Scientific Committee on Consumer Safety

SCCS

OPINION ON

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

(HAA299)

as UV filter in sunscreen products

The SCCS adopted this opinion at its 6th plenary meeting

of 18 June 2014

About the Scientific Committees

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

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

SCCS

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

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

Keywords: SCCS, scientific opinion, cosmetic ingredients, UV filter, 2-(4-(2-(4-Diethylamino-2-hydroxy-benzoyl)-benzoyl)-piperazine-1-carbonyl)-phenyl)-(4-diethylamino-2-hydroxyphenyl)-methanone , HAA299 , Regulation 1223/2009, CAS 75980-60-8 , EC 485-100-6.

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the safety of 2-(4-(2-(4-Diethylamino-2-hydroxy-benzoyl)-benzoyl)-piperazine-1-carbonyl)-phenyl)- (4-diethylamino-2-hydroxyphenyl)-methanone, HAA299 as UV filter in sunscreen products, 18 June 2014, SCCS/1533/14, revision of 23 September 2014.

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1. BACKGROUND

FAT 75'808 is a new notified substance to be used as an UV-filter in sunscreen products.
FAT 75'808 with the chemical name 1,1'-(1,4-piperazinediyl)bis[1-[2-[4-(diethylamino)-2-hydroxybenzoyl]phenyl]-methanone and the CAS No 919803-06-8 has been studied in its micronised form under the name C-1332 as well as in its non-micronised form under the name HAA299.

The first submission for this substance was received from the applicant in April 2009.

2. TERMS OF REFERENCE

1. *Does SCCS consider that the use of FAT 75'808 in its micronised and non-micronised form as an UV-filter in cosmetic products in a concentration up to maximum 10.0 % is safe for the consumers taken into account the scientific data provided?*

2. *Does SCCS have any other scientific concerns for the safe use of the new UV-filter FAT 75'808 in finished cosmetic products?*

3. OPINION

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 not assigned
For the convenience, the trade names HAA299 or FAT 75'808 have been used throughout the whole opinion.

3.1.1.2. Chemical names

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

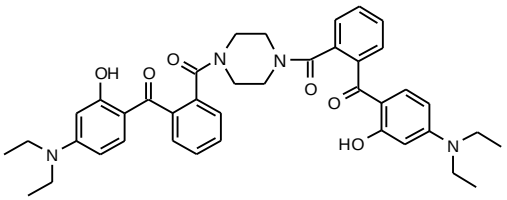
3.1.1.3. Trade names and abbreviations

HAA299, C-1332, FAT 75'808

3.1.1.4. CAS / EC number

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

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

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

3.1.2. Physical form

White-yellowish powder

3.1.3. Molecular weight

Molecular weight: 676.82

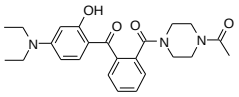
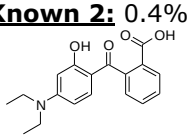
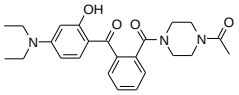
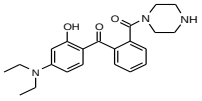
3.1.4. Purity, composition and substance codes

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 structures are included in the Table 1 below.

3.1.5. Impurities / accompanying contaminants

The organic impurities were identified with available reference materials and the amounts calculated against the active ingredient using response factors. The sum of volatile compounds content were at levels up to 0.6%, the balance of the composition comprised also water. The samples were characterized by UV/VIS, IR and ^{1}H and ^{13}-C-NMR spectroscopy. The composition of the non-micronised HAA299-batches used for the toxicological assays is shown in Table 1.

Table 1. Composition of the non-micronised HAA299-batches used for the toxicological assays

Composition of the non-micronised HAA299 batches as used in the studies submitted				
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-ethnaol) = <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%

Analytical methods:

The test article was analysed by HPLC with UV-detection according to HPLC-method LC-ACZ00 (ref D).

1. Quantification of active ingredient with external standard:
   HPLC
2. Water content:
   Karl-Fischer titration
3. Impurities – quantification with external standard:
   HPLC/HPLC-MS

4. Volatile matters/Residual solvents:
   Gas Chromatography

SCCS Comment
Content of inorganic impurities, specifically contents of Ni, Co, Cr, CD, Pb and Hg, in HAA299 should be reported

3.1.6. Solubility

Water solubility: 1.66 ± 1.24 µg/l at 20°C (OECD 105), insoluble (Ref. G)

Table 2. Solubility of HAA299 in water (ref.: G) and other solvents (ref.: J)

Solvent (INCI Name)	Solubility of HAA299	
	FAT 75'808/D % batch VTM07B04	FAT 75'808/B % batch VTM05B10
Water	< 0.001	< 0.001
Propyleneglycol	0.002	0.004
C_{12}-C_{15} Alkyl Benzoate	0.001	0.004
Caprylic / Capric Triglyceride	0.001	0.002
Isopropylmyristate	0.0005	< 0.001
Isopropylpalmitate	0.0004	0.002
Coco Caprylate / Caprate	0.0003	< 0.001
Dicaprylyl Carbonate	0.0007	< 0.001
Butyloctyl Salicylate	0.0014	0.004
Lauryl Pyrrolidone	0.013	0.023
Caprylyl Pyrrolidone	0.027	0.057

3.1.7. Partition coefficient (Log Pow)

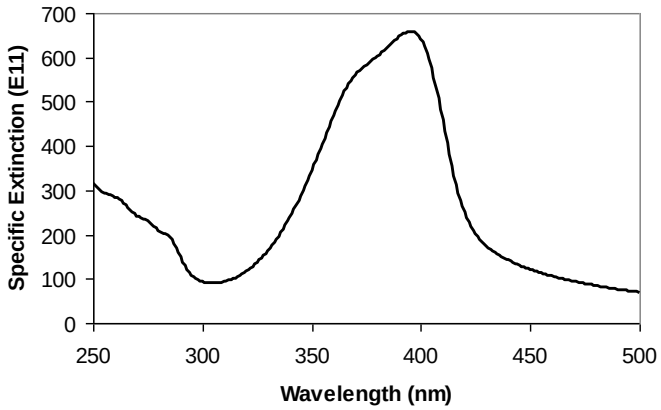
Log $P_{o/w}$ 4.8 at pH = 6.8 (HPLC method, OECD 117) (Ref.: G)

3.1.8. Additional physical and chemical specifications

Melting point:	257.1 °C (OECD no. 102) (Ref.: E)
Boiling point:	/
Flash point:	Based on its chemical structure, FAT 75'808 is not considered as an explosive material. (Ref.: I)
Vapour pressure:	$1.7*10^{-22}$ Pa at 25°C (Ref.: F) (calculated; Modified Watson Correlation method; OECD 104)
Flammability	FAT 75'808 is not considered as highly flammable (Ref.: H)
Density	/
Viscosity	/

pKa /
Refractive index /
pH /

UV – visible absorption spectrum



3.1.9. Homogeneity and Stability

The stability and homogeneity of FAT 75'808 suspended in solvents or mixed into sunscreen ingredients has been assessed in each study enclosed in this dossier. Stability and homogeneity of FAT 75'808 was proven in each dosing forms applied to assess the toxicological endpoints summarised within the dossier.

Some results are detailed below.

Table 3. Homogeneity and stability of HAA299

TYPE OF TEST	***DETERMINATION OF CONCENTRATION, HOMOGENEITY, AND STABILITY OF HAA299***
SAMPLE PREPARATION:	FAT 75'808 was ground to a fine powder and mixed with 0.5% caxboxymethylcellulose (vehicle) to achieve the test-concentrations of 10, 50 and 200 mg/ml.
SAMPLE ANALYSED	Dosage forms of at 10 and 200 mg/ml analysed for stability and homogeneity. Homogeneity: analysed at three different levels of container (top, middle, and bottom) for concentration of FAT 75'808 at day 0 just after preparation and after 9 days storage at +4°C (protected from light). Stability: analysed on day 0 just after preparation, day 4 and 9 days after storage at +4°C (protected from light)
RESULT :	Each dosage form analysed (10 and 200 mg/ml) was shown to be homogeneous at day 0 just after preparation and 9 days after storage at +4°C (protected from light). Each dosage form analysed (10 and 200 mg/ml) was found to be stable over a 9-day storage period at +4°C (protected from light): at 10 mg/mL a deviation of -7% FAT 75'808 was found on day 9 from initial value on day 0. At 200 mg/ml deviation of 5% FAT 75'808 was found on day 9 from initial value on day 0
REMARK	Deviation of actual to nominal concentration was found to be within the range of +/- 10%.

- The stability as well as the concentration and homogeneity of HAA299 (Batch nr. VTM05B10, purity of 99.1%) in the dosage forms of 10 and 200 mg/ml covering all the test concentrations used during the study have been assessed in the 90-day oral toxicity (gavage) study in rats (Ref. 3). The analysis showed that HAA299 suspended in 0.5% carboxymethylcellulose is stable and homogeneous in all the dosage forms under which the test item was applied over a 4- and 9-day storage period at +4°C and protected from light.
- The stability and homogeneity of dosage forms of 10, 50 and 200 mg/ml FAT 75'808/E and FAT 75'808/G (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.
- The stability and homogeneity of dosage forms of 20, 60 and 200 mg/mL FAT 75'808/B (suspension in 0.5% carboxymethylcellulose) performed with HAA299 lot number VTM05B10 (purity 99.1%) was demonstrated in the prenatal developmental toxicity study (Ref. 11). The difference between mean measured concentrations of the administered dosage forms and nominal concentrations were in the range of ± 10%. Each dosage form was demonstrated to be homogenous (CV <5%).
- Stability of the formulated ^{14}C- FAT 75'808 at the time of application used in the percutaneous study (Ref. 12) was checked by HPLC. The test item was shown to be stable, more than 96% of the radioactivity was found in the HPLC radiochromatogram.
- Stability of the formulated ^{14}C- FAT 75'808 at the time of application used in the oral Absorption, Distribution, Metabolism and Excretion study (Ref. 13) was checked by HPLC. The test item was shown to be stable, more than 91% of the radioactivity was found in the HPLC radiochromatogram
- Stability of the formulated ^{14}C- FAT 75'808 at the time of application used in the dermal absorption study (Ref. 24) was checked by HPLC. The test item was shown to be stable, 98% of the radioactivity was found in the HPLC radiochromatogram.

SCCS comment
In some of the studies, the stability of the test substance was controlled under the conditions of the study. No long-term stability data at room temperature or in commercial sunscreen products are available.

3.1.10. Particle size of HAA299

Non-micronised
The particle size distribution of HAA299 = FAT 75'808 was determined according to the European Commission, Document ECB/TM/February 1996: "Particle Size Distribution, Fibre Length and Diameter Distribution" Guidance Document using the laser diffraction method.

FAT 75'808/B is a fine, white-yellowish powder. Under the microscope (with a magnification of 400) squarish, agglutinated and colourless crystalline structures were observed.

By laser diffraction particle size of FAT 75'808/B was shown to range between 0.3 µm and 300 µm with 5% of the particles having a mean particle size of less than 14.1 µm, 10% of the particles with a mean particle size of less than 24.9 µm and 90% of all particles being smaller than 210.4 µm. The mass median diameter (MMD) of the test item indicates that 50 % (by mass) of the particles are smaller than 99.1 µm (Ref.: K).

Micronisation process of HAA299 to C-1332

HAA299 is mixed and homogenized in water together with defined surfactants and antifoaming agents. The resulting homogeneous suspension goes then in a milling step through a stirred media ball mill until the milled product reaches the quality criteria, defined by particles size and E1,1 value of an UV-spectrum. In a final process step the pH value of the product is adjusted. The product is filtered, and, after adding buffer and preservative, thickened to a defined viscosity and packaged. The resulting paste-like product is ready for use for the formulation of commercial sunscreen products.
Currently, the formulation for HAA299 is as follows:

40.0%	HAA299 (active)
7%	Texapon K14S Spezial (surfactant / stabilizer)
1.3%	Buffer/preservative
1%	Silfoam SE2 (defoamer)
0.3%	thickener/solvent for thickener
50.4%	water

Test Articles Used for toxicity testing and Particle Size Characterization

Particle Size Determination and Appearance

The studies included with the submitted dossier were conducted with *non-micronised large particle* sized samples of HAA299. In addition, toxicology information was gained from studies with the *micronised formulation of HAA299* which is representative of the trade product known as C-1332.

For each of the non-clinical studies conducted and submitted within this dossier, the batch used and the purity and particle size range of HAA299 are summarised in table 4 below

Table 4. Summary of HAA299 batches and their particle size distributions

FAT 75'808 Suffix Used	Batch number	Particle size distribution	Comment	Study
/A	HAA299/7-5 (purity 98.7%)	D(0.5) 61.19 µm* d(0.5) 52.88 µm*	Non-micronised (Ref. L) (Ref. L1)..	- Phototoxicity/ Photoallergenicity in guinea pigs
/B	VTM05B10 (purity 99.1%)	5% < 14.1 µm , 10% < 24.9 µm, 50 % < 99.1 µm (MMD), 90% <210.4.	Non-micronised (Ref. K).	- All studies submitted and not listed in this column
/C	HAA299/77 (purity 99.8%)	d(0.5) 6.08 µm*	Non-micronised (Ref. M).	- Acute oral toxicity
/D	VTM07B04 (purity 98.6%)	d(0.1) 5.38 µm* d(0.5) 13.91µm d(0.9) 67.68 µm	Non-micronised (Ref. N).	- Large particle sized reference item in bone marrow micronucleus assay and UDS assay

/E	MGU 789, LA 2397_24 (purity 51.2% HAA-299) from HAA299 lot number VTM07B04	d(0.5) 134 nm* d(0.9) 202 nm	This d(0.5) is within the expected range for the product's specifications (Ref. O)	-Oral gavage dosing toxicity & reproduction-developmental toxicity
/G	MGU 799, LA 2397_31 (purity 51.0% HAA-299) from HAA299 lot number VTM07B04	d(0.5) 138 nm* d(0.9) 207 nm	This d(0.5) is within the expected range for the product's specifications (Ref. P)	- Acute oral toxicity - Oral gavage dosing toxicity and reproduction-developmental toxicity, - Bone marrow micronucleus test
/H	MGU 814, LA 2397_37 (purity 50.1% HAA-299), from HAA299 lot number VTM07B04	d(0.5) 136 nm* d(0.9) 241 nm	This d(0.5) is within the expected range for the product's specifications (Ref. Q)	- Acute Inhalation Toxicity, - UDS assay
Radioalabelled ^{14}C-HAA-299, micronised at the testing laboratory (Harlan)	FAT 75'808/B and[^{14}C] labelled FAT 75'808, batch number 3574052, radiochemical purity 97.7%	d(0.1) 0.38 µm d(0.5) 0.76 µm d(0.9) 1.90 µm	Large, micronised particles	- Percutaneous penetration *in vitro* rat & human skin
Radioalabelled ^{14}C-HAA-299, micronised at the testing laboratory (Harlan)	FAT 75'808/B and[^{14}C] labelled FAT 75'808, batch number 3574052, radiochemical purity 97.7%	d(0.1) 1.30 µm d(0.5) 8.33 µm d(0.9) 19.8 µm	Large, micronised particles	- *In vivo* oral absorption, distribution, metabolism and elimination
Radioalabelled ^{14}C-HAA-299, micronised at the testing laboratory (Harlan)	FAT 75'808/D and [^{14}C] labelled FAT 75'808, batch number 3574052, radiochemical purity 97.7%	D(0.5) 134 nm; 140 nm	This d(0.5) is within the expected range for the product's specifications	- Percutaneous penetration *in vitro* rat & human skin, - *In vivo* dermal absorption rat, - *In vivo* oral absorption, distribution, and elimination
Radioalabelled ^{14}C-HAA-299, micronised at the testing laboratory (Harlan)	Micronised FAT 75'808/D and [^{14}C] labelled FAT 75'808, batch number 3574052, radiochemical purity 97.7	D(0.5) 152 nm; 153 nm	This d(0.5) is within the expected range for the product's specifications	- Percutaneous penetration *in vitro* pre-damaged human skin
* Particle size distribution measured by FOQELS (Fiber Optic Quasi Elastic Light Scattering)				

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 5. 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 5. 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 5. 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
Estimated Weight of one particle	1.70E-21 g
Specific Surface Area	34.3 m^2/g
* Values prepared and summarised by Herzog, B. and Giesinger, J.; Ciba internal report 09 March 2009. ** value representative for the radio-labelled material formulation	

SCCS general comment
The long term stability of HAA299 and the stability in typical sunscreen products were not provided.
According to the information received from the applicant during the public consultation period, HAA299 batches FAT 75'808/A, B, C, and D (not micronised or micronised) were not composed of nano-particles. HAA299 batches FAT 75'808/E, G and H were composed of nano-particles. Therefore, the safety evaluation of HAA299 non-nano batches has been separated from the HAA299 nano-batches

3.2. Function and uses

HAA299 is a UV filter active intended to be used in sunscreen products as skin protectant against UVA-1 rays as shown in the absorption spectrum. HAA299 is most effective as a UV filter when it is milled to a smaller particle size, a process that is referred to as *micronisation*. This process yields C-1332 with median particle size of 120-160 nm.

