Scientific Committee on Consumer Safety

SCCS

OPINION ON

HAA299 (nano)

The SCCS adopted this document
at its plenary meeting on 26 and 27 October 2021

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of eight weeks after its initial publication. Comments received during this time were considered by the SCCS. For this Opinion, no change occurred and conclusions remain unchanged from the preliminary version.

1. ABSTRACT

The SCCS concludes the following:

1. In light of the data provided, does the SCCS consider HAA299 (nano) safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?

The available data indicate that HAA299 (nano) is a practically insoluble material, with very low dermal and oral absorption. Due to the very low systemic availability, the material is unlikely to exert systemic genotoxic or reproductive effects. The NOAEL of 1000 mg/kg/day indicates that the material is of overall low toxicological concern. Given the low dermal penetration, and low systemic toxicity, the calculation of margin of safety (MoS) is not appropriate in this case. The SCCS considers that HAA299 (nano) as covered within the provided characteristics (minimum purity equal to or above 97%, median particle size in terms of particle number equal to or above 50 nm) is safe when used as a UV-filter in dermally-applied cosmetic products up to a maximum concentration of 10%.

Based on the inflammatory effects on the lung after the acute inhalation exposure, the SCCS has concerns regarding the repeated use of products containing HAA299 (nano) in applications that could lead to inhalation exposure. Therefore, the SCCS does not recommend the use of HAA299 (nano) in applications that could lead to exposure of the consumer's lungs via inhalation.

2. In view of the previous SCCS opinion (SCCS/1533/14) does the SCCS consider HAA299 non-nano and nano form safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?

The data considered in this Opinion has not provided any new or additional concern that merits a revision of the previous SCCS opinion (SCCS/1533/14). Therefore, the SCCS considers HAA299, either as non-nano or nano form, safe when used as a UV-filter in dermally-applied cosmetic products up to a maximum concentration of 10%. The SCCS considers that the combined maximum concentration of non-nano and nano forms of HAA299 should not exceed 10% in a cosmetic product.

3. In case the SCCS finds HAA299 (nano) not safe, does it still uphold the conclusions of the SCCS/1533/14 opinion with regard to the safe use of HAA299 non-nano form?

/

4. Does the SCCS have any further scientific concerns (on human health) with regard to the use of HAA299 (nano) in cosmetic products?

This opinion is based on the currently available scientific evidence, which shows an overall very low or lack of dermal absorption of HAA299 (nano) in human skin. If any new evidence emerges in the future to show that HAA299 (nano) used as UV-filter in cosmetic products can penetrate human skin (healthy, compromised, sunburnt or damaged skin) to reach viable cells in higher levels than demonstrated in this submission, then the SCCS may consider revising this assessment.

Keywords: SCCS, scientific opinion, HAA299, nano, CAS 919803-06-8, EC No. 485-100-6, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on HAA299 (nano), preliminary opinion 22 July 2021, final opinion 26-27 October 2021, SCCS/1634/2021

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

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

HAA299 (nano) with the chemical name '2-(4-(2-(4-Diethylamino-2 hydroxy-benzoyl)-benzoyl)-piperazine-1-carbonyl)-phenyl)-(4-diethylamino-2-hydroxyphenyl)-methanone' and INCI name 'Bis-(Diethylaminohydroxybenzoyl Benzoyl) Piperazine' (CAS 919803-06-8) is a cosmetic ingredient with the reported functions of UV-filter. Currently HAA299 normal form and nano form is not regulated under the Cosmetic Regulation (EC) No. 1223/2009.

In 2009, Commission' services received a dossier from industry to support the safe use of HAA299 (micronised and non-micronised) in cosmetic products, which was further substantiated with additional information in 2012. In its corresponding opinion (SCCS/1533/14), the SCCS concluded that *"the use of non-nano HAA299 (micronised or non-micronised, with median particle size distribution around 134 nm or larger, as measured by FOQELS) at a concentration up to 10% as an UV-filter in cosmetic products, does not pose a risk of systemic toxicity in humans".*

In addition, SCCS stated that "*[the Opinion]…covers the safety evaluation of HAA299 in non-nano form. The opinion does not cover the safety evaluation of HAA299 which is composed of nano particles*' and highlighted that '*[the Opinion]…does not apply to inhalation exposure of HAA299 since no information on chronic or sub-chronic toxicity after inhalation is provided".*

With the current submission, received in September 2020, and in view of the previous SCCS opinion (SCCS/1533/14) on the normal form of HAA299, the applicant requests to assess the safety of HAA299 (nano) intended to be used as UV-filter up to a maximum concentration of 10%.

Terms of reference

1. *In light of the data provided, does the SCCS consider HAA299 (nano) safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?*

2. *In view of the previous SCCS opinion (SCCS/1533/14) does the SCCS consider HAA299 non-nano and nano form safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?*

3. *In case the SCCS finds HAA299 (nano) not safe, does it still uphold the conclusions of the SCCS/1533/14 opinion with regard to the safe use of HAA299 non-nano form?*

4. *Does the SCCS have any further scientific concerns (on human health) with regard to the use of HAA299 (nano) in cosmetic products?*

3. OPINION

Preamble:

HAA299 was notified in the form of the following three entities:

- HAA299 neat (non-nano form, bulk powder), the safety of which was previously evaluated by SCCS in 2014 (SCCS/1533/14);
- HAA299 (nano), an aqueous dispersion of nanoparticles formulation with excipients containing the HAA299 active UV filter ingredient that was micronised to nano size;
- HAA299 (non-nano, formulated) as an aqueous dispersion of particles, formulated with excipients containing the HAA299 active UV filter ingredient in non-nano form.

In this evaluation, only the nanoform of HAA299 has been considered, along with some information on the non-nano forms where appropriate.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Primary name: 2-(4-(2-(4-Diethylamino-2-hydroxy-benzoyl)-benzoyl)-piperazine-1-carbonyl)-phenyl-(4-diethylamino-2-hydroxyphenyl)-methanone

INCI name: Bis-(Diethylaminohydroxybenzoyl Benzoyl) Piperazine

3.1.1.2 Chemical names

1,1'-(1,4-piperazinediyl)bis[1-[2-[4-(diethylamino)-2-hydroxybenzoyl]phenyl]-methanone

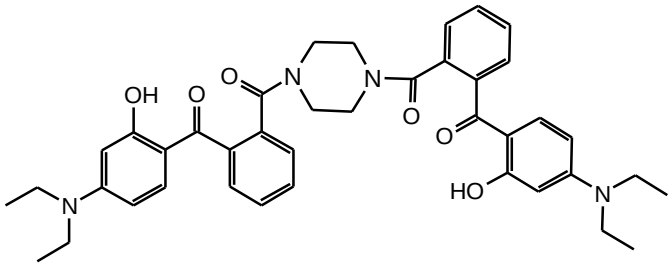
3.1.1.3 Trade names and abbreviations

HAA299 (nano)
C-1332 (micronised)
C-1332
FAT 75808/E
FAT 75’808/G
FAT 75808/H
Dispersion C-1332 OP1/2010
Dispersion C-1332 OP1/2010

3.1.1.4 CAS / EC number

CAS: 919803-06-8
EC number: 485-100-6

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Empirical Formula: $C_{40}H_{44}N_4O_6$

3.1.2 Physical form

HAA299 (nano) is a viscous white dispersion of nanoparticles (paste-like product).

3.1.3 Molecular weight

Molecular weight: 676.82 g/mol

3.1.4 Purity, composition and substance codes

HAA non-nano form:
Four different batches of the non-micronised form, HAA299, were used for studies presented in this dossier and were shown to have a similar analytical profile. The purity was >98% (w/w) expressed as active ingredient and each batch contained up to 0.4% of one or two known by-products, details of their composition are included in the Table 1. below.
The analytical methods used for the determination of active ingredient (HAA299 non-nano) content in technical grade active ingredient show typically a variance of ± 1%. According to the Applicant, this systematic error must be accounted for in the definition of the minimum active ingredient content.

Minimum purity: 97 % HAA299
Typical purity: > 98 % HAA299

Ref: SCCS/1533/14

Composition of HAA299 (nano)

HAA299 (nano) is a formulation containing approx. 50% w/w of the UV active ingredient HAA299 which is mixed with excipients and subsequently micronised to yield the respective nano form. The composition of HAA299 (nano) is given in Table 1:

Table 1. Formulation of HAA299 (nano)

Ingredient	CAS number	Function	Mass fraction (% w/w)
HAA299	919803-06-8	UV absorption	45 – 55 %
Water	7732-18-5	Solvent	31 – 54.5 %
Various, confidential*	Various, confidential*	Excipients*	0.5 – 14 %

*confidential (Ref: BASF Grenzach, Jochen Giesinger, (June 2020) HAA299 (nano) in formulation Particle Size Measurements of Different Batches of Micronised Test Substance _ CONFIDENTIAL, Study Period 2006-2019)

Chemical identity of formulated UV active ingredient HAA299 (nano)

According to the Applicant, HAA299 (nano) will be the marketed product form. It contains the active UV filter ingredient HAA299 in nano size. Results of the characterisation of HAA299 (nano) in three batches are presented in Table 2.

Table 2. Chemical Identity of HAA299 (nano)

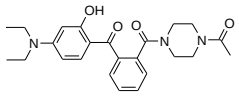
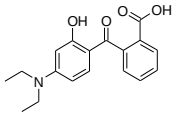
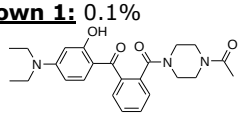
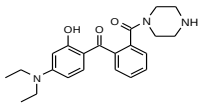
HAA299 (nano) Batch No	Appearance and homogeneity	Identity	Content of active ingredient	Water content
5388F7	After shaking and stirring with a spoon for 5 min the test item was a milky, light yellowish, pasty liquid at room temperature and was apparently homogeneous on a macroscopic scale.	The ^{1}H-NMR and ^{13}C-NMR spectra show the signals expected for the main constituent of the test item along with signals of minor impurities or additives.	53.6 ± 0.8 via UV/Vis spectroscopy	39.3 g/100 g
RIN195_9	After homogenization at room temperature for 3 hours on a shaking device the test item was a light beige, viscous liquid and obviously homogeneous.	The ^{1}H-NMR and ^{13}C-NMR spectra show the signals expected for the given structure. In both spectra there are additional signals with low intensity of unknown side components.	50.2 via UV/Vis spectroscopy	43.2 g/100 g
RIN208_10	After homogenization by stirring with spatula for 5 min the test item was a milky, light yellow pasty liquid and obviously homogeneous.	The ^{1}H-NMR and ^{13}C-NMR spectra show the signals expected for the given structure. In both spectra there are additional signals with low intensity of unknown side components.	51.0 ± 0.2 via UV/Vis spectroscopy	42.2 g/100 g

Ref: BASF (Feb 22, 2018); BASF (Aug 01, 2016)); BASF (Nov 11, 2017)

3.1.5 Impurities / accompanying contaminants

Impurities of HAA299 neat in various batches are presented in Table 3 and in SCCS/1333/14.

Table 3. Composition of HAA299 neat in various batches

FAT 75'808 Suffix Used	Batch number	Measured purity active (% w/w)	Organic Impurities (% w/w)	Others (solvents, volatile matters)
/A	HAA299/7-5	98.7 ± 0.5%.	**Known 1:** 0.1%  All other organic impurities were found to be below the quantification limit (<0.1%)	Water = 0.1% (may change during storage) Volatile compounds: -N-methylpyrrolidone (NMP) = 0.5% - others (ethyl acetate, acetone, 2-methoxy-ethanol) = < 0.1%.
/B	VTM05B10	99.1 ± 0.5%.	**Known 2:** 0.4%  All other organic impurities were found to be below the quantification limit (<0.1%)	Water = < 0.1% (may change during storage) Volatile compounds (N-methylpyrrolidone, ethyl acetate, acetone, 1-propanol, piperazine = 0.1%.
/C	HAA299/77	99.8 ± 0.67%.	All organic impurities were found to be below the quantification limit (<0.1%)	Water = < 0.1% (may change during storage) Volatile compounds (ethyl acetate, acetone, 2-methoxy-ethanol) = <0.1%.
/D	VTM07B04	98.6 ± 0.8%.	**Known 1:** 0.1%  **Known 3:** 0.1%  All other organic impurities were found to be below the quantification limit (<0.1%)	Water: 0.2% (may change during storage) Volatile compounds: N-methylpyrrolidone = 0.04%, ethyl acetate = <0.02%, 1-propanol = 0.04%, methyl-ketone = 0.5%

SCCS comment

The identity of impurities that are present in batches of HAA299 (nano), as notified here, must also be reported in terms of chemical nature and concentration as presented for the non-nano HAA299 in Table 3. The level of N-methylpyrrolidone (NMP), which is a reprotoxicant Cat 1B substance, should be at minimum level. The content of inorganic impurities in HAA299 neat, specifically of Ni, Co, Cr, CD, Pb and Hg, must also be reported.

3.1.6 Solubility

According to the Applicant, HAA299 (nano) is only dispersible in water. The ingredient HAA299 (neat) is not available in its nanoform as pure ingredient. Therefore, the determination of water solubility according to current guidelines is not possible.

For the UV active ingredient, please refer to HAA299 neat (non-nano) and the respective SCCS opinion (SCCS/1533/14): water solubility: 1.66 ± 1.24 µg/L at 20°C (OECD 105), insoluble (<0.001) (Ref. G)

SCCS comment
From the provided information, the SCCS considers that HAA299 nano is practically insoluble in water.

3.1.7 Partition coefficient (Log P_{ow})

Not applicable for HAA299 (nano).
For the non-nano UV active ingredient, LogP_{ow} 4.8 at pH = 6.8 (HPLC method, OECD 117) (SCCS/1533/14)

3.1.8 Additional physical and chemical specifications

According to the Applicant, the pure nano material is not available, as it is produced as a dispersion (see below) and, therefore, the determination of the dissociation constant is not applicable. For the UV active ingredient, please refer to HAA299 neat (non-nano) and respective SCCS Opinion (SCCS/1533/14) for further specifications.
The dissociation constant study does not need to be determined as the substance is not soluble in water.
pH and viscosity are adjusted during the micronisation process by adapting the amount of acid and rheology modifier.
pH: ca 6-8
Viscosity: 200 – 1200 mPa s

Nano specific properties of HAA299 (nano)
Micronisation process of HAA299 neat into HAA299 (nano)
HAA299 (neat) is a non-nano particle size powder when produced. Due to its extremely low solubility in cosmetic oils and water, it cannot be incorporated into a cosmetic formulation. The water phase remains clear even after intense mixing. Therefore, in that powder form HAA299 neat is not suitable for use as UV filter in personal care products.
In order to be used in cosmetic applications, the UV filter HAA299 has to be dispersed in a homogeneous aqueous formulation which can be later easily mixed with the water phase of a cosmetic emulsion. A proprietary process that the Applicants refer as micronisation, wet-milling or wet-grinding, is required to prepare this homogeneous aqueous dispersion of HAA299 UV active ingredient. Because of electrostatic charging and thus for worker safety reasons, it is not possible to mill the bulk material in its dry form. Therefore, the size reduction of the bulk material is processed by wet-milling. HAA299 neat alone is neither soluble nor dispersible in water, thus, prior to the grinding process surfactants and emollients are required to introduce the material into the water phase. At the finalization of the micronisation a thickener is needed to stabilize the resulting preparation from agglomeration of the nanoparticles. The different product forms before and after the micronisation process are illustrated in Figure 1.

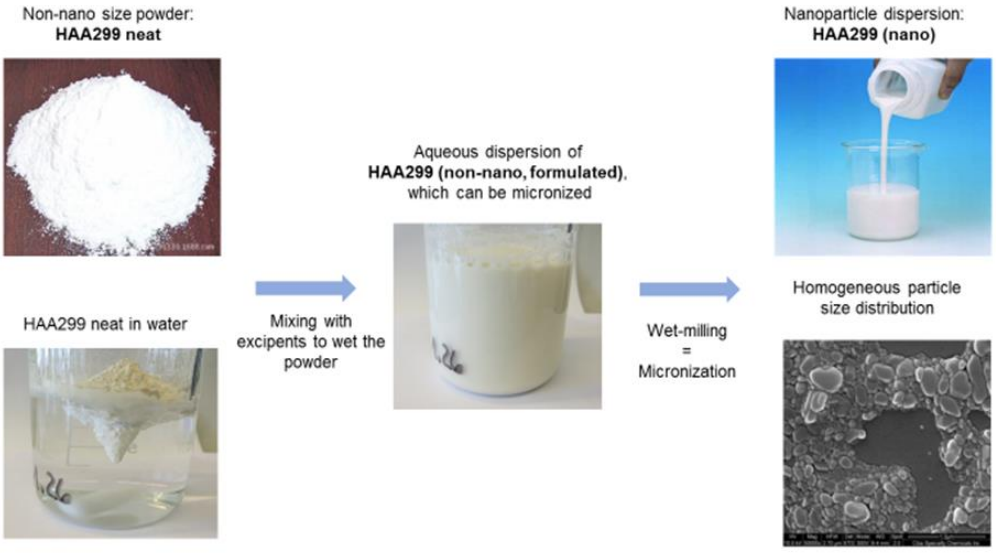


Figure 1: Product form of HAA299 before and after micronisation

The micronisation is a multi-step process during which the size of the HAA299 particles is reduced over time. The milling step involves a stirred media ball mill and is repeated until the micronised product reaches the quality criteria, defined by particle size and specific extinction (E1,1 value) of a UV-spectrum. It is thus possible to vary the particle size of the UV filter as well as the efficiency by the number of milling cycles.
By taking fractions of the dispersion at different stages of the micronisation process and by measuring the UV absorption of those fractions, it can be shown that the UV absorption of HAA299 particles increases with decreasing particle size, leading to a significant advantage to HAA299 (nano) versus HAA299 (non-nano, formulated).
In a final process step the pH value of the product is adjusted. The product is filtered and thickened to a defined viscosity and packaged. The resulting paste-like product HAA299 (nano) is ready for use for the formulation of commercial sunscreen products.

3.1.9 Particle size

The particle sizes of several samples of HAA299 (nano) were measured with the Fiber Optic Quasi Elastic Light Scattering (FOQELS) technique. Figure 2 below shows the analytical result of HAA299 (nano) sample FAT 75’808/H as an example of the technique applied.

