SCCS/1443/11
Revision of 23 July 2013

Scientific Committee on Consumer Safety

SCCS

OPINION ON

2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)

COLIPA n° S79

The SCCS adopted this opinion by written procedure on 18 March 2013

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 four 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, UV-filter, S79, 2,2'-methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol), Directive 76/768/EEC, CAS 103597-45-1, EC 403-800-1

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on 2,2'-methylene-bis-(6(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol), 18 March 2013, revision of 23 July 2013

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1. BACKGROUND

2,2'-methylene-bis-(6(2H-benotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) (CAS No 103597-45-1), Methylene bis-benzotriazolyl tetramethylbutylphenol (INCI) listed in Annex VII as reference number 23, has been used as UV filter in sunscreens, day care products and skin lightening products.

Submission I for Methylene bis-benzotriazolyl tetramethylbutylphenol was submitted in 1998 by COLIPA[1].
The Scientific Committee on Cosmetic Products and Non-Food expressed its opinion (SCCNFP/0080/98) with the following conclusions:
"The SCCNFP is of the opinion that Methylene bis-benzotriazolyl tetramethylbutylphenol is safe for use in cosmetic products as a UV light absorber at a maximum concentration of 10%".

After a SCCNFP opinion, 2,2'-methylene-bis-(6(2H-benotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) S79 was requested for inclusion in Annex VII, part 1 – List of UV filters which Cosmetic Products may contain – to Council Directive 76/768/EEC.

As a result of the recast of the European Cosmetic Directive (76/768/EEC) into the Cosmetic Regulation (EC No. 1223/2009) a new description will be necessary for this chemical. According to the description stated in the Cosmetic Regulation under article 2 this material could fulfil the definition of a nanomaterial. Based on this new definition supplementary data on the material on its nano form, was submitted by the applicant.

According to the applicant, the current submission II takes into account the available information on the nano-form of this ingredient.

2. TERMS OF REFERENCE

1. *Does the SCCS consider 2,2'-methylene-bis-(6(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) in its nano form safe for use as a UV-filter with a concentration up to 10 % in cosmetic products taking into account the scientific data provided?*

2. *Does the SCCS have any further scientific concern with regard to the use of 2,2'-methylene-bis-(6(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) in its nano form in 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

2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)

3.1.1.2. Chemical names

2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)

3.1.1.3. Trade names and abbreviations

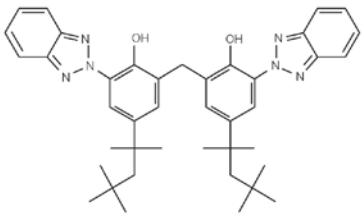
UV-absorbing ingredient (UV filter) (further referred to as MBBT)	**MBBT** Tinosorb® MBBT FAT 75714B CGF-C002089 TKA 40027 CG 30-1881 *
Nano-MBBT:	Tinosorb® M FAT 75'634

* additional trade names are on the market

3.1.1.4. CAS / EC number

CAS: 103597-45-1
EC: 403-800-1

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{41}H_{50}O_2N_6$

3.1.2. Physical form

Yellowish powder (MBBT)
Viscous white dispersion (nano-MBBT)

Comment
Note that this colour change reflects the behaviour which is typical for a nanomaterial in dispersion: A colour change with modified particle size distribution in dispersion is expected to occur from the colour of the bulk component (here yellow) to white with increased fractions of smaller sized particles on physico-chemical grounds.

3.1.3. Molecular weight

Molecular weight: 658.89 Da

3.1.4. Purity, composition and substance codes

MBBT, non-micronised:

Method
In a chemical characterisation study, the purity and thus batch composition of MBBT (FAT 75714B), was determined by means of HPLC-DAD (diode-array detection)(purity assessment) and HPLC-MS (isomer identification). The purity was measured as relative peak area of the DAD signal at a wavelength of 304 nm.

Results
The purity of the UV-absorbing ingredient determined by chromatography is depicted below:

Purity of the UV-absorbing ingredient based on relative peak area in the HPLC-DAD chromatogram

Ingredient Concentration [mg/mL]	**Main Peak** (% area)	**Isomer Peak** (% area)	**Total** (% area)
0.1	98.78	1.22	100
0.4	98.59	1.41	100
Mean			
n.a.	98.69	1.32	100

n.a.: not applicable

Conclusion
The purity of the UV-absorbing ingredient including main compound and isomer determined by chromatography was >99.9 % (area/area).