SCCS comment
Although the applicant states that the micronised HAA299 offers better UV protection than non-micronised HAA299, no evidence for this is provided.

3.3. Toxicological Evaluation

Section: A. HAA299 non-nano batches

A.3.3.1. Acute toxicity

A.3.3.1.1. Acute oral toxicity

Guideline:	OECD Guideline no. 423
Species/strain:	Sprague-Dawley albino rats, 8 week old
Group size:	2 groups, with 3 females
Test substance:	FAT 75'808/C
Batch:	HAA299/77
Purity:	99.8%
Vehicle:	0.5% carboxymethylcellulose
Dose levels:	2000 mg/kg bw; 5 ml/kg body weight
Administration:	Oral gavage
GLP:	In compliance
Study period:	3 – 19 Nov 2004

Single oral gavage dose of HAA299 suspended in 0.5% carboxymethylcellulose, administered at 2000 mg/kg bw (5 ml/kg bw) to two group of 3 females.

Deaths did not occur during the study. Slight piloerection was apparent in all animals up to four hours after treatment. No other clinical signs and no effect on body weight gain were noted over the 14-day observation period. Necropsy of all animals did not reveal abnormal findings or changes related to the test item.

It is concluded that the acute oral LD_{50} is greater than 2000 mg/kg bw.

Ref.: 1

A.3.3.1.2. Acute dermal toxicity

Guideline:	OECD Guideline no. 402
Species/strain:	Sprague-Dawley albino rats, 8 week old
Group size:	2 groups, 5 males and 5 females
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	HAA299 moistened with water
Dose levels:	2000 mg/kg bw
Administration:	Dermal application
GLP:	In compliance
Study period:	18 Apr – 2 May 2006

On the day before treatment, the dorsal area of each animal was clipped (i.e. approximately 5 cm x 7 cm for males and 5 cm x 6 cm for females) using an electric clipper. Only animals with healthy intact skin were used for the study. The test item was applied to the skin of one group of ten Sprague-Dawley rats (five males and five females). HAA299 was moistened with purified water, applied in a single dermal dose of 2000 mg/kg bw as a

uniform layer, and occluded for 24-hours to approximately 10% of the body surface area. Clinical signs, mortality and body weight gain were checked for a period of 14 days following the single application of the test item.

No deaths, no clinical signs and no cutaneous reactions were observed over the 14-day observation period. Body weight gain was not affected during the study and necropsy did not reveal observable changes.

It was concluded that the acute dermal LD_{50} is greater than 2000 mg/kg.

Ref.: 2

A.3.3.1.3. Acute inhalation toxicity

/

A.3.3.2 Irritation and corrosivity

A.3.3.2.1. Skin irritation

Guideline:	OECD guideline no. 404
Species/strain:	New Zealand White rabbits
Group size:	3 males
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	None
Dose level:	500 mg
Dose volume:	/
Observation:	4 h
GLP:	In compliance
Study period:	4 – 9 April 2006

Method
An acute dermal irritation/corrosion study was performed in three male New Zealand rabbits (2 to 4 months old). A single dose of 500 mg HAA299 neat was applied to the closely-clipped skin of one flank. The test item was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.

Result
No cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours were 0.0 for erythema and 0.0 for edema.

Conclusion
Under the experimental conditions, the test item HAA299 was non-irritant when applied topically to rabbits.

Ref.: 4

A.3.3.2.2. Mucous membrane irritation

Guideline:	OECD guideline no. 405
Species/strain:	New Zealand White rabbit.

Group size:	3 males
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	None
Dose level:	100 mg
Dose volume:	/
Observation:	Up to 72 h
GLP:	In compliance
Study period:	11 – 16 April 2006

Method
A single dose of 100 mg of HAA299 in its original form was introduced into the conjunctival sac of the left eye and the lower and upper eyelids were held together for about 1 second. The right eye was not treated and served as control. The eyes of the three animals were not rinsed for approximately 24 hours after instillation of the test article. Indication of pain did not occur in any animal upon instillation of the test article or shortly thereafter. Residual test article was not noted in the treated eyes of any of the animals at the 1-hour observation interval. Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration. The mean values of the scores for chemosis, redness of the conjunctiva, iris lesions and corneal opacity were calculated for each animal.

Result
A slight or moderate chemosis (grade 1 or 2), a moderate redness of the conjunctiva (grade 2) and a clear discharge (grade 1) were observed in all animals at the 1-hour reading. A slight redness of the conjunctiva (grade 1) persisted at the 24-hour reading in 1/3 animals. A slight corneal opacity (grade 1) was recorded in 1/3 animals at the 24-hour reading only.

Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for chemosis, 0.0, 0.3 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and 0.0 for iris lesions and 0.3, 0.0 and 0.0 for corneal opacity.

Conclusion
Under the experimental conditions used, HAA299 was slightly irritant to the eyes of rabbits.
Ref.: 5

A.3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD Guideline no. 429
Species/strain:	Female CBJ/A mice, 9 weeks old
Group size:	7 groups 4 mice per group
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	Propylene glycol
Concentration:	0, 1.0, 2.5, 5.0, 10, 25%
Positive control:	alpha hexacinnamaldehyde (HCA)
GLP:	In compliance
Study period:	29 March – 10 April 2006

Methods

Female CBJ/A mice were assigned to one of 7 groups of 4 mice per group. Propylene glycol was used as vehicle for HAA299. A homogeneous suspension was obtained at the maximum concentration of 25%, after grinding the test item with a mortar and pestle. HAA299 was used at doses of 0 (vehicle, negative control), 1.0, 2.5, 5.0, 10 or 25% in propylene glycol, and 25% alpha hexacinnamaldehyde (positive control).

During the induction phase, the test item, vehicle or reference item was applied over the ears (25 µl per ear) for 3 consecutive days (days 1, 2 and 3). After 2 days of resting, the proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of tritiated methyl thymidine (day 6). The obtained values were used to calculate stimulation indices (SI). The irritant potential of the test item was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6.

Results
Adverse signs of toxicity or body weight change did not occur. The stimulation index (SI) was below 3 for each group given HAA299 and irritation was not seen based on the absence of increased ear thickness. The results are summarised in the table 6. The HCA positive control group showed the test system was sensitive for the assay.

Table 6. Stimulation index

Group % FAT 75808	SI Value
1.0	0.5
2.5	1.02
5.0	1.42
10	1.11
25	0.93
25% HCA	14.27

Conclusion
Under the experimental conditions, the test item HAA299 did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.

Ref.: 6

A.3.3.4. Dermal / percutaneous absorption

Large, micronised particles
***In vitro* percutaneous penetration, rat and human cadaver skin ex vivo**

Guideline:	OECD no. 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 male and one female donor (age 61 – 81 years)
Membrane integrity:	Rat skin membranes with K_p >3.5x10^{-3} cm/h and human skin membranes with K_p > 2.5x10^{-3} cm/h were excluded
Number of membranes:	For rat: 7 membranes. For human 5 membranes (2 membranes excluded)

Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	97%
Test item:	[^{14}C] labelled HAA299
Mean particle size:	d(0.5) = 0.76 µm
Dose volume:	2 mg/cm²
Receptor fluid:	6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v)
Method of Analysis:	24 hours under non-occluded conditions
GLP:	In compliance
Study period:	3 – 15 Aug 2006

Methods

The percutaneous penetration of micronised HAA299 was determined *in vitro* using split-thickness skin membranes from rat and human skin. The [^{14}C] labelled HAA299 was synthesized using ^{14}C labelled Piperazine with a radiochemical purity $\geq$97%. Labelled batch number 3574052. The skin membranes were set up in flow-through diffusion cells, the formulated [^{14}C] HAA299 was applied onto the skin membranes at a finite dose of 13 µl/cm² and the perfusates collected at defined time intervals. One dose level of 2 mg HAA299/cm^2 was used reflecting a concentration of 10% test item in the final formulation. The mean particle size of the test item was found to be d(0.5) = 0.76 µm

The integrity of each skin membrane was determined by applying 50 µl tritium water (about 200,000 dpm) to the skin membrane surface and occluding the donor chamber with adhesive tape. The cumulative penetration was determined over a time period of 6 hours by collecting hourly fractions. The permeability coefficient (K_p) of each skin membrane was calculated for the 3 - 6 hours interval. Rat skin membranes with K_p >3.5x10^{-3} cm/h and human skin membranes with K_p >2.5x10^{-3} cm/h were excluded from the subsequent experiment. After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

The formulated [^{14}C] HAA299 was applied onto skin membranes of 200 µm thickness at a concentration of 105 mg/cm³ leading to an area concentration of 2131 µg/cm². Seven and six replicates were used for rat and human skin membranes, respectively. The exposure of the test item was performed under non-occluded conditions over an exposure time of 24 hours. During the exposure period the receptor fluid (6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) dissolved in physiological saline (0.9% NaCl w/v)) was collected in hourly intervals between 0-6 hours and thereafter in 2 hours intervals until the end of the experiments. At the end of the experiment the remaining [^{14}C] HAA299 was removed from the skin membranes by rinsing the skin membranes four times with acetonitrile. The skin membranes were removed from the diffusion cell and consecutively stripped until the stratum corneum was removed from the skin membrane.

Twenty-four hours after application the perfusate sampling was terminated and each skin membrane surface rinsed four times with about 0.5 ml acetonitrile. All skin membrane rinse fractions were combined according to the individual cells. The skin membranes were removed from the diffusion cell and consecutively stripped until the stratum corneum was removed from the skin membrane; 3 to 5 strips were taken. The stripping tapes were combined into one specimen and aliquots were measured for radioactivity after mixing with tissue solubilizer (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 150 ml acetonitrile and the radioactivity in the cell wash was determined by LSC.

Results

After application of [^{14}C] HAA299 an average of only 0.16% (range 0.06 – 0.27; SD = 0.09) of the applied dose penetrated through the *rat* skin membrane within 24 hours. The mean

Flux was calculated to be 0.543 µg/cm^2/h. At the end of exposure 51% of the applied dose could be washed off from the skin membranes. After skin membrane rinse 42.7% of the dose remained in/on the skin membrane and the major part of this remaining test item was located in the stratum corneum, i.e. 42.3% of the applied dose was determined in tape strips. Only a negligible amount, i.e. 0.36% of the applied dose, was found in the remaining skin membrane after tape stripping.

For *human* skin membranes the penetration of the test item resembles very closely to that observed in rat skin membrane. Within 24 hours of exposure only 0.10% (range 0.08 – 0.13%; SD = 0.02) of the applied dose penetrated totally through human skin membranes. The mean Flux was calculated to be 0.317 µg/cm^2/h. The bulk of the applied dose, i.e. 92%, could be washed off 24 hours after start of exposure. In stratum corneum 2.8% of the applied dose was found and only 0.03% in the remaining skin membrane after tape stripping.

The amount of test item in lower skin layers was lower in human skin membranes as compared to rat skin membranes, respectively.

The results are summarised in the Tables 7 and 8.

Table 7. Skin absorption of HAA299 with rat and human skin membranes

Test system	**Rat Skin Membrane**		**Human Skin Membrane**	
Applied Dose [µg/cm^2]	2131		2131	
Applied Volume [µl]	13		13	
Application Area [cm^2]	0.64		0.64	
Concentration [mg/cm^3]	105		105	
Penetration within	% of dose	µg/cm^2	% of dose	µg/cm^2
6 h	0.11	2.24	0.07	1.42
12 h	0.13	2.77	0.09	1.95
24 h	0.16	3.48	0.10	2.22
Flux [µg/cm^2/h]	0.543*		0.317*	

* steady state seen during the first 6 hours after dosing

Table 8. Recovery [% of Dose]*

Skin Membrane:	**Rat**	**Human**
Applied dose [µg/cm^2]	2131	2131
Perfusates	0.16 (0.09)	0.10 (0.02)
Remaining skin membrane	0.36 (0.36)	0.03 (0.02)
Total Absorbed	**0.52**	**0.13**
Skin membrane rinse	51.35 (9.58)	91.99 (11.76)
Tape strips	42.34 (9.61)	2.81 (1.26)
Diffusion cell wash	3.12 (2.78)	1.44 (1.14)
Recovery	**97.33** (1.94)	**96.37** 13.08)
* Values are mean (± standard deviation)		

Conclusion
The study authors concluded that *large micronised* HAA299 particles, applied to rat and human skin membranes, penetrated at a limited extent through the skin membranes. The total absorption, based on the amount penetrated through the skin membrane (perfusate) and the amount measured in the remaining skin membrane layers after tape stripping, was 0.52% and 0.13% of the applied dose, or 11.1 µg-eq/cm^2 and 2.8 µg-eq/cm^2 for rat and human skin membranes respectively.

Ref.: 12

SCCS comment
The number of membranes used for dermal absorption was lower than that recommended in SCCS Notes of Guidance.

Micronised particles
***In vitro* percutaneous penetration, rat and human cadaver skin ex vivo**

Guideline:	OECD no. 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 individual (male age 78 years) and female (age 89 years)
Membrane integrity:	Rat skin membranes with K_p >3.5x10^{-3} cm/h and human skin membranes with K_p >2.5x10^{-3} cm/h were excluded
Number of membranes:	For rat: 7 membranes. For human 6 membranes (1 human membrane was damaged
Test substance:	FAT 75'808/D
Batch:	VTM07B0, 3574052
Purity:	98.6%
Test item:	[^{14}C] labelled HAA299
Mean particle size:	d(0.5) = 140 nm and 134 nm
Dose volume:	2 mg/cm²
Receptor fluid:	6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v)
Method of Analysis:	24 hours under non-occluded conditions
GLP:	In compliance
Study period:	6 May – 28 Oct 2008

Methods
The [^{14}C] labelled HAA299 was synthesized using ^{14}C labelled Piperazine with a radiochemical purity $\geq$97% and specific activity of 2242 MBq/mmol (60.6 mCi/mmol) or 3309 kBq/mg (89.43 µCi/mg). The radiochemical was repurified at the testing laboratory.

