Product was a non-primary irritant and a non-primary sensitizer to the skin.

Ref. 50

SCCS comment

Adequate information on skin sensitization has not been provided. Although some statements were given on skin sensitization of P1 and P3, original study reports were not provided. Information on material characterisation for the materials used in the studies was only provided for P4, and not for P1-P3. The statements provided are not supported by experimental data. For P2, information has not been provided at all. This information, including original study reports, should be provided. For P4, a HRIPT report is available: see Human data (section 3.4.9).

3.4.4 Repeated dose toxicity

Data on the repeated dose toxicity have not been provided for any of the materials included in this dossier.

3.4.5 Mutagenicity/genotoxicity

3.4.5.1 Mutagenicity/genotoxicity *in vitro*

According to the Notifier, P1 does not induce germ cell mutagenicity.

For P2 and P3, data on mutagenicity/ genotoxicity have not been provided.

P4:

Genotoxicity was evaluated by Micronucleus Test following OECD/OCDE Guidelines for testing of chemicals n° 487. The product as manufactured was not mutagenic at any concentration assayed in presence of metabolic activation, neither without metabolic activation (See full report below).

Micronucleus test *in vitro*

Results on a micronucleus test *in vitro* have been provided for colloidal platinum dispersion Platinum Matrix EM (NP0003, batch 50804031)(P4). The results as reported by the Notifier are presented below.

Materials and methods

Cytotoxicity assay in human lymphocytes

Lymphocytes isolation has been performed through Ficoll-PlaqueTM plus gradient centrifugation. Total isolated mononuclear cells were resuspended in DMEM culture medium with 10% FCS (endotoxin free) and were seeded (2.28×10^6 cells/cm²) in FALCON 75 cm² plastic bottles. These bottles were previously coated for three hours with Type B gelatin (2% solution, endotoxin free). Cells were incubated at 37°C for 20 minutes to allow adhesion of mononucleated cells. After this process, they were collected and cells present in these supernatants were seeded again in a gelatin-coated T75 plastic bottle (2.28×10^6 cells/cm²). Cells were incubated at 37°C for 30 minutes to allow adhesion of mononucleated cells. Supernatants containing lymphocytes were collected together, counted and plated in 24-well assay plates at 100,000 cells/well with complete RPMI 1640 medium (10% FCS, 1% glutamine, 1% penicillin/streptomycin, 50 µg/mL gentamicin and 10 nM Hepes). After 24 hours, lymphocyte proliferation was stimulated by 1 mg/mL phytohemagglutinin (PHA) for 24 hours at 37°C.

In order to set up experimental concentrations for a micronucleus test (MNT), a maximum acceptable toxic concentration (MATC) of the compound was tested in isolated human

Opinion on Platinum (nano)

lymphocytes. Lymphocytes were seeded in 24-well plates (100,000 cells/well). A range of different concentrations of test compound was applied and incubated for 6 hours. After incubation, WST-1 was added to reveal the cytotoxicity effect.

Cytotoxicity assay results allowed setting up concentrations for MNT.

CHO-K1 culture and treatment

CHO-K1 cells were seeded in 24-well plates (250,000 cell/well) with Ham's F-12 medium (10% FCS). After 24 hours, cells were treated for 6 hours with different concentrations of the compounds and positive (20 µg/mL cyclophosphamide, 1 µg/mL mitomycin C) or negative (free medium) controls of MNT assay (with and without S9 microsomal fraction). After incubation, cells were washed with PBS buffer and incubated with 5 µg/mL of cytochalasin B for 36 hours.

Micronucleus Test (MNT)

Samples were centrifuged at 1200 rpm for 5 minutes. Supernatant was discarded and pellet was resuspended in hypotonic medium (0.075M KCl) and incubated at 37°C for 10 minutes. Then, samples were centrifuged at 800 rpm for 5 minutes. Supernatant was discarded and two cell extensions for every sample were performed on microscope slides. Samples were dyed with 10 µg/mL acridine orange, a nucleic acid selective fluorescent cationic dye. Samples were quantified for MN with a fluorescence microscope. For each slide, 1000 cells were counted, two slides for each sample. Cells were classified between mononucleated, binucleated and multinucleated. MN were counted in a total of 2000 binucleated cells to test genotoxicity, following OECD Guideline (draft) 487. Criteria followed to select cells and MN were as described in Fenech 2000 and Bonassi *et al.*, 2000).

OECD Guideline (draft) 487 was followed to calculate CBPI (cytokinesis-block proliferation index) and micronucleus.

Results

Cytotoxicity assay in human lymphocytes

Within the range of concentrations tested, Platinum Matrix EM (NP0003) did not present cytotoxicity in human lymphocytes. It presented values at 450 nm similar to the ones of negative control (CTRL) and far from those of positive control (SDS 0.02%).

Micronucleus Test (MNT) of the compound in CHO-K1 cells

After a first experiment performed in lymphocytes we observed a very low number of binucleated cells and micronucleus in culture. Furthermore, this experiment finished on May 15th, 2008 did not follow the acceptance criteria established in the guideline. For these reasons, the initial study Director decided in agreement with the Sponsor a modification of the protocol.

In this case the micronucleus test was performed using the CHO-K1 cells as a cellular model to test genotoxicity. This model is also accepted by OECD Guideline (draft) 487.

We tested different concentrations of Platinum Matrix EM (NP0003) that were set up from cytotoxicity results obtained in lymphocytes. For MNT in CHO-K1 cells, the OECD Guideline (draft) 487 was followed.

CHO-K1 cells were incubated for 6 hours with three different concentrations of Platinum Matrix EM (NP0003) (1, 0,5, 0,25%). Incubations were performed with and without S9 microsomal fraction (metabolic competence).

Results for Platinum Matrix EM (NP0003) are shown in Tables below.