Comment
The SCCS notes that around 1.5 % of the content has been identified as an isomer, full characterisation of which has not been provided. However, since toxicological studies were

performed with the test item containing the above mentioned isomer, the SCCS considers the toxicological evaluation covers the potential toxicity of the isomer.

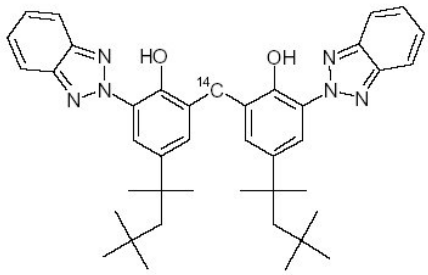
MBBT, micronised:
Tinosorb® M
Preparation containing approx. 50 % w/w of the UV-absorbing ingredient 2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)

Typical composition of the formulated UV-filter Tinosorb® M, containing micronized (ball-milled) MBBT as the UV absorbing compound.

Ingredient	CAS Number	Function	Mass Fraction (% w/w)
MBBT	103597-45-1	UV-Absorption	46.0 – 55.0
Decyl Glucoside	68515-73-1	Surfactant	6.0 - 10.0
Propylene Glycol	57-55-6	Wetting Agent	0.2 – 0.6
Xanthan Gum	11138-66-2	Stabilizer, Thickener	0.1 – 0.5
Water	7732-18-5	Vehicle	40.0 – 42.0

For the different batches of Tinosorb® M used in the toxicology studies, see Annex I

^{14}C-radio-labelled MBBT (purity 99.1 %; position of the label, see in figure below) was mixed with non-radio-labelled MBBT (purity ≥ 99.5 %) and milled to produce batches of radio-labelled nano-MBBT.



3.1.5. Impurities / accompanying contaminants

Impurities of the MBBT

Method
Typical impurities of MBBT were determined by means of HPLC-DAD and HPLC-MS with the concentration limit for the determination of impurities set to 0.08 % (area/area). This investigation covered known impurities and possible impurities which could come from the last steps of synthesis. In addition, volatile impurities were assessed by means of thermo-desorption coupled with GC/MSD. The water content was determined by Karl Fischer titration and a metal analysis was performed by means of ICP-OES.

Results
In this chemical characterisation study, only one relevant impurity peak was detected by HPLC and identified as an isomer of the UV-absorbing ingredient. The water content was determined to be <0.1 %. All measured trace metals were below their quantitation limit. The volatile impurities Isopropanol, Dibutylamine, Xylene (mixture of *ortho*-, *meta*- and *para*-isomers) and Formaldehyde were identified and shown to have a concentration in or below the ppm range. Additional impurities could be identified on a qualitative basis by means of NIST library fits only.

Impurity	Concentration
Water	< 0.1 %
Trace metals[a]	< LOQ
Isopropanol	2.2 µg/g
Dibutylamine	< 0.5 µg/g[c]
Xylene[b]	6.8 µg/g
Formaldehyde	0.93 µg/g
Cis-3-Nonen-1-ol	n.a.
Dodecane	n.a.
9-Decyn-1-ol	n.a.
4-tert-Buthyl-o-xylene	n.a.

a: As, Cd, Cr, Cu, Fe, Hg, Na, Ni, Pb, Sb, Se, Sn, Zn
b: mixture of ortho-, meta- and para-isomers
c: value below estimated limit of detection
n.a.: not applicable
LOQ: limit of quantitation ranging from <1 - <10 mg/kg

Ref: ciba (2004a)

Impurities of Tinosorb® M
These were not separately reported. Upon request the applicant commented:

“The nanomaterial is produced by the physical process of milling technical MBBT. It is not heated and no chemical reaction is performed, it is not dissolved and re-crystallized again. The additional components (decyl glucoside, propylene glycol, xanthan gum and water) are added for milling purposes and to stabilize the resulting micro dispersion. They are considered to be formulants. The whole milling process: dispersion in de-ionized water, adding decyl glucoside as dispersant, pre-milling in a corundum disc mill, main milling with a ball mill, stabilizing the microfine dispersion with xanthan gum is therefore regarded to be a formulation process.