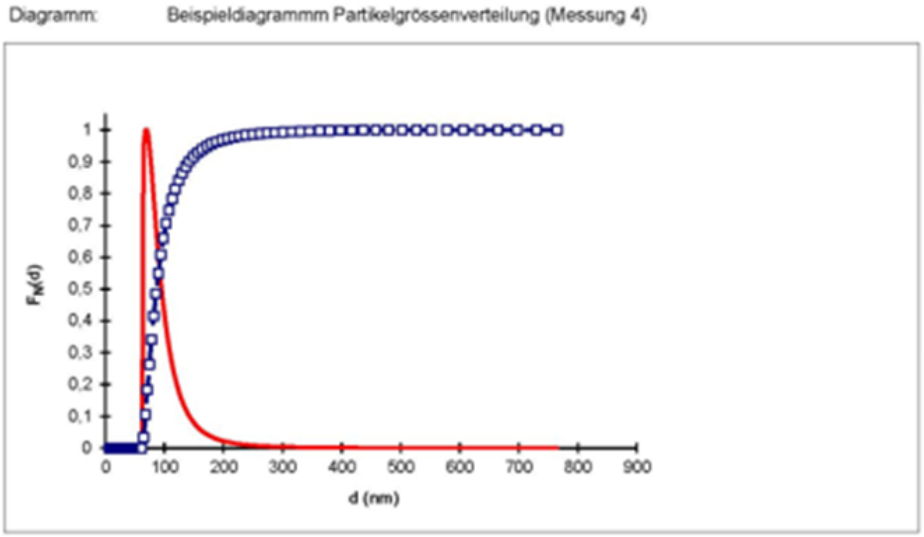


Figure 2: Particle number distribution of HAA299 (nano) sample FAT 75'808/H

The red solid line is the particle size distribution, the blue dotted line is the cumulative particle size distribution, d (0.5) = 75 ± 28 nm. This means that 50% of the particles in terms of particle number are smaller than 75 nm. About 60 – 70% of the particles are below a size of 100 nm. This represents a nanomaterial according to Cosmetic Regulation (EC) No 1223/2009, which provides a definition of a nanomaterial (NM).

The particle size of HAA299 (nano) was also measured according to ISO 13320: Particle size analysis – Laser diffraction methods – Ref. Nr. 13320/2020 (E), and results are presented in Figure 3.

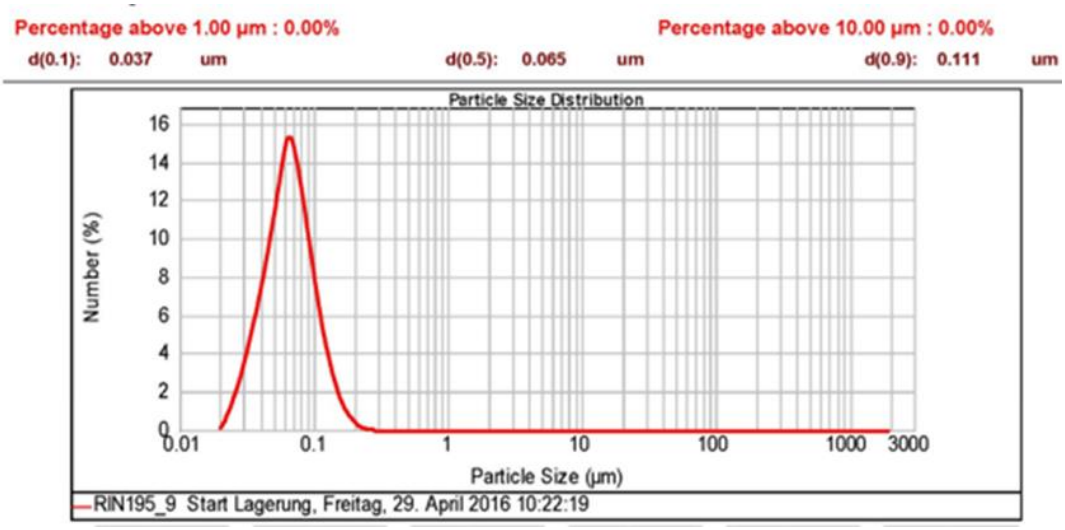


Figure 3: Particle number distribution of HAA299 (nano) batch RIN195_9

Specification particle size of expected commercial product HAA299 (nano) is:
d_{50} > 100 nm according to volume distribution
d_{50} > 50 nm according to number distribution

SCCS comment
Explanation is needed on the provided d_{50} values according to volume and number distribution, these values should be a specific number (not > or < a number). It should also be indicated what would be a sufficient particle size reduction in the milling process to reach a certain product specification. The SCCS has, therefore, considered that the value of d50 (expressed as number based particle size distribution) is equal to or greater than 50nm. The SCCS will, however, need the full range of intended particle size distribution from the milling process.

Zeta-Potential
-40mV at pH 7.

Particle Size characterisation of HAA299 (nano) in the batches used for non-clinical studies

Toxicological data for the HAA299 (nano) were obtained from non-clinical studies to address the following endpoints:
- Acute oral and inhalation toxicity
- Skin and eye irritation*
- Dermal / percutaneous penetration*
- Repeated dose toxicity (subacute toxicity)
- Mutagenicity and genotoxicity*
- Reproductive toxicity
- Toxicokinetics*

For several of these endpoints (*), comparative studies with HAA299 (nano) and HAA299 (non-nano, formulated) were performed to assess the impact of the particle sizes on the toxicological outcome. For each of the non-clinical studies conducted and submitted within this dossier, the batch used, the purity and particle size range of HAA299 particles are summarised in Table 4 and in Table 5.

Table 4. Overview of HAA299 (nano) batches used in toxicological studies and their particle size distributions

Product form	Test substance name (Batch no.)	Batch description	Particle size distribution	Study
HAA299 (nano)	FAT 75'808 /E (MGU 789)	Micronised formulation containing excipients and 51.2% HAA299 in nano form. For preparation HAA299 (lot no. VTM07B04; purity: 98.6%) was used.	Volume/mass distribution: d(0.5) 134 nm d(0.9) 202 nm Number distribution: **d(0.5) 65 nm** d(0.9) 89 nm	-Repeated dose (subacute)&Reproductive/ Developmental toxicity CIT 2009; 34039 RSR
HAA299 (nano)	FAT 75'808 /G (MGU 799, LA 2397_31)	Micronised formulation containing excipients and 51.0% HAA299 in nano form. For preparation HAA299 (lot no. VTM07B04; purity: 98.6%) was used.	Volume/mass distribution: d(0.5) 138 nm d(0.9) 207 nm Number distribution: **d(0.5) 84 nm** d(0.9) 123 nm	- Repeated dose (subacute) & Reproductive/ Developmental toxicity CIT 2009; 34039 RSR - Acute oral toxicity CIT 2009; 34033 TAR - Genotoxicity *in vivo* (MNT) CIT 2009; 34083MAS

Product form	Test substance name (Batch no.)	Batch description	Particle size distribution	Study
HAA299 (nano)	FAT 75'808 /H (MGU 814, LA 2397_37)	Micronised formulation containing excipients and 50-51% HAA299 in nano form. For preparation HAA299 (lot no. VTM07B04; purity: 98.6%) was used.	Volume/mass distribution: d(0.5) 136 nm d(0.9) 241 nm Number distribution: **d(0.5) 75 nm** d(0.9) 106 nm	- Acute Inhalation Toxicity, RCC 2008; B78625 --- Mutagenicity/ Genotoxicity *in vivo* (UDS) Pasteur 2009; FSR-IPL 080310
Radiolabelled HAA299 (nano)	FAT 75808 (3574052 (radiolabelled HAA299); VTM05B10 (non-radiolabelled HAA299))	Micronised formulation containing excipients and HAA299 in nano form ([^{14}C-labeled and non-labelled). For preparation ^{14}C-HAA299 (lot no. 3574052; radiochemical purity: 97.7%) and non-labelled HAA299 (lot. no. VTM07B04; purity: 98.6%) were used.	Study specific size measurements (n°1) Volume/mass distribution mean $d_{0.5}$= 137 nm mean $d_{0.9}$=255 nm Number distribution: **mean d(0.5) 64 nm** mean d(0.9) 94 nm	- Dermal / Percutaneous absorption (rat & human skin *in vitro*, rat *in vivo*) Harlan 2009; B27628 Harlan 2009; B70582 Harlan 2009; B27617 - Toxicokinetics (*In vivo*, oral, rat) Harlan 2009; A89291
			Study specific size measurements (n°2) Volume/mass distribution mean $d_{0.5}$=153 nm; mean $d_{0.9}$=254 nm Number distribution: **mean d(0.5)= 55 nm** mean d(0.9)= 72 nm	- Dermal / Percutaneous absorption (human skin predamaged *in vitro*) Harlan 2009; C22987
HAA299 (nano)	C-1332 micronised (RIN195_9)	Micronised formulation containing excipients and 50.2 % HAA299 in nano form. For preparation HAA299 (lot no. 00015HN9; purity: 98.7%) was used.	Volume/ mass distribution d(0.5) 110 nm d(0.9) 181 nm Number distribution: **d(0.5) 65 nm** d(0.9) 111 nm	- Eye irritation (BCOP, EpiOcular ™) BASF 2017 63V0349/16A140 BASF 2017 62V0349/16A141 - Skin irritation (EpiDerm™) BASF 2017 68V0349/16A142

Product form	Test substance name (Batch no.)	Batch description	Particle size distribution	Study
HAA299 (nano)	C-1332 micronised (RIN208_10)	Micronised formulation containing excipients and 51.0% HAA299 in nano form. For preparation HAA299 (lot no. 00015HN9; purity: 98.7%) was used.	Volume/ mass distribution d(0.5) 110 nm d(0.9) 180 nm Number distribution: **d(0.5) 65 nm** d(0.9) 111 nm	- Mutagenicity/ Genotoxicity *in vitro* (MNT, HPRT) BASF 2019 33M0349/16M411 BASF 2019 50M0349/16M413
HAA299 (nano)	C-1332 micronised (5388F7)	Micronised formulation containing excipients and 52.5% HAA299 in nano form. For preparation HAA299 (lot no. 00015HN9; purity: 98.7%) was used.	Volume/ mass distribution d(0.5) 120 nm d(0.9) 265 nm Number distribution: **d(0.5) 62 nm** d(0.9) 109 nm	- Mutagenicity/ Genotoxicity *in vitro* (MNT) BASF 2019 33M0349/16M411
Particle size distribution measured by Malvern 2000 or FOQELS (Fiber Optic Quasi Elastic Light Scattering)				

For a number of toxicological studies, the non-nano form of HAA299 has been used. The comparative non-nano HAA299 was taken before the start of the milling process, i.e. at the step of the slurry preparation. In this dossier it is referred to as HAA299 (non-nano, formulation). As the batches were prepared without micronisation, they are not within the product specification regarding particle size distribution.

Table 5. Overview of HAA299 (non-nano, formulated) batches used in toxicological studies and their particle size distributions

Product form	Test substance name (Batch no.)	Batch description	Particle size distribution	Comment	Study
HAA299 (non-nano, formulated)	C-1332 non-micronised (RIN197_1)	Non-micronised formulation containing excipients and 44.1 % HAA299 in non-nano form. For preparation HAA299 (lot no. 00015HN9; purity: 98.7%) was used.	Volume/mass distribution $d_{0.5}$=10.3 µm $d_{0.5}$=19.8 µm Number distribution: **d(0.5)= 2.8 µm** d(0.9)= 7.3 µm	This d(0.5) is **NOT** within the range for the product specification of HAA299 (nano)	- Eye irritation (BCOP, EpiOcular™) BASF 2017 63V0350/16A139 BASF 2017 62V0350/16A143 - Skin irritation (EpiDerm™) BASF 2017 68V0350/16A144
HAA299 (non-nano, formulated)	C-1332 non-micronised (RIN226)	Non-micronised formulation containing excipients and 49.6% HAA299 in non-nano form. For preparation HAA299 (lot no. 00015HN9; purity: 98.7%) was used.	Volume/mass distribution $d_{0.5}$=10.3 µm $d_{0.9}$=20.4 µm Number distribution: **d(0.5)= 2.8 µm** d(0.9)= 7.2 µm	This d(0.5) is **NOT** within the range for the product specification of HAA299 (nano)	- Mutagenicity/ Genotoxicity *in vitro* (MNT, HPRT) BASF 2018 33M0350/16M412 BASF 2018 50M0350/16M414

Particle size distribution measured by Malvern 2000 or FOQELS (Fiber Optic Quasi Elastic Light Scattering)

SCCS comment
The Applicant should provide a clear product specification for HAA299 (nano) which is intended to be marketed.

3.1.10 Microscopy

Scanning electron microscopy (SEM) image of HAA299 (nano) at 50 000 times magnification is presented in Figure 4.

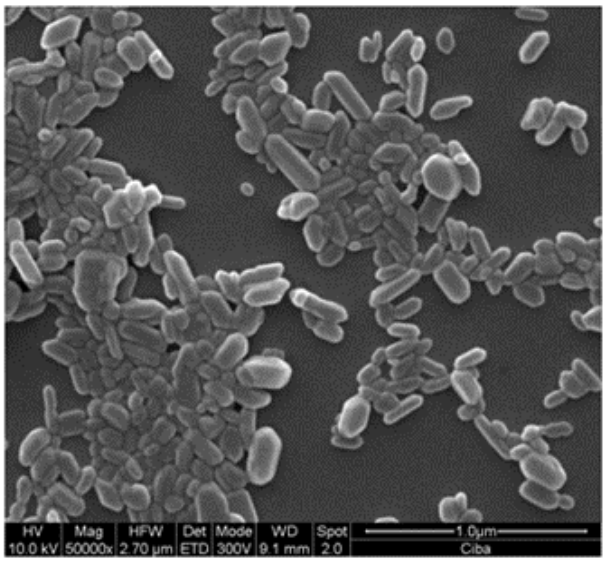


Figure 4: Scanning electron microscopy (SEM) image of HAA299 (nano) at 50 000 times magnification

3.1.11 Crystal structure

/

3.1.12 UV absorption

The specific extinction (E1%, 1cm) of micronised HAA299 with different particle sizes is shown in Figure 5. It shows the dependence of the absorption potential on the particle size of the material used, indeed the (E1%, 1cm) value increases with decreasing particle size. Best E efficiency is achieved, when the particle size is 120 nm (by mass/ volume distribution), which corresponds to a particle size of around 60 nm based on number evaluation.

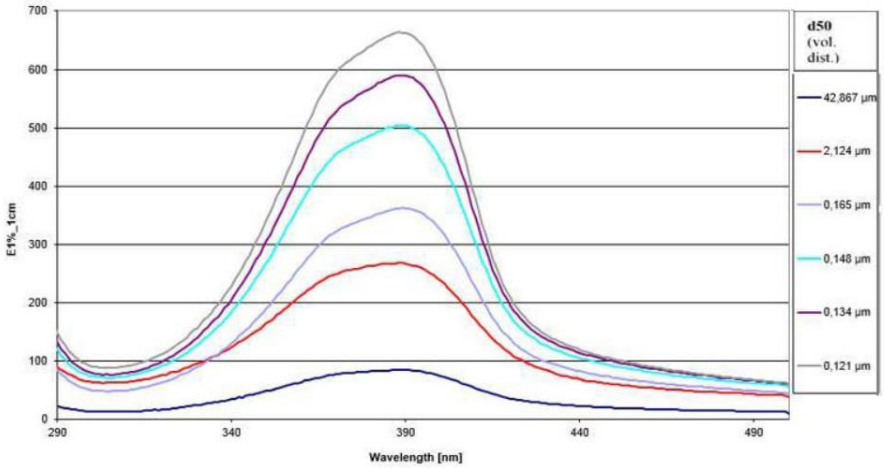


Figure 5. Specific extinction E1%, 1cm of micronised HAA299 particles in formulation with different particle sizes (d_{50} / volume distribution)

3.1.13 Surface characteristics

Specific Surface Area: 34.3 m^2/g

3.1.14 Droplet size in formulations

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3.1.15 Other parameters of characterisation

Particle Dosing Estimation of micronised HAA299
The physical characterization of the particles present in micronised HAA299 was further defined to allow an estimation of the test system dosages calculated as number of particles and surface area of particles administered. These parameters are suggested as the more representative exposure estimate than mass of test item applied. The physical characterization parameters are summarised in Table 6. For the particle size of the micronised test item, the mean (136 nm) of median particle sizes of the various batches of micronised HAA299 was used. Due to its larger particle size, one batch of the test item was micronised at the testing laboratory. Calculation of particle dosages or exposures performed with these parameter values are assumed to be representative of the various batches of micronised HAA299 used in the toxicology studies reported herein. In the following study summaries, the particle doses are derived from the values shown in Table 6. For some of the studies, the particle size distributions were not available until after the study had been completed so the final report does not contain this information.

Table 6. Particle parameters for micronised HAA299

Parameter	Value*
Assumptions	1) 30%** concentration of monodisperse particles of size d(0.5); 2) Values are representative for other HAA299 micronised dispersions used in toxicology studies.
d(0.5)	$1.36E^{-07}$ m
Surface Area	$5.81E^{-14}$ m^2/g
Volume	$1.35E^{-21}$ m^3
Density	1288 kg/m^3
Number particles per cm^3	$1.77E^{+20}$ / cm^3
Estimated Weight of one particle	**$1.70E^{-21}$ g**
Specific Surface Area	34.3 m^2/g
* Values prepared and summarized by Herzog, B. and Giesinger, J.; Ciba internal report 09 March 2009. ** value representative for the radio-labelled material formulation	

SCCS comment
The Applicant has only provided point values for each of the parameters in Table 6 above, whereas a range of these values within the intended specification are needed.

3.1.16 Homogeneity and stability

The intended commercial formulation HAA299 (nano), containing the nano form of the UV active HAA299 and its excipients, was subject to a storage investigation under ambient conditions. Two different batches were analysed by HPLC for active UV ingredient content, initially and after four years of storage. The results showed the chemical stability of the preparation by recovering 100.0 % and 99.8 % of the initial active UV ingredient content, respectively (see Table 7).