Opinion on Platinum (nano)

-S9	Negative		M02508	M02508	M02508	
	Control	MMC	CPh	1%	0,50%	0,25%
CBPI	1,86	1,44	1,84	1,75	1,79	1,82
MN	66	243	71	71	61	57
B	2000	1518	2137	1958	2052	2078
MN (%)	33	160	33	36	30	27

Table 3.- Results obtained for Platinum Matrix EM (NP0003) compound without S9 microsomal fraction. CBPI: Cytokinesis-Blocked Proliferation Index; MN: Micronucleus; B: number of binucleatedcells analysed. Negative control: free medium-treated cells;MMC: Mitomycin C-treated cells and CPh: Cyclophosphamide-treated cells, positive controls; M02508: Platinum Matrix EM (NP0003)-treated cells

+S9	Negative		M02508	M02508	M02508
	Control	CPh	1%	0,50%	0,25%
CBPI	1,70	1,63	1,61	1,65	1,62
MN	105	154	70	66	62
B	2295	2423	2184	2148	2005
MN (%)	46	64	32	31	31

Table 4: Results obtained for Platinum Matrix EM (NP0003) compound with S9 microsomal fraction. CBPI: Cytokinesis-Blocked Proliferation Index; MN: Micronucleus; B: number of binucleatedcells analysed. Negative control: free medium-treated cells; CPh: Cyclophosphamide-treated cells, positive control; M02508: Platinum Matrix EM (NP0003)-treated cells

In order to count MN, CBPI index must be around 2, indicating that a representative number of binucleated cells have been counted for the results to be significant. We can observe that all controls, negative and positive have a CBPI around 2, meaning that experiments have been performed correctly. Moreover, results of controls were as expected. Mitomycin C, as positive control, presented a high number of MN without S9 fraction, and Cyclophosphamide was only positive (ie: high number of MN) in presence of S9 fraction because it requires metabolic activation to be genotoxic.

Platinum Matrix EM (NP0003) compound presented a CBPI around 2 for all tested concentrations (1, 0.5, 0.25%) and MN determination was similar to negative control. Similar results were obtained in presence of S9 fraction, thus indicating that Platinum Matrix EM (NP0003) was not genotoxic at these concentrations.

Conclusions by the Notifier

Under the conditions used in this study, performed according to OECD/OCDE Guideline for testing of chemicals n° 487 (*In vitro* micronucleus test), Platinum Matrix EM (NP0003) compound was not found mutagenic at any concentration assayed in presence of metabolic activation. Without metabolic activation Platinum Matrix EM (NP0003) neither was found mutagenic.

Ref. 51

SCCS comments

- There is a considerable discrepancy between preliminary cytotoxicity assessment, which was performed on human lymphocytes and the main MN test that was performed on CHO-K1 cells. Interpretation of the results is difficult, considering the different properties of both cell types, and additionally taking into account the 6 h exposure time

Opinion on Platinum (nano)

- of the lymphocytes. Preliminary cytotoxicity study should be performed on CHO cells to determine the cytotoxicity range as required by OECD TG 487.
2. As evidenced in Table 4 of the Notifier's report, in control cells (apparently cultured only in the presence of culture medium) a high MN frequency was observed (46%) which exceeded the values for the test substance by over 50%. This indicates that the cell test system was of limited reliability.
 3. The data are presented in the form of mean values only without providing detailed information for all individual cultures.
 4. No historical data have been provided to verify the test system response in the presented report.
 5. No data have been provided on the stability of the nanoparticle suspensions in culture media (especially with S9-mix) during the exposure period.
 6. No internalization of the nanoparticles by the cells was investigated, hence there is no appropriate evidence of cell exposure.
 7. The concentrations of HEPES (10 nM) or phytohemagglutinin (1 mg/mL) used in the cell cultures were rather unusual.

Cytotoxicity and micronucleus test

Other additional information is provided for colloidal platinum dispersion aXonnite Platinum (Pt), Sample code: NI - 0778 -17. Both a cytotoxicity test *in vitro* (agar diffusion) and a genotoxicity test (micronucleus test) have been performed (Table 11).

Table 11: Design and summary of the results of the cytotoxicity and genotoxicity test for colloidal platinum dispersion aXonnite Platinum (Pt), Sample code: NI - 0778 -17:

No.	Parameter	Test method	Requirement	Result
1.*	Cytotoxicity <i>in vitro</i>	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

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 12.

Opinion on Platinum (nano)

Table 12: Results of the *in vitro* micronucleus test (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)	3,27% ± 0,54% (NO)

Test without metabolic activation, long-term		
Cell culture control	Positive control	Tested sample
2,92% ± 1,18%	67,80% ± 7,22% (YES)	3,68% ± 2,35% (NO)

Test with metabolic activation S9, short-term		
Cell culture control	Positive control	Tested sample
2,27% ± 0,99%	14,38% ± 1,45% (YES)	2,04% ± 0,36% (NO)

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

Ref. 52

SCCS comments

The information provided in the additional study on cytotoxicity and genotoxicity is not acceptable and not sufficient. The results of the whole study (on both cytotoxicity and genotoxicity testing) are not reliable for the following reasons:

SCCS comments on the cytotoxicity study on aXonnite Platinum (P1):

- 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 first page of the Notifier's report there is information indicating that the concentration of 100 ppm was tested while on the second 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 platinum nanomaterials may vary and can be below 100 µg/mL, depending on cell types and particle sizes (Hashimoto *et al.*, 2016, Konieczny *et al.*, 2013, Sahin *et al.*, 2018, Almeer *et al.*, 2018, Jeyaraj *et al.*, 2019).

Ref. 53, 54, 55, 56, 57

- No information on control substances used is given, neither positive nor negative.
- No data are provided on the stability of the silver 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 platinum nanomaterials. 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. 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

Opinion on Platinum (nano)

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 platinum dispersions in nano form.

SCCS comments on the genotoxicity study on aXonnite Platinum (P1):

1. It is not clear to the SCCS why an ISO guideline for testing medical devices was followed, while cosmetic ingredients should be tested using OECD TG test guidelines or EU methods (see SCCS 1611/19)
2. L929 fibroblasts are not suggested in OECD TG 487: the choice of the cell line was not justified
3. No data on positive control substances were given (concentrations, vehicles, etc.)
4. No data have been provided which are necessary to demonstrate that the cells in culture have divided, so that a substantial proportion of the cells scored have 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.
5. 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.