The purity of the UV absorbing ingredient as well as the identity and amount of impurities of MBBT used for the preparation of Tinosorb® M microfine material are consequently identical with those of MBBT in the non-micronized form.”

3.1.6. Solubility / dissolution

UV-absorbing ingredient (UV filter): extremely low solubility in water (< 5 ng/L at 25 °C)
increased solubility in water when formulated in a sun lotion (1.0 – 3.2 mg/L, temperature unspecified)
solubility in fat simulant (170 mg/100 g at 37 °C)

Formulated micronised UV filter: dispersible in water (temperature unspecified) soluble in polar cosmetic oils at 25 °C;

Comment

Solubility is given for the non-micronised form only and not for the nano form. However, by definition of the physico-chemical term 'solubility' this does not depend on the size distribution of the compound, if appropriate procedures are applied. It is not clear to what extent the other substances of the nano-formulation influence the solubility of nano-MBBT compared to non-nano MBBT.

3.1.7. Partition coefficient (Log P_{ow})

UV-absorbing ingredient (UV filter): 12.7 at 25 °C (calculated value) determined according to OECD 107

Formulated micronised UV filter: not applicable

Comment

Log Pow is based on distribution of solubilised substances in aqueous and organic phases and as such does not apply to insoluble particles. However, considering the fat solubility of MBBT, the SCCS considers that the partition coefficient should be similar for both the non-micronized and micronized forms.

3.1.8. Additional physical and chemical specifications: nano specific properties

The UV-absorbing ingredient MBBT is micronised by means of pre-milling with a corundum disc mill followed by the main milling step which is conducted with a ball mill (Herzog et al., 2004a). This milling process is performed in deionised water using Decyl Glucoside as dispersant. After micronisation, Xanthan Gum is added to the dispersion in order to prevent particle sedimentation. The standard composition of the resulting formulated micronised UV filter Tinosorb® M is specified below:

Physic-chemical property	UV-absorbing ingredient (UV filter)	Formulated micronised UV filter
Physical state	solid	liquid (aqueous dispersion)
pH range	not applicable (solid)	pH 10.5-12 at 25 °C determined with glass electrode according to DIN 19268
Odour	characteristic odour organoleptic determination	trace characteristic odour organoleptic determination
Melting point	195.7 °C	not applicable (liquid)
Boiling point	not applicable (solid)	approximately 100 °C determined according to OECD 103
Vapour pressure	6×10^{-13} Pa at 25°C	not determined
Relative density	No data	determined according to OECD 109 1.04 – 1.12 g/cm³ at 25 °C
Dynamic viscosity	Not applicable (solid)	200 – 1000 mPa × s at 25 °C determined according to DIN 53019
Flammability	not flammable	not highly flammable
Auto flammability	No data	not self-igniting
Explosiveness	not explosive	not explosive
Dry content	No data	55.5 – 59.5 % determined by means of IR drying of 1 g at

		135 °C for 60 minutes

Comment

From the provided description of the production process, it is the understanding of the SCCS that micronisation and addition of Xanthan Gum is part of the manufacturing process of the nano-sized material.

The SCCS notes that the density of MBBT (1160 kg/m³ from the CAS registry) differs from the density of the formulation. The dry content of the raw material should be 100% as no solvent is contained. The vapour pressure of the raw material is very low and thereby the vapour pressure of the emulsion is dominated by high vapour pressure components like water. While boiling does not occur for MBBT, the formulation boils with water as the ingredient with the lowest boiling temperature.

3.1.9. Size and size distribution

Methods

The particle size of Tinosorb® M (batch 1365CL6VY) was measured with the fiber-optic quasi-elastic light scattering (FOQELS[1]) technique. From the measured scattering data the volume weighted particle size distribution was calculated and a median value of particle size distribution d(0.5) was derived. With the FOQELS technique, the particle size distribution could be resolved to sizes in the ultrafine range, i.e. well below 100 nm. Based on the derived particle size, specific surface area and number concentration were calculated.