Table 7: Analytical results of active ingredient determinations in HAA 299 (nano) initially and after 4 years storage under ambient conditions

Product form	Batch name	HAA299 content at T=0	HAA299 content at T=4 years	HAA299 recovery
HAA299 (nano)	Dispersion C-1332 OP1/2010	48.9 w%	48.9 w%	100.0%
HAA299 (nano)	Dispersion C-1332 OP2/2010	49.4 w%	49.3 w%	99.8%

In addition, stability and homogeneity of HAA299 (nano) was proven in each dosing form applied to assess the toxicological endpoints summarized within the dossier.
For chemical stability of HAA299 neat, the UV active ingredient HAA299 in non-nano powder form, please refer to SCCS/1533/14, revision of 23 September 2014.

Taken from SCCS/1533/14

- The stability and homogeneity of dosage forms of 10, 50 and 200 mg/ml FAT 75'808/E and FAT 75'808/G (HAA299 nano, suspension in 0.5% carboxymethylcellulose) performed with HAA299 lot number VTM07B04, purity of 98.6% was demonstrated in

the combined repeated dose study with the reproduction/developmental toxicity screening (Ref. 21), good homogeneity (CV<2%) of each dosage form analysed just after preparation was demonstrated. The test concentrations in the administered dosage forms analysed in weeks 1, 6 and 9 remained within the range of variation [-5% to +3%] when compared to the nominal values.

Ref: SCCS/1533/14; BASF, 2014 (25.08.2014)

3.2 TOXICOKINETICS

3.2.1 Dermal/percutaneous absorption

***In vitro* percutaneous penetration through intact rat and human skin**

Guideline:	OECD TG 428
Species/strain:	Four male rats (HanBrl: WIST (SPF) (8-9 weeks old) Human full thickness skin was obtained from the dorsal upper leg of two individuals (male aged 78 years and female aged 89 years)
Membrane integrity:	Rat skin membranes with Kp >3.5x10-3 cm/h and human skin membranes with Kp >2.5x10-3 cm/h were excluded
Number of membranes:	For rat: 7 membranes. For humans: 6 membranes (1 human membrane was damaged)
Exposed skin area:	0.64 cm^2
Test substance:	HAA299 (nano): FAT 75'808, a water-based formulation of micronised HAA299 with [^{14}C] label and without label (FAT 75'808/D))
Batch:	VTM07B04 (not labelled), 3574052 (labelled)
Purity:	98.6% (non-radiolabelled test item)
Radiochemical purity:	97.7% (radiolabelled test item)
Mean particle size:	Radiolabelled test item
Number distribution:	d(0.5): 64 nm and d(0.9): 94 nm
Volume distribution:	d(0.5): 137 nm and d(0.9): 255 nm
Target dose level:	approx. 2 mg/cm²
Test concentration:	2175 µg/cm²
Receptor fluid:	6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v)
Exposure conditions:	Application for 24 hours; non-occluded
GLP:	In compliance
Study period:	6 May – 28 Oct 2008, report dated 2009

Methods
Full thickness skin was removed from 4 male rats and stored frozen until prepared for use. Human full thickness skin was obtained post-mortem from the dorsal upper leg of 2 individuals and stored frozen until use. Skin membranes of each species were prepared by removing subcutaneous fat and the upper 200 µm by dermatome. From each species 7 membranes in cells were prepared and finally, 7 rat skin membranes and 6 human skin membranes were used.
The integrity of each skin membrane was determined by tritiated water.
The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions.
Twenty-four hours after application the perfusate sampling was terminated. The skin membranes were removed from the diffusion cell and stratum corneum was removed by 15 tape strips which were combined into 5 consecutive groups. The skin membranes remaining

after stripping were digested and the radioactivity was determined in samples by liquid scintillation counting (LSC).

Results

The test item HAA299 (nano); i.e. micronised labelled and non-labelled HAA299 in a formulation, was shown to have a radiochemical purity of 98.14%, determined by HPLC at the time of application. The mean permeability coefficient (Kp) value of the membranes chosen was 0.62 ± 0.15 x 10^{-3} cm/h for the rat and 1.4 ± 0.80 x 10^{-3} cm/h for the human skin. The applied dose was determined to be 2175 µg/cm^2. Based on test item found in the perfusate, the percutaneous penetration rate in rat and human skin was below reliably quantifiable concentrations as shown in Table 8.

Table 8. Skin absorption of HAA299 (nano) in rat and human skin membranes

Harlan No B27628	**Rat Skin Membrane**		**Human Skin Membrane**	
Applied Dose [µg/cm^2]	2175		2175	
Applied Volume [µL]	13		13	
Application Area [cm^2]	0.64		0.64	
Concentration [mg/cm^3]	107.07		107.07	
Penetration within	% of dose	µg/cm^2	% of dose	µg/cm^2
6 h	< 0.01	*0.011	< 0.01	*0.031
12 h	< 0.01	*0.018	< 0.01	*0.055
24 h	< 0.01	*0.082	< 0.01	*0.083
Flux[µg/cm^2/h]- measured	n.a.**		n.a.**	
Flux[µg/cm^2/h]- estimated+	0.065		0.090	
*value calculated from the measured dpm, all of which are below LOQ of about 0.1 µg-equivalents **values were below LOQ +estimated by replacing <LOQ values with the LOQ and calculating flux.				

The estimated flux for rat and human skin was 0.065 µg/cm^2/h, based on the penetration rate at steady state between 1-16 hours and calculated by using the corresponding limit of quantification (LOQ) values instead of the measured values, all of which were below LOQ. Similarly, the flux for human skin was estimated to be 0.090 µg/cm^2/h for 1-10 hours based on the LOQ values. The distribution and recovery of the labelled test item at test termination is summarized in Table 9.

Table 9. Distribution and recovery of HAA299 (nano) in rat and human skin experiments

Recovery [% of Dose SD given in brackets]*		
Skin Membrane:	**Rat**	**Human**
Applied Dose [µg/cm^2]	2175	2175
Perfusates	<0.01 (<0.01)#	<0.01 (<0.01)#
Remaining Skin membrane	0.51 (0.25)	0.02 (0.02)
Total absorbed (%)	0.52	0.03
As µg a.i./cm^2	11.3	0.65
Skin membrane Rinse	64.63 (17.29)	90.16 (4.21)
Tape Strips	29.28 (12.17)	2.04 (1.2)
Diffusion cell wash	5.14 (4.01)	9.81 (3.44)
Recovery	99.58 (4.79)	102.04 (2.03)
*Values are mean (+ standard deviation) #Calculated from measured dpm values, most of which were below LQ of about 0.04 µg a.i. equivalents.		

The very low absorption and percutaneous penetration of the test item allowed only an estimation of a flux parameter because the values were below LOQ for the samples. Penetration through rat skin membranes was slightly higher than through human skin membranes. Similarly, larger amounts of the test item were found in the tape strips from rat

skin compared to human skin membranes, whereas the human skin showed 90% removable in surface wipe and membrane rinses. Both test systems showed recovery of more than 99% of applied dose and the test item was shown to remain stable during the 24-hour exposure period.

Conclusion
It was concluded that the cumulative penetration of formulated HAA299 (nano) through rat and human skin membrane was <0.01% of the applied dose for both types of skin membranes during 24 hours of exposure. Together with the amount measured in the remaining skin membrane after tape stripping, the total amount of absorbed radioactivity within 24 hours accounted for 0.52% of the applied dose in rat skin membrane and 0.03% of the applied dose in human skin membrane. Thus, in conclusion, HAA299 (nano) did not penetrate through rat and human skin membranes.

Ref: Harlan Ltd, CH-4452 Itingen, Switzerland, March 09, 2009

SCCS comment
The number of membranes and donors (the latter in case of human skin) used for dermal absorption was lower than that recommended in the SCCS Notes of Guidance for both rat und human skin samples. The amount considered absorbed (i.e. the amount from perfusate and remaining skin after tape stripping) amounted to 0.52% in rat skin and 0.03 % in human skin.

***In vitro* percutaneous penetration, pre-damaged human cadaver skin ex vivo**

Guideline:	OECD Guideline no. 428
Species/strain:	Human full thickness skin was obtained from the dorsal upper leg of 3 individuals
Membrane integrity:	Use of skin membranes with the highest Kp was intended.
Number of membranes:	7 membranes, 2 males (78 and 17 years), 1 female (83 years)
Exposed skin area:	0.64 cm^2
Test substance:	HAA299 (nano): FAT75'808, a water-based formulation of micronised HAA299 with [^{14}C] label and without label (FAT 75'808/D)
Batch:	VTM07B04 (not labelled), 3574052 (labelled)
Purity:	98.6% (non-radiolabelled test item)
Radiochemical purity:	97.7% (radiolabelled test item)
Mean particle size:	Radiolabelled test item
Number distribution:	d(0.5): 55 nm and d(0.9): 72 nm
Volume distribution:	d(0.5): 153 nm and d(0.9): 254 nm
Target dose level:	approx. 2 mg/cm²
Dose volume:	2116 µg/cm²
Receptor fluid:	6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v)
Exposure conditions:	Application for 24 hours; non-occluded
GLP:	In compliance
Study period:	27 Oct – 3 Nov 2008

Methods
Tape-stripped human skin membranes were used to model a compromised stratum corneum with impaired barrier properties. Human full thickness skin was obtained post-mortem from the dorsal upper leg or abdominal area of three individuals and stored frozen until preparation of membranes (3 tape strips to prepare pre-damaged skin, removal of the upper 200 µm).
The integrity of the skin membrane was determined by applying 50 µl tritiated water (about 200,000 dpm) to the skin membrane surface. The donor chamber was covered with adhesive

tape (occluded conditions). The cumulative penetration was determined over 6 hours by collecting hourly fractions. The Kp of each skin membrane was calculated for the 3 - 6 hours interval. Seven samples of the skin membranes with the highest Kp were taken for the subsequent experiment (except for one cell showing a distinctly high Kp value, i.e. 6.1 $x10^{-3}$ cm/h). Kp values <$2.5x10^{-3}$ cm/h were considered indicator of undamaged skin.
The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions. Receptor medium (perfusate) from each cell was collected separately at ambient temperature in 1-hour intervals for the 0 - 6 hour period (6 intervals), and in 2-hour intervals for the remaining exposure period (6 – 24 h, 9 intervals).
The skin membranes were removed from the diffusion cell and consecutively stripped until the stratum corneum was removed from the skin membrane, i.e. 15 tape strips were needed. Up to five consecutive stripping tapes were combined into one specimen and aliquots were measured for radioactivity after mixing with tissue solubiliser (Solvable). The skin membranes remaining after stripping were digested in Solvable and the radioactivity was determined by LSC. The diffusion cells were then washed with ethanol and the radioactivity in the cell wash was determined by LSC.

Results
The test item HAA299 (nano), i.e. micronised labelled and non-labelled HAA299 in a formulation, was shown to have a radiochemical purity of 98.49%, determined by HPLC at the time of application. The test item was shown to remain stable during the exposure period as indicated by the >95% radiochemical purity determined by HPLC analysis of the skin membrane rinses collected. Seven cells with the highest Kp were used for the study (except for one cell showing a distinctly high Kp value, i.e. 6.1 x 10^{-3} cm/h), assuming that the damage of the skin membrane led to an increase in permeability. Overall, the mean Kp value of the membranes chosen was 2.36 ± 0.23 $x10^{-3}$ cm/h. The applied dose was determined to be 2116 µg/cm^2. Based on test item found in perfusate the percutaneous penetration rate was very low or below reliably quantifiable concentrations as shown in Table 10.

Table 10. Skin absorption of HAA299 (nano) in pre-damaged human skin membranes

	Human Skin Membrane	
Applied Dose [µg/cm^2]	2116	
Applied Volume [µL]	13	
Application Area [cm^2]	0.64	
Concentration [mg/cm^3]	104.18	
Penetration within	% of dose	µg/cm^2
6 h	< 0.01	*0.037
12 h	< 0.01	*0.077
24 h	< 0.01	*0.165
Flux[µg/cm^2/h]- calculated	0.009*	
Flux[µg/cm^2/h]- estimated+	0.09+	
*value calculated from the measured dpm, all of which are below LOQ of about 0.09 µg-equivalents +estimated by replacing <LOQ values with the LOQ and calculating flux		

The mean flux reflecting the penetration rate under steady-state conditions could not be calculated as all measured values were below LOQ. However, using the LOQ values the calculation gives a worst-case scenario for the estimated flux of 0.09 µg/cm^2/h. The distribution and recovery of the labelled test item at test termination is summarized in Table 11.

Table 11. Distribution and recovery of HAA299 (nano) in pre-damaged human skin experiments

Recovery [% of Dose, SD given in brackets]*	
Skin Membrane:	**Human**
Applied Dose [µg/cm^2]	2116
Perfusates (0-24h)	<0.01 (0.0)#
Remaining Skin membrane	0.04 (0.09)
Total absorbed (%)	**0.04**
As µg a.i./cm^2	**0.85**
Skin membrane Rinse	82.45 (6.89)
Tape Strips	5.18 (3.83)
Diffusion cell wash	7.56 (3.78)
Recovery	**95.24** (2.64)
*Values are mean (± standard deviation) #Calculated from measured dpm values, most of which were below LQ of about 0.09 µg a.i. equivalents.	

Conclusion
The cumulative penetration of formulated HAA299 (nano) through pre-damaged human skin membrane was less than 0.01% of the applied dose during 24 hours of exposure. The calculated mean flux using the LOQ values was below 0.09 µg/cm^2/h through pre-damaged human skin membranes. For the pre-damaged human skin membranes, 82.45% of administered dose could be removed by the skin rinse after the exposure period. An additional amount of 5.18% was located in/on the stratum corneum and 0.04% was found in lower skin layer.

Ref: Harlan Ltd, CH-4452 Itingen, Switzerland, March 11, 2009

SCCS comment
The number of membranes and donors used for dermal absorption was lower than that recommended in SCCS Notes of Guidance for both rat und human skin samples. The SCCS notes that there is no standard protocol for the investigation of dermal penetration through damaged skin and no common understanding of "damaged skin", which could be wounded, physically damaged, sunburnt, etc. In this particular study, skin was artificially damaged by tape stripping (3 tape strips, removal of the upper 200 µm of the skin). However, the study indicates that *in vitro* penetration through tape stripped predamaged human skin might be lower than through rat skin *in vitro*.

***In vivo* rat dermal absorption study**

Guideline:	OECD Guideline TG 427
Species/strain:	Male Wistar (HanBrl:WIST (SPF) about 8 weeks old
Groups:	4 males, sacrificed after 6 h, 24 h, 48 h and 72 h
Test substance:	HAA299 (nano): FAT75'808, a water-based formulation of micronised HAA299 with [^{14}C] label and without label (FAT 75'808/D))
Batch:	VTM07B04 (not labelled), 3574052 (labelled)
Purity:	98.6% (non-radiolabelled test item)
Radiochemical purity:	97.7% (radiolabelled test item)
Mean particle size:	Radiolabelled test item

Number distribution: d(0.5): 64 nm and d(0.9): 94 nm
Volume distribution: d(0.5): 137 nm and d(0.9): 255 nm
Dose: 2.1 mg/cm²; 10 cm^2; 200 µl
Exposure conditions: Application for 6 hours; non-occluded
GLP: In compliance
Study period: 28 May – 30 Oct 2008

Methods
For the preparation of the final test item formulation HAA299 (nano), [^{14}C] labelled HAA299 was synthesized using ^{14}C-labelled Piperazine with a radiochemical purity >97% and specific activity of 3309 kBq/mg. The radiochemical was repurified and the final dosing suspension was prepared as a mixture of non-labelled and labelled HAA299 to give [^{14}C]-labelled HAA299 with a final specific radioactivity of about 20 kBq/mg. After removing residual solvent, the mixture was micronised in a micro-mill with additions of excipients and water using the process that is used to prepare the commercial product intended to be sold for formulation of consumer sunscreens.
The rats were dosed (200 µl, applied to an area of 10 cm² each) at one nominal dose level of 2.1 mg HAA299/cm^2. Sixteen male rats were assigned to groups consisting of 4 animals each. Before dosing, a double 'O'-ring, one glued on the top of the other, with an inside area of approximately 10 cm^2 (Ø 36 mm) was glued to the shaved skin using cyanoacrylate adhesive. The application suspension (200 µl) was applied to the skin inside the 'O'-ring using a syringe and spread evenly. In order to prevent uncontrolled loss of the test item the 'O'-ring was covered with a permeable tape (non-occlusive conditions).
The exposure time to the formulated test item was 6 hours for all animals. At the end of the exposure period the remaining test item was removed from the application site and collected by washing with mild soap solution (4 times) followed by one time with tap water using soft cotton swabs and then analysed for the radiochemical. Four animals each were sacrificed at 6, 24, 48, and 72 hours after start of application, respectively.
Urine and faeces were collected for the following intervals: 0-6, 6-24, 24-48, and 48–72 hours after application. The dermal penetration profile was also evaluated by skin stripping the dosed site after each group's termination. The upper skin layer, i.e. stratum corneum and the fur grown during the experiment were removed from the application site by gluing a tape with cyanoacrylate adhesive on the top of the treated skin area. After a drying period of about 10 minutes the tapes were snatched off from the application site. This procedure was repeated one to two times until the stratum corneum was removed from the application site. The tape strips of the treated skin area were combined to one specimen according to each animal and were dissolved in tissue solubiliaer. Termination of rats in each group was followed by radiochemical analysis of collected samples of blood, gastro-intestinal tract, carcass, and skin from a treated and non-treated area.

Results
The purity of the test item HAA299 (nano), i.e. micronised labelled and non-labelled HAA299 in a formulation, at the time of application was 98.1%. Stability of the test item formulation was evaluated by analysis of the skin wash samples, that revealed more than 99% of the radioactivity was found as unchanged HAA299, indicating the dermally applied [^{14}C]-HAA299 remained unchanged during the time of exposure. The applied dosage was 2128 µg/cm^2. The results are presented in Tables 12 and 13.