The dosing suspension was prepared as a mixture of non-labelled and labelled test material to give [^{14}C]-labelled HAA299 with a final specific radioactivity of about 20 kBq/mg (0.54 µCi/mg). After removing residual solvent from the mixture it was micronised in a micro-mill with additions of a surfactant (sodium myreth sulfate) and silicon defoaming agent using the Ciba-patented process that is used to prepare the commercial product sold for formulation of consumer sunscreens.

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 from the full thickness sections and then from the stratum corneal aspect removing the upper 200 µm by dermatome. The membranes were then cut into pieces (ca. 1.8 x 1.8 cm) and mounted in flow-through diffusion cells each consisting of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber

was 0.64 cm^2. From each species 7 membranes in cells were prepared and the cells placed in manifolds and connected to a peristaltic pump. For an equilibration period of 1 hour, saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 ml/h.

The integrity of each skin membrane was determined by applying 50 µl tritium 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 permeability coefficient (K_p) of each skin membrane was calculated for the 3 - 6 hours interval. Rat skin membranes with K_p>3.5x10^{-3} cm/h and human skin membranes with K_p > 2.5x10^{-3} cm/h were excluded from the subsequent experiment. After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

A target dose level of 2 mg/cm² was selected for both rat and human skin based on the topical application rate of final sunscreen formulations assumed to be used by humans. A 13 µl aliquot of the dosing solution was applied manually to each skin membrane preparation. The amount applied to each cell was shown to be 1392 µg/cell or 2175 µg/cm^2 by determination of the radioactivity content of three control doses taken prior to the first, in the middle, and after the last administration for each dose level.

The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions. The very low solubility of HAA299 in usual substances led to use a receptor fluid (perfusate) of 6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v); it was delivered at a flow rate of about 3 ml/h during the testing period. The perfusate from each cell was collected separately at ambient temperature in 1-hour intervals for the 0 to 6 hour period (6 intervals), and in 2-hour intervals for the remaining exposure period (9 intervals).

Twenty-four hours after application the perfusate sampling was terminated and each skin membrane surface rinsed three times with a shower gel solution (1%) in water followed by one time with 0.5 ml purified water for each chamber. All skin membrane rinse fractions were combined according to the individual cells. 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 solubilizer (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 dosing mixture was shown to have a radiochemical purity of 98.14%, determined by HPLC at the time of application and a concentration of 106 mg [^{14}C]-HAA299/ml determined by LSC. Mean particle size (d(0.5)) was 140 nm and 134 nm in the two measurements taken. 7 rat skin membranes and 6 human skin membranes were used. The applied dose was determined to be 2175 µg/cm^2 (total particles dosed 1.3x10^{18}/cm^2, surface area of particles dosed 0.07 m^2/cm^2.

Based on test item found in perfusate the percutaneous penetration rate in rat and human skin was below reliably quantifiable concentrations as shown in table 9.

Table 9. Skin absorption of HAA299 with rat and human skin membranes

HAA299	**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 LOQ values instead of the measured values, all of which were below LQ. 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 summarised in Table 10.

Table 10. Distribution and recovery of the test item

	Recovery [% of Dose]*	
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 limits of quantification for the samples. Penetration through rat skin membranes was slightly higher than through human skin membranes.

Similarly, larger amounts of test item in the tape strips from rat skin compared to human skin membranes was found, 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
The study authors concluded that the cumulative penetration of formulated HAA299 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 formulated as micro suspension according to technical specification did not penetrate through rat and human skin membranes.

Ref.: 22

SCCS comment
The number of membranes used for dermal absorption was lower than that recommended in SCCS Notes of Guidance.

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

Guideline:	OECD no. 428
Species/strain:	Human full thickness skin was obtained from the dorsal upper leg of three individuals (male age 78 and 17 years, female age 83 years)
Membrane integrity:	Human skin membranes with K_p >2.5x10^{-3} cm/h were excluded
Number of membranes:	7 membranes, 3 persons (2 from males 78 and 17 year, 1 female 83 years)
Test substance:	FAT 75'808/D
Batch:	VTM07B04, 3574052
Purity:	98.6%
Test item:	[^{14}C] labelled HAA299
Mean particle size:	d(0.5) = 153 nm and 152 nm
Dose volume:	2 mg/cm²
Receptor fluid:	6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v)
Method of Analysis:	24 hours under non-occluded conditions
GLP:	In compliance
Study period:	27 Oct – 3 Nov 2008

The experiment was performed to investigate changes in absorption through skin that may occur if the skin is damaged before application, e.g. by sunburn, dryness, irritation, atopic and eczematous status, or abrasions such as those from shaving. Tape-stripped human skin membranes were used to model a compromised stratum corneum with impaired barrier properties. This will allow uptake of small particle sized test material through impaired skin to be covered in the human safety assessment.

Methods
The [^{14}C] labelled HAA299 was synthesized using ^{14}C labelled Piperazine with a radiochemical purity $\geq$97% and specific activity of 2242 MBq/mmol (60.6 mCi/mmol) or 3309 kBq/mg (89.43 µCi/mg). The radiochemical was repurified at the testing laboratory.

The dosing suspension was prepared as a mixture of non-labelled and labelled test material to give [^{14}C]-labelled HAA299 with a final specific radioactivity of about 20 kBq/mg (0.54 µCi/mg). After removing residual solvent from the mixture it was micronised in a micro-mill with additions of a surfactant (sodium myreth sulfate) and silicon defoaming agent using the Ciba-patented process that is used to prepare the commercial product sold for formulation of consumer sunscreens.

Human full thickness skin was obtained post-mortem from the dorsal upper leg or abdominal area of three individuals and stored frozen until use. Skin membranes were prepared by removing subcutaneous fat from the full thickness sections. The human cadaver skin was tape stripped 3 times, the upper 200 µm were removed by dermatome and then circular samples cut into pieces (ca. 1.8 x 1.8 cm) and mounted in flow-through diffusion cells each consisting of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm^2. Fourteen membranes in cells were prepared and the cells placed in manifolds and connected to a peristaltic pump. For an equilibration period of 1 hour, saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 ml/h.

The integrity of the skin membrane was determined by applying 50 µl tritium 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 permeability coefficient (K_p) 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. K_p values $<2.5x10^{-3}$ cm/h were considered indicator of undamaged skin and were excluded from the experiment. After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

A target dose level of 2 mg/cm² was selected based on the topical application rate of final sunscreen formulations assumed to be used by humans. A 13 µl aliquot of the dosing solution was applied manually to each skin membrane preparation. The amount applied to each cell was shown to be 1354 µg/cell or 2116 µg/cm^2 by determination of the radioactivity content of three control doses taken prior to the first, in the middle, and after the last administration for each dose level.

The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions. The very low solubility of HAA299 in usual substances led to use a receptor fluid (perfusate) of 6% (w/v) polyethoxyoleate (PEG 20 oleyl ether) in physiological saline (0.9% NaCl w/v); it was delivered at a flow rate of about 3 ml/h during the testing period. The perfusate from each cell was collected separately at ambient temperature in 1-hour intervals for the 0 to 6 hour period (6 intervals), and in 2-hour intervals for the remaining exposure period (6 – 24 h, 9 intervals).

Twenty-four hours after application the perfusate sampling was terminated and each skin membrane surface rinsed three times with a shower gel solution (1%) in water followed by one time with 0.5 ml purified water for each chamber. All skin membrane rinse fractions were combined according to the individual cells. 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 solubilizer (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 dosing mixture was shown to have a radiochemical purity of 98.49%, determined by HPLC at the time of application and a concentration of 104 mg [^{14}C]-HAA299/ml determined by LSC. Mean particle size d(0.5) was 153 nm and 152 nm in the two measurements taken. 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 assuming that the damage of the skin membrane led to an increase of permeability. The applied dose was determined to be 2116 μg/cm^2 (total particles dosed 1.3x10^{18}, surface area of particles dosed 0.07 m^2/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 11.

Table 11. Skin absorption of HAA299 with pre-damaged human skin membranes

HAA299	**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		

Table 12. Distribution and recovery of the test item

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HAA299	**Recovery [% of Dose]***
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.

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 summarised in table 12.

Conclusion
The study authors concluded that the cumulative penetration of formulated ^{14}C-HAA299 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.: 23

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

Guideline:	OECD no. 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:	FAT 75'808/D
Batch:	VTM07B04, 3574052
Purity:	98.6%
Test item:	[^{14}C] labelled HAA299
Mean particle size:	d(0.5) = 153 nm and 152 nm
Dose:	2.1 mg/cm²; 10 cm^2; 200 µl
Exposure time	6 hours under non-occluded conditions
GLP:	In compliance
Study period:	28 May – 30 Oct 2008

Methods
The [^{14}C] labeled HAA299 was synthesized using ^{14}C labelled Piperazine with a radiochemical purity $\geq$97% and specific activity of 2242 MBq/mmol (60.6 mCi/mmol) or 3309 kBq/mg (89.43 µCi/mg). The radiochemical was repurified at the testing laboratory.

The dosing suspension was prepared as a mixture of non-labelled and labelled test material to give [^{14}C]-labelled HAA299 with a final specific radioactivity of about 20 kBq/mg (0.54 µCi/mg). After removing residual solvent from the mixture it was micronised in a micro-mill with additions of a surfactant (sodium myreth sulfate) and silicon defoaming agent using the Ciba-patented process that is used to prepare the commercial product sold for formulation of consumer sunscreens.

The rats were dosed (200 µl each) at one nominal dose level of 2.1 mg HAA299)/cm^2 (number of particle 1.2 x 10^{18}, surface area 0.07 m^2/cm^2) applied to an area of 10 cm². 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 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 dose 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 solubilizer.

Termination of rats in each group was followed by radiochemical analysis of collected samples of blood, plasma, gastro-intestinal tract, carcass, and skin from treated and non-treated area.

Results

Purity of the dosing formulation at the time of application was shown to 98.1% [^{14}C] HAA299. Stability of the dosing 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]HAA-299 remained unchanged during the time of exposure. The applied dosage was 2127 μg/cm^2. The results are presented in Tables 13 and 14.

98.5% of the applied dose could be dislodged from the application site at the end of exposure. 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% for the dose level.

Dermal absorption was generally low. The amount totally absorbed during the 6-h exposure period was 1.133 µg/cm² corresponding to 0.05% ($\pm$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%. Within 72 hours, an increase in systemic absorption was observed with a maximum rate of 0.43% (48 h time point is disregarded by SCCS, see Comment).

The concentrations of radioactivity in blood during exposure were below the limit of quantification (LOD, 0.180 ppm HAA288) at all sampling time points. The systemically absorbed dose was very slowly excreted with urine and faeces.

Table 13. Mass balance at different time points

Mass balance [µg HAA299 equivalents/cm²]				
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**			

*See comment by SCCS

Table 14. HAA299 distribution after dermal application

Mass balance [percent 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
Faeces				
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[1]	< 0.01	< 0.01	< 0.01	< 0.01
Skin Non-Treated Area[1]	< 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
Dislodged Dose[2]	**99.19**	**99.26**	**97.90**	**97.68**
Total Recovery	**99.53**	**100.17**	**99.83**	**99.15**

[1] Residues determined in the taken part of the specimen
[2]Dislodged dose; mean = 98.51%
*See comment by SCCS

Conclusion
The study authors concluded that micronised HAA299 penetrated to a very low extent through rat skin after dermal application.

Ref.: 24

SCCS comment
The results presented in Table 13 regarding "Total Systemic Absorption" after 48 h and in Table 14 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 did the other rats. The applicant did not comment on this. The SCCS opinion is that the results (System Absorption = 0.76%) of the 48 h time point should be disregarded.

SCCS general comment
Four skin absorption studies, three *in vitro* studies and one *in vivo* study, have been performed by the applicant. All studies were made in the same laboratory. In the two *in vitro* studies with rat skin, the total absorptions were 0.52% and 0.52% after 24h compared with 0.43% (total excreted and remaining in the body) after 72 h in the *in vivo* rat study. Thus, on the basis of rat studies the dermal absorption is around 0.5%.

In the three *in vitro* studies with human skin the absorption after 24 h was 0.13% (large micronised particles), 0.03% (micronised particles) and 0.04% (micronised particles, pre-damaged skin). It is noted that the amounts in the perfusates were 0.10%, <0.01%, and <0.01% in the three experiments. The difference in absorption cannot be explained by "out layers".

The purity of the test item used in the skin absorption studies ranged between 97% to 98.6%. Thus, it is uncertain to what extent the impurity play a role in the observed absorption.

The SCCS is of the opinion that rat skin dermal absorption is around 0.5% of the applied amount.

The human dermal skin absorption is likely to be lower than the rat skin absorption. In terms of risk assessment, the human skin absorption will represent an uncertain small systemic exposure dose (SED) and will not allow a valid calculation of margins of safety.

A.3.3.5. Repeated dose toxicity

A.3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

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A.3.3.5.2. Sub-chronic (90 days) oral toxicity

Guideline:	EEC Directive No. 2001/59, B26, 21th August 2001; EEC Directive No. 2004/73, B43, 29 April 2004 and EPA, Guideline 799, 9620-62-158, 15 August 1997 (neurotoxicological investigations)
Species/strain:	Sprague-Dawley rats, 6 week old at start of study
Group size:	Control. 18 males and 18 females, Low and middle dose 14 males and 14 females, High dose 20 males and 20 females.
Test substance:	FAT 75'808/B
Batch:	VTM05B10

Purity:	99.1%
Vehicle:	0.5% [w/v] carboxymethylcellulose in purified water
Dose levels:	0, 50, 250 and 1000 mg/kg bw/day
Dose volume:	5 ml/kg bw
Route:	Oral administration (gavage)
Administration:	13 week
GLP:	In compliance
Study period:	06 June – 12 October 2006

Methods

The test material was a suspension of HAA299 in the vehicle (0.5% [w/v] carboxymethylcellulose in purified water). The homogeneity and stability (over 4 and 9 days) of the test item in the dosage forms were checked and the actual concentrations were verified in weeks 1, 4, 8 and 13 during the administration period, using validated analytical method. Results of these analyses showed homogeneity, stability, and dosed concentrations were each within an acceptable range of -4 to +2%.

The dose-levels used in this 13-week study (50, 250 and 1000 mg/kg bw/day) were selected on the basis of the results of a 14-day dose range-finding study (CIT/Study No. 31455 TSR) in which no signs of toxicity were observed during the in-life phase after daily oral gavage dosing with 100, 750, or 1500 mg/kg bw/day.

The test system was rat strain Sprague-Dawley, Crl CD® (SD) IGS BR, Caesarian Obtained, Barrier Sustained-Virus Antibody Free (COBS-VAF®) from Charles River Laboratories France, l'Arbresle, France. On the first day of treatment the animals were 6 weeks old and had a mean body weight of 196 g (range: 178 g to 217 g) for the males and 169 g (range: 143 g to 185 g) for the females.

Daily dosing was by oral gavage of 50 or 250 mg/kg bw/day for 13 consecutive weeks of test material suspension under a constant dosage-volume of 5 ml/kg bw/day to 14 male and 14 female rats (10 principal and 4 satellite animals per sex and per group). Another group of 20 males and 20 females (16 principal and 4 satellite animals per sex) were treated under the same experimental conditions at the dose-level of 1000 mg/kg bw/day. One other group of 18 males and 18 females (16 principal and 2 satellite animals per sex) received the vehicle alone under the same experimental conditions and acted as a control group. At the end of the treatment period, all surviving animals were sacrificed, with the exception of six principal animals per sex from the group treated at 1000 mg/kg/day and from the control group, which were sacrificed after an additional 4-week treatment-free period.