The described treatment of FOQELS measurements was validated by disc centrifugation studies and confirmed by atomic force microscopy (Herzog et al., 2004b).

Results

Determination of the Tinosorb® M particle size distribution by means of FOQELS technique revealed a d(0.5) of 150 ± 9 nm (mass based) as an average value with standard deviation of 10 individual measurements. With this particle size and the density of the particles of 1160 kg/m³ a specific surface area of 34.5 m²/g was obtained. The derived number of particles in 1 cm³ at an active concentration of 50 % was 2.44×10^{20}.

Particle size, surface area and mass concentration of Tinosorb® M

Endpoint	**Datum**
Particle size (d(0.5)-value) mass based	150 ± 9 nm
density	1160 kg/m³
number of particles	2.44×10^{20} cm^{-3}

Additional information was sent on request, with regard to the particle size on a number basis. The sample investigated with the FOQELS method was Tinosorb M-PGL, ITEM: 0758792,which can be regarded as a typical production batch.

d(0.5) = 74 sd 42 nm (number based)

[1] The FOQELS (fiber-optic quasi-elastic light scattering) is a dynamic light scattering technique which is based on measurement of the intensity fluctuations of the back-scattered light of a particle dispersion. For a detailed description of the methodology please refer to Herzog *et al.* (2004b).

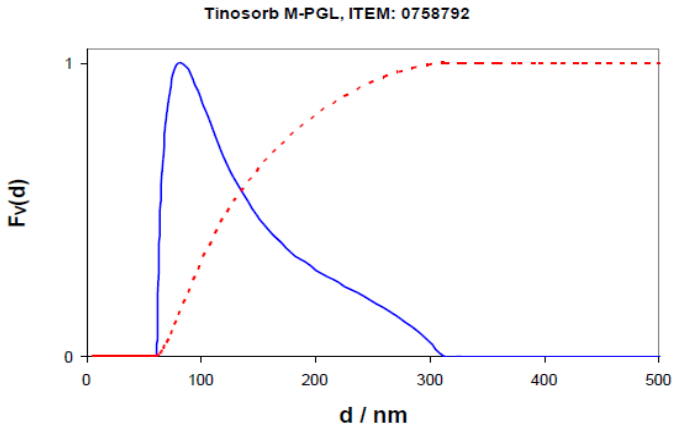


Figure 1: Particle volume distribution of Tinosorb M Lot 1365CL6VY; the blue solid line is the particle size distribution, the red dotted line is the cumulative particle size distribution, **d(0.5) = 188 ± 24 nm**

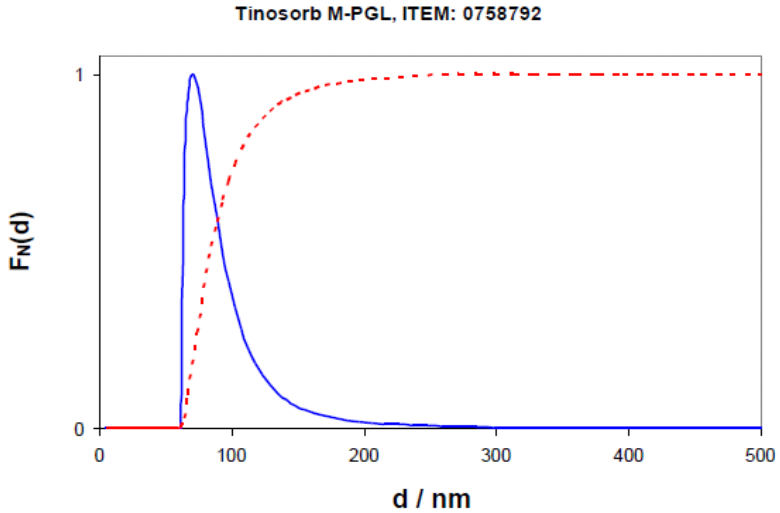


Figure 2: Particle number distribution of Tinosorb M Lot 1365CL6VY; the blue solid line is the particle size distribution, the red dotted line is the cumulative particle size distribution, **d(0.5) = 74 ± 42 nm**

The d(50) particle sizes on mass and on number basis, are reported below, for the different batches in the dossier:

Batch	d50 (laserdiff.) V	d50 (laserdiff.)N
Tinosorb M Lot.01722CL4	117nm	61nm
Tinosorb M Lot. 01635cl4	124nm	62nm
Tinosorb M Lot.01636cl4	123nm	66nm
Tinosorb M Lot.01637cl4	125nm	62nm
Tinosorb M Lot.01723cl4	117nm	61nm
Tinosorb M Lot.01724cl4	125nm	66nm
Tinosorb M Lot. 5915252	124nm	66nm
Tinosorb M Lot.5915253	126nm	66nm
Tinosorb M Lot.5915254	126nm	66nm
Tinosorb M Lot.5915255	127nm	66nm
Tinosorb M Lot.5915257	117nm	64nm
Tinosorb M Lot.5915258	126nm	66nm

Comment
The d(50) particle sizes on mass and on number basis were determined by laser diffraction and FOQELS for a non-radiolabelled nano MBBT batch termed "cold", which was used as a reference for comparison with the batch of radiolabelled “hot” nano MBBT that was used in the toxicological studies. However, the resulting values from laser diffraction and FOQELS are considerably different. This might be due to the difficulty to calibrate optical techniques in view of the size dependency of their sensitivity. Herzog et al, have provided some evidence to show that small particles can be detected by FOQELS which is generally considered the more suitable technique for smaller particle fractions.

Sample	d50 (Laserdiff.) V	d50 (Laserdiff.) N	d50 (Foqels) V	d50 (Foqels) N
Nano MBBT cold (15420CL2AA)	114nm	84nm	97nm	49nm

Overview on particle sizes for the different batches (ref submission II combined)

Overall Tinosorb® M batches, a mean particle size d(0.5) of 122 ± 0.005 nm (n = 32) with a range of 115 – 129 nm and a specific surface area of 56.0 ± 2.9 m^2/g (n = 32) with a range of 52 - 67 m^2/g was determined. The MBBT batches displayed a mean particle d(0.5) size of 207 ± 70 µm (n = 3) with a range of 142 – 281 µm and a specific surface area of 0.21 ± 0.03 m^2/cm^3 (n = 3) with a range of 0.19 - 0.25 m^2/cm^3.

Comment
It is not reported whether these particle size distributions are mass or number based. Most likely they are mass based.

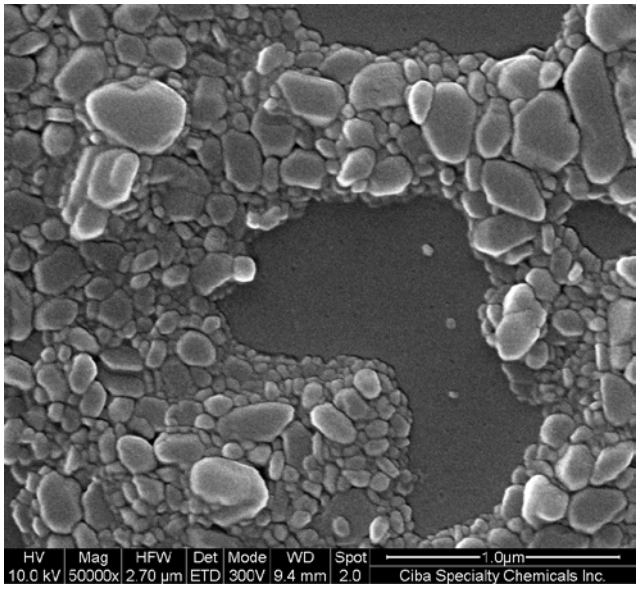
3.1.10. **Microscopy**

3.1.10.1 scanning electron microscopy

Method
The particle size distribution of Tinosorb® M (Batch 1365CL6VY) was visualized by means of scanning electron microscopy (SEM).

Results
Below, a SEM image of Tinosorb® M particles at 50000 times magnification is presented. The particle sizes observed with this technique are in agreement with the FOQELS presented in section 3.1.9 for the same batch.