Overall SCCS comment on mutagenicity/genotoxicity

The SCCS is of the opinion that mutagenicity/genotoxicity data on platinum nanomaterials provided by the Notifiers are not sufficient to exclude potential mutagenic/genotoxic effects. The provided studies were not performed or reported according to GLP.

Only results on chromosomal aberrations have been provided for P4 and these alone are not sufficient to exclude mutagenicity/genotoxicity. According to the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS 1611/19), data on gene mutation in mammalian cells and uptake by cells are additionally required.

Mutagenicity/genotoxicity data from *in vitro* studies would be required for all colloidal platinum dispersions as part of the base set requirements for assessment of safety of cosmetic products (including proper material characterisation). Any existing data from *in vivo* studies (conducted prior to 11 March 2013) with relevant, well-characterised materials should also be provided.

3.4.6 Carcinogenicity

According to the Notifier, P1 is not carcinogenic.

For P2 and P3, data on carcinogenicity have not been provided.

P4:

In cases where a considerable oral intake is expected, or when the data on dermal/percutaneous absorption indicate a considerable penetration of the ingredients through the skin (taking into account the toxicological profile of the substance and chemical structure), further toxicological investigations may become necessary, together with specific additional genotoxicity, and/or mutagenicity data.

This does not apply as demonstrated by nonexistence of dermal absorption.

SCCS comment

Only statements are given without supporting original study reports with experimental data. The absence of dermal absorption of platinum P4 dispersion has not been demonstrated. As described in the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19), information on carcinogenicity is required if significant systemic exposure or genotoxicity cannot be excluded. The SCCS notes that information has not been provided to exclude systemic availability via the relevant uptake route(s), or genotoxicity, to allow discounting the need for information on carcinogenicity.

3.4.7 Reproductive toxicity

According to the Notifier, P1 does not induce reproductive toxicity.

For P2 and P3, data on reproductive toxicity have not been provided.

P4:

In cases where a considerable oral intake is expected, or when the data on dermal/ percutaneous absorption indicate a considerable penetration of the ingredients through the skin (taking into account the toxicological profile of the substance and chemical structure), further toxicological investigations may become necessary, together with specific additional genotoxicity, and/or mutagenicity data.

This does not apply as demonstrate by nonexistence of dermal absorption.

SCCS comment

Only statements are given without supporting original study reports with experimental data. The absence of dermal absorption of platinum P4 dispersion has not been demonstrated. As described in the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19), information on reproductive toxicity is required if systemic exposure cannot be excluded. The SCCS notes that information that would have made it possible to draw conclusions on reproductive toxicity has not been provided, namely information on excluding systemic availability via the relevant uptake route(s).

3.4.8 Photo-induced toxicity

For P1-P3, information on photo-induced toxicity has not been provided.

P4:

No photo reactivity is expected due to the chemical nature of the material; there is no presence of functional groups susceptible of photo-chemical transformation.

SCCS comment

Platinum nanoparticles have a reactive surface, absorb UV (216 and 264 nm), and have (photo) catalytic activity. Therefore, information on photo-induced toxicity (and at least UV extinction spectra, see Notes of Guidance, SCCS/1602/18) should be provided for all of the notified nanomaterials that are used in cosmetic products intended for use on sun-exposed skin.

3.4.9 Human data

P4:

Human subject repeat insult patch test skin irritation/ sensitization evaluation (Occlusive Patch)

Opinion on Platinum (nano)

Method: HRIPT - Human Repeat Insult Patch Test study
Subjects: 50 humans (33 women, 17 men)
Test substance: Acetyl tertapeptide-17 (and) Colloidal platinum
Batch: Lot NP0003, batch C1010223
Concentrations: 'As is', 0004–0006% in water with 0.4-0.6% phenoxyethanol
Route: topical on the back, under occlusive patch (Webril) 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: 2010
Published: No

Results and conclusion according to the notification:

According to the submitted table with individual responses, no adverse reactions of any kind were noted during the induction or the challenge phase of this study.

HRIP demonstrated good tolerability of the product on 50 volunteers. The test material (AMA Laboratory No.:M3008: Client NO: INF, Platinum Matrixem, Lot # NP0003, batch # C1010223, when tested under occlusion as described herein, may be considered as a NON-PRIMARY IRRITANT and NON-PRIMARY SENSITIZER to the skin according to the reference.

SCCS comment

While skin sensitisation to metallic platinum (probably from released ions) is extremely rare, if at all, the limited information from the submitted study does not indicate a sensitising potential of the test article. However, the sample size for a completely negative test among 50 participants is considered too small to yield an acceptable confidence limit.

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

Ref. 50

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

Safety evaluation was not possible for any of the notified materials because of the lack of data on dermal penetration, adequate information to allow derivation of a toxicological point of departure (PoD) and lack of evidence to exclude genotoxicity/ mutagenicity.

3.6 DISCUSSION

The information provided by the Notifiers is not sufficient to allow a safety evaluation of the 12 notified platinum nanomaterials. At request of SCCS, a number of additional files have been provided, but additional information was still not sufficient for adequate risk assessment.

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 a literature search performed by the Commission was considered by the SCCS. However, it was not possible to relate the toxicological data obtained from this information with the types of the 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 nano platinum materials included in this Opinion.

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

Opinion on Platinum (nano)

- Data relevant to the notified platinum nanomaterials in regard to 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 notified platinum nanomaterials for the relevant uptake route(s).
- Data relevant to the notified nanomaterials regarding toxicity and the methodology used for acute toxicity, irritation/sensitisation, and mutagenicity/genotoxicity. This should be supplemented with reproductive toxicity and carcinogenicity if a significant systemic exposure is indicated.

In the absence of sufficient data to allow safety assessment, the SCCS has considered the different aspects of Pt nanomaterials that could raise a concern over consumer safety. 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 A (Platinum and Colloidal Platinum) and B (Acetyl tetrapeptide-17 (and) Colloidal Platinum) 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?*

Having considered all the information provided by the Notifiers, and that obtained from other sources, the SCCS is of the opinion that it is not possible to carry out a safety assessment of any of the notified platinum nanomaterials due to limited or missing essential information. The limited amount of data provided by the Notifiers also does not correspond to the requirements and data standards as indicated in the SCCS Guidance (SCCS 1611/19), and the SCCS Memorandum (SCCS/1524/13).