Results

There were no unscheduled deaths or premature sacrifices during the treatment period or treatment-free periods and no treatment-related clinical signs occurred during the study. There were no test material-related effects of any dosage on body weight, body weight gain, food consumption and behavioural signs by functional observational battery. No relevant ophthalmological findings were noted at the end of the treatment period. There were no relevant differences in the number and duration of estrous cycles. Hematological, blood biochemistry and urinary parameters were not affected by treatment with the test item. Circulating levels of thyroid hormones, or in progesterone, estradiol, or testosterone levels in treated animals were considered to be similar in weeks 6 and 13 in comparison to controls. There were no macroscopic or microscopic findings indicative of toxicity of HAA299 at the end of the treatment and treatment-free periods.

Plasma analysis, performed two hours after treatment on day 1, as well as 24 hours after treatment in weeks 6 and 13, did not find quantifiable levels of test material in any of the dose groups at any of the time points sampled.

Conclusion
Under the experimental conditions of this study, the No Observed (Adverse) Effect Level (NOEL/NOAEL) of HAA299 given by oral route to rats for 13 weeks at constant dose-levels can be established at 1000 mg/kg/day.

Ref.: 3

SCCS comment
The absence of effect observed up to the highest dose may be related to the low bioavailability.

A.3.3.5.3. Chronic (> 12 months) toxicity

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A.3.3.6. Mutagenicity / Genotoxicity

A.3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD no. 471
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 *Escherichia coli,* WP2*uvr*A
Assay conditions:	Plate incorporation and pre-incubation assay without and with S9-mix from rat livers (Aroclor 1254 induced). Three plates were investigated per test concentration. Two independent experiments were performed
Test substance:	FAT 75'808/A
Batch:	HAA299/7-5
Purity:	98.7%
Vehicle:	DMSO
Concentrations:	50 – 5000 µg/plate with and without metabolic activation
GLP:	In compliance
Study period:	27 Jul – 14 Sep 2005

Methods
HAA299 was tested in the Ames test using four histidine-requiring *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* strain WP2*uvr*A. The substance was suspended in DMSO at 200 mg/ml and tested with a confirmatory experiment, in the absence or presence of Aroclor-induced rat liver S9, at doses ranging from 50 to 5000 µg/plate (with and without S9). After a preliminary toxicity assay, both experiments were performed according to the direct plate incorporation method except for the second test with S9 mix, which was performed according to the pre-incubation method (60 minutes, 37°C). Positive control substances appropriate to each test system component were used concurrently in the test item experiments.

Results
In a preliminary toxicity test with strain TA100, bacteriostatic activity of 52.5% and 30.5%, respectively, compatible with the maximum level acceptable being 75% was observed at 5000 and 1500 µg/plate. The selected treatment-levels were 50, 150, 500, 1500 and 5000 µg/plate, for both mutagenicity experiments with and without S9 mix. At 5000 µg/plate, a

marked decrease in the number of revertant colonies in the *Salmonella* strains TA 1535 and TA 98 and in the *Escherichia coli* strain was observed. For the other doses, no evidence of any increase in the number of revertants, both with or without S9 mix, was recorded. The number of revertants for the vehicle and positive controls was as specified in the acceptance criteria and the study was considered valid.

Conclusion
Under the applied experimental conditions, HAA299 did not show mutagenic activity in the bacterial reverse mutation test with *Salmonella typhimurium* and *Escherichia coli*.

Ref.: 14

SCCS comment
Negative results may be due to lack of bacterial uptake of HAA299 particles.

***In vitro* Mammalian Chromosome Aberration Test**

Guideline:	OECD Guideline no. 473
Species/strain:	Human lymphocytes from 2 healthy donors (one male and one female for each experiment)
Replicates:	Two independent experiments
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	DMSO
Concentrations:	1.56 to 200 µg/ml for the first experiment (± S9) 6.25 to 200 µg/ml for the second experiment (± S9)
Treatment:	Cells were harvested 3, 20, and 44 hours after start of treatment
GLP:	In compliance
Study period:	11 May – 11 Aug 2006

Methods
An *in vitro* cytogenetics assay was conducted with human lymphocytes exposed to HAA299 with and without exogenous rat S9 derived metabolic activation system**.**

The test item was tested in two independent experiments, both with and without a liver metabolizing system (S9 mix), obtained from rats previously treated with Aroclor 1254. The highest dose-level for treatment in the first experiment was selected on the basis of pH, osmolality and solubility. For selection of the dose-levels for the second experiment, any toxicity indicated by the reduction of mitotic index (MI) in the first experiment was also taken into account.

For each culture, heparinized whole blood was added to culture medium containing a mitogen (phytohemagglutinin) and incubated at 37°C, for 48 hours.

In the first experiment, lymphocyte cultures were exposed to the test or control items (with or without S9 mix) for 3 hours then rinsed. Cells were harvested 20 hours after the beginning of treatment, corresponding to approximately 1.5 normal cell cycles.

The second experiment was performed as follows:

- without S9 mix, cells were exposed continuously to the test or control items until harvest
- with S9 mix, cells were exposed to the test or control items for 3 hours and then rinsed.

Cells were harvested 20 hours and 44 hours after the beginning of treatment, corresponding to approximately 1.5 normal cell cycles and 24 hours later, respectively. One and a half hours before harvest, each culture was treated with a colcemid solution (10 µg/ml) to block cells at the metaphase-stage of mitosis. After hypotonic treatment (KCl 0.075 M), the cells were fixed in a methanol/acetic acid mixture (3/1; v/v), spread on glass slides and stained with Giemsa. All the slides were coded for scoring.

HAA299 was suspended in dimethylsulfoxide (DMSO). The dose-levels of the positive controls were as follows:

- without S9 mix, Mitomycin C: 3 µg/ml (3 hours of treatment) or 0.2 µg/ml (continuous treatment),
- with S9 mix, Cyclophosphamide: 12.5 or 25 µg/ml.

Results

In the culture medium, the dose-level of 200 µg/ml obtained using a maximum treatment volume of 1% (v/v) showed a slight precipitate. At this dose-level, the pH and the osmolality values were equivalent to those of the vehicle control cultures.

The dose-levels used for treatment were from 1.56 to 200 µg/ml for the first experiment and from 6.25 to 200 µg/ml for the second experiment, both with and without S9 mix. A slight precipitate was observed at the end of the treatment period, generally at dose-levels ≥ 25 µg/ml.

Experiments without S9 mix:

Except for some sporadic decreases in mitotic indices which were not clearly dose-related, no noteworthy toxicity was observed in either experiment and at either harvest time.

The dose-levels selected for metaphase analysis were as follows:

- 50, 100 and 200 µg/mL, for the 3-hour and the 20-hour treatments, the latter being the highest achievable and precipitating dose-level,
- 200 µg/mL, for the 44-hour treatment, this dose-level being the highest achievable and precipitating dose-level.

No significant increase in the frequency of cells with structural chromosomal aberrations was noted after 3-, 20- as well as 44-hour treatments.

Experiments with S9 mix:

Except for some sporadic decreases in mitotic indices which were not clearly dose-related, no noteworthy toxicity was observed in either experiment and at either harvest time.

The dose-levels selected for metaphase analysis were as follows:

- 50, 100 and 200 µg/mL, for the 20-hour harvest time in both experiments, the latter being the highest achievable and precipitating dose-level,
- 200 µg/mL, for the 44-hour harvest time, this dose-level being the highest achievable and precipitating dose-level.

No significant increase in the frequency of cells with structural chromosomal aberrations was noted in either experiment and at either harvest time. No increase in polyploidy was observed. The frequency of cells with structural chromosome aberrations of the vehicle and positive controls was as specified in acceptance criteria.

Conclusion

Under the experimental conditions used, HAA299 did not induce chromosome aberrations in cultured human lymphocytes.

Ref.: 15

SCCS comment

Negative results may be due to lack of cellular uptake of HAA299 particles.

Mouse lymphoma (*tk* locus) *in vitro* mutation

Guideline:	OECD no. 476	
Species/strain:	Mouse lymphoma L5178Y cell line	
Replicates:	Two independent experiments	
Test substance:	FAT 75'808/B	
Batch:	VTM05B10	
Purity:	99.1%	
Vehicle:	DMSO	
Concentrations:	0, 3.13, 6.25, 12.5, 25, 50 and 100 µg/mL: for both experiments ± S9-mix	
Positive control:	Without S9-mix:	methyl methane sulfonate (MMS)
	With S9-mix:	cyclophosphamide (CPA)
Treatment:	Experiment I:	3 hours treatment ± S9-mix
	Experiment II:	24 hours treatment without S9-mix
GLP:	In compliance	
Study period:	27 Jun – 14 Aug 2006	

Methods
HAA299 was evaluated in mouse lymphoma L5187Y cells for effects at the *tk*-locus with and without exogenous metabolic activation. The test item was suspended in DMSO at a concentration of 20 mg/ml.

After a preliminary toxicity test, HAA299 was tested in two independent experiments, with and without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254.

Approximately 0.5 x 10^6 (3-hour treatment) or 0.15 x 10^6 (24-hour treatment) cells/ml in 20 ml culture medium with 5% horse serum were exposed to the test or control items, in the presence or absence of S9-mix (final concentration of S9-mix 2%), at 37°C. For the 24-hour treatment, the incubation at 37°C was performed with a gentle shaking. Cytotoxicity was measured by assessment of adjusted relative total growth (Adj. RTG). The number of mutant clones (differentiating small and large colonies) was checked after the expression of the mutant phenotype. The test item was suspended in dimethylsulfoxide (DMSO).

The dose-levels for the positive controls were as follows:
- without S9 mix: methylmethane sulfonate (MMS), used at a final concentration of 25 µg/ml (3-hour treatment) or 5 µg/ml (24-hour treatment),
- with S9 mix: Cyclophosphamide (CPA), used at a final concentration of 3 µg/ml.

Results
Since the test item was non-toxic, poorly soluble, the highest dose-level was based on the level of precipitate. The selected dose-levels were as follows: 0, 3.13, 6.25, 12.5, 25, 50 and 100 µg/ml: for both experiments with and without S9 mix. Precipitate was noted in culture medium at the end of treatment mainly at dose-levels ≥ 25 µg/ml.

Experiments without S9 mix:
Cytotoxicity:
Following the 3-hour as well as the 24-hour treatments, no toxicity was noted in test item treated cultures as shown by the Adj. RTG values which were very consistent with the vehicle control values.

Mutagenicity:

Following the 3-hour treatment as well as the 24-hour treatments, no noteworthy increase in the mutant frequency was observed in test item treated cultures.

Experiments with S9 mix:
Cytotoxicity:
In either experiment, no toxicity was noted in test item treated cultures as shown by the Adj. RTG values which were very consistent with the vehicle control values.

Mutagenicity:
In either experiment, no noteworthy increase in the mutant frequency was observed in test item treated cultures.

The cloning efficiencies CE2 and the mutant frequencies of the vehicle and positive controls were as specified in acceptance criteria.

Conclusion
Under the experimental conditions, HAA299 did not show any mutagenic activity in the mouse lymphoma assay.

Ref.: 16

SCCS comment
Negative results may be due to lack of cellular uptake of HAA299 particles.

A.3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

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A.3.3.7. Carcinogenicity

/

A.3.3.8. Reproductive toxicity

A.3.3.8.1. Two generation reproduction toxicity

/

A.3.3.8.2. Teratogenicity

Guideline:	OECD no. 414
Species/strain:	Sprague-Dawley strain (Rj Han. SD)
Group size:	24 mated females
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	0.5% carboxymethylcellulose in purified water
Dose levels:	0 (vehicle control) 100, 300 or 1000 mg/kg bw/day
Dose volume:	5 ml/kg bw/day
Route:	Oral administration (gavage)
Administration:	Day 6 to day 20 *post-coitum* inclusive
GLP:	In compliance
Study period:	17 Jul – 14 Aug 2006

Methods
The objective of this study was to evaluate the potential toxic effects of HAA299 on the pregnant female and on embryonic and fetal development following daily oral administration (gavage) to pregnant female rats during the period of organogenesis (from implantation to closure of the hard palate: day 6 to day 20 *post-coitum* (*p.c.*) inclusive).

Three groups of 24 mated female rats of the Sprague-Dawley strain [Rj Han. SD, Indemn of Organism Pathogen Specific Han (IOPS Han)] (11 weeks old at the start of treatment) received HAA299 by daily oral administration at 100, 300 or 1000 mg/kg bw/day at a constant dosage volume of 5 ml/kg/day; the once-daily doses were administered from day 6 to day 20 *p.c.*. Another group of 24 mated females of the same strain received the vehicle alone (0.5% carboxymethylcellulose) under the same experimental conditions and served as the control group. Dosages were selected based on a dose-range finding study with doses of 100, 300 and 1000 mg/kg bw/day in which adverse effects did not occur in dams or their embryos and fetuses. The dose formulation was a suspension of the test material at 20, 60 or 200 mg/ml in aqueous 0.5% carboxymethylcellulose prepared for up to 9 days treatment and shown by analytical verification to remain stable under refrigeration for this length of time.

Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designated intervals. On day 21 *p.c.*, the dams were sacrificed and subjected to a macroscopic post-mortem examination. The gravid uteri were weighed and the fetuses were removed by hysterectomy. The following litter parameters were recorded: numbers of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses. The fetuses from the first 20 pregnant females were weighed, sexed and subjected to external, soft tissue or skeletal examinations. The placentas were examined for grossly observable changes.

Results
Unscheduled deaths did not occur in any of the dosed presumed pregnant females of any group. Body weight, body weight change, and food consumption were not affected by the test material when compared to control group. The gravid uterine weights in the test material-dosed dams were not significantly different from control group dams. No test material related clinical signs were noted during the study. One female treated at 1000 mg/kg bw/day had opacity of the right eye from day 19 *p.c.* until sacrifice on day 21 *p.c.* Given the low incidence of this finding, it was considered to be spontaneous in origin. No abnormalities were observed in treated females at macroscopic *post-mortem* examination.

Litter data. All pregnancy parameters (numbers of *corpora lutea*, implantations and fetuses and the extent of pre- and post-implantation losses) were similar to control values for all test item-treated groups. All groups had between 46 and 52% of male fetuses. Mean fetal body weight was similar to the mean control weight for all treated groups.

One fetus in the control group, one fetus in the group treated at 100 mg/kg bw/day and one fetus in the group treated at 1000 mg/kg bw/day had gastroschisis (a congenital fissure in the abdominal wall usually accompanied by protrusion of the viscera). As the incidence between each of the test item-treated groups and the control group was the same, this malformation was considered not to be related to treatment. No treatment-related variations were observed on external examination.

One fetus in the group treated with 300 mg/kg bw/day had a dilated 3rd cerebral ventricle. As this was not observed in any other group, it was considered to be spontaneous in origin. One fetus in the group treated at 100 mg/kg bw/day was missing the right kidney and ureter and another fetus in the same group had a small, malpositioned kidney. As these

observations were not recorded in the groups treated at 300 or 1000 mg/kg bw/day they were considered not to be related to treatment.

There were no skeletal malformations in fetuses from the groups treated with the test item. All skeletal variations recorded in the treated groups were observed at incidences similar to the controls and were not related to treatment.

Conclusion
HAA299 was well tolerated by the dams, with no adverse effects at any dose-level. Fetal examination resulted in no treatment-related malformations or variations at any dose-level. Under the experimental conditions of this study, the No Observed (Adverse) Effect Level (NOEL/NOAEL) for both the maternal toxicity and the developmental toxicity was identified at 1000 mg/kg bw/day.