SEM picture of Tinosorb® M particles (Lot. 1365CL6VY)



3.1.11. crystal structure

/

3.1.12. UV absorption

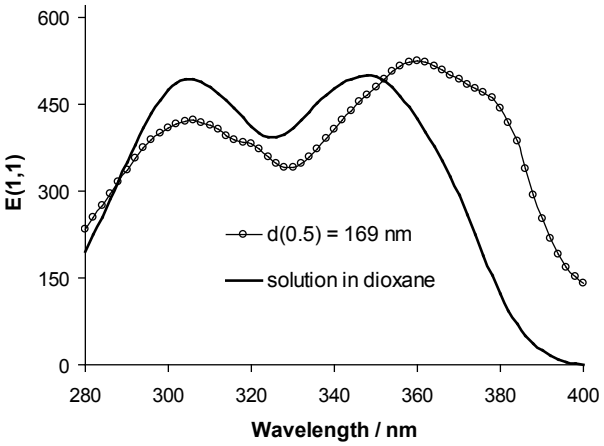
Author:	Herzog et al. (2004a)
Title:	New UV absorbers for cosmetic sunscreens – a breakthrough for the photoprotection of human skin
Guideline:	/
Batch:	1365CL6VY
GLP:	/
Reference:	Chimia 58: 554-559

Method
UV spectra were recorded with the UV-absorbing ingredient MBBT (d(0.5): 169 nm volume based) and with MBBT dissolved in Dioxane.

Since for organic coloured pigments the UV absorption often increases with smaller particles while the scattering shows a maximum at a certain particle size, the dependence of UV attenuation on particle size was assessed using MBBT dispersions with different particle sizes and an UV/Vis spectrometer with integration sphere accessory.

Results
The UV spectra of micronised MBBT and MBBT dissolved in Dioxane are shown in Figure 1.

Figure 3: UV spectra of MBBT

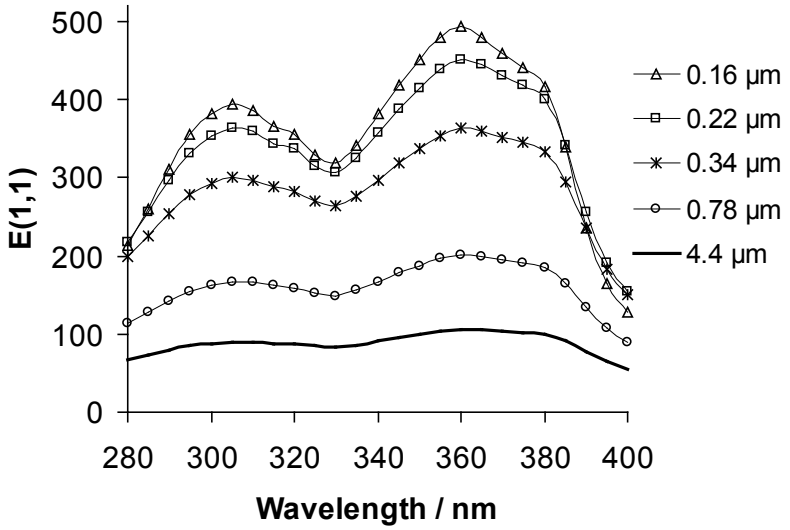


The absorption spectrum of MBBT in solution shows a double band structure. The band at 350 nm can be attributed to a ππ*-charge transfer state. This is favoured by the planar orientation enforced by the intramolecular hydrogen bond, made possible by the hydroxy group in ortho-position. The band at about 305 nm arises from a local transition within the benzotriazole moiety. The absorption spectrum of micronised particulate MBBT in dispersion shows distinctive differences to that in solution, which are not explained by the additional scattering effect: The longer wavelength band is shifted to 360 nm and is followed by a shoulder at 380 nm. The short wavelength band at 305 nm shows no significant shift, but there is an additional shoulder at 320 nm. These spectral changes are attributed to electronic interactions of the chromophores in the particles (Horn and Rieger, 2001) and depend on the particle size (Herzog et al., 2004b).

The specific extinction E(1,1) as a function of wavelength is shown in Figure 3.4 for different particle sizes. There is a strong effect of particle size on the extinction. The smaller the particles become, the more efficient they absorb UV are due to a larger surface area and a more even distribution in the vehicle as compared to larger particles. In terms of the specific extinction at 360 nm, the MBBT particles at the end of the milling process show about 90 % of the highest possible performance.