Table 12. Mass balance [µg HAA299 equivalents/cm²] at different time-points

	Dose Level	2127.9 µg/cm²			
	Sacrifice Time Point	6 h	24 h	48 h	72 h
Systemic Absorption	0-6 h	0.060	0.238	3.856	0.142
	6-24 h	-	0.501	7.253	1.052
	24-48 h	-	-	1.947	0.956
	48-72 h	-	-	-	2.260
	Cage Wash	0.063	0.204	0.364	0.613

	Residues	1.010	1.506	2.767	4.089
	Total	1.133	2.438	16.187	9.113
Application Site		27.264	17.115	24.833	22.294
Dislodged Dose		2089.419	2112.047	2083.162	2078.415
Sum		2117.817	2131.600	2124.181	2109.822
Penetration Rate [µg·cm^{-2}·h^{-1}] = 0.1889					

Table 13: Distribution of HAA299 (nano) after dermal application [% of dose]

Dose Level	2127.9 µg/cm²			
Sacrifice Time Point	6 h	24 h	48 h	72 h
Urine				
0 - 6 h	<0.01	<0.01	0.18	< 0.01
6 -24 h	-	0.01	0.28	0.04
24 - 48 h	-	-	<0.01	<0.01
48 -72 h	-	-	-	< 0.01
Subtotal	<0.01	0.02	0.47	0.05
Feces				
0 - 6 h	< 0.01	< 0.01	< 0.01	< 0.01
6 - 24 h	-	0.01	0.06	<0.01
24 - 48 h	-	-	<0.08	0.04
48 -72 h	-	-	-	0.10
Subtotal	< 0.01	0.01	0.14	0.16
Cage Wash	< 0.01	< 0.01	0.02	0.03
Total Excretion	**<0.01**	**0.04**	**0.63**	**0.24**
Residues				
Whole Blood*	< 0.01	< 0.01	< 0.01	< 0.01
Skin Non-Treated Area*	< 0.01	< 0.01	< 0.01	< 0.01
Gastrointestinal Tract	<0.01	0.01	0.04	0.10
Remaining Carcass	0.04	0.05	0.08	0.09
Subtotal	0.05	0.07	0.13	0.19
Systemic Absorption	**0.05**	**0.11**	**0.76**	**0.43**
Skin Stripping	1.27	0.80	1.16	1.04
Remaining Treated Skin	0.02	<0.01	<0.01	<0.01
Application Site	**1.28**	**0.80**	**1.17**	**1.05**
Skin Wash	94.43	91.48	93.34	95.11
Cover and O-Ring	3.76	7.77	4.56	2.57

The vast majority of the applied dose could be dislodged from the application site at the end of exposure (i.e. 97.68 - 99.26%). After the washing procedure, a maximum of 1.28% of the dose remained in/on the treated skin area. For this dose level, this remaining radioactivity in/on the application side was located mainly in the stratum corneum.
The amount of radioactivity determined in the stratum corneum remained roughly the same with ongoing time (0.80% at 24 hours, 1.16% at 48 hours, 1.04% at 72 hours). Only small amounts were determined in the lower skin layer (corium and subcutis), i.e. less than 0.02% of the dose level tested.
Dermal absorption was generally low. The amount totally absorbed during the 6-h exposure period was 1.133 µg/cm² corresponding to 0.05% (± 0.02%) of dose applied. The calculated penetration rate during the 6-hour exposure time accounted for 0.19 µg·cm^2/h. Dermal absorption after 24 h was 2.438 µg/cm² corresponding to 0.11% (± 0.05%). Within 72 hours, an increase in systemic absorption was observed with a maximum rate of 0.43% (± 0.26%). A higher systemic absorption (0.76% of the applied dose) was found within 48 hours, mainly due to excessively higher urinary excretion amounts of one animal (1.82%) compared to the other animals in this group, showing only 0.01-0.03% of the applied dose in urine. Thus, the biological relevance of this finding is questionable.
The concentrations of radioactivity in blood during exposure were below the limit of quantification (LOD, 0.180 ppm HAA299) at all sampling time points. The systemically absorbed dose was very low, however, a slow excretion via urine and faeces is indicated.

Conclusion
It was concluded that formulated HAA299 (nano) penetrated to a very low extent through rat skin after dermal application under the chosen testing conditions.

Ref: Harlan Ltd, CH-4452 Itingen, Switzerland, March 10, 2009

SCCS comment
The results presented in the Table 12 regarding "Total Systemic Absorption" after 48 h and in Table 13 urine excretion after 48 h is misleading. The excretion measured in the 4 rats was: 1.82, 0.03, 0.02, and 0.01 µg/cm² which gave a mean of 0.47 µg/cm² (S.D. = 0.90 µg/cm²). Thus, one rat (rat number 9) excreted about 100 times more than the other rats. The SCCS is of the opinion that the results (System Absorption = 0.76%) of the 48 h time point should be disregarded.

Applicants overall conclusion on dermal / percutaneous absorption
HAA299 (nano) with a mean d(0.5) of 55-65 (number distribution) or 140-150 nm (volume distribution), which represents a typical formulation and size distribution intended to be sold for the preparation of consumer sunscreens, showed a very limited dermal absorption in guideline *in vitro* studies with rat and human skin and in an *in vivo* study in rats.
The non-occluded application of HAA299 (nano) for 24 hours on rat skin resulted in an overall absorbed fraction of 0.52 % of the applied dose. In comparison, the application of HAA299 (non-nano, formulated) with a d(0.5) of 760 nm (volume distribution) in a similar formulation (water plus excipients) and under similar testing conditions showed the same overall absorbed fraction of 0.52 % (see RCC 2006, Nr. A67127; cited in SCCS/1533/14, A.3.3.4 (Ref. 12)). Thus, the differences in particle size (nano versus non-nano form) does not have any influence on the dermal absorption potential of HAA299 in rat skin *in vitro*. The *in vitro* findings for HAA299 (nano) were confirmed *in vivo*, showing an overall systemic exposure of 0.43 % of the applied dose within 72 hours after a 6-hour dermal exposure period at comparable test concentrations.
In vitro testing with human skin revealed a lower absorption of HAA299 (nano) compared to rat skin. When HAA299 (nano) was applied non-occluded to intact skin (Kp = 1.4 x 10-3 cm/h) for 24 hours, a very low absorbed fraction of 0.03 % was detected mainly in viable skin layers. In a comparable study, using pre-damaged skin by additional tape stripping (Kp = 2.36 x 10-3 cm/h), the absorbed fraction of applied HAA299 (nano) was comparable (0.04 %). HAA299 (non-nano with a similar formulation) with a d(0.5) of 760 nm (see above) showed a slightly higher absorption potential in human skin (overall absorbed fraction 0.13 %) than HAA299 (nano; see SCCS/1533/14, A.3.3.4 (Ref. 12)). Accordingly, the dermal absorption of HAA299 in general is found to be very low, and the nano form of HAA299 does not show a higher dermal absorption potential than the respective non-nano form on human skin *in vitro*.

SCCS overall comment on dermal / percutaneous absorption
Three *in vitro* studies and one *in vivo* study were submitted by the applicant. All studies were conducted in the same laboratory.
In the *in vitro* study using intact rat skin, the total absorption was 0.52 % after 24 h compared to 0.43 % (total excreted and remaining in the body) after 72 h in the *in vivo* rat study. Thus, based on the rat studies the dermal absorption is around 0.5 %.
In the *in vitro* study using intact human skin the absorption after 24 h was 0.03 % and in the *in vitro* study using damaged human skin the absorption was 0.04 %. It is noted that the amounts in the perfusates were <0.01 % in intact and pre-damaged human skin, respectively. The SCCS notes that at present there is no standard protocol for the investigation of dermal penetration through damaged skin and no common understanding of "damaged skin", which could be wounded, physically damaged, sunburnt, etc. Therefore, the

results of the study using damaged human skin will not be taken into account for this evaluation.
Based on the available data, the SCCS considers that systemic availability after dermal application is very low.

3.2.2 Toxicokinetics

***In vivo* rat oral absorption, distribution, and elimination**

Guideline:	OECD Guideline no. 417
Species/strain:	Male HanRcc:WIST (SPF): approx. 7 weeks old, approx. 200 g
Group size:	4 males
Test substance:	HAA299 (nano): FAT 75'808, a water-based formulation of micronised HAA299 with [14C] label and without label (FAT 75'808/D)
Batch:	VTM07B04 (not labeled), 3574052 (labeled)
Purity:	98.6% (non-radiolabeled test item)
Radiochemical purity:	97.7% (radiolabeled test item)
Mean particle size:	Radiolabeled test item Number distribution: d(0.5): 64 nm and d(0.9): 94 nm Volume distribution: d(0.5): 137 nm and d(0.9): 255 nm
Dose levels:	100 mg/kg bw (nominal dose); 107.9 mg/kg bw (actual dose mean); 106 - 110 mg/kg bw (actual dose range)
Dose volume:	3.5 ml/kg bw
Route:	Oral administration (gavage)
Administration:	Single dose
GLP:	In compliance
Study period:	8 - 30 Oct 2008

Methods
In vivo absorption, distribution, and elimination of HAA299 (nano), i.e. micronised HAA299 in a formulation, was evaluated over 96-hours after a single oral gavage dose in male Wistar rats. For the preparation of the final test item formulation HAA299 (nano), [14C] labelled HAA299 was synthesized using 14C labelled Piperazine with a radiochemical purity >97% and specific activity of 3309 kBq/mg. The radiochemical was repurified and the final dosing suspension was prepared as a mixture of non-labelled and labelled HAA299 to give [14C]-labelled HAA299 with a final specific radioactivity of about 20 kBq/mg. After removing residual solvent, the mixture was micronised in a micro-mill with additions of excipients and water using the process that is used to prepare the commercial product intended to be sold for formulation of consumer sunscreens.
An aliquot of about 1 g of the micro-suspension was diluted with about 8 g purified water to prepare the oral gavage mixture dosed at 3.5 ml/kg bw. Samples of the dosing formulation were analysed for particle size and a mean d(0.5) of 64/137 nm (number/volume distribution) was determined. A group of 4 rats received a nominal dose of 100 mg/kg bw (mean actual dose administered = 107.9 mg/kg bw) by oral gavage.
Urine and faeces samples were collected from each rat during four 24-hour periods. Blood and plasma were collected from each animal 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hours after dosing. The study was terminated 96-hours after dosing and samples of liver, kidney, renal fat and muscle and the remaining carcass were retained and processed for determination of radioactivity. For recovery analyses, cages were rinsed separately, and the cage washes were analysed for radioactivity.

Results
All four animals survived the study period, gained weight, and did not show signs of toxicity or adverse effects. The concentrations of radioactivity determined in blood or in any tissue or organ investigated generally were below the limit of quantification. When considering the concentrations measured, the overall mean fraction in carcass and tissues accounted to about 0.03% of the applied dose. A mean fraction of 0.04% of the total amount administered was excreted via urine, whereas excretion by faeces accounted to 97.30% (Table 13). Within 48 hours after administration, almost the complete dose was excreted via faeces, accounting for 97.27 % of the applied dose. The mean total recovery was 97.37% of administered radioactivity during the study period.
Based on the radioactivity excreted with the urine and the remaining radioactivity in the carcass and tissues, the extent of absorption of HAA299 accounted for only 0.07 % of the administered dose.

Table 14: Excretion of radioactivity after single oral administration of [14C] FAT 75´808 to male rats

Excretion [% of dose]		
Urine		
	0 - 24 h	**0.03**
	24 - 48 h	**<0.01**
	48 - 96 h	**<0.01**
	Subtotal	**0.04**
Feces		
	0 - 24 h	**90.73**
	24 - 48 h	**6.54**
	48 - 96 h	**0.03**
	Subtotal	**97.30**
Cage Wash		**<0.01**
Total Excretion		**97.35**

Conclusion
After oral administration of HAA299 (nano), i.e. micronised labelled and non-labelled HAA299 in a formulation, the test substance was poorly absorbed from the gastro-intestinal tract, and the extent of absorption accounted to only 0.07 % of the administered dose. HAA299 was predominantly excreted with the faeces, whereas only 0.04 % of the dose was excreted with the urine. All selected tissues and organs did not show significant quantities of HAA299. Thus, absorption of orally administered HAA299 (nano) from the gastrointestinal tract was minimal, which resulted in a low bioavailability.

Ref: Harlan Ltd, CH-4452 Itingen, Switzerland, March 05, 2009

Applicants overall conclusion on toxicokinetics
HAA299 (nano) with a mean d(0.5) of 64/137 nm (number/volume distribution), representing a typical formulation and size distribution intended to be sold for the preparation of consumer sunscreens, was poorly absorbed from the gastro-intestinal tract after oral (gavage) administration to male rats at 100 mg/kg bw. The extent of absorption according to the fraction detected in urine, tissues and carcass accounted to only 0.07 % of the administered dose. In comparison, single oral administration of HAA299 (non-nano, formulated) with a d(0.5) of 8.33 µm (volume distribution) in 0.5% carboxymethyl cellulose and 0.4% Tween 80 at the same dose to male rats, resulted in a higher absorption rate of 1.93 % (1.75% in urine and 0.18% in the carcass) of the applied dose (Harlan 2009, No. A89291; cited in SCCS/1533/14, A.3.3.9 (Ref. 13)). However, based on the low purity of the radiolabelled test item in the application medium (91.4%), the absorbed radioactivity was likely caused by impurities and the actual absorption of HAA299 may have been significantly lower than the

fraction detected in this study. Although, the fraction of HAA299 (non-nano, formulated) absorbed is not clearly established, bioavailability of nano and non-nano forms of HAA299 can be considered as very low after oral exposure. Furthermore, systemic exposure of HAA299 at toxicologically relevant doses is not to be expected independent from its form as nano or non-nano material.

SCCS comment
The SCCS notes that that oral absorption of HAA299 (nano)-related radioactivity in vivo in rats is very low (0.07% of the administered radioactivity).

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

The following information was provided by the Applicant:
HAA299 (nano) is a formulated micronised UV filter intended to be used in sunscreen products as skin protectant against UVA1-visible rays. The HAA299 active ingredient will be commercialised in a water-based preparation, as it is most effective as an UV filter when it is milled to a smaller particle size, a process we refer to as micronisation. This micronisation process results in HAA299 (nano), a formulation containing the nano form of the active UV filter HAA299 and excipients. The energy intensive, proprietary micronisation process yields an aqueous dispersion of HAA299 nanoparticles with median particle size of >100 nm according to volume evaluation and of > 50 nm according to number evaluation.

3.3.2 Calculation of the SED/LED

The systemic exposure dose (SED) is not calculated because it is likely to be very low, considering the very low dermal/oral absorption.

The estimated daily exposure shown below is in terms of externally applied amount of a typical sunscreen formulation:

Amount of sunscreen applied daily	18 g
Concentration HAA299 (nano) in sunscreen	10 %
Estimated daily exposure	**1.8 g/d**

3.4 TOXICOLOGICAL EVALUATION

3.4.1 Irritation and corrosivity

3.4.1.1 Skin irritation

Irritation test Reconstructed Human Epidermis (RhE) according to OECD 439

Guideline/method:	OECD TG 439 (July 2015), Commission Regulation (EU) No 640/2012, B.46
Name of test substance:	C-1332 (micronised)
Test-substance No.:	16/0349-1
Batch identification:	RIN195_9

CAS No.:	919803-06-8
Content:	w (active ingredient): 50.2 % water content: 43.2 g/100 g
Physical state / color:	Liquid / white, milky
Identity:	Confirmed
Homogeneity:	The homogeneity was provided by stirring the test substance intensely with a spatula.
Storage stability:	The stability under storage conditions over the study period was guaranteed by the sponsor, and the sponsor holds this responsibility. The test facility is organizationally independent from the BASF SE sponsor division.
RhE model:	EpiDerm™ 200 kit, Skin Irritation Test, 24 EPI-200 tissues (reconstructed epidermis): surface 0.6 cm² cultured in Millicells® Ø 1cm
Supplier:	MatTek *In Vitro* Life Science Laboratories, Bratislava, Slovakia
Group size:	3
Test substance:	C-1332 (micronised), substance No. 16/0349-1
Batch:	RIN195_9
Appearance:	Liquid/white, milky of which homogeneous suspension was provided by stirring intensely
Purity:	50.2% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 65 nm and d(0.9): 111 nm Volume distribution: d(0.5): 110 nm and d(0.9): 181 nm
pH:	~9.
Vehicle:	applied undiluted as 50.2% dispersion in water
Positive control:	5% (w/v) sodium dodecyl sulfate in water
Negative control:	PBS, sterile
Storage conditions:	Room Temperature
Dose:	30 µL of the undiluted test item, positive or negative controls
Exposure time:	One hour
Post incubation period:	42 hours
Route:	Topical application on skin construct
Read out:	Measurement metabolic activity by reduction of mitochondrial dehydrogenase activity, measured by reduced formazan production after incubation with a tetrazolium salt (MTT).
Supplier:	MatTek *In Vitro* Life Science Laboratories, Bratislava, Slovakia / Sigma, Germany
Read out time:	After post incubation of 42 hours
Controls:	Negative control (PBS, sterile), Positive control (5% (w/v) sodium dodecyl sulfate in water), product control on MTT reduction.
GLP:	In compliance
Date report:	05 October 2017
Study period:	11 Oct – 14 Oct 2016
Published:	No

Methods
The test item HAA299 (nano), i.e. micronised HAA299 in a formulation also named C-1332 (micronised), was applied on the EpiDerm™ three-dimensional human epidermis model. Three tissues were treated with the test item, the positive control (5% (w/v) sodium dodecyl sulfate in water) and the negative control (PBS, sterile), respectively. Thirty microliters of the undiluted test item, positive or negative controls were concurrently applied using a pipette. A nylon mesh was placed carefully onto the tissue surface afterwards. The tissues were kept under the laminar flow hood at room temperature for 25 minutes overall and for 35 minutes in the incubator. The tissues were washed with sterile PBS to remove residual test material 1 hour after start of application. Rinsed tissues were blotted on sterile absorbent paper and transferred into new 6-well plates, pre-filled with 0.9 mL fresh medium. When all tissues were rinsed, the surface of each tissue was carefully dried with a sterile cotton swab. Subsequently,

the tissues were incubated (37°C) for 24 ± 2 hours. Then, tissues were transferred into new 6-well plates pre-filled with 0.9 mL of fresh medium and placed into the incubator for additional 18 ± 2 hours post-incubation period. Finally, the assay medium was replaced by 0.3 mL MTT solution and the tissues were incubated in the incubator for 3 hours. After incubation, the tissues were washed with PBS to stop the MTT-incubation. The formazan formed was extracted by incubation of the tissues in isopropanol. The optical density at a wavelength of 570 nm (OD_{570}) of the extracts was determined spectrophotometrically. Blank values were established with isopropanol for each microtiter plate.
The irritant potential of the test item was predicted from the mean relative tissue viabilities compared to the negative control tissues concurrently treated with sterile PBS. A chemical is considered as "irritant", if the mean relative tissue viability with a test material is less than or equal to 50%, whereas borderline results (45%-55%) need to be reassessed by repetition(s) of the test. To exclude direct MTT reduction, the test item was added to 0.9 mL MTT solution and the mixture was incubated in the dark at about 37 °C for 3 hours. A negative control (de-ionized water) was tested concurrently.