To enable safety assessment by the SCCS, the Notifiers need to provide the necessary information, a summary of which is provided in Annex I.

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

In the absence of sufficient data to allow safety assessment, the SCCS has considered the different aspects of platinum nanomaterials 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 platinum, colloidal platinum, and acetyl tetrapeptide-17 colloidal platinum (nano) in cosmetic products can pose a risk to the consumer. The SCCS will be ready to assess any evidence provided to support safe use of the materials in cosmetic products.

5. MINORITY OPINION

None.

6. REFERENCES

1. Notification_1002908.pdf
2. Notification_1002908_69005_safety_file_2017-5-10-9-58-50.pdf
3. Notification_1002908_69005_spec_file_2017-5-10-9-58-50 (1).pdf
4. Notification_1002908_69005_tox_profile_file_2017-5-10-9-58-50 (1).pdf
5. Notification_1003132.pdf
6. Notification_1003132_69005_safety_file_2017-5-10-9-58-50.pdf
7. Notification_1003132_69005_spec_file_2017-5-10-9-58-50.pdf
8. Notification_1003132_69005_tox_profile_file_2017-5-10-9-58-50.pdf
9. Notification_1002945.pdf
10. Notification_1002945_112784_safety_file_2017-5-31-7-51-9.pdf
11. Notification_1002945_112784_spec_file_2017-5-31-7-51-9.pdf
12. Notification_1002945_112784_tox_profile_file_2017-5-31-7-51-9.pdf
13. Notification_1002946.pdf
14. Notification_1002946_112784_safety_file_2017-5-31-7-51-9.pdf
15. Notification_1002946_112784_spec_file_2017-5-31-7-51-9.pdf
16. Notification_1002946_112784_tox_profile_file_2017-5-31-7-51-9.pdf
17. Notification_1003541.pdf
18. Notification_1003541_112784_safety_file_2017-5-31-7-51-9.pdf
19. Notification_1003541_112784_spec_file_2017-5-31-7-51-9.pdf
20. Notification_1003541_112784_tox_profile_file_2017-5-31-7-51-9.pdf
21. Notification_1002990.pdf
22. Notification_1002990_99860_safety_file_2017-7-28-9-38-49.pdf
23. Notification_1002990_99860_spec_file_2017-7-28-9-38-49.pdf
24. Notification_1002990_99860_tox_profile_file_2017-7-28-9-38-49.pdf
25. Notification_1002991.pdf
26. Notification_1002991_99860_safety_file_2017-7-28-9-38-49.pdf
27. Notification_1002991_99860_spec_file_2017-7-28-9-38-49.pdf
28. Notification_1002991_99860_tox_profile_file_2017-7-28-9-38-49.pdf
29. Notification_1003464.pdf
30. Notification_1003464_126191_safety_file_2018-12-30-14-29-11 (2).pdf
31. Notification_1003464_126191_spec_file_2018-12-30-14-29-11 (2).pdf
32. Notification_1003464_126191_tox_profile_file_2018-12-30-14-29-11 (2).pdf
33. Notification_1003465.pdf
34. Notification_1003465_126191_safety_file_2018-12-30-14-29-11.pdf
35. Notification_1003465_126191_spec_file_2018-12-30-14-29-11.pdf
36. Notification_1003465_126191_tox_profile_file_2018-12-30-14-29-11.pdf
37. Notification_1003414.pdf
38. Notification_1003414_72372_safety_file_2018-11-14-10-27-29.pdf
39. Notification_1003414_72372_spec_file_2018-11-14-10-27-29.pdf
40. Notification_1003414_72372_tox_profile_file_2018-11-14-10-27-29.pdf
41. Notification_1003374.pdf
42. Notification_1003374_86357_safety_file_2018-10-17-14-19-59.pdf
43. Notification_1003374_86357_spec_file_2018-10-17-14-19-59.pdf
44. Notification_1003374_86357_tox_profile_file_2018-10-17-14-19-59.pdf
45. Notification_1003374_86357_other_file_2018-10-17-14-19-59.pdf* (not accessible)
46. Notification_1001866.pdf
47. Notification_1001866_42328_safety_file_2015-3-5-15-8-57.pdf
48. Notification_1001866_42328_spec_file_2015-3-5-15-8-57.pdf
49. Notification_1001866_42328_tox_profile_file_2015-3-5-15-8-57.pdf
50. Notification_1001866_42328_exposure_file_2015-3-5-15-8-57.pdf
51. Advanced *in vitro* cell technologies, S.L. (ADVANCELL), Parc Científic de Barcelona, CT770 – Final Draft Report, July 29, 2008
52. National Medicines Institute, Official Medicines Control Laboratory, Department Of Biochemistry And Biopharmaceuticals, Test Report: NI - 0778 – 17, 23.08.2017
53. Almeer RS, Ali D, Alarifi S, Alkahtani S, Almansour M. *Green platinum nanoparticles interaction with HEK293 cells: cellular toxicity, apoptosis, and genetic damage.* Dose

Opinion on Platinum (nano)