Comment by SCCS
The authors of the dossier did not specify how they determine the 'highest possible performance'. In general and as stated in (Horn and Rieger, 2001) it remains a complex issue to relate the UV absorption of nano-emulsions to the bulk absorption properties.

Figure 4: UV spectra of MBBT dispersions at different particle sizes



3.1.13. zeta potential

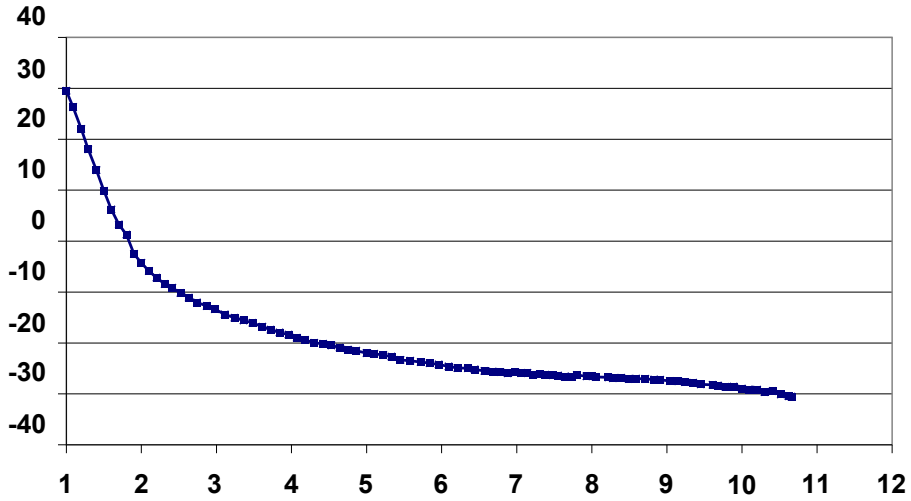
Methods

The ζ-potential of Tinosorb® M (batch 1365CL6VY) containing 10 % UV-absorbing ingredient MBBT in micronised form was measured with a Matec MBS-8000 ESA (electroacoustic spectroscopy) system. Titration was performed with 2M HCl to assess the ζ-potential as a function of the pH value.

Results

At neutral pH, the ζ-potential was determined to be -25 mV, which is of advantage for the stability of the dispersion. The ζ-potential as function of pH is shown below:

ζ-potential of Tinosorb® M particles containing 10 % MBBT



Conclusion
The stability of micronised Tinosorb® M particles in dispersion is enhanced by their negative ζ-potential at physiological pH.

Comment
The formulation of Tinosorb® M contains 50% nano-MBBT, however it seems that the zeta potential was measured at in use concentration (10%).

3.1.14. droplet size in formulations

Title: RK11_033 Sonnenschutzspray TGV. Droplet sizing of sunsprays containing Tinosorb® M (UV Filter – MBBT) using laser diffraction system (Malvern Mastersizer)
Reference: RK11/033
Guideline: /
GLP: /
Batch/Lot n°: 0004327851

Methods
Particle/droplet size measurements were performed by means of laser diffraction (Malvern Mastersizer) when spraying UV protective propellant sprays or pump sprays containing the UV-absorbing ingredient MBBT in the micronised form (Tinosorb® M). The sun spray formulations listed below were sprayed into a test chamber and droplet sizes were measured at a distance of about 30 cm from the spray head.

Test samples:
1. Formulation UV11060-1-1 (8 % MBBT)/30 % propane/butane
2. Formulation UV11060-1-1 (8 % MBBT)/40 % propane/butane
3. Formulation UV11060-2-1 (10 % MBBT)/30 % propane/butane
4. Formulation UV11060-2-2 (10 % MBBT)/40 % propane/butane
5. Formulation UV11060-1-1 (8 % MBBT)/pump spray
6. Formulation UV11060-2-2 (10 % MBBT)/pump spray

Results
The mean droplet size (d(0.5); volume distribution) for all spray formulations (propellant sprays and pump sprays) was found to be >200 µm for all aerosol formulations and >80 µm for the pump spray. The droplet fraction below 10 µm was in all cases, for both types of spray, <1 % (volume distribution).

Comment
The submitted data is not considered sufficient. In case of sprayable applications, appropriate data are needed on the physics of the spray generation and the atmospheric conditions during use (including behaviour of the aerosols in time).