Results
The relative mean viability of the test item-treated tissues determined after exposure with the test item was 69.5% (Table 15). Application of the positive control showed a relative mean viability of 3.3% and the mean OD_{570} of the negative control fulfil the acceptance criteria, which overall confirms the validity of the chosen testing conditions.

Table 15: Relative viability of EpiDerm™ tissue samples

Group	**Relative viability (mean ± SD, n = 3) [% of NC]**
NC (PBS)	100 ± 8.8
HAA299 (nano)	69.5 ± 15.6
PC (5% SDS)	3.3 ± 0.3

n: number of samples, NC: negative control, PBS: Phosphate buffered saline, PC: positive control, SD: standard deviation, SDS: Sodium dodecyl sulfate.

Conclusion
Based on the observed results, the test item HAA299 (nano), i.e. micronised HAA299 in a formulation, does not show a skin irritation potential in the EpiDerm™ *in vitro* skin irritation test under the test conditions chosen. Based on this validated stand-alone *in vitro* test, HAA299 (nano) is not expected to be irritating to skin when applied undiluted. Overall, HAA299 (nano) is considered non-irritating to the skin under the given use conditions.

Ref: BASF SE, 05.10.2017

SCCS comment
The assay performed is in accordance with the SCCS acceptance criteria. One of the three tissues exposed to the test substance showed a low viability of 53%. However, the overall mean viability is 69.5% (SD 15.6%), which is within the acceptance criteria.
Although the assay with the RhE model is not yet validated/evaluated for nanomaterials, the SCCS considers the results of this assay plausible, and considers that the test substance is not an irritant under the test conditions used.

3.4.1.2 Mucous membrane irritation / eye irritation

Bovine Corneal Opacity and Permeability Test (BCOP Test)

Guideline:	OECD Guideline no. 437
Test type:	Bovine Corneal Opacity and Permeability Test (BCOP Test)
Test substance:	HAA299 (nano)
Batch:	RIN195_9
Purity:	50.2% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 65 nm and d(0.9): 111 nm Volume distribution: d(0.5): 110 nm and d(0.9): 181 nm
Vehicle:	applied undiluted
Positive control:	100% ethanol / 100% dimethylformamide
Negative control:	de-ionized water
GLP:	In compliance
Study period:	03 Nov 2016

Methods
Isolated corneas (free of defects such as opacity, scratches, pigmentation etc.) from bovine eyes were treated with the test item HAA299 (nano), i.e. micronised HAA299 in a formulation (also named C-1332 (micronised)). Before treatment, corneas were mounted in a vertical position in cornea holders consisting of anterior and posterior chambers filled to excess with pre-warmed Eagles's MEM (without phenol red) at about 32 °C. After an equilibration period the medium was replaced with fresh pre-warmed medium and initial corneal opacity readings were taken for each cornea with an opacitometer. Corneas, showing macroscopic tissue damage or an opacity value < 550 opacity units were discarded. Each treatment group (test item, positive and negative controls) consisted of 3 corneas. After removal of the medium in the anterior chamber, 750 µL of the undiluted liquid test item, de-ionized water (negative control) or 100% ethanol / 100% dimethylformamide (positive controls) were applied directly to the epithelial surface of the cornea using a pipette (open chamber method). The corneas were incubated in a horizontal position at about 32 °C for approximately 10 minutes. Positive and negative controls were then removed, and the epithelium was washed at least 3 times with Eagle's MEM (containing phenol red) and once with Eagle's MEM (without phenol red). Both chambers were then refilled with fresh Eagle's MEM (without phenol red). Because the test item could not be removed by a syringe, the epithelium was rinsed with the open chamber method. The corneas were incubated for further 2 hours at about 32 °C.
Corneal opacity readings were taken for each cornea with an opacitometer after a visual assessment. For determination of permeability, the medium in the anterior chamber was replaced by 1 mL sodium fluorescein solution (4 mg/mL) and incubated for 90 ± 5 min in a horizontal position at about 32 °C. The amount of sodium fluorescein that permeated through the corneas into the posterior chamber was measured spectrophotometrically. Three aliquots per cornea were transferred to a 96-well microtiter plate and the optical density (OD490) was determined. The mean corneal opacity and permeability values of each treatment group were used to calculate an *In Vitro* Irritancy Score (IVIS). An IVIS > 65 results in a prediction of an ocular corrosive or a severe irritant.

Results
The mean corneal opacity and permeability values and the calculated IVIS values are given in Table 16. The mean IVIS of the group treated with the test item was 8.0 and did not identify HAA299 (nano) to pose a risk of serious damage to eyes. Treatment with the positive control ethanol resulted in opacity, permeability and IVIS values within a 2 SD range of the Laboratory's historical mean value, meeting the acceptance criteria and confirming the validity of the test conditions chosen. The mean permeability value of the positive control dimethylformamide was slightly out of the historical 2 SD range (0.345-0.824). However, as all other quality criteria of the test were met, this deviation is not considered to have any influence on the validity of the study.

Table 16: Opacity, permeability and *in vitro* irritancy score (IVIS) after 10 minutes of exposure followed by a 2-hour post incubation period

Group	**Mean opacity change (± SD; n = 3)**	**Mean permeability score (± SD; n = 3)**	**Mean IVIS (± SD; n = 3)**
HAA299 (nano)	5.3 ± 2.0	0.181 ± 0.070	8.0 ± 2.3
NC (de-ionized water)	4.2 ± 3.4	0.003 ± 0.001	4.2 ± 3.4
PC (Ethanol)	27.9 ± 1.9	0.822 ± 0.314	40.2 ± 2.8
PC (DMF)	101.2 ± 3.1	0.888 ± 0.318	114.5 ± 6.5
[1] n: number of samples, NC: negative control, PC: positive control, SD: standard deviation			

Conclusion
Based on the observed results, the test item HAA299 (nano), i.e. formulation of micronised HAA299, does not cause ocular corrosion or severe irritation in the Bovine Corneal Opacity and Permeability Test (BCOP Test) under the test conditions chosen. However, the chosen test method does not allow for the evaluation of eye irritation, and the result does not exclude a putative irritation potential of the test item.

Ref: BASF SE, 2017

EpiOcular™ Eye Irritation Test

Guideline:	OECD Guideline no. 492
Test system:	EpiOcular™
Test substance:	HAA299 (nano)
Batch:	RIN195_9
Purity:	50.2% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 65 nm and d(0.9): 111 nm Volume distribution: d(0.5): 110 nm and d(0.9): 181 nm
Vehicle:	applied undiluted
Controls:	positive control (methyl acetate); negative control (sterile de-ionized water)
GLP:	In compliance
Study period:	18 Oct – 20 Oct 2016

Methods
The test item HAA299 (nano), i.e. micronised HAA299 in a formulation (also named C-1332 (micronised)), was applied on the EpiOcular™ three dimensional non-keratinized tissue construct composed of normal human derived epidermal keratinocytes used to model the human corneal epithelium (OCL-200 Tissue; Lot 23740; MatTek *In Vitro* Life Science Laboratories, Bratislava, Slovakia). Two tissues were treated with each: the test item, the positive (methyl acetate) or the negative (sterile de-ionized water) control. After a pre-incubation period, the tissues were pre-treated with 20 µL of PBS in order to wet the tissue surface. The tissues were incubated at standard culture conditions at 37°C for 30 minutes. Using a pipette, 50 µL of the undiluted liquid test item, the negative or the positive control was applied covering the whole tissue surface. After application, the tissues were placed into the incubator until the total exposure time of 30 minutes was completed. To remove the test item, the tissues were washed with sterile PBS and immersed into 12-well plates, pre-filled with 5 mL/well pre-warmed medium (post-soak immersion). After 12 minutes, each tissue was dried on absorbent paper and transferred to fresh 6-well plates filled with 1 mL/well pre-warmed medium. For post-incubation, the tissues were incubated at standard culture conditions for 2 hours. After the post-incubation period, tissues were incubated with 0.3 mL MTT solution for 3 hours before the tissues were washed with PBS to stop the MTT-incubation. The formazan formed was extracted by incubation of the tissues in isopropanol. The optical density at a wavelength of 570 nm (OD570) of the extracts was determined

spectrophotometrically. Blank values were established with isopropanol for each microtiter plate.
The irritant potential of the test item was predicted from the mean relative tissue viabilities compared to the negative control tissues concurrently treated with sterile water. A chemical is considered as "irritant", if the mean relative tissue viability with a test material is less than or equal to 60%, whereas borderline results (55%-65%) need to be reassessed by repetition(s) of the test. To exclude direct MTT reduction, the test item was added to 0.9 mL MTT solution and the mixture was incubated in the dark at about 37 °C for 3 hours. A negative control (de-ionized water) was tested concurrently.

Results
The mean viabilities of the different dose-groups are summarized in the Table 17. The mean viability of the test-substance treated tissues was 47.1%. All the acceptance criteria were met in this test, and the positive control demonstrated appropriate sensitivity of the tissues used under test conditions (relative viability < 50%, expected tissue viability of approximately 25%), which overall confirms the validity of the chosen testing conditions.

Table 17: Relative viability of EpiOcular™ tissue samples

Group	**Relative viability**	
	Mean viability (n = 2) [% of NC]	**Inter-tissue variability [%]**
NC (de-ionized water)	100	12.4
HAA299 (nano)	47.1	15.6
PC (Methyl acetate)	27.8	3.7

n: number of samples, NC: negative control, PC: positive control

Conclusions
Based on the observed results, it was concluded, that test item HAA299 (nano), i.e. micronised HAA299 in a formulation, shows an eye irritation potential in the EpiOcular™ *in vitro* eye irritation test under the test conditions chosen.

Applicants overall conclusion on mucus membrane irritation
HAA299 (nano) has been tested in two *in vitro* tests, i.e. a BCOP and an EpiOcular™ test. Based on the IVIS score of 8.0 in the BCOP, HAA299 (nano) was identified to be no ocular corrosive or severe irritant. However, this score was within the range (> 4.5-<45), representing a group for which no prediction can be made for eye irritation, making further testing with another suitable method necessary. As a follow-up study, application of HAA299 (nano) in the EpiOcular™ test resulted in a mean viability of 47.1%, which identified the formulation tested to have a potential for eye irritation (<=60% viability).
For comparison of the eye irritation potential between the nano and non-nano form of HAA299, an identical formulation containing non-micronised HAA299 and the same excipients, was tested in a BCOP and in an EpiOcular™ test under comparable testing conditions. Both, the IVIS score (4.1; predicted as borderline) and the mean viability (45.3%, predicted as eye irritant) of HAA299 (non-nano, formulated) was comparable to the results obtained for the formulation using the nano form of HAA299.
Overall, it is concluded, that the eye irritating effects of HAA299 (nano) results from excipients of the formulation and not from the active UV filter ingredient HAA299. Furthermore, the presence of HAA299 in a nano or a non-nano form does not have any impact on its eye irritation potential.

SCCS comment
Although it is possible that the eye irritation potential is due to the excipients, the test findings do not rule out an eye irritation potential from the HAA299 (nano) itself.

3.4.2 Skin sensitisation

No skin sensitization study is available for HAA299 (nano).
However, according to the Applicant, when considering the skin sensitization data of HAA299 neat and the very limited dermal absorption capacity of both the nano and non-nano form of HAA299, HAA299 (nano) is not considered to induce skin sensitization. It is currently unclear, if HAA299 neat is not a skin sensitizer due to the lack of a dermal penetration potential to reach the cellular targets of the immune system or due to the absence of an intrinsic potential to activate the immune system. However, the risk of skin sensitization due to HAA299 (nano) is low, when used as a UV-filter in cosmetic products in a concentration up to a maximum of 10 %.

SCCS comment
No skin sensitisation study has been provided for HAA299 nano whereas the data from LLNA on non-nano form have shown lack of skin sensitisation. A stable dispersion can be tested in an *in vitro* skin sensitisation test, but this has not been validated for nano particles and the experience with such assays for nanomaterials is yet extremely limited.
Although the argument used by the Applicant to consider skin sensitisation data from non-nano form to extrapolate to HAA299 (nano) is not supported, the SCCS agrees that the extremely low dermal penetration and the large molecular size of HAA299 makes it unlikely to be a skin sensitiser.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

Guideline:	OECD Guideline no. 423
Species/strain:	Sprague-Dawley female rats, 8 weeks old
Group size:	3 groups with 3 females
Test substance:	FAT 75'808/G: HAA299 (nano)
Batch:	MGU 799
Purity:	51% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 84 nm and d(0.9): 123 nm Volume distribution: d(0.5): 138 nm and d(0.9): 207 nm
Vehicle:	Water
Dose levels:	One group received 300 mg/kg bw and two groups received 2000 mg/kg bw (expressed as active ingredient HAA299), total particles 2.420, surface area 14 m^2
Administration:	Oral gavage (10 ml/kg bw)
1 GLP:	In compliance
Study period:	4 Mar – 1 Apr 2008

Methods
The test item HAA299 (nano), i.e. micronised HAA299 in a formulation (also named FAT 75'808/G), was prepared in purified water as test vehicle and was administered by oral gavage with a volume of 10 ml/kg body weight to 3 groups of three fasted female Sprague-Dawley rats. The dose is expressed as active ingredient HAA299 and is corrected for the 51% active material content of the test item. All dosed animals were observed up to 14 days after dosing for clinical signs, mortality, and body weight gain and then subjected to necropsy.

Results
Mortality and clinical signs of toxicity did not occur during the study in any of the animals. When compared to historical control data, a slightly lower body weight gain was noted between day 1 and day 8 in 1 female given 300 mg/kg and in 1 female given 2000 mg/kg (returning to normal afterwards) and between day 8 and day 15 in 1 female given 300 mg/kg. The body weight gain of the other animals given 300 or 2000 mg/kg was not affected by the treatment with HAA299 (nano). At necropsy, no apparent abnormalities were observed in any animals.

Conclusion
Under the experimental conditions of this study, the oral LD50 of HAA299 (nano), i.e. the formulated and micronised nano UV active ingredient HAA299, was higher than 2000 mg/kg in rats.

Ref: CIT F-Evreux. (27 February 2009)

SCCS comment
The SCCS has noted that the acute oral LD50 of HAA299 (nano) is >2000 mg/kg in rat.

3.4.3.2 Acute inhalation toxicity

Guideline:	OECD Guideline no. 403
Species/strain:	Wistar [HanRcc:WIST(SPF)], male rats (9 weeks old) and female rat (10 weeks old)
Group size:	15 males and 15 females
Test substance:	FAT 75'808/H: HAA299 (nano)
Batch:	MGU 814, LA 2397_37
Purity:	50.1% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 75 nm and d(0.9): 106 nm Volume distribution: d(0.5): 136 nm and d(0.9): 241 nm Mass median aerodynamic diameters (MMADs) = 1.0 µm
Vehicle:	Water excipients
Dose levels:	20% FAT 75'808/H or 20% FAT 75808 placebo plus 80% purified water, the target concentration as active ingredient of UV filter HAA299 was 10%. Median droplet volume: 5.24×10^{-13}. Particles a.i./droplet 9.27×10^{7}
Administration:	4 hour, nose-only inhalation, mean aerosol concentration of 4.721 mg/L air
GLP:	In compliance
Study period:	21 Apr – 6 May 2008

Methods
The test item HAA299 (nano), also named FAT 75'808/H is a formulation containing 50.1% HAA299, which is prepared by micronisation with further excipients. A placebo group, receiving the formulation without the UV active ingredient HAA299 (FAT 75'808 placebo also named FAT 75'808/I), was used to identify any effects related to the excipients as such. For animal application, the test item HAA299 (nano) and the placebo formulation were prepared as aqueous dilutions at a ratio (w/w) of 20% FAT 75'808/H or 20% FAT 75808 placebo plus 80% purified water. The target concentration of the active ingredient HAA299 was 10%.
Both the test item and placebo aerosols were generated at ambient conditions using a cyclone glass atomiser that was operated at maximum throughput. Animals were confined separately in restraint tubes that were positioned radially in the nose-only, flow-past exposure chamber. The test system was Wistar rats. At the beginning of the experimental phase, male animals were 9 weeks old and females were 10 weeks old. The study did also examine lung inflammatory response markers via broncho-alveolar lavage fluid (BALF) sampling.

In each dose group, animals were subdivided in three satellite groups of five male and five female animals. The first satellite group was sacrificed about 14 hours post end of exposure for broncho-alveolar lavage fluid (BALF) and plasma sampling, the second being assigned to interim pathology at approximately 24 hours post end of exposure (test day 2), and the third being assigned to pathology at 14 days post exposure (test day 15). All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. until interim sacrifice or over a 15-day observation period. Body weights were recorded prior to exposure on test day 1 in all animals, and during the observation period on test days 4, 8 and 15 in all animals assigned to sacrifice on day 15.
The BALF examinations comprised total and differential cell counts and the determination of total protein, TNFα and IL-6. In addition, total protein was determined in blood plasma from the animals assigned to BALF sampling. Pathology examinations comprised complete macroscopic pathology, the determination of lung weight and histopathology of the lungs and tracheobronchial lymph nodes on days 2 and 15.