- Response 2018; 16(4), 1559325818807382.
doi: 10.1177/1559325818807382.
54. Hashimoto M, Kawai K, Kawakami H, Imazato S. *Matrix metalloproteases inhibition and biocompatibility of gold and platinum nanoparticles*. J. Biomed. Mater. Res. Part A 2016; 104(1), 209–217.
doi: 10.1002/jbm.a.35557
55. Jeyaraj M, Gurunathan S, Qasim M, Kang MH, Kim JH. A *Comprehensive review on the synthesis, characterization, and biomedical application of platinum nanoparticles*. Nanomaterials (Basel). 2019 Dec 2;9(12) : 1719.
doi: 10.3390/nano9121719.
56. Konieczny P, Anna GG, Skalniak L, Koziel J, Francesca LF, Crosera M, Cierniak A, Zuba S, Borowczyk J, Laczna E, Drukala J., Pyza E., Semik D., Woznicka O., Klein A., Jura J. *Effects triggered by platinum nanoparticles on primary keratinocytes*. Int. J. Nanomed. 2013; 8, 3963–3975.
doi: 10.2147/IJN.S49612
57. Sahin B, Aygün A, Gündüz H, Sahin K, Demir E, Akocak S, Sen F. *Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 Cell Line*. Colloids Surf. B Biointerfaces 2018; 163, 119–124.
doi: 10.1016/j.colsurfb.2017.12.042
58. Report on dermal absorption of aXonnite Platinum produced by NanoTech ordered by NanoTech on 2016.04.21 (Warsaw, 2016.09.20)
59. Overview report ACC chemicals (Notifier P1) on Colloidal platinum (nano)/ platinum (nano)

7. 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 A1. Summary of material characterisation data on platinum (nano), colloidal platinum (nano) and Acetyl tetrapeptide-17 Colloidal Platinum (nano) as provided by Notifiers in this Opinion.

Information required^a	aXonnite platinum	APS-WM100	/	Platinum matrixEM (PF)
Chemical identity	Y	Y	Y	Y
Chemical composition	Y	Y	N	Y
Particle size ^b	Y	Y	Y	Y
Morphology	Y/N	Y/N	N	Y
Surface characteristics	N	N	N	Y
Solubility	Y	Y	Y	Y
Surface area	Y	N	Y	Y
Catalytic activity	N	N	N	Y/N
Concentration ^c	Y	Y	N	Y
Dustiness ^c				
Density and pour density ^d	Y	N	N	Y
Redox potential	N	N	N	Y
pH ^e	Y	N	N	Y
Viscosity ^f	Y	N	N	Y
Stability	Y/N	N	N	Y/N
UV absorption	N	N	N	N
Other	N	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.

Opinion on Platinum (nano)

Table A2. Summary of toxicological information on platinum (nano), colloidal platinum (nano) and Acetyl tetrapeptide-17 Colloidal Platinum (nano) as provided by Notifiers in this Opinion.

Information required ^a	aXonnite platinum	APS-WM100	/	Platinum matrixEM (PF)
Likelihood and extent of internal exposure via skin, lung or oral route considering use type	N	N	N	N
Dermal absorption for dermally-applied products	Y	N	N	Y
Biokinetic behaviour, aggregation/agglomeration considered during tests?	N	N	N	N
Acute toxicity	Y	N	N	N
Irritation and corrosivity	Y	N	N	Y
Skin sensitisation	Y	N	Y/N	Y
Mutagenic/genotoxicity ^a	Y/N	N	N	Y
Repeated dose toxicity	N	N	N	N
Phototoxicity – for products intended for use in sunlight exposed skin	N	N	N	N
Human data	N	N	N	Y
Reproductive toxicity ^b	Y/N	N	N	Y/N
Carcinogenicity ^c	Y/N	N	N	Y/N
Other relevant information	N	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 nuclear 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.

Opinion on Platinum (nano)

Table A3. Comparison of exposure information for platinum (nano), colloidal platinum (nano) and Acetyl tetrapeptide-17 Colloidal Platinum (nano) 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	aXonnite platinum	APS-WM100	/	Platinum matrixEM (PF)
Category of cosmetic products in which the ingredient is intended for use	Y	Y	Y	Y
Category of the ingredient in the finished cosmetic product	Y	Y	Y	Y
Quantity of the product used in each application	Y	Y	Y	Y
Frequency of use	N	N	N	N
Total area of skin contact	N	N	N	N
Duration of exposure	N	N	N	N
Foreseeable misuse which may increase exposure	N	N	N	N
Consumer target groups (e.g. children, people with sensitive, damaged or comprised skin) where specifically required	N	N	N	N
Quantity likely to enter the body (fraction absorbed) for each target group	N	N	N	N
Estimated dermal exposure based on the intended use of the product	N	N	N	N
Estimated oral exposure based on the intended use of the product	N	N	N	N
Estimated inhalation exposure based on the intended use of the product	N	N	N	N
Exposure calculation for each target group	N	N	N	N
Other relevant information	N	N	N	N

Opinion on Platinum (nano)

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).

8. ANNEX II

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

As the information and data provided by the Notifiers were insufficient to perform a safety assessment, additional information on platinum nanomaterials was obtained from the published literature.

From the current evaluation, and the relevant information from published literature, the SCCS has concluded that there is a basis for concern that the use of platinum, colloidal platinum, and acetyl tetrapeptide-17 colloidal platinum (nano) in cosmetic products can pose a risk to the consumer because of the following considerations:

Physicochemical aspects

1. Platinum, colloidal platinum and Acetyl tetrapeptide-17 Colloidal Platinum is comprised of particles that are in the nanoscale. The size varies between 1.5 and 100 nm for the various forms of Pt nanoparticles (Table 3 of the current Opinion). In the literature (as reviewed in Czubacka and Czerczak (2019)), sizes of even below 1 nm (sub-nanometer scale) have been reported.
2. Platinum is an insoluble and persistent material. In the files provided by the Notifiers, the solubility/ dissolution is reported as below 0.01 mg/l.
3. Platinum metal (as nanomaterial) is generally well known for its ability of catalysing partial oxidation, hydrogenation (Cheng *et al.*, 2009) and dehydrogenation (Xu *et al.*, 2008)(due to a large surface area), which is crucial for many industrial processes (Gopal *et al.*, 2013), and it exhibits some specific properties such as a large surface area and good resistance to corrosion and chemical attacks (Sahin *et al.*, 2018). Therefore, any systemically available Pt nanomaterial may induce surface-catalysed oxidative reactions under biological conditions inside the cells, which may lead to harmful effects.