Ref.: 11

SCCS comment
The absence of effect observed up to the highest dose may be related to the low bioavailability.

A.3.3.9. Toxicokinetics

Large, micronised particles

Guideline:	OECD no. 417
Species/strain:	Male HanRcc:WIST (SPF): Wistar rats about 7 weeks old, approx 200 g
Group size:	Group 1: 4 males, for mass balance Group 2: 9 males, for blood kinetics
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Test item:	[^{14}C] labelled HAA299 radiolabel purity 91.4%
Mean particle size:	d(0.5) = 8.33 µm
Vehicle:	0.5% carboxymethylcellulose and 0.4% Tween 80 in purified water
Dose levels:	100 mg/kg bw
Dose volume:	5 ml/kg bw
Route:	Oral administration (gavage)
Administration:	Single dose
GLP:	In compliance
Study period:	24 Oct 2006 – 26 Feb 2009

Methods
The *in vivo* absorption, distribution, metabolism, and elimination of micronised (d(0.5) = 8.33 µm) radiolabeled HAA299 was evaluated in male Wistar rats. The experiment was performed over 96-hours after a single oral gavage dose with the following objectives:

1) to estimate for the oral route the rate and extent of intestinal absorption of the test item,
2) to investigate the blood kinetics,
3) to determine the pattern of tissue distribution of the test item,
4) to determine the rates and routes of excretion of the test item, and
5) to investigate the metabolite pattern in urine and faeces extracts.

The [^{14}C] labelled HAA299 was synthesized by PerkinElmer Life Science Products (Boston, MA, USA) as HAA299, [Piperazine-^{14}C] with a radiochemical purity 91.4% and specific activity of 2242 MBq/mmol (60.6 mCi/mmol) or 3309 kBq/mg (89.43 µCi/mg), and labelled

batch number 3574052. The material was diluted with the nonradiolabeled test item to a final specific radioactivity of 147 kBq/mg (3.97 µCi/mg). After removing of residual solvent the mixture was micronised in a planet micro mill with addition of 3 g carboxymethylcellulose (CMC) solution [0.5% CMC and 0.4% Tween 80, v/v] and zirconium milling balls. The resulting micro suspension had a total volume of 10 ml and a concentration of 18.1 mg HAA299/ml suspension. Determined by laser particle sizer, about 50% of the particles were below 8.33 µm in diameter and 10% less than 1.30 µm in diameter.

The test system was 13 male HanRcc:WIST (SPF): Wistar rats, outbred, SPF-quality of approximately 200 g body weight corresponding to about 7 weeks of age. An acclimatization period was at least five days to the laboratory environment and included at least one day to the metabolism cages. The animals were kept in rooms maintained at standard conditions, i.e. a temperature of 22±3°C, a relative humidity of 30-70% and a 12 hours light/dark cycle.

The animals were divided into one group of 4 rats for mass balance (Group 1) and a second group of 9 animals for determination of blood kinetics (Group 2); each group received a single dose of 100 mg HAA299/kg bw.

Group 1: Urine and faeces samples for each of four 24-hour periods were collected individually and separately. Study termination at 96-hours after dosing. Cages were rinsed separately and the rinsates analysed for collected radioactivity. From each animal, in addition to the blood and plasma collected, samples of each of the following were taken and weighed: liver, kidney, fat, and muscle; the remaining carcass was retained and processed for determination of radioactivity. Metabolites in urine and faeces were investigated after processing the samples separately for the 0-24 hours and 24-48-hours collection periods. For urine, aliquots representing 10% of the total volume of each animal of Group 1 were pooled according to sampling time.

Group 2: Blood kinetics were determined from serial blood samples of approximately 0.3 ml each withdrawn sublingual from 3 individual animals at the selected time points and collected into heparinized tubes. After taking aliquots of whole blood, it was separated into plasma and red blood cells by centrifugation at about 1500-2000 g for 10 min. Three animals each were sacrificed by exsanguination after anesthesia with carbon dioxide at 24, 36 and 48 hours after administration. Terminal blood was collected into heparinized tubes and worked up analogously to the sublingual blood samples. The remaining specimens were stored frozen.

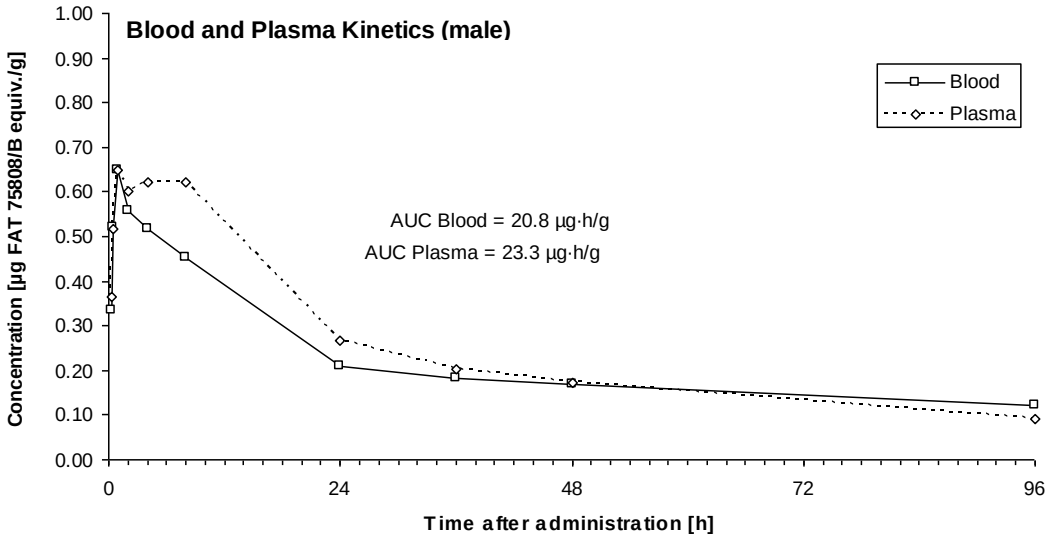
Results

All 13 animals survived their specified study period and did not show signs of toxicity or adverse effects. Administered doses across both groups ranged from 99.7 to 104.8 mg HAA299 /kg bw; the mean values were 102.3 mg/kg (2893 kBq/rat) for Group 1 and 102.9 mg/kg (3050 kBq/rat) for Group 2. The test item purity was 91.4% determined by HPLC analysis of the dosing mixture and remained stable during the 96-hours exposure period.

Absorption and Excretion. After oral administration the radioactivity was very poorly absorbed from the gastrointestinal tract into systemic circulation. The extent of absorption, calculated based on the urinary excretion and the remaining amount in the carcass and tissues, accounted for 1.93% of the administered dose.

The maximum concentration level in blood and plasma was achieved 1 hour after administration, accounting for 0.649 and 0.650 µg HAA299 equivalents/g, respectively. After reaching the maximum the concentration in blood decreased rapidly with an initial half life (1-24 h) of 14.8 hours, whereas the concentration in plasma remained almost constant until 8 hours post dosing. Thereafter the concentration in plasma decreased also with an

initial half-life (8-36 h) of about 16.9 hours. The AUC values (0-96 h) were calculated to be 20.8 and 23.3 µg·h/g for blood and plasma, respectively. The blood/plasma ratio was 1:1.1.



As summarised in table 15 below, 91.03% of the administered test item was excreted with the faeces, which represented 98.1% of the recovered radioactivity within 48 hour after dosing. Only a very small amount of radioactivity was excreted with the urine, i.e. 1.75% of the dose or 1.89% of the radioactivity recovered.

Table 15. Excretion of HAA299 after oral administration

Excretion [% of dose]		
Urine		
	0 - 24 h	**1.66**
	24 - 48 h	**0.07**
	48 - 96 h	**0.03**
	Subtotal	**1.75**
Faeces		
	0 - 24 h	**87.62**
	24 - 48 h	**3.37**
	48 - 96 h	**0.04**
	Subtotal	**91.03**
Cage Wash		**0.02**
Total Excretion		**92.80**

Distribution. The very low extent of absorption resulted in generally very low residues measurable in the tissues. The LOQ was 0.02 µg-equivalents per gram for each tissue, except for the remaining carcass with LOQ of 0.013 µg-equivalents per gram. Expressed as percent of dose applied, blood, kidney, fat and muscle were below 0.01%, while liver was

0.02 % and carcass 0.15% of dose. Table 16 below summarizes the findings for the tissue distribution samples collected.

Table 16. Tissue distribution of HAA299 after oral administration

Residues 96 hours after administration					
[Harlan A89291]	**µg-equivalents per g**			**percent of dose**	
	Mean	SD	LOQ	**Mean**	SD
Dose [mg/kg]	**102.3**	1.7	--		
Blood	**0.122**	0.029	0.024	**<0.01**	<0.01
Plasma	**0.091**	0.024	0.023	--	--
Liver	**0.415**	0.067	0.024	**0.02**	<0.01
Kidneys	**0.649**	0.236	0.026	**<0.01**	<0.01
Fat	**0.066**	0.011	0.024	**<0.01**	<0.01
Muscle	**0.173**	0.032	0.024	**<0.01**	<0.01
Carcass	**0.149**	0.031	0.013	**0.15**	0.03
		Total Residues		**0.18**	0.04

Metabolite pattern- Urine. Due to very low amount of radioactivity found in the urine at time interval 24-48 hours the metabolite fraction for this fraction was not analysed. Chromatography revealed a simple metabolite pattern consisting of a cluster of very polar fractions and one defined metabolite fraction. Unchanged HAA299 was not found in urinary metabolite pattern as checked by co-chromatography with unlabeled test item. However, for the assessment of the urinary metabolite pattern it has to be considered that the total amount of urinary excretion was significantly lower than the total amount of radiolabeled impurities (about 7% of dose) that were administered with the test item. Comparing the urinary metabolite pattern with the pattern of impurities given in the analysis of the administration suspension it is very likely that the radioactivity found in the urine originated from the test item impurities.

Metabolite pattern- Faeces. For the investigation of the fecal metabolites the 0–24 and 24-48 hours faeces were each pooled separately and extracted with acetonitrile/water 80/20 (v/v), acetonitrile, and tetrahydrofurane (THF). About 98% of the faeces radioactivity (Table 17) was extractable at room temperature (Extract 1 and Extract 2).

Table 17. Extractable HAA299 from faeces after oral administration of HAA299

		Percent of pooled faeces				**Percent of dose**		
Time interval	Designation	Total	**Extract 1**	**Extract 2**	Non-extractable	Total	**Extract 1**	**Extract 2**
0-24 h	F1	100.0	**98.3**	**0.2**	1.6	87.6	**86.1**	**0.2**
24-48 h	F2	100.0	**98.0**	**<0.1**	2.0	3.4	**3.3**	**<0.1**

The Extract 1 of both time intervals was quantitatively analysed by HPLC and revealed almost only unchanged HAA299; i.e., totally 84.8 % (0 – 48h) of dose. In the first time interval (0-24 hours) 5 additional more polar metabolite fractions were found but did not exceed 0.6 % of the dose. Again the pattern of these polar metabolite fractions resembled closely the impurities of the test item.

Conclusion
The study author concludes that *large micronised* HAA299 was poorly absorbed from the gastro intestinal tract into system circulation after oral administration. The apparent extent of absorption, calculated based on the radioactivity excreted with the urine and the remaining radioactivity in the carcass and tissues, accounted for 1.93% of the administered dose. However, indications are given, that absorbed radioactivity was caused by the impurities of the test item and the actual absorption of HAA299 may be significantly lower than 2% of the dose. Almost the complete dose was excreted unabsorbed with the faeces as unchanged parent, accounting for 90.99 % of dose within 48 hours after administration.
Ref.: 13

SCCS comment
The label purity of the test item given by oral gavage was 91.4%. Thus, it is uncertain if the 1.93% of the radioactivity found in urine (1.75%) and carcass (0.18%) represent impurities or HAA299. The majority of the radioactivity in the urine was excreted within the first 24 hours.

In vivo rat oral absorption, distribution, and elimination

Guideline:	OECD 417
Species/strain:	Male HanRcc:WIST (SPF): Wistar rats about 8 weeks old, approx 200 g
Group size:	4 males
Test substance:	FAT 75'808/D
Batch:	VTM07B04, Labelled 3574052
Test item:	[^{14}C] labelled HAA299 radiolabel purity 98.8%
Mean particle size:	d(0.5) = 140 nm
Vehicle:	0.5% carboxymethylcellulose and 0.4% Tween 80 in purified water
Dose levels:	Mean 107.9 mg/kg bw (106 - 110 mg/kg bw)
Dose volume:	3.5 ml/kg bw
Route:	Oral administration (gavage)
Administration:	Single dose
GLP:	In compliance
Study period:	8 - 30 Oct 2008

Methods
The *in vivo* absorption, distribution, and elimination of micronised (d(0.5) = 140 nm), radiolabelled HAA299 was evaluated in male Wistar rats. The experiment was performed over 96-hours after a single oral gavage dose to achieve the following 4 objectives:

1) to estimate for the oral route the rate and extent of intestinal absorption of the test item,
2) to investigate the blood kinetics,
3) to determine the pattern of tissue distribution of the test item and
4) to determine the rates and routes of excretion of the test item.

The [^{14}C] labeled HAA299 was synthesized by PerkinElmer LAS, Inc. (Boston, MA, USA) as HAA299 [Piperazine-^{14}C] with a radiochemical purity of 98.8% and specific activity of 2242 MBq/mmol (60.6 mCi/mmol) or 3309 kBq/mg (89.43 µCi/mg). The dosing suspension was prepared as a mixture of non-labelled and labelled test material to give [^{14}C]-labelled HAA299 with a final specific radioactivity of about 20 kBq/mg (0.54 µCi/mg). After removing residual solvent from the mixture it was micronised in a micro-mill with additions of a surfactant (sodium myreth sulfate) and silicon defoaming agent using the Ciba-patented process that is used to prepare the commercial product sold for formulation of consumer sunscreens.

An aliquot of about 1 g of the microsuspension 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 indicated the d(0.5) = 137 nm and 140 nm for the two samples. Group of 4 rats received 107.9 mg/kg bw (range: 105 mg/kg bw (No. particles/kg bw $6.24x10^{19}$, surface area 0.73 m^2) to 110 mg/kg bw (No. particles/kg bw $6.47x10^{19}$, surface area 0.75 m^2)) by oral gavage.

Urine and faeces samples were cpollected for each rats during four 24-hour periods. The study was terminated 96-hours after dosing. Cages were rinsed separately and the rinsates analysed for collected radioactivity. From each animal, in addition to the blood and plasma collected, samples of each of the following were taken and weighed: liver, kidney, fat and muscle; the remaining carcass was retained and processed for determination of radioactivity.

Results
All four animals survived the study period, gained weight, and did not show signs of toxicity or adverse effects. The extent of absorption, calculated based on the radioactivity excreted with the urine and the remaining radioactivity in the carcass and tissues, accounted for only 0.07 % of the administered dose. The concentrations of radioactivity determined in blood or in any tissue or organ did not show measurable quantities of test item.

The elimination is shown in Table 18. Urine, representing absorbed radioactive material, was 0.04% of the total amount administered and carcass and tissue about 0.03% while excretion by faeces was 97.30%. Total recovery was 97.35% of administered radioactivity during the study period.