Results
There were no clinical signs, no premature deaths and no effects on body weight during the study. In BALF collected 24 hours after end of exposure, total cell count (neutrophil numbers), total protein and TNFα were distinctly and statistically significantly higher in both sexes of test item-treated animals than in placebo control animals, while total protein levels in plasma did not distinguish the two groups. The changes in BALF were consistent with the histopathology findings of diffuse alveolar histiocytosis seen in all test item-treated animals assigned to interim pathology at approximately 24 hours post end of exposure (test day 2). These findings were occasionally accompanied by minimal granulocytic infiltration.
By test day 15, these histopathology findings were no longer evident. In addition, on test day 2, lung weights and lung to terminal body weight ratios were slightly but statistically significantly higher in both sexes of test item-treated animals compared control animals. By test day 15, statistically significant differences were limited to lung to terminal body weight ratio in females of the test item group. These differences were considered as of minor toxicological relevance in the absence of any histopathological findings on test day 15.
Macroscopic pathology findings attributable to treatment with the test item HAA299 (nano) or placebo were not evident. Increases of total cell count (neutrophil numbers) and total protein in BALF and of absolute and relative lung weight, and the histopathology findings of diffuse alveolar histiocytosis and minimal granulocytic infiltration seen in test item group on test day 2 were attributed to the treatment with the test item. Increase in neutrophil numbers in BALF on test day 2 was considered as indicative of an inflammatory reaction.
According to the Applicant, pulmonary inflammatory responses are not expected with use of HAA299 (nano) in sunscreens or other products from spray-on dispensers. Spray applicators are generally pump-type dispensers with some increase in marketed products using fine-spray aerosol type dispensers. In each of these applicators, the droplet sizes (aerosol) are designed to be at least 30 times larger than those used in this rat inhalation test. However, toxicological studies assessing the repeated inhalative exposure to HAA299 (nano) are currently not available.

Conclusion
For HAA299 (nano), it is concluded that the inhalation LC50 is greater than the highest technically achievable aerosol concentration level of 4.7 mg/l air. A notable but reversible lung inflammatory response occurred but is considered a normal non-allergenic type response to exposure to particulate material.

SCCS comment
Although the inflammatory response was reported as reversible, the study used single exposure for 4 hours, and the conclusions have not taken into account the likely repeated applications (that would be the case when the substance was used in sprayable sunscreen formulations). Therefore, based on the submitted data, the SCCS cannot exclude concerns in regard to use in sprayable products.
Units for 'median droplet volume' should be provided.

3.4.4 Repeated dose toxicity

3.4.4.1 Repeated dose (28 days) dermal toxicity

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3.4.4.2 Repeated dose oral toxicity

Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Guideline:	OECD Guideline no. 422
Species/strain:	Sprague-Dawley Crl CD® Rj Han: SD, 10 weeks old
Group size:	5 groups with 10 males and 10 females
Test substance:	FAT 75'808/E and FAT 75'808/G: HAA299 (nano)
Batch:	MGU 789 and MGU799
Purity:	51% HAA299 (active) and excipients
Particle size:	FAT 75'808/E Number distribution: d(0.5): 65 nm and d(0.9): 89 nm Volume distribution: d(0.5): 134 nm and d(0.9): 202 nm FAT 75'808/G Number distribution: d(0.5): 84 nm and d(0.9): 123 nm Volume distribution: d(0.5): 138 nm and d(0.9): 207 nm
Vehicle:	Water
Dose levels:	0, 100, 500 and 2000 mg/kg bw/day, respectively, or as active ingredient (HAA299) 0, 50, 250 and 1000 mg/kg bw/day
Treatment duration:	for the males: - for 15 days before mating, - during the mating period (up to 3 weeks), until sacrifice (i.e. at least 4 weeks in total) for the females: - 15 days before mating, - during the mating period (up to 3 weeks), - during pregnancy and lactation, until day 5 post-partum
Administration:	Oral gavage (5 ml/kg bw). pH = 6 in all formulations tested
GLP:	In compliance
Study period:	22 Jan – 25 Mar 2008

Methods
The objective of the study was to evaluate the potential systemic and reproductive toxic effects of the test item HAA299 (nano, following daily oral gavage administration to male and female rats from before mating, through mating and, for the females, through gestation until day 5 post-partum during lactation period. Dosage formulations of the test item were prepared as suspensions in the vehicle (purified water).
A placebo group was used to identify any effects related to the excipients. A second control group of 10 males and 10 females was treated with distilled water for comparison with placebo-treated group or test-item treated groups.
10 weeks old sexually mature Sprague-Dawley rats were used in the study. Each of the 5 study groups received randomly assigned 10 animals per sex.
Clinical signs and mortality were checked daily. Detailed clinical observations were performed once before the start of the dosing period and then weekly. Body weight and food consumption were recorded weekly until mating and then at designated intervals throughout gestation and lactation. A Functional Observation Battery including motor activity was performed at the end of the study on the first five males and the first five females to deliver in each group. Blood

samples were taken, and urine was collected from the same animals for analysis of hematology, blood biochemistry and urinary parameters at the end of the study.
The parent males were sacrificed 2 weeks after the end of the mating period and the parent females were sacrificed on day 6 post-partum.
A complete macroscopic post-mortem examination was performed on all animals.
The body weight and principal organ weights (adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes, and thymus) were recorded and selected organs/tissues were preserved from first 5 sacrificed as scheduled males and females. A microscopic examination was performed on selected organs for five males in control and high-dose groups, with particular attention paid to the male gonads for spermatogenesis staging and morphological structure. Furthermore, microscopic examinations on selected organs from the first five females to deliver in the control purified water, placebo mix and high-dose groups and on all macroscopic lesions were performed.

Results
There were no unscheduled deaths at any dose-level. Six mated females, one given the placebo, one treated at 50 mg/kg bw/day, two treated at 250 mg/kg bw/day and two treated at 1000 mg/kg bw/day were sacrificed on day 25 post-coitum due to absence of delivery.
Sporadic clinical signs were observed and represented hypersalivation (ptyalism) in 1/10 males and 2/10 females at 1000 mg/kg/day and 1/10 males at 250 mg/kg/day active ingredient HAA299. Reflux at dosing was observed in 2/10 females at 1000 mg/kg/day and in 1/10 females at 250 mg/kg/day, 2/10 in the placebo and 1/10 in the purified water control groups, respectively. Loud breathing was observed in 1/10 females given 1000 mg/kg/day on lactation days 4 and 5. All clinical signs are considered to relate to treatment with the test item, however they are non-adverse in view of the very limited incidence and the fact, that they were isolated. Consequently, there were no clinical signs observed during the study that could be considered to have toxicological importance.
In all HAA299 (nano) treated groups, mean body weights and body weight gains values were similar to controls given purified water. There were no treatment-related effects on haematology, blood biochemistry or urinary parameters.
Only very rare exceptions of abnormal scores or presence/absence of abnormal/normal behaviour were recorded during the Functional Observation Battery. As these effects were isolated and without dose-relationship they were not considered to give evidence of neurotoxicity or to be related to treatment with the placebo mix or the test item.
At the post-mortem examinations of the F0 generation parent animals, test item treatment-related macroscopic observations were not revealed. None of the differences in organ weights noted between the test item-treated and the placebo groups were considered to have any toxicological importance. The organ weights of test-item treated animals showed some statistically significant differences from the placebo control group among males and females. However, the differences could not be clearly assigned to the test item as they did not show a relationship to dose. Importantly, in all cases, histological evidence for an adverse effect was not found.
No treatment-related histopathological findings were noted. All the microscopic findings encountered were recognised as commonly observed changes in the untreated rat of this strain and age kept under laboratory conditions. Moreover, their incidence, severity and morphological characteristics were approximately similar in both control and treated animals and showed no indication of treatment or dose-relationship.
Minimal to marked, generally unilateral, seminiferous tubule atrophy was recorded in 1 placebo group rat, in 1 low dose rat (50 mg/kg bw/day) and 2 high dose rats (1000 mg/kg bw/day). This change was considered unrelated to treatment with the test item as it is considered as a spontaneous change occasionally seen in untreated rats, that it was poorly dose-related, generally unilateral, and also noted in one rat given placebo mix.

Conclusion
When administered daily at dose-levels of 50, 250 or 1000 mg a.i./kg/day by oral gavage to male and female Sprague-Dawley rats, for 2 weeks before mating, during mating, gestation and until day 5 post-partum, HAA299 (nano), i.e. micronised HAA299 in a formulation, did

not elicit signs of systemic toxicity. Non-adverse clinical signs of hypersalivation, reflux at dosing and loud breathing were sporadically observed at 250 and/or 1000 mg a.i./kg/day. No histopathological lesions considered to be reflective of systemic toxicity were observed. Based on the experimental conditions of this study, the highest dose-level tested (1000 mg a.i./kg/day) was determined as No Observed Adverse Effect Level (NOAEL).

Ref: CIT F-Evreux, 20 March 2009

SCCS comment
In this repeated dose toxicity study (45 days), one male rat, in the highest dose, was found to show some deviation of the control parameters indicative for the presence of an inflammatory reaction (increase in WBC and PMN) while other blood values were within normal ranges. However, the histopathology did not show any organ with a severe inflammation that might explain the WBC, PMN and RTC increases in the blood.
Overall, the SCCS considers 1000 mg/kg as the NOAEL.

3.4.4.3 Sub-chronic (90 days) toxicity (oral, dermal)

No sub-chronic toxicity data are available for HAA299 (nano).

Applicants' overall conclusion on repeated dose toxicity
Repeated dose toxicity of HAA299 (nano), formulated as the intended commercial product for formulation of consumer sunscreens, was evaluated in a combined subacute repeated dose and reproductive toxicity screening study in Sprague-Dawley rats. Besides of non-adverse sporadic cases of in-life findings (hypersalivation, reflux at dosing and loud breathing), no treatment related effects on body weight, food consumption, haematology, blood biochemistry or urinary parameters were observed. Motor activity and parameters tested during the Functional Observation Battery did not show any effects related to the administration of HAA299 (nano). Organ weights were not affected by treatment and no test item-related macroscopic findings and histopathological lesions were observed. When comparing this study to repeated dose toxicity data for HAA299 neat, no evident increase in systemic toxicity was observed between the nano and non-nano form of HAA299. Although no comparative sub-chronic (13 week) study is available for HAA299 (nano), the sub-acute (4 week) application of the nano form provides evidence for an absence of systemic toxicity even after a longer application duration. This is supported by the toxicokinetic data, showing, that HAA299 (nano) is not taken up orally more efficiently than HAA299 (formulated, non-nano). Since low bioavailability might account for the absence of systemic toxicity, the sub-chronic application of HAA299 (nano) is not expected to show such effects. Taking together both repeated dose toxicity studies, testing the nano and the non-nano form of HAA299, does not show any toxicity after repeated exposure.

Overall SCCS comment on repeated dose toxicity
No sub-chronic toxicity data were provided for HAA299 (nano). However, from the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422), a NOAEL value of 1000 mg/kg could be derived.

3.4.5 Reproductive toxicity

3.4.5.1 Combined Repeated Dose and Reproduction/Developmental Toxicity study

The repeated dose toxicity part of the study is described under 3.4.4.2. Other aspects (reproductive and developmental toxicity) of this study are described in this section.

When administered daily at dose-levels of 50, 250 or 1000 mg a.i./kg/day by oral gavage to male and female Sprague-Dawley rats, for 2 weeks before mating, during mating, gestation and until day 5 post-partum, HAA299 (nano), i.e. micronised HAA299 in a formulation, C-1332 there were no effects on mating, fertility, delivery, pup survival or development during 5 days of lactation. No histopathological lesions considered to be reflective of systemic toxicity including male and female reproductive organs were observed.

Table 18: Summary of the delivery data

Dose-level (mg/kg/day)	0 (purified water)	0 (placebo mix)	50	250	1000
Number of pregnant females surviving delivery	10	9	9	8	8
Mean duration of gestation (days)	22.0	21.9	21.8	21.8	21.9
Mean number of *corpora lutea*	17.8	15.1	18.1	18.3	16.6
Mean number of implantations	16.1	13.3	16.4	17.8	15.0
Mean pre-implantation loss (%)	9.6	13.2	7.1	2.8	8.3
Mean number of pups delivered	13.0	12.3	15.3	15.8	13.4
Mean post-implantation loss (%)	17.3	8.6	7.5	11.3	11.2

Applicants' conclusion on reproduction and developmental toxicity
Based on the experimental conditions, the dose-level of 1000 mg a.i./kg/day was defined as No Observed Adverse Effect Level (NOAEL) for parental toxicity, reproductive performance (mating and fertility) and development.

SCCS comment
Based on the results on fertility and developmental parameters assessed in the repeated dose toxicity study (45 days) with the Reproduction/Developmental Toxicity Screening Test (3.4.4.2), 1000 mg a.i./kg/day is considered to be the $NOAEL_{repro}$. For other endpoints see section 3.4.4.2.

3.4.5.2 Teratogenicity

No teratogenicity data are available for HAA299 (nano). Some developmental aspects on HAA299 neat have been addressed in the study discussed in SCCS/1533/14, A.3.3.8.2.

SCCS comment
No teratogenicity data are available for HAA299 (nano).

Overall SCCS conclusion on reproductive toxicity
The SCCS considers a dose of 1000 mg a.i./kg/day as a $NOAEL_{repr}$ for reproductive and developmental toxicity endpoints based on the results of the repeated dose toxicity study (45 days) with the Reproduction/Developmental Toxicity Screening Test (3.4.4.2).

3.4.6 Mutagenicity/genotoxicity

3.4.6.1 Mutagenicity/genotoxicity *in vitro*

IN VITRO GENE MUTATION TEST IN CHO CELLS (HPRT LOCUS ASSAY)

Guideline:	OECD Guideline no. 476
Species/strain:	Chinese hamster ovary (CHO) cells
Replicates:	Two independent experiments
Test substance:	HAA299 (nano)
Batch:	RIN208_10
Purity:	51.0% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 65 nm and d(0.9): 111 nm
Volume distribution:	d(0.5): 110 nm and d(0.9): 180 nm
Vehicle:	ultrapure water
Concentrations:	7.8 to 2000 µg/mL (± S9)
Exposure period:	4 hours
GLP:	In compliance
Study period:	05 Mar – 19 Jul 2018

Methods
The test item HAA299 (nano), i.e. micronised HAA299 in a formulation (also named C-1332 (micronised)), was assessed for its potential to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Two independent experiments were performed with (1^{st} experiment) and without (1^{st}, 2^{nd} experiment) the addition of liver S9 mix from phenobarbital- and β- naphthoflavone induced rats.
The test item was incubated at 0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000 or 2000 µg/mL based on a dose selection according to the current OECD guideline. The vehicle (ultrapure water) was used as a negative control and ethyl methanesulfonate (400 µg/mL; without S9 mix) and 7,12-dimethylbenz[a]anthracene (1.25 µg/mL; with S9 mix) were used as positive controls. Following attachment of the cells for 20 - 24 hours, cells were treated with the test item for 4 hours in the absence and presence of metabolic activation. Subsequently, cells were cultured for 6 - 8 days and then selected in 6-thioguanine-containing medium for another week. Finally, the colonies of each test group were fixed with methanol, stained with Giemsa and counted.
Cytotoxicity was determined by the morphology assessment of the cells and the relative survival after the exposure period via the cloning efficiency 1 (adjusted by cell loss). The determination of the cloning efficiency 2 assessed the viability after the expression period. Absolute/relative cloning efficiencies and the relative survival were calculated for each test group accordingly. Furthermore, the pH, osmolality and the test substance solubility were assessed in the test cultures. The mutant frequency was determined by the number of mutant colonies versus the number of seeded cells. For the determination of particle size distribution via analytical ultracentrifugation in culture medium, samples of 6 concentrations and the respective vehicle control groups each were taken before administration and at the end of treatment period from both experimental parts of the 1st experiment and from the experimental part without S9 mix in the 2nd experiment.

Results
Precipitation was observed macroscopically in the culture medium at all test concentrations used, whereas the osmolality and pH values were not influenced.
In the 1st experiment (without S9 mix), the particle size distribution analysis showed no significant content of HAA299 nanoparticles. Therefore, this experimental part was considered invalid and a repeat experiment (2nd experiment) was performed. In the presence of S9 mix, a determination of the nanoparticle content was not possible due to interaction of HAA299 particles with the S9 particles. In the 2nd experiment (without S9 mix), concentrations above the limit of quantification were found in the 40-100 nm diameter range at test concentrations of 250 µg/mL and above. The respective fraction ranged between 14-23% and 13-14% after cell culture incubation. It was found, that the fraction of 40-100 nm HAA299 particles remained stable during incubation and that a significant fraction of the total dose remains dispersed below 100 nm.
No cytotoxicity was observed up to the highest concentrations evaluated for gene mutations in both experiments with and without S9 mix. The vehicle control showed mutant frequencies within the range expected for the CHO cell line and both positive control substances (EMS and DMBA) led to the expected statistically significant increase in the frequencies of forward mutations
Incubation with the test item did not cause any biologically relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in two experiments performed independently of each other. The mutant frequencies at any concentration were close to the range of the concurrent vehicle control values and within the 95% control limit of the historical vehicle control data (MFcorr.: 0.00 – 6.49 per 10^6 cells without S9 mix; MFcorr.: 0.00 – 7.43 per 10^6 cells with S9 mix).

Conclusion
The test item HAA299 (nano), i.e. formulation of micronised HAA299, is not mutagenic in the HPRT locus assay in CHO cells in the absence and the presence of metabolic activation under the experimental conditions of this study.

Ref: BASF SE (2019), 24. Jun. 2019

SCCS comment
The experiment with S9-mix where one concentration resulted in micronucleus frequencies significantly higher compared to control was performed only once. Although the test results are negative, no evidence is provided for cellular internalization of HAA299 (nano). The SCCS therefore considers the study results as inconclusive.