Toxicological aspects

Genotoxicity/ mutagenicity:

In vitro studies have demonstrated that platinum nanoparticles (PtNPs) can induce *DNA damage and apoptosis*, and could be used as anticancer agents. However, PtNPs did not cause significant cytotoxicity. Pelka *et al.* (2009) demonstrated that PtNPs caused *DNA strand breaks* in human colon carcinoma cells (HT29). Asharani *et al.* (2010) suggested that p53 activation in Pt-NP-treated cells is due to genotoxic stress, with the subsequent activation of p21 leading to a proliferating cell nuclear antigen-mediated growth arrest and apoptosis. Gehrke *et al.* (2011) described the DNA-damaging properties of PtNPs as related to the association of Pt with DNA. DNA strand breaks mediated by metallic PtNPs were caused by the platinum ions forming during the incubation of cells. The study was carried out on human colon carcinoma cells. Lebedová *et al.* (2018) showed that 50 nm platinum nanoparticles caused a small increase in DNA damage in comparison to the 5 nm-sized ones. Nejdl *et al.* (2017) reported inhibition of the activity of Taq DNA polymerase and DNA structural damage, and concluded that this effect, together with the transition of PtNPs into platinum ions (Pt^{2+}), caused *mutagenicity* and an increased DNA damage in comparison to cisplatin. Cytotoxicity of PtNPs may increase by the encapsulation in liposomes.

General toxicity:

In several studies the toxic effects of Pt-NPs have been reported (Table A4).

Table A4: Summary of toxicity studies with Pt nanomaterials

Reference	NP	Size (nm)	Route/exposure/animal	Dose	Toxicity
Adeyemi <i>et al.</i> , 2016	Pt	1-6	Oral gavage for 5 days in Wistar rats	9.75 mg/kg/day	Altered gene expression associated with inflammation in stomach tissue
			Oral gavage for 30 days in Wistar rats	10, 50, and 50 mg/kg	Lower organ weights Morphological lesions including inflammation in heart, kidney and liver indicating translocation from GI tract to other organs Cellular degeneration
Adeyemi <i>et al.</i> , 2018	Pt	not mentioned	Oral gavage for 7 days in Wistar rats	25 and 50 mg/kg	Oxidative stress in plasma
Park <i>et al.</i> 2010a	Pt	21	Intratracheal instillation in ICR mice	1 mg/kg	Lung inflammation
Yamagishi <i>et al.</i> , 2013a;b;	Pt	<1 and <8	Single IV in BALB/c mice	5-20 mg/kg	Accumulation of PtNP's in heart, liver and spleen Nephrotoxicity Hepatotoxicity

Opinion on Platinum (nano)

			IP (twice a week, 4 weeks) in C57BL/6 mice	10 mg/kg	Nephrotoxicity
Katsumi <i>et al.</i> , 2014;	Pt	30 and 106	IV	50 µg/ kg	Hepatotoxicity
Brown <i>et al.</i> , 2018	Pt	70	IV	10 mg/kg	Nephrotoxicity Hepatotoxicity
Park <i>et al.</i> 2010b	Pt	20	Single oral dose in ICR mice	0.25, 0.5 and 1 mg/kg during premating, gestation and lactation periods	Increase in offspring mortality, decreased infant growth

In summary (as mentioned in Table A4), there is evidence in published studies that PtNPs can cause *inflammatory effects and oxidative stress* after oral and intratracheal exposure in test animals. In addition, PtNPs have been reported to cause morphological lesions, including inflammation and cellular degeneration as well as oxidative stress in plasma. *Hepatotoxicity and nephrotoxicity* has been reported after intravenous injection of PtNPs. *Nephrotoxicity* was also observed after intraperitoneal administration of PtNP's. Also indications for developmental effects have been reported after oral exposure to PtNP's.

In vitro toxicity studies. PtNPs (polyvinylpyrrolidone-coated platinum nanoparticles of 5.8 and 57 nm) have been reported to trigger toxic effects on primary keratinocytes, decreasing cell metabolism (Konieczny *et al.*, 2013). Human bronchial epithelium (BEAS-2B) and human lung alveolar type II epithelioid cells (A549) were treated with platinum nanoparticles (25 nm, 8.13 mg/m³) and after 24 h, a loss of viability was observed in A549 cells (Kachi *et al.*, 2011). A remarkable increase in IL-8 due to exposure in both cell lines has been observed, which was dependent on the time of exposure which, in turn, was related to the deposited mass (Diabaté *et al.*, 2009). High doses of PtNPs (70 nm, citrate coated) induced HepG2 cytotoxicity in a HepG2 liver model (Labrador-Rached *et al.*, 2018). Buchtelova *et al.*, (2017) demonstrated small PVP-capped PtNPs (ca. 10 nm, ca. 14 nm, > 20 nm) to be cytotoxic to 3 different types of malignant cell lines (GI-ME-N – neuroblastoma, MDA-MB-231 – breast, LNCaP – prostate).

Exposure aspects

4. The frequency of use of the products containing PtNPs can be relatively high as the uses are intended for a wide range of products. They are also used in many types of technologies and consumer products (cosmetics, supplements, food additives) (Gehrke *et al.*, 2011; Horie *et al.*, 2011), electrocatalysis, data storage systems, new electronic devices, electrochemical biosensors, chemisensors, fluorescent and

Opinion on Platinum (nano)

refractometric sensors (Obreja *et al.*, 2008) but also in medicine, diagnostics and treatment (Elder *et al.*, 2007; Kawasaki *et al.*, 2007). Platinum nanoparticles (PtNPs) have also found a number of biomedical applications, e.g., in diagnostic mediators, medical implants, drug delivery vehicles and photothermal therapy compounds (Johnstone *et al.*, 2016; Wang *et al.*, 2017), as well as in enhanced radiosensitisation (Porcel *et al.*, 2010), detection of cancer cells (Asharani *et al.*, 2010), and to provide bond strength between tooth structures (Hoshika *et al.*, 2010) and bacterio-toxic effects (Gopal *et al.*, 2013).

Dermal penetration

After dermal administration, PtNPs have been shown to *penetrate through the skin barrier*. A study by Mauro *et al.* (2015) showed dermal penetration of PtNPs (5.8 ± 0.9 nm) when applied to the outer surface of the skin (the human abdominal full thickness skin obtained as a surgical waste) for 24 h. Even a minor injury to the skin barrier caused significant increase of the metal penetration.