Table 18. Excretion of HAA299

Urine	Time period	[% of dose]
	0 – 24 h	0.03
	24 – 48 h	<0.01
	48 – 72 h	<0.01
	72 – 96 h	<0.01
	Subtotal	0.04
Faeces		
	0 - 24 h	90.73
	24 - 48 h	6.54
	48 - 72 h	0.03
	72 - 96 h	<0.01
	Subtotal	97.30
Cage Wash		<0.01
Total Excretion		97.35

Conclusion
The study authors concluded that orally administered ^{14}C labelled micronised HAA299 was not absorbed from the gastrointestinal tract into systemic circulation.

Ref.: 25

SCCS comment
The purity of the test item given by oral gavage was 98.8%. Thus, it is uncertain if the 0.07% of the radioactivity found in urine (0.04%) and carcass and tissue (0.03%) represent

impurities or HAA299. The majority of the radioactivity in the urine was excreted the first 24 hours.

SCCS general comment
All studies involving radioactive ^{14}C-HAA299 were performed in the same commercial laboratory. In the study with "*large micronised*" particles of d(05) 8.33 µm the total absorption was calculated to about 1.93% while the impurity was about 8%. In the last experiment with *micronised* particles of d(0.5) = 140 nm the total absorption was calculated to 0.07% while the impurity was about 1%. Thus, it is uncertain whether the difference in absorption is due to the particle size or the amount of impurity.

A.3.3.10. Photo-induced toxicity

A.3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Guideline:	/
Species/strain:	Hartley albino guinea pigs, males or nulliparous non-pregnant females
Group size:	Control 5 animals, UV exposed 10 (with 2 additional animals)
Test substance:	FAT 75'808/A
Batch:	HAA299/7-5
Purity:	98.7%
Vehicle:	Olive oil
Dose levels:	30%
Dose volume:	0.5 ml
Route:	Topical application
Administration:	/
UV source:	A lamp with mercury steams (BIOTRONIC U.V. 312-365 nm - VILBER LOURMAT) which emitted between 312 and 365 nm.
UV intensity:	The source placed at 4 cm of the back of the guinea pigs, emitted about 5.2 mW/cm² at 365 nm (UVA) and about 4.5 mW/cm² at 312 nm UVB).
GLP:	In compliance
Study period:	3 Aug – 8 Oct 2005

Methods
The test item was HAA299 prepared as a suspension in olive oil; a preliminary test with the test item indicated 30% as the highest practical test item concentration for use in the definitive test.

Assessment of the phototoxic potential
Before treatments, the 17 guinea pigs were clipped and depilated at shoulder region, on both flanks. The day of treatments, animals were weighed and anaesthetized. On both sides of the spinal column, two areas (of about 15 cm²) were defined and received the following treatments :

- on the right area of the animals from the groups 1 and 2 : 0.5 ml of test item applied by gentle digital massage (until complete penetration)
- on the left area of the animals from the groups 1 and 2 : no treatment.

30 minutes later, the right and left areas of the 12 animals from group 2 were exposed to a non erythematogenous UVA + UVB dose i.e. 14 Joules/cm² of UVA and 0.2 Joules/cm² of UVB. The 2 areas of the 5 animals from group 1 were not exposed to UV radiation

24 and 48 hours after the end of UV exposures, local skin reactions were observed and graded, for each animal.

Assessment of the photoallergic potential
Four days before the end of a rest period (D-4), all the guinea pigs were clipped again and depilated (D-3 and D5) using cold wax strips (Vichy) at shoulder region.

Induction phase
On D1, at the end of a rest period of 10 days, the 17 guinea pigs received 4 intradermal injections of 0.1 ml of a 1 : 1 mixture (v/v) of Freund's Complete Adjuvant and distilled water.

On D2, D4, D9 and D11, the animals were weighed and anaesthetised. The treated area previously defined on the animals from the 2 groups received 0.5 ml of the test item, applied by gentle digital massage (until complete penetration).

30 minutes after application, the treated area of the animals from the group 2 was exposed to UVA/UVB (at the same dose than for the phototoxicity test). The treated area of the animals from group 1 was not exposed to UV.

Challenge test
4 days before the end of a rest period of 11 days, the 17 animals were clipped again and depilated (D18 and D19) with cold wax strips at dorsal lumbar region.
On D24 (a t the end of the rest period), they were weighed and anaesthetised.
Two areas (of about 15 cm²) were defined on both sides of the spinal column at posterior lumbar level and received the following treatments :
- on the right area of the animals from the groups 1 and 2 : 0.5 ml of test item applied by gentle digital massage (until complete penetration)
- on the left area of the animals from the groups 1 and 2 : no treatment.

30 minutes later, the right and left areas of the animals from group 2 were exposed to a non erythematous dose of UVA radiation alone (7.2 Joules/cm²) and a non erythematous dose of UVB radiation alone (0.2 Joules/cm²). The 2 areas of the animals from group 1 were not exposed to UV radiation.

On D3, D5, D10 and D12 during the induction phase and 24, 48 and 72 hours after the end of UV exposures during the challenge test, local reactions were observed and graded, for each animal.

Results
Phototoxic potential
The formation of barely to clearly visible erythema (indexes 1 and 2) was noted on 80 % of the animals from the group 2 on the skin areas untreated + exposed and 0 % of the animals on the skin areas treated + exposed. As no index differing from 2 units at least is observed between the 2 skin areas of the animals from group 2, the test item diluted at 30 % with olive oil is not phototoxic. The reactions noted are attributed to UV radiation. No irritation was noted on the skin of the animals from group 1 treated with the test item used diluted at 30 % with olive oil.

Photoallergic potential
The formation of barely visible erythema (index 1) was noted on 10% of the animals from group 2 on the skin areas untreated + exposed and 0% of the animals on the skin areas of the animals on the skin areas treated + exposed. As no index differing from 2 units at least is observed between the 2 skin areas of the animals from group 2, the test item diluted at 30% with olive oil is not photosensitizing. No irritation was noted on the skin of the animals from group 1 treated with the test item used diluted at 30% with olive oil.

Conclusions

Under the experimental conditions used, HAA299 diluted at 30 % with olive oil may be considered as devoid of phototoxic and photoallergic potentials in guinea pigs.

Ref.: 7

A.3.3.10.2.	Photomutagenicity / photoclastogenicity

Bacterial Reverse Mutation Test

Guideline:	OECD no. 471
Species/strain:	*Salmonella typhimurium*, TA98, TA100, TA102, TA1537
Assay conditions:	Experiment I: Plate incorporation ± S9-mix Experiment II: Pre-incubation assay ± S9-mix from rat livers (Aroclor 1254 induced). Three plates were investigated per test concentration. Two independent experiments were performed S9-mix from rat livers, Aroclor 1254 induced
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	DMSO
Concentrations:	Experiment I: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate
UV source:	A Xenon-lamp (Sunset CPS, ATLAS, D-63558 Gelnhausen) that emits a continuous spectrum of simulated sunlight
UV intensity:	Intensity of irradiation was 0.1 – 0.3 mW/cm^2
GLP:	In compliance
Study period:	16 May – 6 Jun 2006

Methods

The study was performed to investigate the potential of HAA299 to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1537, TA 98, TA 100, and TA 102. These strains were chosen since they tolerate relatively high doses of UV irradiation used to assess the possible photomutagenic potential of sunblockers.

The test material was dosed as a suspension in dimethyl sulfoxide (DMSO). The irradiation was performed with a Xenon-lamp that emits a continuous spectrum of simulated sunlight. The intensity of irradiation was 0.1 – 0.3 mW/cm^2 and each bacterial strain received its respective amount of tolerable UVA and UVB exposure.

The assay was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate in the presence and absence of metabolic activation system (rat S9 mix). In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. Minor toxic effects, evident as a reduction in the number of revertants, were observed in strain TA 1537 from 1000 µg/plate up to 5000 µg/plate and in strain TA 102 at 5000 µg/plate in the pre-experiment. The following concentrations were used in the main tests:

Experiment I: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in both experiments.

Results
No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with HAA299 under irradiation with artificial sunlight at any dose level.

There was also no tendency of an increased number of revertants with increasing concentrations in the range below the generally acknowledged border of biological relevance. In the pre-experiment and experiment I, the data in the negative and solvent control without irradiation of strain TA 102 were slightly above the historical control range. Since this deviation was rather small, this effect is considered to be based upon biologically irrelevant fluctuations in the number of colonies.

Appropriate reference mutagens (Sodium azide, NaN3, 4-nitro-o-phenylene-diamine, 4-NOPD, methyl methane sulfonate, MMS, 8-Methoxypsoralen (8-MOP)) were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion
Under the experimental conditions used of *Salmonella typhimurium* photomutagenicity assay, HAA299 did not induce gene mutations by base pair changes or frame-shifts in the genome.

Ref.: 17

SCCS comment
Negative results may be due to lack of bacterial uptake of HAA299 particles.

Photo- Chromosome Aberration Test with V79 Chinese Hamster cells

Guideline:	OECD no. 473
Cells:	Chinese hamster V79 cells
Replicates:	Duplicate cultures in three independent experiments
Test substance:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Solvent:	DMSO
Concentrations:	0.9 – 30 µg/ml without and with S9-mix
Treatment:	24 h treatment without S9-mix, harvest time immediately after the end of treatment 4 h treatment with S9-mix, harvest time 24 h after the start of treatment
UV source:	A Xenon-lamp (Sunset CPS, ATLAS, D-63558 Gelnhausen) with an additional special filter glass, emitting visible light and UVA/UVB light (ratio: about 30:1) > 290 nm that emits a continuous spectrum of simulated sunlight
UV intensity:	Intensity of irradiation was125 – 200 mJ/cm^2
GLP:	In compliance
Study period:	4 May – 26 Sep 2006

Methods
HAA299 suspended (pre-experiment) or dissolved (Exp. IA, IB and II) in DMSO, was assessed for its potential to induce structural chromosomal aberrations in V79 Chinese Hamster cells in the absence and presence of artificial sunlight in three independent experiments.

The cultures were pre-incubated with the test item for 30 minutes where after they were exposed to 125 mJ/cm^2 UVA (Exp. IA, IB, and II) or 200 mJ/cm^2 UVA (Exp. II). Three hours after start of treatment, the cultures were washed. Corresponding cultures with the test item were kept in the dark for the 3 hrs exposure period. The cells were harvested 18 hrs (Exp. I) and 28 hrs (Exp. II) after start of treatment with the test item. In the cytogenetic experiments for each experimental group two parallel cultures were set up. Per culture at least 100 metaphase plates were scored for structural chromosome aberrations.

Dose selection for the cytogenetic experiments was performed considering the toxicity data. In the pre-test on toxicity, precipitation of the test item after 4 hrs treatment was observed at 15.6 µg/ml and above, in the absence and presence of irradiation. Since no relevant toxicity was observed in the pre-test, the test item was tested up to a concentration exhibiting clear test item precipitation. Therefore, 30 µg/ml (with and without irradiation) was chosen as top treatment concentration in Experiment IB. The positive controls did not show clastogenic responses so the experiment was repeated with the same exposures and the second time gave acceptable outcome. Due to technical reasons the experimental part with irradiation was repeated twice with the same top concentration. A confirmatory Experiment IA without irradiation was performed to confirm the results obtained in Experiment IB.

Dose selection of Experiment II was also influenced by test item toxicity. In the range finding experiment no clearly reduced cell numbers were observed after 24 hrs exposure up to the highest concentration. Therefore, 30 µg/ml were chosen as top treatment concentration for continuous exposure in the absence and presence of irradiation.

Results
No toxic effects indicated by clearly reduced mitotic indices or cell numbers of below 50 % of control were observed up to the highest applied concentration being far in the range of test item precipitation.

Neither statistically significant nor biologically relevant increases in the number of cells carrying structural chromosomal aberrations, exclusive gaps, were observed, in the absence and the presence of artificial sunlight. The aberration rates of the test groups treated with the test item were clearly within the respective historical control data ranges. In the absence and the presence of artificial sunlight, no biologically relevant increase in the frequency of polyploid metaphases was found after treatment with the test item as compared to the rates of the solvent controls. Appropriate mutagens were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

Conclusion
Under the experimental conditions used, HAA299 did not induce structural chromosome aberrations in V79 cells after irradiation with artificial sunlight when tested up to cytotoxic and/or precipitating concentrations.

Ref.: 18

SCCS comment
Negative results may be due to lack of cellular uptake of HAA299 particles.

Unscheduled DNA synthesis in hepatocytes, rat
See section B3.3.6.2

Bone marrow micronucleus test, mouse
See section B3.3.6.2

A.3.3.11. Human data

/

A. 3.3.12. Special investigations

In vitro androgen receptor binding assay

Guideline:	/
Cytosolic AR:	Prostate glands of young adult rats (8 rats) (HsdRCCHan:WIST)
Test item:	FAT 75'808/B
Batch:	VTM05B10
Purity:	99.1%
Replicate:	2 independent assays with duplicates in each assay
Vehicle:	DMSO
Doses:	0.5 nM to 0.5 mM
Positive control:	^{3}H-methyl trienolone (R1881)
GLP:	In compliance
Study period:	12 Jul – 27 Nov 2006

Methods
The purpose of the study was to investigate whether HAA299 can bind to the rat androgen receptor (AR). Cytosolic preparations were prepared from the prostates of 8 HsdRCCHan :WIST rats, which had been dosed with the GnRH antagonist (Antarelix). The cytosolic preparations were incubated with the test substance (HAA299) at a range of concentrations (0.5nM to 0.5mM) and a fixed concentration (5nM) of the radiolabelled androgen, ^{3}H-methyl trienolone, in order to determine the ability of HAA299 to displace the ^{3}H-methyl trienolone. Quantitation of displacement was then used to determine the intrinsic activity of HAA299 to interact with the androgen receptor (AR). Two independent assays with duplicates in each assay were performed.

Results
HAA299 exhibited no displacement of ^{3}H-methyl trienolone at any of the concentrations tested. Visual examination showed that some test substance had co-precipitated with the methyl trienolone-hydroxyapatite complex as the precipitate had a yellow colour. Displacement of ^{3}H-methyl trienolone was observed following incubation with increasing concentrations of non-radioactive methyl trienolone (positive control) but not with DMSO (vehicle control). The results showed a good correlation in both assays and between duplicates in each assay.

Conclusion
Inability to displace ^{3}H-methyl trienolone from cytosolic preparations of rat prostate tissue, indicated that, at concentrations up to $5x10^{-4}$ M, the test substance (FAT 75808/B) does not possess intrinsic potential to bind to the rat androgen receptor in the *in vitro* androgen receptor binding assay.

Ref.: 8

In vitro estrogen receptor binding assay

Guideline:	/
Cytosolic ER:	Uteri of immature rats (20 rats) (HsdRCCHan : WIST)
Test item:	FAT 75'808/B
Batch:	VTM05B10

Purity:	99.1%
Replicate:	2 independent assays with duplicates in each assay
Vehicle:	DMSO
Doses:	0.5 nM to 0.5 mM
Positive control:	^{3}H-estradiol
GLP:	In compliance
Study period:	5 – 8 Sep 2006

Methods
The purpose of the study was to investigate whether FAT 75808/B can bind to the rat estrogen receptor (ER). Cytosolic preparations were prepared from the uteri of 20 immature HsdRCCHan : WIST rats. These were incubated with the test substance (HAA299) at a range of concentrations (0.5nM to 0.5mM) and a fixed concentration (5nM) of the radiolabelled estrogen, ^{3}H-estradiol, in order to determine the ability of HAA299 to displace the 3H-estradiol.

Quantitation of displacement was then used to determine the intrinsic activity of HAA299 to interact with the estrogen receptor (ER). Two independent assays with duplicates in each assay were performed.