IN VITRO MICRONUCLEUS ASSAY IN V79 CELLS (CYTOKINESIS BLOCK METHOD)

Guideline:	OECD Guideline no. 487
Species/strain:	V79 cells
Replicates:	Nine independent experiments
Test substance:	HAA299 (nano) Batch: RIN208_10 (Exp. 1-5, 8, 9) 5388F7 (Exp 6, 7)
Purity:	51.0-52.5% HAA299 (active) and excipients
Particle size (RIN208_10):	Number distribution: d(0.5): 65 nm and d(0.9): 111 nm
Volume distribution:	d(0.5): 110 nm and d(0.9): 180 nm
Particle size (5388F7):	Number distribution: d(0.5): 62 nm and d(0.9): 109 nm Volume distribution: d(0.5): 120 nm and d(0.9): 265 nm
Vehicle:	ultrapure water
Concentrations:	7.8 to 2000 µg/mL (± S9)
Exposure/preparation interval:	Without S9: 4/24 hours, 24/24 hours

	With S9: 4/24 hours, 4/44 hours
GLP:	In compliance
Study period:	05 Mar 2018 – 12 Jun 2019

Methods
The test item HAA299 (nano), i.e. micronised HAA299 in a formulation (also named C-1332 (micronised)), was assessed for its clastogenic or aneugenic activity based on the formation of micronuclei in V79 cells *in vitro*. Seven independent experiments using batch RIN208_10 were carried out with or without the addition of liver S9 mix from induced rats and 2 further experiments after the addition of S9 were carried out using a comparable batch 5388F7.
The test item was incubated at 0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 µg/mL (Exp. 1-3, 8, 9) and 500, 750, 1000, 1250, 1500, 1750, 2000 µg/mL (Exp. 4-7) based on a dose selection according to the current OECD guideline. The vehicle (ultrapure water) was used as a negative control and ethyl methanesulfonate (500 and 600 µg/mL; without S9 mix) and cyclophosphamide (0.5 and 1 µg/mL; with S9 mix) were used as positive controls.
Before treatment, cells (3 - $5x10^5$ cells per culture) were seeded in 25 cm^2 cell culture flasks, containing MEM with 10% FCS, and were incubated at 37°C with 5% (v/v) CO2 and ≥ 90% relative humidity. After the attachment period (about 20 - 24 hours after seeding), the medium was removed and treatment medium (MEM medium with/without 10% FCS dependent on the presence of S9) was added. At the end of the 4-hour exposure period, the medium was removed, cultures were rinsed and MEM (10% FCS) supplemented with CytB (3 µg/mL) was added for further cultivation according to the respective recovery time. In the case of 24-hour continuous exposure, CytB was added to the treatment medium at the start of treatment, and cell preparation was started directly at the end of exposure. At 44 hours preparation interval, CytB was added 24 hours before preparation of the cultures.

For cell harvest and staining, single cell suspensions were prepared from each test group by enzymatic dissociation and the cell number per flask of each cell suspension was determined using a cell counter. $5x10^4$ cells per slide were centrifuged at 600 rpm for 7 minutes onto slides using a Cytospin centrifuge. At least two slides per flask were prepared. After drying, the slides were fixed in 90% (v/v) methanol for 10 minutes and stained with a mixture of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and propidium iodide (PI) to differentiate between DNA (DAPI) and cytoplasm (PI). The cytospin slides were scored by fluorescence microscopy and a sample of at least 1000 cells for each culture was analysed for micronuclei, i.e. 2000 cells for each test group.
Cytotoxicity was determined by morphology assessment of the cells, cell counts of cell suspensions before preparing the cytospin slides and the cytokinesis-block proliferation index (CBPI) in at least 1000 cells per culture (2000 cells per test group) to calculate the % cytostasis (relative inhibition of cell growth compared to the respective vehicle control).
Two experiments were determined to be invalid, i.e. 4 hours exposure, 24 hours harvest time, without S9 mix (Exp. 1) and 4 hours exposure, 44 hours harvest time, with S9 mix (Exp. 4).
For the determination of the particle size distribution via analytical ultracentrifugation, culture medium samples of six concentrations and the respective vehicle control were taken before administration and at the end of treatment period in experiments 1-3. In the 1st experiment (with S9), the particle size distribution analysis was not possible due to interaction of the test substance with the S9 particles. Therefore, in all other experiments performed with S9 mix no particular size distribution was determined.

Results
Precipitation was observed macroscopically in the culture medium at all test concentrations used, whereas the osmolality and pH values were not influenced. A significant fraction of the total mass dose <100 nm was observed at the beginning of the exposure in the test medium. In the 1st experiment without S9 mix, the fraction of HAA299 particles in a range of 40-100 nm was between 10% and 42% of the nominal dose before and 13 -19% after incubation. This fraction was comparable in the follow-up experiments without S9 mix, and ranged between 12% and 35% before incubation, 12-14% after 4/24h incubation (Exp. 2) and 12-24% after 24/24h incubation (Exp. 3). It was demonstrated that the fraction of 40-100 nm

particles was found to remain stable during cell culture incubations. Thus, a significant fraction of the total dose remains dispersed below 100 nm under testing conditions.
After exposure in the presence of S9 (Exp. 1), a shift to larger sizes of the detected particles was found, but they generally remain stable in the same size range as before incubation. The S9 mix consists of micronised biological matter, which represents a substantial and non-constant background, and has a significant affinity with other particles. Thus, a determination of the nanoparticle content was not possible due to interaction of HAA299 particles with the S9 particles. Accordingly, follow-up samples with S9 mix were not analyzed in the 2nd experiment.
According to the proliferation index (CBPI) and cell counts, no clear cytotoxicity was observed up to the highest applied test substance concentration. The vehicle controls gave frequencies of micronucleated cells within the historical negative control data range for V79 cells. Both positive control substances, ethyl methanesulfonate (EMS) and cyclophosphamide (CPP), led to the expected increase in the number of cells containing micronuclei.

In the absence of metabolic activation (Exp. 2), HAA299 (nano) showed mean micronucleus rates ranging between 1.0 - 1.5% and the concurrent vehicle control value was 1.0%, which is slightly above the 95% control limit (0.1% - 0.9%) of the historical vehicle control data range. Furthermore, the micronucleus rate at the highest concentration tested (1.5%) was slightly above and the maximum historical control limit of 1.3%. No statistical significance or dose dependency was observed. The experiment was repeated (Exp. 8) using the same testing conditions and the micronucleus rates ranged between 0.3 - 0.5%. Cultures continuously treated with HAA299 (nano; Exp. 3) showed micronucleus frequencies range of 0.6 - 1.4%. The micronucleus rate (1.4%) at the test substance concentration of 31.3 µg/mL was above the 95% historical control limit (0.1% - 0.9%) and the maximum historical control limit (1.3%). The value of the concurrent vehicle control was 0.9%. Nevertheless, none of the increased values were statistically significant or dose dependent. This experimental part was repeated (Exp. 9) in which micronucleus rates between 0.1 - 0.6% were found. All values were not statistically significant as compared to their respective vehicle control value and a dose response was also not observed.
In the presence of metabolic activation (Exp.1) treated cultures showed mean micronucleus rates ranging between 0.2 - 1.6% and the concurrent vehicle control value was 0.6%. The mean micronucleus rate of the test substance at 1000 µg/mL (1.6%) was statistically significant higher compared to the concurrent vehicle control and above the 95% historical control limit (0.1% - 1.0%) and the maximum historical control limit (1.5%). A significant dose-related increase in the percentage of micronucleated cells by testing for linear trend was observed. In a confirmatory experiment (Exp. 4), micronucleus rates between 0.3 - 0.8% were counted from 1250.0 µg/mL onward up to 2000.0 µg/ml. 1000.0 µg/mL was not scorable for micronuclei due to the bad quality of the slides. When testing a comparable test substance batch (Exp. 6), micronucleus values from 0.3% to 0.6% were counted. Neither a statistical significance nor a dose dependency was obtained in the latter two experiments.
Cultures harvested after 44h (Exp. 3) showed micronucleus frequencies ranging between 1.0 - 2.0% after test substance treatment. Concentrations of 15.6, 500, 1000 and 2000 µg/mL showed a statistically significant increase in the percentage of micronucleated cells compared to their respective vehicle control value. A dose response was not observed by the linear trend-test. In the confirmatory experiment (Exp. 5), the micronucleus rates ranged between 1.0 - 1.4% and the respective vehicle control was 1.2%. No statistical increase and no dose-dependent effect was observed. When testing a comparable test substance batch (Exp. 7), the micronucleus rates ranged between 0.4 - 0.6%. All values were not statistically increased compared to the vehicle controls and the linear trend test was not significant.

Conclusion
Several experimental parts within this study were repeated in order to address the biological relevance of the obtained results. The experimental parts addressing the mutagenicity in the absence of S9 mix showed two inconclusive results in Exp. 2 and 3. In case of Exp. 2 not only the values from the HAA299 (nano) treated cultures were above the 95% control limit of the historical data but also the concurrent vehicle control value. In Exp. 3 non-significant

increases were observed without a dose relationship. Neither of the effects could be reproduced using the same batch in Exp.8 and 9. Thus, these effects are considered to have no biological relevance.
Similarly, in the presence of S9 mix significant increases in the micronucleus rates were observed after treatment with HAA299 (nano) at both 24 and 44 h preparation intervals (Exp. 1 and 3). However, these increases could not be reproduced in 4 independently performed experiments (Exp. 4-7) using 2 batches of the compound. Accordingly, these effects are considered to have no biological relevance.
Thus, under the experimental conditions chosen here, the test item HAA299 (nano), i.e. formulation of micronised HAA299, has not the potential to induce micronuclei (clastogenic and/or aneugenic activity) in V79 cells in the absence and presence of metabolic activation.

Ref: BASF SE (2019), 29.Oct. 2019

SCCS comment
Experiments 2 and 3 performed in the absence of S9 mix showed inconclusive results, however, neither of the effects could be reproduced using the same batch in experiments 8 and 9. Similarly, in the presence of S9 mix significant increases in the micronuclei frequency were observed after treatment with the test substance at both 24 and 44 h preparation intervals. Additionally, a significant concentration-related increase in the percentage of micronucleated cells by testing for linear trend was observed in the presence of S9 mix. These increases, however, could not be reproduced in 4 independently performed experiments (experiments 4-7) using 2 batches of the compound, thus the effects in experiments 1 and 3 are biologically not relevant.
In conclusion, although the results are negative, in the absence of any evidence for cellular internalisation of HAA299 (nano), the study is considered by the SCCS as inconclusive.

3.4.6.2 Mutagenicity/genotoxicity *in vivo*

***In vivo/in vitro* Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes**

Guideline:	OECD Guideline no. 486
Species/strain:	Male Fischer rats (5 – 6 weeks old)
Group size:	3 males (for dose group 2000 mg/kg: 3 additional satellite animals/sex/time-point allocated for determination of plasma level of the test item)
Vehicle:	Water
Treatment:	Once by oral gavage at 10 ml/kg bw
Positive controls:	Dimethylhydrazine, 10 mg/kg bw - 2-4 hour expression time 2-acetamidofluorene, 25 mg/kg - 12-16 hour expression time
GLP:	In compliance
Study period:	8 - 28 Apr 2008

Micronised

Test substance:	FAT 75’808/H: HAA299 (nano)
Batch:	MGU 814, LA 2397_37
Purity:	51.2 % HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 75 nm and d(0.9): 106 nm Volume distribution: d(0.5): 136 nm and d(0.9): 241 nm
Dose levels:	0, 1000 and 2000 mg/kg bw/day test substance

Non-micronised

Test substance:	FAT 75’808/D: HAA299 neat
Batch:	VTM07B04
Purity:	98.6%

Dose levels: 2000 mg/kg bw/day
Mean particle size: Volume distribution: d(0.5): 13.9 µm and d(0.9): 67.7 µm

Methods
The objective of this study was to evaluate the potential of HAA299 (nano), i.e. micronised HAA299 in a formulation, to induce DNA damage or increased repair synthesis of the genome. The non-nano form HAA299 neat was tested in parallel, which contained the active ingredient at significantly larger particle sizes with no further excipients.
The test item HAA299 (nano): FAT 75'808/H contained 51.2% HAA299 and was prepared by micronising HAA299 with further excipients. A subsequent analysis of the test item sample indicated a d(0.5) = 75/136 nm (number/volume distribution). The non-nano form HAA299 neat (FAT 75'808/D) represents the reference item of this study with a purity of 98.6% and a the median particle size of d(0.5) = 13.9 µm (volume distribution). This reference substance was not micronised and was used as a comparison to assess, if any observed effects may be attributable to particle size differences. In a placebo group, animals were treated with the formulation without HAA299 (FAT 75'808/I; placebo) to address potential effects from the excipients used. A further control group received the vehicle (water) alone. Dosage forms were prepared by dilution with distilled water except the reference item was suspended in CMC (0.5% in distilled water).
Male Fischer rats were used with a weight of approximately 200 g and age of 5-6 weeks at the time of expression time sampling. Based on a preliminary toxicity test, the selected doses were 1000 and 2000 mg/kg body weight of the test item HAA299 (nano), 2000 mg/kg of the reference item HAA299 neat or 1000 mg/kg body weight of the placebo. Dosing was once by oral gavage at 10 ml/kg body weight and each group consisted of 3 males. Positive control substances were dimethylhydrazine at 10 mg/kg for the 2-4 hour expression time and 2-acetamidofluorene at 25 mg/kg for the 12-16 hour expression time.
Blood samples were collected after sacrifice for determination of test item concentrations. Hepatocytes were collected after liver perfusion and removed to culture well-plates for radiolabelling; 12 culture wells per animal were prepared as slides. Autoradiography was conducted with 6 slides, 6 were held as backup if needed, and where possible 50 cells per slide from 3 slides per animal were evaluated for grain counting classified as nuclear (NC) or cytoplasmic (CC) grain counts, and the net nuclear grains (NNG) per cell was determined (NNG = NC-CC).
For the determination of systemic exposure, plasma samples were obtained individually in six satellite groups of three male rats each, receiving either the vehicle, HAA299 (nano) or HAA299 neat at 2000 mg/kg. Animals were sacrificed 2 or 12 hours after treatment for plasma collection and analysed via HPLC-MS after precipitation and separation of serum proteins.

Results
The test item HAA299 (nano), the reference item HAA299 neat and the placebo (formulation without HAA299) did not cause any increased net nuclear grain counts, did not increase the frequency of cells in repair or did not induce cellular proliferation as seen in frequency of cells in S-phase. The viability of the hepatocytes was not affected due to the *in vivo* treatment either with the test item, placebo or reference item. Treatment with the positive controls each provided adequate responses, which confirmed appropriate testing conditions.

Plasma samples analysis showed, that the active ingredient HAA299 could be detected at very low concentrations after administration of the nano or non-nano form of HAA299. A maximum level of 6.5 ng/mL was observed in one animal of the test group receiving HAA299 (nano) at the 2h sampling time (overall range 2.9-6.5 ng/mL), which only slightly exceeded the limit of quantification ((LOQ of 1.3 ng/mL plasma). Comparable low plasma levels of HAA299 (2.8 - 3.6 ng/mL) were observed after application of the non-nano form HAA299 neat. In placebo group samples, HAA299 was also detectable at very low concentrations (2.9 ng/mL). HAA299 concentrations in animals treated with HAA299 containing test material were slightly higher after 2 hours and comparable to the concentrations found in placebo samples after 12-hours treatment. Due to these very low levels, a reliable quantification was not possible. These

results demonstrate that HAA299 either in a nano or non-nano form is not able to enter systemic circulation in toxicologically significant amounts.

Conclusion
It is concluded that HAA299 (nano), i.e. micronised HAA299 in a formulation, and the non-nano form HAA299 neat did not reveal any genotoxic activity under the test conditions. Regarding the concentration levels in plasma of animals treated with nano and non-nano HAA299, no evidence of exposure was demonstrated.

Ref: Institute Pasteur de Lille. Lille, France, January 07, 2009

SCCS comment
The UDS assay is questionable for its usefulness for cells other than liver, as well as having limited sensitivity. Based on the DNA lesion and tissue specificity, it is widely accepted that a negative result in the UDS alone is insufficient to rule out the *in vivo* genotoxic potential (EFSA, 2017).
The SCCS has noted that regarding the concentration levels in plasma of the animals treated orally with nano and non-nano HAA299, the systemic exposure appears to be very low.
The SCCS has also noted with surprise that HAA299 was still detectable at very low concentrations in the placebo group samples (animals treated with the formulation without HAA299). This unexpected finding has not been explained.
In the view of these shortcomings, and especially in the absence of evidence of target cell exposure, the SCCS considers the study as inconclusive.

Bone Marrow Micronucleus Test by Oral Route in Mice

Guideline:	OECD Guideline no. 474
Species/strain:	Swiss Ico: OF1 mice, 6 weeks old.
Group size:	5 animals/sex (for dose group 2000 mg/kg: 8 animals/sex + 3 additional satellite animals/sex allocated for determination of plasma level of the test item)
Administration:	Oral gavage, 2 times, separated by 24 hours, the animals were sacrificed 24 hours after last treatment
Dose volume:	10 mL/kg bw
Route:	Oral administrations, pH between 6 and 7
Vehicle:	Water
Positive control:	Cyclophosphamide (50 mg/kg bw)
GLP:	In compliance
Study period:	5 Mar 2008 – 13 Mar 2009

Micronised

Test substance:	FAT 75’808/G: HAA299 (nano)
Batch:	MGU 799, LA 2397_31
Purity:	51% HAA299 (active) and excipients
Particle size:	Number distribution: d(0.5): 84 nm and d(0.9): 123 nm
Volume distribution:	d(0.5): 138 nm and d(0.9): 207 nm
Dose levels:	0, 500, 1000 and 2000 mg/kg bw/day (based on active ingredient)

Non-micronised

Test substance:	FAT 75’808/D: HAA299 neat
Batch:	VTM07B04
Purity:	98.6%
Dose levels:	2000 mg/kg bw/day
Mean particle size:	Volume distribution: d(0.5): 13.9 µm and d(0.9): 67.7 µm

Methods
HAA299 (nano), representing micronised HAA299 in a formulation, with a d(0.5) = 84/138 nm (number/volume distribution), was tested in Swiss Ico: OF1 mice for its potential to induce chromosomal or mitotic apparatus damage in bone marrow cells.
The test item HAA299 (nano), also named FAT 75'808/G, was suspended in the vehicle (water) to achieve concentrations of 50, 100 and 200 mg/mL and then homogenized using a magnetic stirrer. By using a dosing volume of 10 mL/kg bw, the target dose-levels were 500, 1000 and 2000 mg/kg/day. The concentration and dose-levels are expressed as active item, taking into account the HAA299 content of 51%.
The non-nano form HAA299 neat (FAT 75'808/D) represents the reference item of this study with a purity of 98.6% and a median particle size of d(0.5) = 13.9 µm (volume distribution). The reference substance was not micronised and was used for comparison to assess, if any observed effects may be attributable to particle size differences. Dosage preparation was made by suspension in the vehicle in order to achieve the concentration of 200 mg/mL and then homogenization using a magnetic stirrer. Using a treatment volume of 10 mL/kg, the target dose-level was 2000 mg/kg/day.
In a placebo group, animals were treated with the formulation without HAA299 (FAT 75'808/F; placebo) to address potential effects from the excipients used. The placebo was administered in its original form using a treatment volume of 10 mL/kg.
One group of five animals per sex received the positive control test item (Cyclophosphamide) once by oral route at the dose-level of 50 mg/kg bw. Two groups of five animals per sex received either vehicle or placebo as negative control test items. The high dose test item (HAA299 (nano)) group and the reference item (HAA299 neat) group consisted of 8 animals per sex and additionally satellite groups of 3 animals per sex were retained for blood sampling after dosing and determination of HAA299 concentrations in plasma. Blood samples for these determinations were taken at 1 hour (satellite animals) and 24 hours (at terminal sacrifice on 3 out of 8 animals of each sex) after the second treatment. The blood plasma samples were analysed via HPLC-MS after precipitation and separation of serum proteins.
Additional 2 groups of five animals per sex were given oral administrations of HAA299 (nano) at the 2 lower doses.
At the time of sacrifice (24 hours after the last dose), femurs of the animals were removed, and the bone marrow was flushed out using foetal calf serum. After centrifugation, the supernatant was removed and the cells in the sediment were resuspended by shaking. A drop of this cell suspension was placed and spread on a slide. The slides were air-dried and stained with Giemsa. The slides were coded so that the scorer is unaware of the treatment group of the slide under evaluation ("blind" scoring).
For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. Polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

Results
Neither mortality nor clinical signs were observed in the animals of either sex given the test item HAA299 (nano), the reference item HAA299 neat or the placebo formulation (excipients without HAA299). The mean values of MPE as well as the PE/NE ratio in the groups treated with HAA299 (nano), HAA299 neat or placebo were comparable to those of the vehicle group.