According to the Notifiers of the current Opinion, the results of the conducted experiment indicates a lack of penetration of PtNPs, applied in the form of aXonnite Platinum, because the amount of nano-platinum in the acceptor chamber did not change after 2, 4, 6, 8, 10 and 24h relative to the background value, i.e. pure water. However, the SCCS considered the way of presentation of the data very confusing, which did not allow interpretation of the results. In the absence of clarification, or new data, the SCCS could only conclude that the provided data showed skin penetration of platinum P4 particles.

Toxicokinetics/ distribution

A short-term biodistribution study by Brown *et al.* (2018) showed that no PtNPs were observed in the plasma after 24 h after intravenous administration of 10 mg Pt/kg bw in mice, but that PtNPs were found in all of the organs investigated (liver, spleen, kidney and lungs). The long-term biodistribution study showed that PtNPs had either been excreted from the system or deposited in tissues/organs. The biggest depositions of the studied nanomaterial was observed in the liver and spleen.

Conclusion

Platinum (Pt) is an insoluble, and persistent material, which in non-nano form is inert and not likely to degrade/ionise under physiological conditions. However, Pt nanoparticles may surface-catalyse oxidative reactions, which under biological conditions may lead to harmful effects.

PtNPs are known to be absorbed by the respiratory and digestive tract routes, and there are indications that PtNPs can also penetrate skin (Mauro *et al.*, 2015; Czubacka and Czerczak, 2019). The available information also suggests that any systemically available PtNPs will likely accumulate in the liver and spleen and may also reach other internal organs, such as lungs, kidneys or heart. Toxicokinetics of platinum nanoparticles is also shown to be strongly dependent on the particle size.

Only few studies regarding platinum nanomaterial toxicity have been conducted but these are not in line with the accepted protocols for toxicological testing (e.g. OECD test guidelines). In particular, there are limited data on toxicological consequences following dermal exposure and inhalation exposure.

Taking together the insoluble, persistent and surface-reactive nature, the potential for systemic exposure to PtNPs due to dermal penetration, and the reported toxicological effects, the use of nano-form of platinum in cosmetic products can raise a concern in regard to consumer safety. The SCCS will be ready to assess any evidence provided to support safe use of the material in cosmetic products.

References:

- Adeyemi OS., Sulaiman F.A., Akanji M.A., Oloyede H.O.B., Sulaiman A.A., Olatunde A. *et al.* (2016). Biochemical and morphological changes in rats exposed to platinum nanoparticles. *Comp Clin Pathol.* 2016;25:855–64. <https://doi.org/10.1007/s00580-016-2274-5>.
- Adeyemi O.S., Olajide I.O., Adeyanju A.A., Awakan O.J., Otohinoyi D.A. (2018). Modulation of rat plasma kynurenine level by platinum nanoparticles and likely association with oxidative stress. *Bioint Res Appl Chem.* 2018;8(4):3364–7.
- Asharani P.V., Xinyi N.G., Hande P.M., Valiyaveettil S. (2010). DNA damage and p53-mediated growth arrest in human cells treated with platinum nanoparticles. *Nanomedicine.* 2010; 5(1):51–64. <https://doi.org/10.2217/nnm.09.85>.
- Brown A.L., Kai M.P., DuRoss A., Sahay G., Sun C. (2018). Biodistribution and toxicity of micellar platinum nanoparticles in mice via intravenous administration. *Nanomaterials.* 2018;8(6):410. <https://doi.org/10.3390/nano8060410>.
- Buchtelova H., Dostalova S., Michalek P., Krizkova S., Strmiska V., Kopel P. *et al.* (2017). Size-related cytotoxicological aspects of polyvinylpyrrolidone-capped platinum nanoparticles. *Food Chem Toxicol.* 2017;105:337–46. <https://doi.org/10.1016/j.fct.2017.04.043>.
- Cheng H., Xi C., Meng X., Hao Y., Yu Y., Zhao F. (2009). Poly-ethylene glycol-stabilized platinum nanoparticels: The efficient and recyclable catalysts for selective hydrogenation of o-chloronitrobenzene to o-chloroaniline. *Journal of Colloid and Interface Science* 2009; 336: 675-678.
- Czubacka E., Czerczak S. (2019). Are platinum nanoparticles safe to human health? *Medycyna Pracy* 2019, 70(4): 487-495. <https://doi.org/10.13075/mp.5893.00847>
- Diabaté S., Weiss C., Mülhopt S., Paur H-R, Niedetzky V., Seipenbusch M. (2009). Biological effects in human lung cells exposed to platinum nanoparticles aerosol. European Aerosol Conference; 2009 Sept 11; Karlsruhe, Germany.
- Elder A., Yang H., Gwiazda R., Teng X., Thurston S., He H. *et al.* (2007). Testing nanomaterials of unknown toxicity: An example based on platinum nanoparticles of different shapes. *Adv Mater. Special Issue: Special Section on Bionanotechnology* 2007;19(20):3124–29. <https://doi.org/10.1002/adma.200701962>.
- Gehrke H., Pelka J., Hartinger C.G., Blank H., Bleimund F., Schneider R. *et al.* (2011). Platinum nanoparticles and their cellular uptake and DNA platinination at non-cytotoxic concentrations. *Arch Toxicol.* 2011;85(7):799–812. <https://doi.org/10.1007/s00204-010-0636-3>.
- Gopal J., Hasan N., Manikandan M., Wu H. (2013). Bacterial toxicity/compatibility of platinum nanospheres, nanocuboids and nanoflowers. *Sci Rep.* 2013;3:1260. <https://doi.org/10.1038/srep01260>.
- Horie M., Kato H., Endoh S., Fujita K., Nishio K., Komaba L.K. *et al.* (2011). Evaluation of cellular influences of platinum nanoparticles by stable medium dispersion. *Metalomics.* 2011;3(11) : 1244–52. <https://doi.org/10.1039/c1mt00060h>.
- Hoshika S., Nagano F., Tanaka T., Ikeda T., Wada T., Asakura K. *et al.* (2010). Effect of application time of colloidal platinum nanoparticles on the microtensile bond strength to dentin. *Dental Mater J.* 2010;29(6):682–9, <https://doi.org/10.4012/dmj.2009-125>.