Results
HAA299 exhibited no displacement of ^{3}H-estradiol at any of the concentrations tested. At the higher concentrations (5×10^{-5} upwards) there was an unusual observation of an apparent increase in the binding of ^{3}H-estradiol. Visual inspection showed precipitation of test substance at these concentrations and therefore it is likely that there has been a coprecipitatation of the test substance and ^{3}H-estradiol.

Displacement of ^{3}H-estradiol was observed following incubation with increasing concentrations of non-radioactive estradiol (positive control) but not with DMSO (vehicle control). Consistent results were obtained between duplicates within each assay and across both assays.

Conclusion
Inability to displace ^{3}H-estradiol from cytosolic preparations of rat uterine tissue, indicated that, at concentrations up to 5×10^{-4}M, the test substance (HAA299) does not possess intrinsic potential to bind to the rat estrogen receptor in the *in vitro* estrogen receptor binding assay.

Ref.: 9

***In vivo* uterotrophic assay by oral gavage to immature rats**

Guideline:	/
Strain of animals:	Female rats (Alpk: HsdRCCHan IST strain)
Groups:	10 animals, 5 study groups
Test item:	FAT 75’808/B
Batch:	VTM05B10
Purity:	99.1%
Vehicle:	0.5% carboxymethylcellulose in water
Doses:	250, 500 and 1000 mg/kg bw
Administration:	Orally by gavage for 3 consecutive days
Positive control:	β-estradiol (0.4 mg/kg bw/day)
GLP:	In compliance
Study period:	27 Sep – 13 Oct 2006

Methods
The effect of HAA299 on uterine growth in immature female rats was studied. Groups of ten immature female AlpkHsdRCCHan : WIST strain rats (19-20 days of age at the start of the study) received an oral dose of 0 (vehicle control), 250, 500 or 1000 mg/kg bw of HAA299 once a day for 3 consecutive days. As a positive control, one group of rats received an oral dose of 0.4 mg β-estradiol/kg bw once a day for 3 consecutive days. The vehicle used for the test substance and β-estradiol was 0.5% carboxymethylcellulose in water. The bodyweight of each rat was recorded daily, and detailed clinical observations were made at the same time. At the end of the study (approximately 24 hours after administration of the final dose), all of the animals were killed. The uterus was removed from each animal and trimmed of any fat and adhering non-uterine tissue. The uterus was then opened with a small incision, squeezed and blotted onto filter paper to remove any excess fluid and the uterine wet weight was recorded.

Results
No dosing related clinical signs were noted following oral administration of HAA299 at 250, 500 or 1000 mg/kg bw/day. Body weights were not significantly affected during the study although bodyweights at the low and mid dose group were minimally reduced compared to control on days 2 and 3.

There was no effect of HAA299 administration, at any dose level up to 1000 mg/kg on mean uterine weight. Mean uterine weight for the positive control group showed a 2.7-fold increase relative to the vehicle control demonstrating the sensitivity of the system to measure the expected response to a known positive control agent.

Conclusion
There were no changes to clinical condition, body weight or uterine weight following oral gavage administration of HAA299 to the immature rat at doses up to 1000 mg/kg for three consecutive days. There was no evidence of an uterotrophic response to this test substance.

Oral gavage administration of β-estradiol to the immature rat for three consecutive days resulted in a marked increase in uterine weight, demonstrating a positive uterotrophic response with this substance.

Ref.: 10

Section: B. HAA299 Nano-batches

B.3.3.1. Acute toxicity

B.3.3.1.1. 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
Batch:	MGU 799
Purity:	51% (excipients sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol)
Particle size:	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 2.4^{20}, surface area 14 m^2
Administration:	Oral gavage (10 ml/kg bw)
GLP:	In compliance

Study period: 4 Mar – 1 Apr 2008

Methods
The test item was prepared in purified water 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. Mortality did not occur in any of the rats. 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. However, when compared to historical control data, a slightly lower body weight gain was noted between day 1 and day 8 in 1/3 females given 300 mg/kg and in 1/3 females given 2000 mg/kg (it return to normal thereafter) and between day 8 and day 15 in 1/3 females 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 the test item. At necropsy, no apparent abnormalities were observed in any animals.

Conclusion
Under the experimental conditions of this study, the oral LD_{50} of the test item HAA299 was higher than 2000 mg/kg in rats.

Ref.: 19

B.3.3.1.2. Acute dermal toxicity

/

B.3.3.1.3. Acute inhalation toxicity

Guideline:	OECD Guideline no. 403
Species/strain:	Wistar [HanRcc:WIST(SPF)], male rats (9 week old) and female rat (10 weeks old)
Group size:	15 males and 15 females
Test substance:	FAT 75'808/H
Batch:	MGU 814
Purity:	50.1% (excipients sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol)
Particle size:	d(0.5) = 136 nm, mass median aerodynamic diameters (MMADs) 1.0 µm
Vehicle:	Water and sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol
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: $5.24x10^{-13}$. Particles a.i./droplet $9.27x10^{7}$
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, FAT 75'808/H contained 50.1% HAA299 prepared by micronisation with excipients sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol. A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of sodium myreth sulfate, silicon defoamer, xantham gum, and butylene glycol, labelled FAT 75'808 placebo (synonymous with FAT 75'808/I), was prepared. The test item and the placebo 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 as active ingredient of UV filter HAA299 was 10%.

Both the placebo and the test item 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 concentration of the placebo and test item dilutions were determined gravimetrically and/or by chemical analysis; the particle size distribution determined gravimetrically; and temperature, relative humidity and oxygen concentration were measured on test atmosphere samples collected directly from the aerosol delivery tube in the breathing zone of the animals.

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 examined 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 14 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 to be 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 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 to be indicative of an inflammatory reaction.

The study authors write that pulmonary inflammatory responses are not expected with use of HAA299 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.

Conclusion
The study authors concluded that for micronised HAA299 the inhalation LC_{50} 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.

Ref.: 20

B.3.3.2 Irritation and corrosivity

B.3.3.2.1. Skin irritation

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B.3.3.2.2. Mucous membrane irritation

/

B.3.3.3. Skin sensitisation

/

B.3.3.4. Dermal / percutaneous absorption

/

B.3.3.5. Repeated dose toxicity

B.3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

See Section B.3.3.8

B.3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

/

B.3.3.5.3. Chronic (> 12 months) toxicity

/

B.3.3.6. Mutagenicity / Genotoxicity

B.3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

/

B.3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Unscheduled DNA synthesis in hepatocytes, rat

Guideline:	OECD guideline no. 486
Species/strain:	Male Fischer rats (5 – 6 weeks old)
Group seizes:	3 males
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
Batch:	MGU 814, LA 2397_37
Purity:	51.1%
Dose levels:	0, 1000 and 2000 mg/kg bw/day
Particles per kg bw:	5.89×10^{20}, and 1.18×10^{21}
Mean particle size:	d(0,5) 136 nm

Non-micronised

Test substance:	FAT 75'808/D
Batch:	VTM07B04
Purity:	98.6%
Dose levels:	2000 mg/kg bw/day
Mean particle size:	d(0.5) = 13.9 µm

Method

The objective of this study was to evaluate the potential of HAA299 containing micronised small particle sizes, to induce DNA damage or lead to increased repair synthesis of the genome. Tested in parallel was HAA299 which contained the active ingredient in large particle sizes.

The test item, FAT 75'808/H, contained 50.1% HAA299 of batch number MGU 814, LA 2397_37 prepared by micronizing HAA299 VTM07B04 (FAT 75'808/D) with excipients sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol. A subsequent analysis of the test item sample indicated a d(0.5) = 136 nm

The Reference material, FAT 75'808/D was from HAA299 batch VTM07B04 with a purity of 98.6%; the median particle size, d(0.5) = 13.9 µm. This substance was not micronised and was used as a comparison to assess if any observed effects may be attributable to particle size differences. The Reference material was administered at a dose equivalent to that of the group receiving the highest dose of test item.

A placebo group was used to address effects from the excipients; accordingly, a mixture of sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol, labelled as FAT 75'808/H (placebo) was administered at a dose equivalent to that of the group receiving the lower dose of test item. A 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).

The test system was male Fischer rats, weighing approximately 200 g and 5-6 weeks of age at time of expression time sampling. Based on a preliminary toxicity test, the selected doses of the test item were 1000 and 2000 mg/kg body weight, 2000 mg/kg for the reference item and 1000 mg/kg body weight for the placebo. Dosing was once by oral gavage at 10 ml/kg body weight and each group consisted of 3 males. The particle equivalent dosages from the micronised HAA299 are shown in the table below. 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).

Results
The test item, placebo and reference item did not cause increased net nuclear grain counts, did not increase the frequency of cells in repair, or 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 article, placebo or reference item. The positive control items each gave responses.

Plasma samples analysis showed HAA299 could be detected at very low concentrations, the maximum level of 6.5 ng/ml, only slightly exceeding the limit of quantification ((LOQ of 1.3 ng/ml plasma). However HAA299 was also detectable in placebo samples at very low concentrations. In two of three samples from the high dose group at the 2 hours sampling time, HAA299 concentrations were slightly higher than the concentrations of the placebo samples. Concentrations were at the level of the placebo samples after 12-hours treatment. Due to the very low levels reliable quantification was not possible. The study authors consider that the results demonstrate HAA299 is not able to penetrate the systemic circulation in toxicologically significant amounts.

Conclusion
The study authors concluded that *micronised* HAA299 and *non-micronised* HA299 did not reveal any genotoxic activity under the test conditions. Regarding the concentration levels in plasma of animals treated with *micronised* HAA299 at 2000 mg/kg bw, no evidence of exposure was demonstrated.

Ref.: 27

Bone marrow micronucleus test, mouse

Guideline:	OECD guideline no. 474
Species/strain:	Swiss Ico: OF1 mice, 6 weeks old.
Group size:	5 males and 5 females (2000 mg/kg 8 males and 8 females, the 3 satellite animals 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
GLP:	In compliance
Study period:	5 – 13 Mar 2008

Micronised

Test substance:	FAT 75'808/G
Batch:	MGU 799. LA 2397_31
Purity:	51.0% (according to the last analytical certificate)
Dose levels:	0, 500, 1000 and 2000 mg/kg bw/day
Particles per kg bw:	$2.94x10^{20}$, $5.87x10^{20}$, and $1.17x10^{21}$
Mean particle size:	d(0.5) = 138 nm and d(0.9) = 207 nm

Non-micronised

Test substance:	FAT 75'808/D
Batch:	VTM07B04
Purity:	98.6%
Dose levels:	2000 mg/kg bw/day
Mean particle size:	d(0.5) = 13.9 µm

Method

Dosage formulation of the test item (FAT 75'808/G Batch: MGU 799. LA 2397_31) (d(0.5) = 138 nm and d(0.9) = 207 nm) was by suspending it in the vehicle (water) to achieve the concentrations of 50, 100 and 200 mg/ml and then homogenizing using a magnetic stirrer. At a dosing volume of 10 ml/kg bw, the target dose-levels as test item were 500, 1000 and 2000 mg/kg/day

The Reference material, FAT 75'808/D was from HAA299 lot VTM07B04 with a purity of 98.6%; the median particle size, d(0.5) = 13.9 µm. This substance was not micronised and was used as a comparison to assess if any observed effects may be attributable to particle size differences. Dosage form preparation was 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

A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol, labelled FAT 75'808/F (placebo). The placebo was administered in its original form using a treatment volume of 10 ml/kg.

The study was conducted in Swiss Ico: OF1 mice. A preliminary toxicity test was performed to define the dose-levels to be used for the cytogenetic study. In the main study, one group of five males and five females received the positive control test item (Cyclophosphamide) once by oral route at the dose-level of 50 mg/kg bw. An additional three groups of five males and five females mice were given oral administrations of test item, placebo, or reference material at dosages cited above. The high dose test item group and the reference material group retained satellite groups of 3 male and 3 female mice for blood sampling after dosing and determination of test item in plasma. Blood samples for these determinations were taken from 3 mice per sex at 1 hour (satellite animals) and 24 hours (at terminal sacrifice on 3 out of 8 animals of each sex) after the second treatment.

At the time of sacrifice (24 hours after the last dose), all the animals were killed by CO_2 inhalation in excess. The femurs of the animals were removed and the bone marrow was flushed out using fetal 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; the 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 placebo, the reference item or the test item.

After oral dosing with micronised and not micronised test item 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, indicating very low systemic exposure. The study authors consider these results demonstrate HAA299 was not able to penetrate the systemic circulation in toxicologically significant amounts.

The mean values of MPE as well as the PE/NE ratio in the groups treated with HAA299 or with the placebo were comparable to those of the vehicle group. Cyclophosphamide induced statistically significant increases ($p<0.01$ in males and $p<0.05$ in females) in the frequency of MPE.

Conclusion
The study authors concluded that the *micronised* HAA299 and *non-micronised* HAA299 did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two oral administrations of mice, at a 24-hour interval, at the dose-levels of 2000 mg/kg bw/day.

Ref.: 26

SCCS comment
It is not clear if the test item concentrations represent concentration of active substance.

In the reference it is stated "The concentration and dose-levels of the test item were expressed as active item" [page 11]
In the dossier it is written: "the target dose-levels as test item were 500, 1000 and 2000 mg/kg/day, respectively, or as active ingredient of UV filter HAA299: 250, 500 and 1000 mg/kg/day, respectively." [page 63]

SCCS comment on mutagenicity
Non-micronised HAA299 was tested *in vitro* for mutagenic activity in a bacterial reverse mutation tests, a mutagenicity test in mammalian cells (mouse lymphoma assay) and a mammalian test for chromosome aberration. All tests were negative.

Micronised and *non-micronised* HAA299 were tested with rats in an *in vivo* unscheduled DNA synthesis test and with mouse in an *in vivo* micronucleus test. All tests were negative.
The question may, however, be raised if the negative results involving oral administration of HAA299 to rats and mice is due to lack of systemic exposure and the negative *in vitro* tests with HAA299 to lack of bacteria/cell absorption.

SCCS general comment

No conclusion can be drawn with regard to genotoxicity potential of HAA299.

B.3.3.7. Carcinogenicity

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B.3.3.8. Reproductive toxicity

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
Batch:	MGU 789 and MGU799
Purity:	51% (excipients sodium myreth sulfate, silicon defoaming agent, xanthan gum and butylene glycol)
Particle size:	FAT 75'808/E d(0.5) = 134 nm d(09) = 202 nm; FAT 75'808/G 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 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 toxic effects of the test item, FAT 75'808 as a micronised small particle, 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) to achieve the concentrations of 20, 100 and 400 mg/ml and then homogenizing using a magnetic stirrer. At a dosing volume of 5 ml/kg bw/day, the target dose-levels as test item were 100, 500 and 2000 mg/kg bw/day, respectively, or as active ingredient of UV filter HAA-299: 50, 250 and 1000 mg/kg bw/day, respectively.

A placebo group was used to identify any effects related to the excipients; accordingly, a mixture of sodium myreth sulfate, silicon defoamer, xantham gum, and butylene glycol was prepared and labelled FAT 75'808/F (placebo mix). The placebo was diluted in distilled water and administered at a dose of 400 mg/ml, which was equivalent to that of the group receiving the highest dose of test item.

A control group of 10 males and 10 females were treated with distilled water during the study and served as comparison for placebo-treated group or test-item treated groups.

The test system was rats of strain Sprague-Dawley. At the beginning of the treatment period the animals were 10 weeks old. The animals were sexually mature and the females

were virgin. Each of the 5 study groups received 10 males and 10 females randomly assigned to treatment groups.