Cyclophosphamide induced statistically significant increases in the frequency of MPE and both, vehicle and positive controls were consistent with historical control data. Thus, the sensitivity and validity of the test system under the chosen experimental conditions was demonstrated. After oral dosing with HAA299 (nano) or HAA299 neat, the active ingredient HAA299 could be detected in plasma at 1- and 24-hours after dosing. However, plasma concentrations were too low (below the limit of quantification of 1 ng/ml) to allow reliable quantification of the analytes. These results demonstrate that HAA299 either in a nano or non-nano form is not able to enter systemic circulation in toxicologically significant amounts.

Conclusion
In conclusion, HAA299 (nano), i.e. micronised HAA299 in a formulation, and the non-nano form HAA299 neat, did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells under the testing conditions chosen. Regarding the concentration levels in plasma of animals treated with nano and non-nano HAA299, no evidence of exposure was demonstrated.

Ref: CIT F-Evreux, (March 13, 2009)

SCCS comment
The SCCS has noted that regarding the concentration levels in plasma of animals treated orally with nano and non-nano HAA299, the systemic exposure appears to be very low.
In the absence of evidence of target cell exposure, the SCCS considers that the results of the study are inconclusive.

Applicants overall conclusion on mutagenicity / genotoxicity
The HAA299 (nano), i.e. micronised HAA299 in a formulation, was tested in two *in vitro* mutagenicity/genotoxicity tests, i.e. a HPRT test and a MN test.
Incubation of Chinese hamster ovary (CHO) cells with HAA299 (nano) did not result in any biologically relevant increase in the mutant frequencies. Thus, HAA299 (nano) was identified to induce no gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus. Analytical assessment of HAA299 particles in the test culture revealed a stable and significant fraction of the total dose, that remained dispersed below 100 nm in particle size at least for incubations without S9 mix. In support, testing of non-micronised HAA299 in the same formulation (HAA299 non-nano, formulated) under comparable testing conditions, did confirm the absence of a biologically relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolising system (See 7 ANNEX I). Further studies with the non-nano form HAA299 neat (already submitted), did not demonstrate any mutagenic potential (see SCCS/1533/14). In a bacterial reverse mutation test (AMES) according to OECD Guideline no. 471 and in a mouse lymphoma mutation assay according to OECD Guideline no. 476, HAA299 neat was not mutagenic (LEMI 2005 (No. 2005-EAJ733-2) and CIT 2006 (No. 31633 MLY) cited in SCCS/1533/14, A.3.3.6.1 (Ref. 14, 16)). Thus, HAA299 in a nano or non-nano form was shown to have no mutagenic potential.
The potential clastogenic and aneugenic activity of HAA299 (nano) was evaluated *in vitro* by assessment of induction of micronuclei in V79 cells. No biologically relevant increase of micronucleus rates was observed in the absence and presence of metabolic activation. In line with the findings in the HPRT test, HAA299 particles were determined to remain dispersed below 100 nm in particle size at least for incubations without S9 mix in the test culture before and after incubation. Comparable testing of HAA299 (non-nano, formulated) under similar conditions did also not result in any biologically significant increase of the percentage of micronucleated cells (See 7 ANNEX I). Testing of the non-nano form HAA299 neat in a mammalian chromosomal aberration test in human lymphocytes according to OECD Guideline N° 473 (already submitted) revealed no significant increase in the frequency of cells with structural chromosomal aberrations (CIT 2006 (No. 31632 MLH) cited in SCCS/1533/14, A.3.3.6.1 (Ref. 15)). Furthermore, no increase in polyploidy was observed. Thus, HAA299 in nano or non-nano form was shown to have no potential for a clastogenic or aneugenic activity.
It is currently not identified, if the absence of mutagenicity/genotoxicity of nano or non-nano HAA299 is due to the lack of cellular/nuclear uptake or the lack of substance specific properties for such adverse effects. However, all *in vitro* genotoxicity studies performed with HAA299 in the nano or non-nano form did not show any mutagenic and genotoxic potential. Therefore, particle size did not have an impact on this potential. Furthermore, the formulation in a commercial product intended to be sold for consumer sunscreens did not result in any relevant mutagenic/genotoxic potential. Taking this into consideration, HAA299 does not pose any risk for mutagenicity/genotoxicity when used as an UV filter in cosmetic products, independent from its form as nano or non-nano material.

Comparative assessment of mutagenicity/genotoxicity of HAA299 (nano) and the non-nano form HAA299 neat was performed in two *in vivo* studies, i.e. an unscheduled DNA synthesis (UDS) test in rats (OECD guideline no. 486) and a murine bone marrow micronucleus test (OECD guideline no. 474). Both forms of HAA299 did not cause any increased net nuclear grain counts, did not increase the frequency of cells in repair, or did not induce cellular proliferation in the UDS test. Further, both forms of HAA299 did not significantly increase the frequency of micronucleated polychromatic erythrocytes in murine bone marrows when compared to the respective vehicle control group. Accordingly, HAA299 did not reveal any genotoxic activity independent of the form administered. In both *in vivo* genotoxicity studies, no evident difference in the systemic uptake of HAA299 in its nano or non-nano form was observed.
As discussed above, the reason for absent mutagenic/genotoxic potential of nano or non-nano HAA299 can either be due to the lack of bioavailability or the lack of substance specific properties for such adverse effects. However, the *in vivo* data with orally applied nano and non-nano HAA299 allow to assess HAA299 to pose no risk for mutagenicity/genotoxicity in use as an UV filter in cosmetic products.

SCCS overall comment on mutagenicity/genotoxicity
HAA299 (nano) did not induce any biologically meaningful increase in mutant frequency in the *in vitro* HPRT test on CHO cells and in the micronucleus test on V79 lung fibroblasts. However, in view of the lack of any evidence of cell internalisation of the test item, the results can only be regarded as inconclusive. This is clearly delineated in the SCCS GUIDANCE ON THE SAFETY ASSESSMENT OF NANOMATERIALS IN COSMETICS (SCCS/1611/19) "…for *in vitro* genotoxicity studies, it is necessary to demonstrate uptake of the nanoparticles in the cell and preferably the nucleus to demonstrate exposure of cellular target structures (e.g. DNA). If such exposure cannot be demonstrated, a negative outcome of such assay might be meaningless, as the target exposure will not be known".

HAA299 (nano) was tested in two *in vivo* studies using UDS test and bone marrow micronucleus test. In both studies, systemic exposure to HAA299 (nano) was shown to be very low. Based on that information and in the absence of evidence of target cell exposure, the SCCS is of the opinion that the results of the studies are inconclusive. However, taking into account the very low bioavailability of HAA299 (nano) at local and systemic levels, the mutagenicity risk to the consumer is considered to be negligible.

3.4.7 Carcinogenicity

No data available.

3.4.8 Photo-induced toxicity

No phototoxicity, photoirritation and photosensitization studies are available for the HAA299 (nano). However, the non-nano form HAA299 neat (FAT 75'808/A, batch number HAA299/7-5, purity 98.7%) as a 30% suspension in olive oil has been tested in Hartley albino guinea pigs and did not induce any phototoxic or photoallergenic reactions (see SCCS/1533/14, A.3.3.10.1. (Ref. 7)). The dermal absorption capacity of the HAA299 (nano) was very limited and even lower than in experiments performed with a formulated non-nano form. Taking into account the absence of a phototoxic, skin sensitizing and photosensitizing potential of the non-nano form HAA299 neat and the very limited dermal absorption capacity of both the nano and non-nano form of HAA299, HAA299 (nano) is considered to show neither any phototoxic nor any photosensitization potential, when used as a UV-filter in cosmetic products in a concentration up to a maximum of 10 %.

SCCS comment
Because HAA299 (nano) is intended to be used in sunscreen products as skin protectant against UVA1 to visible rays, a phototoxicity study should be submitted. The SCCS acknowledges that there is as yet no validation of and very limited experience with the *in-vitro* phototoxicity testing of nanomaterials and that the very low dermal absorption renders a phototoxic potential of HAA299 (nano) unlikely.

3.4.8.1 **Phototoxicity/photo-irritation and photosensitisation**

3.4.8.2 **Photomutagenicity/photoclastogenicity**

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SCCS comment
No photomutagenicity or photoclastogenicity tests are available for HAA299 (nano).

3.4.9 Human data

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3.4.10 Special investigations

/

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

The calculation of margin of safety (MoS) is not justified given the low dermal penetration when applied on human skin, and in consideration of the quite high NOAEL for oral administration of HAA299(nano) to rats at 1000 mg/kg bw/day. Therefore, no concern is raised with regard to systemic toxicity.

3.6 DISCUSSION

PHYSICOCHEMICAL PROPERTIES

The identity of impurities that are present in batches of HAA299 (nano) must also be reported in terms of chemical nature and concentration. The level of N-methylpyrrolidone (NMP), which is a reprotoxicant Cat 1B substance, should be at minimum level. The content of inorganic impurities in HAA299 neat, specifically of Ni, Co, Cr, CD, Pb and Hg, must also be reported.
Explanation is needed on the provided d50 values according to volume and number distribution - these values should be a specific number (not > or < a number). It should also be indicated what would be a sufficient particle size reduction in the milling process to reach a certain product specification.

Explanation is needed on the provided d_{50} values according to volume and number distribution - these values should be a specific number (not > or < a number). It should also be indicated what would be a sufficient particle size reduction in the milling process to reach a certain

product specification. The SCCS has, therefore, considered that the value of d50 (expressed as number based particle size distribution) is equal to or greater than 50nm. The SCCS will, however, need the full range of intended particle size distribution from the milling process. The Applicant should provide a clear product specification for HAA299 (nano) that is intended to be marketed.

TOXICOKINETICS

Three *in vitro* studies and one *in vivo* study were submitted by the applicant. In the *in vitro* study using intact rat skin, the total absorption was 0.52 % after 24 h compared with 0.43 % (total excreted and remaining in the body) after 72 h in the *in vivo* rat study. Thus, based on the rat studies the dermal absorption is around 0.5 %. However, since human *in vitro* studies are available with very low absorption, the SCCS considers that the absorption in humans will be lower. Very low dermal absorption *in vivo* is also confirmed by *in vivo* dermal studies in rats.
Based on the available data, the SCCS considers that systemic availability after dermal application is very low. Furthermore, the SCCS notes that oral absorption of HAA299 (nano)-related radioactivity *in vivo* in rats is very low (value of 0.07% of the administered radioactivity).

FUNCTION AND USES

HAA299 (nano) is a formulated micronised UV filter containing the nano form of the active UV filter HAA299 and excipients.

TOXICOLOGICAL EVALUATION

Irritation and corrosivity

Skin irritation
HAA299 (nano) did not show a skin irritation potential in an EpiDerm™ in vitro skin irritation test under the test conditions chosen.
Although the assay with the RhE model is not yet validated/evaluated for nanomaterials, the SCCS considers the results of this assay plausible and considers that the test substance is not an irritant under the test conditions used.

Mucous membrane irritation / eye irritation
HAA299 (nano) did not cause ocular corrosion or severe irritation in an Bovine Corneal Opacity and Permeability Test (BCOP Test) under the test conditions chosen, however an eye irritation potential was observed in the EpiOcular™ *in vitro* eye irritation test under the test conditions chosen.
Although it is possible that the eye irritation potential in the EpiOcular™ *in vitro* eye irritation test is based on the excipients, the test findings do not rule out an eye irritation potential from the HAA299 (nano) itself.

Skin sensitization
No skin sensitisation study has been provided for HAA299 nano whereas the data from LLNA on the non-nano form have shown lack of skin sensitisation. Although the argument used by the Applicant to consider skin sensitisation data from non-nano form to extrapolate to HAA299 (nano) is not supported, the SCCS agrees that the extremely low dermal penetration and the large molecular size makes a skin sensitising potential unlikely.

Acute toxicity

Oral toxicity

The SCCS has noted that the acute oral LD50 of HAA299 (nano) is >2000 mg/kg in rat.

Inhalation toxicity
On the basis of the submitted data, the SCCS cannot exclude concerns in regard to use of sprayable products.

Repeated dose oral toxicity
In the repeated dose toxicity study (45 days) submitted by the Applicant, one male rat, in the highest dose, was found to show some deviation of the control parameters indicative for the presence of an inflammatory reaction (increase in WBC and PMN) while other blood values were within normal ranges. However, the histopathology did not show any organ with a severe inflammation that might explain the WBC, PMN and RTC increase in the blood.
Overall, the SCCS considers 1000 mg/kg as the NOAEL.

Sub-chronic toxicity
No sub-chronic toxicity data were provided for HAA299 (nano). However, from the Combined Repeated Dose Toxicity Study (45 days), with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422), a NOAEL value of 1000 mg/kg could be derived.

Reproductive toxicity
A dose-level of 1000 mg a.i./kg/day was defined as NOAEL for fertility and developmental effects.
No teratogenicity data are available for HAA299 (nano).

Mutagenicity/genotoxicity
HAA299 (nano) did not induce any biologically meaningful increase in mutant frequency in the *in vitro* HPRT test on CHO cells and in the micronucleus test on V79 lung fibroblasts. However, in view of the lack of any evidence of cell internalisation of the test item the results should be regarded as inconclusive. As clearly delineated in the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19) "…for *in vitro* genotoxicity studies, it is necessary to demonstrate uptake of the nanoparticles in the cell and preferably the nucleus to demonstrate exposure of cellular target structures (e.g. DNA). If such exposure cannot be demonstrated, a negative outcome of such assay might be meaningless, as the target exposure will not be known".
HAA299 (nano) was tested in two *in vivo* studies using UDS test and bone marrow micronucleus test. In both studies, systemic exposure to HAA299 (nano) was proven to be very low. Based on the information and in the absence of evidence of target cell exposure the SCCS is of opinion that the results of the studies are inconclusive.
However, taking into account the very low bioavailability of HAA299 (nano) at local and systemic levels, the mutagenicity risk of HAA (nano) to the consumer is considered to be negligible.

Carcinogenicity
No data were provided for carcinogenicity of HAA (nano).

Photo-induced toxicity
Because HAA299 (nano) is intended to be used in sunscreen products as skin protectant against UVA1 to visible rays, a phototoxicity study should be submitted. The SCCS acknowledges that there is as yet no validation of and very limited experience with the in-vitro phototoxicity testing of nanomaterials and that the very low dermal absorption renders a phototoxic potential of HAA (nano) unlikely.

Photomutagenicity/photoclastogenicity
No photomutagenicity or photoclastogenicity tests are available for HAA299 (nano).

4. CONCLUSION

1. In light of the data provided, does the SCCS consider HAA299 (nano) safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?

The available data indicate that HAA299 (nano) is a practically insoluble material, with very low dermal and oral absorption. Due to the very low systemic availability, the material is unlikely to exert systemic genotoxic or reproductive effects. The NOAEL of 1000 mg/kg/day indicates that the material is of overall low toxicological concern. Given the low dermal penetration, and low systemic toxicity, the calculation of margin of safety (MoS) is not appropriate in this case. The SCCS considers that HAA299 (nano), as covered within the provided characteristics (minimum purity equal to or above 97%, median particle size in terms of particle number equal to or above 50 nm), is safe when used as a UV-filter in dermally-applied cosmetic products up to a maximum concentration of 10%.

Based on the inflammatory effects on the lung after the acute inhalation exposure, the SCCS has concerns regarding the repeated use of products containing HAA299 (nano) in applications that could lead to inhalation exposure. Therefore, the SCCS does not recommend the use of HAA299 (nano) in applications that could lead to exposure of the consumer's lungs via inhalation.

2. In view of the previous SCCS opinion (SCCS/1533/14) does the SCCS consider HAA299 non-nano and nano form safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?

The data considered in this Opinion has not provided any new or additional concern that merits a revision of the previous SCCS Opinion (SCCS/1533/14). Therefore, the SCCS considers HAA299, either as non-nano or nano form, safe when used as a UV-filter in dermally-applied cosmetic products up to a maximum concentration of 10%. The SCCS considers that the combined maximum concentration of non-nano and nano forms of HAA299 should not exceed 10% in a cosmetic product.

3. In case the SCCS finds HAA299 (nano) not safe, does it still uphold the conclusions of the SCCS/1533/14 opinion with regard to the safe use of HAA299 non-nano form?

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4. Does the SCCS have any further scientific concerns (on human health) with regard to the use of HAA299 (nano) in cosmetic products?

This Opinion is based on the currently available scientific evidence, which shows an overall very low or lack of dermal absorption of HAA299 (nano) in human skin. If any new evidence emerges in the future to show that HAA299 (nano) used as UV-filter in cosmetic products can penetrate human skin (healthy, compromised, sunburnt or damaged skin) to reach viable cells, in higher levels than demonstrated in this submission, then the SCCS may consider revising this assessment.

5. MINORITY OPINION

None.

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