Opinion on Platinum (nano)

Johnstone T.C., Suntharalingam K., Lippard S.J. (2016). The next generation of platinum drugs: targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs. *Chem Rev.* 2016;116(5): 3436–86. <https://doi.org/10.1021/acs.chemrev.5b00597>.

Kachi H., Noda M., Wataha J.C., Nakaoki Y., Sano H. (2011). Colloidal platinum nanoparticles increase mitochondrial stress induced by resin composite components. *J Biomed Mater Res B App Biomater.* 2011;96(2), 193–8. <https://doi.org/10.1002/jbm.b.31750>.

Katsumi H., Fukui K., Sato K., Maruyama S., Yamashita S., Mizumoto E. et al. (2014). Pharmacokinetics and preventive effects of platinum nanoparticles as reactive oxygen species scavengers on hepatic ischemia/reperfusion injury in mice. *Metalomics.* 2014;6(5):1050–6. <https://doi.org/10.1039/c4mt00018h>.

Kawasaki H., Yonezawa T., Watanabe T., Arakawa R. (2011). Platinum nanoflowers for surface-assisted laser desorption/ionization mass spectrometry of biomolecules. *J Phys Chem C.* 2007;111(44):16279–83. <https://doi.org/10.1021/jp075159d>.

Konieczny P., Goralczyk A.G., Szmyd R., Skalniak L., Koziel J., Larese F.F. et al. (2013). Effects triggered by platinum nanoparticles on primary keratinocytes. *Int J Nanomed.* 2013;8:3963–75. <https://doi.org/10.2147/IJN.S49612>.

Labrador-Rached C.J., Browning R.T., Braydich-Stolle L.K., Comfort K.K. (2018). Toxicological implications of platinum nanoparticle exposure: Stimulation of intracellular stress, inflammatory response and akt signaling *in vitro*. *J Toxicol.* 2018;1367801. <https://doi.org/10.1155/2018/1367801>

Lebedová J., Hedberg Y.S., Wallinder I.O., Karlsson H.L. (2018). Size-dependent genotoxicity of silver, gold and platinum nanoparticles studied using the mini-gel comet assay and micronucleus scoring with flow cytometry. *Mutagenesis.* 2018;33(1):77–85. <https://doi.org/10.1093/mutage/gex027>

Mauro M., Crosera M., Bianco C., Adami G., Montini T., Fornasiero P. et al. (2015). Permeation of platinum and rhodium nanoparticles through intact and damaged human skin. *J Nanopart Res.* 2015;17:253. <https://doi.org/10.1007/s11051-015-3052-z>

Nejdl L., Kudr J., Moullick A., Hegerova D., Ruttkay-Nedecky B., Gumulec J. et al. (2017). Platinum nanoparticles induce damage to DNA and inhibit DNA replication. *PLoS ONE.* 2017;12(7):e0180798. <https://doi.org/10.1371/journal.pone.0180798>.

Obreja L., Pricop D., Foca N., Melnig V. (2008). Alcoholic reduction platinum nanoparticles synthesis by sonochemical method. *Mater. Plast.* 2008;47(1):42–7. <https://www.revmaterialeplastice.ro/Articles.asp?ID=2504>, <https://revmaterialeplastice.ro/pdf/OBREJA%20LAURA%202010.pdf>

Park E-J, Kim H., Kim Y., Park K. (2010a). Intratracheal instillation of platinum nanoparticles may induce inflammatory response in mice. *Arch Pharm Res.* 2010a;33(5):727–35, <https://doi.org/10.1007/s12272-010-0512-y>.

Park E.J., Kim H., Kim Y., Park K. (2010b). Effects of platinum nanoparticles on the postnatal development of mouse pups by maternal exposure. *Environ Health Toxicol.* 2010b;25(4):27986, 279-286. <https://www.eahrt.org/upload/pdf/eht-25-4-279.pdf>

Pelka J., Gehrke H., Esselen M., Türk M., Crone M., Bräse S. et al. (2009). Cellular uptake of platinum nanoparticles in human colon carcinoma cells and their impact on cellular redox systems and DNA integrity. *Chem Res Toxicol.* 2009; 22(4):649–59. <https://doi.org/10.1021/tx800354g>.

Opinion on Platinum (nano)

Porcel E., Liehn S., Remita H., Usami N., Kobayashi K., Furusawa Y. et al. (2010). Platinum nanoparticles: a promising material for future cancer therapy? *Nanotechnology*. 2010;21(8):85103. <https://doi.org/10.1088/0957-4484/21/8/085103>.

Şahin B., Aygün A., Gündüz H., Şahin K., Demir E., Akocak S. et al. (2018). Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 cell line. *Coll Surf B Biointerfaces*. 2018; 163:119–24. <https://doi.org/10.1016/j.colsurfb.2017.12.042>.

Xu J. et al. (2008). Enhanced dehydrogenation of LiBH₄ catalyzed by carbon-supported Pt nanoparticles. *Chem. Commun* 2008; 44: 5740-5742.

Yamagishi Y., Watari A., Hayata Y., Li X., Kondoh M., Tsutsumi Y. (2013a). et al. Hepatotoxicity of sub-nanosized platinum particles in mice. *Pharmazie*. 2013a;68(3):178–82. <https://doi.org/10.1691/ph.2013.2141>.

Yamagishi Y., Watari A., Hayata Y., Li X., Kondoh M., Yoshioka Y. et al. (2013b). Acute and chronic nephrotoxicity of platinum nanoparticles in mice. *Nanoscale Res Lett*. 2013b;8(1):395. <https://doi.org/10.1186/1556-276X-8-395>.

Wang Z., Chen L., Huang C., Huang Y., Jia N. (2017). Albumin-mediated platinum nanocrystals for *in vivo* enhanced computed tomography imaging. *J Mater Chem B*. 2017;5: 3498–510. <https://doi.org/10.1039/C7TB00561J>.