Daily dosing, at approximately the same time each day, was by gastric intubation as follows:
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 inclusive.

Clinical signs and mortality were checked daily. 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 animals were paired for mating and the dams were allowed to litter and rear their progeny until day 5 *post-partum*. The total litter sizes and numbers of pups of each sex were recorded after birth, pup's clinical signs were recorded daily and pup body weights were recorded on days 1 and 5 *post-partum*. Calculation of reproductive success indices were made for parameters pre- and post-implantation loss, mating, fertility, and gestation.

The parent males were sacrificed 2 weeks after the end of the mating period. The body weight and principal organ weights (adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes, and thymus) were recorded, a complete macroscopic *post-mortem* examination was performed and selected organs/tissues were preserved. 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.

The parent females were sacrificed on day 6 *post-partum* and a macroscopic examination of the principal thoracic and abdominal organs was performed, with particular attention paid to the reproductive organs. In females which were apparently non pregnant, the presence of implantation scars on the uterus was checked using ammonium sulphide staining technique. A microscopic examination was performed 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.

Pups, including those found dead, were carefully examined for gross external abnormalities and a macroscopic *post-mortem* examination was 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. No clinical signs were recorded prior to the sacrifice of these animals which were all confirmed not pregnant at necropsy.

In all HAA299 treated groups, mean body weights and body weight gains values were similar to controls given purified water. There were no treatment-related effects on hematology, blood biochemistry or urinary parameters.

Only very rare exceptions of abnormal scores or presence/absence of abnormal/normal behavior 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.

Single females through all groups showed slightly abnormal estrous cycles with long periods of diestrus. As all these females mated after 12 to 18 days of pairing and were pregnant, these slight disturbances were considered unrelated to treatment with the test item.

Reproductive data evaluation showed that neither the mating nor the fertility parameters were adversely affected by the test item treatment or administration of the placebo. However, post-implantation losses were highly variable. Low post-implantation loss recorded in the group treated with the placebo mix was considered to be fortuitous in origin.

The observation of the pups after birth did not reveal any treatment related effect on pups mortality, body weight gains or sex ratio; furthermore, gross malformations were not found in any of the pups.

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 be of toxicological importance. The organ weights of test-item treated animals showed some statistically significant differences from the placebo control group among males and female. 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 recognized 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 one rat from group 2 (placebo), one rat from group 3 (50 mg/kg bw/day) and two rats from group 5 (1000 mg/kg bw/day). The study authors write that this change was considered unrelated to treatment with the test item as it is considered to be 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

The study authors concluded that based on the experimental conditions of this study, the dose-level of 1000 mg a.i./kg/day was considered to be the No Observed Adverse Effect Level (NOAEL) for parental toxicity and the No Observed Effect Level (NOEL) for reproductive performance (mating and fertility).

Ref.: 21

SCCS comment

The absence of effect observed up to the highest dose may be related to the low bioavailability.

B.3.3.8.1. Two generation reproduction toxicity

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B.3.3.8.2. Teratogenicity

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B.3.3.9. Toxicokinetics

B.3.3.10. Photo-induced toxicity

B.3.3.10.1. Phototoxicity / photoirritation and photosensitisation

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B.3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

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B.3.3.11. Human data

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B.3.3.12. Special investigations

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C.3.3.1. Safety evaluation (including calculation of the MoS)

Safety evaluation of HAA299 batches containing non-nano particles can be performed on the basis of the submitted dossier:
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 low observed systemic toxicity (NOEL/NOAEL for oral administration of HAA299 to rats is 1000 mg/kg bw/d).
Therefore no concern is raised with regard to systemic toxicity.

Safety evaluation of HAA299 batches containing nano particles cannot be performed on the basis of the submitted dossier, because the dossier included only acute oral toxicity, acute inhalation toxicity, repeated dose toxicity and 2 *in vivo* mutagenicity tests.

C.3.3.2. Discussion

As a complete dossier of HAA299 nano-batches for safety evaluation, according to SCCS Notes of Guidance (SCCS/1501/12) and the SCCS nano Guidance (SCCS/1524/13 Revision of 27 March 2014) was not available for safety evaluation, the following discussion deals only with HAA299 non-nano batches.

General considerations
HAA299 is to be used as an UV-filter in sunscreen products. The applicant has submitted studies performed with in principle three different formulations, *non-micronised* (d(0.5) = 5.38 – 99.1 µm, purity 98.6 – 99.8%), *large micronised* particles ^{14}C-HAA299 (d(0.5) = 0.76 µm and 8.33 µm, radiochemical purity 91.4%), *micronised* (d(0.5) = 134 – 138 nm purity 50.1 – 51.2% and a ^{14}C HAA299 with d(0.5) = 134 – 153 nm, radiochemical purity 98.8%).

Physico-chemical properties
HAA299 is a white-yellowish powder. Under the microscope, squarish, agglutinated and colourless crystalline structures were observed. The micronised formulation is representative of the trade product known as C-1332.

No data on long-term stability at room temperature or in typical sunscreen products was provided.

Acute toxicity
The acute oral and dermal toxicity of HAA299 is low (LD_{50} greater than 2000 mg/kg bw).

Irritation
Non-micronised HAA299 was non-irritant when applied topically to rabbits. Non-micronised HAA299 was slightly irritant when administered by ocular route to rabbits.

Sensitisation
Non-micronised HAA299 did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay.
SCCS cannot draw a valid conclusion from the tests on skin sensitisation potential since it is not known if HAA299 penetrated the skin to reach the cellular targets of the immune system. In view of this, sensitisation potential of HAA299 cannot be ruled out.

Dermal absorption
Four skin absorption studies, three *in vitro* studies and one *in vivo* study have been performed by the applicant. In the two *in vitro* studies with rat skin, the total absorptions were 0.52% and 0.52% after 24h compared with 0.43% (total excreted and remaining in the body) after 72 h in the *in vivo* rat study. Thus, on the basis of rat studies the dermal absorption is around 0.5%.

In the three *in vitro* studies with human skin the absorption after 24 h was 0.13% (*large micronised* particles), 0.03% (*micronised* particles) and 0.04% (*micronised* particles, pre-damaged skin). It is noted that the amounts in the perfusates were 0.10%, <0.01%, and <0.01% in the three experiments. The difference cannot be explained by "out layers".

The purity of the test item given ranged between 97% to 98.6%. Thus, it is uncertain to what extent the impurity played a role in the observed absorption.

The SCCS is of the opinion that rat skin dermal absorption is around 0.5% (10 µg/cm^2) of the applied amount. The human dermal skin absorption is likely to be lower than the rat skin absorption. In terms of risk assessment, the human skin absorption will represent a small systemic exposure dose (SED) and will not allow a valid calculation of margins of safety.

Subchronic toxicity
Sprague Dawley rats received non-micronised HAA299 by oral gavage for 13 weeks. Under the experimental conditions of this study, the No Observed (Adverse) Effect Level (NOEL/NOAEL) of HAA299 was 1000 mg/kg bw/day (highest dose tested).

SCCS considers that the absence of effect observed up to the highest dose may be related to the low oral bioavailability of the substance.

Reproductive toxicity
The *teratogenicity* of *non-nano* HAA299 was tested by oral administration on days 6 – 20 post-coitum. The HAA299 was well tolerated by the dams, with no (adverse) effects at any dose-level. Fetal examination resulted in no treatment-related malformations or variations at any dose-level. NOEL/NOAEL for both the maternal toxicity and the developmental toxicity was identified at 1000 mg/kg bw/day (highest dose tested).
SCCS considers that the absence of effect observed up to the highest dose may be related to the low oral bioavailability of the substance.

A further study was performed with nano form of HAA299: the objective of the study was to evaluate the potential toxic effects following daily oral gavage administration to male and female rats from 15 days before mating, through mating (up to 3 weeks) and in addition for the females, during pregnancy and lactation, until day 5 post-partum inclusive. The NOEL/NOAEL of nano HAA299 for parental toxicity and for reproductive performance (mating and fertility) was 1000 mg/kg bw/day (highest dose tested).

Mutagenicity / genotoxicity
Non-micronised HAA299 was tested *in vitro* for mutagenic activity in a bacterial reverse mutation tests, a mutagenicity test in mammalian cells (mouse lymphoma assay) and a mammalian test for chromosome aberration. All tests were negative.

Non-micronised HAA299 was also tested with rats in an *in vivo* unscheduled DNA synthesis test and with mouse in an *in vivo* micronucleus test. Both tests were negative.

SCCS considers that the negative results in the *in vivo* tests involving oral administration of HAA299 to rats and mice may be due to lack of systemic exposure and the negative *in vitro* tests with HAA299 to lack of bacteria/cell absorption.

No conclusion can be drawn with regard to genotoxicity potential of HAA299 particles on the basis of the submitted studies.

Carcinogenicity
No data available.

Toxicokinetics
Two studies have been submitted. In the first study with *large micronised* ^{14}C-HAA299 particles (d(0.5) = 8.33 µm, purity: 91.4%), the *in vivo* absorption, distribution, metabolism, and elimination was evaluated in male Wistar rats. The experiment was performed over 96-hours after a single oral gavage dose (100 mg/kg bw). The study authors conclude that *large micronised* HAA299 was poorly absorbed from the gastro intestinal tract into system circulation after oral administration. The apparent extent of absorption, calculated based on the radioactivity excreted with the urine and the remaining radioactivity in the carcass and tissues, accounted for 1.93% of the administered dose. However, indications are given, that absorbed radioactivity was caused by the impurities of the test item and the actual absorption of HAA299 may be significantly lower than 2% of the dose. Almost the complete dose was excreted unabsorbed with the faeces as unchanged parent, accounting for 90.99 % of dose within 48 hours after administration.

The design of the second study was similar to the first study except that *micronised* ^{14}C-HAA299 particles (d(0.5) = 140 nm, purity: 98.8%) was used. The extent of absorption, calculated based on the radioactivity excreted with the urine and the remaining radioactivity in the carcass and tissues, accounted for only 0.07 % of the administered dose. The concentrations of radioactivity determined in blood or in any tissue or organ did not show measurable quantities of test item. The study authors concluded that orally administered

^{14}C labelled micronised HAA299 was not absorbed from the gastrointestinal tract into systemic circulation.

SCCS noted that the purity of the test item in the first experiment given by oral gavage was 91.4%. Thus, it is uncertain if the 1.93% of the radioactivity found in urine (1.75%) and carcass (0.18%) represent impurities or HAA299. The majority of the radioactivity in the urine was excreted the first 24 hours. The purity of the test item in the second experiment was 98.8%. Thus, it is uncertain if the 0.07% of the radioactivity found in urine (0.04%) and carcass and tissue (0.03%) represent impurities or HAA299. It is uncertain whether the difference in absorption is due to the particle size or the amount of impurity.

Photo-induced toxicity
The studies were performed with *non-micronised* HAA299 (purity: 98.7 – 99.1%)
Phototoxic potential were studied with guinea pigs after dermal application with HAA299 diluted at 30 % with olive oil and UV irradiation (312 – 365 nm; 14 Joules/cm^2 UVA, 0.2 Joules/cm^2 UVB). No irritation was noted on the skin of the animals treated with HAA299 after UV irradiation, while clearly visible erythema (index 1 and 2) was observed on the UV irradiated animals without HAA299. Studies of the *photoallergic potential* involved an induction phase were the animals received intradermal injections of Freunds Complete Adjuvant and topical application of HAA299 followed by UV irradiation. The challenge test involved application of HAA299 again and UV irradiation. No irritation was noted on the skin of the animals treated with the HAA299. The study authors concluded that under the experimental conditions used, HAA299 diluted at 30 % with olive oil may be considered as devoid of phototoxic and photoallergic potentials in guinea pigs.

Non-micronised HAA299 have been tested for *photogenotoxicity in vitro* in the *Salmonella* assay (Xenon lamp with continuous spectrum of simulated sunlight, 0.1 – 0.3 mW/cm^2) as well as for chromosome abbreviation with V79 Chinese Hamster cells (Xenon lamp with continuous spectrum of simulated sunlight, 125 – 200 mJ/cm^2). All tests were negative.

SCCS considers that the tests performed indicated that HAA299 has no phototoxic, photoallergic or photogenotoxic potential.

Special investigation (studies of potential endocrine activity)
In vitro androgen receptor binding assay
Inability to displace ^3H-methyl trienolone from cytosolic preparations of rat prostate tissue, indicated that, at concentrations up to 0.5 mM, HAA299 does not possess intrinsic potential to bind to the rat androgen receptor in the *in vitro* androgen receptor binding assay.

In vitro estrogen receptor binding assay
Inability to displace ^3H-estradiol from cytosolic preparations of rat uterine tissue, indicated that, at concentrations up to 0.5 mM, HAA299 does not possess intrinsic potential to bind to the rat estrogen receptor in the *in vitro* estrogen receptor binding assay.

In vivo uterotrophic assay by oral gavage to immature rats
There were no changes to clinical condition, body weight or uterine weight following oral gavage administration of HAA299 to the immature rat at doses up to 1000 mg/kg bw/day for three consecutive days. There was no evidence of an uterotrophic response to this test substance.

SCCS considers that no endocrine activity was observed in the three experiments above. It cannot be excluded that the lack of activity is due to lack of absorption in the cellular system and after oral administration.

General comment by SCCS

MoS represents the ratio between the No Observed (Adverse) Effect Level (NOEL/NOAEL) and systemic exposure dose (SED). The SCCS is of the opinion that the submitted data are not appropriate for calculation of MoS.

The uncorrected NOEL/NOAEL (assuming 100% absorption) of HAA299 is 1000 mg/kg bw/d based on a 13 week study with oral gavage. Two toxicokinetic studies have been performed. In a study with "large micronised" particles of d(05) = 8.33 µm the total absorption was calculated to 1.93% while the impurity content was about 8% of the test material. In another experiment with micronised particles of d(0.5) = 140 nm, the total absorption was calculated to 0.07% while the impurity content was about 1% of the test material. It is uncertain whether the difference in absorption is due to the particle size or the amount of impurity. Thus, an appropriate correction factor for the limited oral bioavailability cannot be derived unless the applicant can identify the radioactive substances in the urine from the toxicological study and verify if the substances represents impurities or metabolites of HAA299.

4. CONCLUSION

4.1. Does SCCS consider that the use of HAA299 in its micronised and non-micronised form as an UV-filter in cosmetic products in a concentration up to maximum 10.0 % is safe for the consumers taken into account the scientific data provided?

This opinion covers the safety evaluation of HAA299 (micronised or non-micronised), which is not composed of nano particles. The opinion does not cover the safety evaluation of HAA299 which is composed of nano particles.

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 low observed systemic toxicity (NOEL/NOAEL for oral administration of HAA299 to rats is 1000 mg/kg bw/d).

The SCCS is of the opinion 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.

The results of genotoxicity testing were negative *in vitro* as well as *in vivo* but exposure of target cells was not proven. However, in light of the low bioavailability, the mutagenicity risk for the consumer is considered negligible.

This opinion does not apply to inhalation exposure of HAA299 since no information on chronic or sub-chronic toxicity after inhalation is provided.

4.2. Does SCCS have any other scientific concerns for the safe use of the new UV-filter HAA299 in finished 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 in human skin. If any new evidence emerges in the future to show that HAA299 used as UV-filter in cosmetic products can penetrate human skin (healthy, compromised, sunburnt or damaged skin) to reach viable cells, then the SCCS may consider revising this assessment.

5. MINORITY OPINION

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