3.1.15. Homogeneity and Stability

Stability in dose formulation

Author: Harlan Laboratories Ltd. (2009)

Title:	Percutaneous penetration of ^{14}C-FAT 75714 (MBBT) formulated as Tinosorb® M through pre-damaged human split-thickness skin membranes *(in vitro*)
Reference:	Study report number C27241
Guideline:	OECD 428
GLP:	In compliance
Batch/Lot n°:	3389-279

Methods
In a percutaneous penetration study, the stability of ^{14}C-FAT 75714 (radiochemical purity: 99.1 %), i.e. of the UV-absorbing ingredient MBBT in micronized form (d(0.5): 89 nm), suspended in formulation and water was determined at the time of application and in the skin rinse fluid (after 24h of exposure) by means of a HPLC method with UV detection. The radioactivity signal was monitored with a radioactivity flow monitor.

Results
HPLC analysis after administration on split-thickness skin membranes revealed a radiochemical purity of the formulated micronized test item of 98.93 %. In agreement with this outcome, the radiochemical purity of the test item in the skin membrane rinse was determined to be 98.89 %. Test item stability in the dose formulations was additionally confirmed by the UV-based chromatogram at 280 nm.

General comments on physico-chemical characterisation

- Not all batches of nano-MBBT used in the toxicity studies have been characterised.
- The SCCS notes that around 1.5 % of the content has been identified as an isomer, full characterisation of which has not been provided.
- Considering the fat solubility of MBBT, the SCCS considers that the partition coefficient should be similar for both the non-micronized and micronized forms.
- In the majority of studies, it is not indicated whether the reported particle sizes are mass or number based.
- Determination of the 'highest possible performance' on UV absorption was not specified. In general and as stated in (Horn and Rieger, 2001) it remains a complex issue to relate the UV absorption of nano-emulsions to the bulk absorption properties.
- The submitted data on droplet size is not considered sufficient. In case of sprayable applications, appropriate data are needed on the physics of the spray generation and the atmospheric conditions during use (including behaviour of the aerosols in time)

3.2. Function and uses

2,2'-methylene-bis-(6(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) (CAS No 103597-45-1), Methylene bis-benzotriazolyl tetramethylbutylphenol (INCI) is used in nano form as UV filter in sunscreens, day care products and skin lightening products at a maximum concentration of 10%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

No studies provided with nano-sized material

For studies with MBBT: see annex II

3.3.1.2. Acute dermal toxicity

No studies provided with nano-sized material

For studies with MBBT: see annex II

3.3.1.3. Acute inhalation toxicity

Title:	4-hours acute inhalation toxicity study in rats
Guideline:	OECD 403
GLP:	In compliance
Test item:	FAT 75'634 (containing 50 % (w/w) MBBT); microfine material; d(0.5) = 128 nm
Batch/lot n°:	00292CL7

Method
In a limit test, a group of 15 albino rats [HanRCC:WIST (SPF)] per sex was nose-only exposed to a 20 % (w/w) aqueous dilution of FAT 75'634, *i.e.* the formulated micronised UV filter Tinosorb® M. This tested aqueous dilution corresponds to a 10 % dilution of the UV-absorbing ingredient MBBT. The animals were exposed at the highest technically achievable aerosol concentration of 4.877 ± 0.255 mg/L (n = 8; determined by chemical analysis) for a single period of 4 hours. The resulting MBBT concentration was 0.488 mg/L. Two gravimetric measurements of particle size distribution performed during the exposure produced mass median aerodynamic diameters (MMADs) of 1.42 and 1.44 µm with geometric standard deviations (GSDs) of 2.10 and 2.10, thus confirming that the aerosol particles were within the target size range of 1 – 4 µm and were deposited in all regions of the rat respiratory tract. A further group of 15 rats per sex was exposed to an aqueous dilution of the control item, FAT 75'634 Placebo, at similar aerosol generation conditions.

Each dose group was subdivided in three satellite groups of 5 rats per sex, the first satellite group being assigned to sacrifice at approximately 14 hours post end of exposure for bronchoalveolar lavage fluid (BALF) and plasma sampling (day 1), the second being assigned to interim pathology at approximately 24 hours post end of exposure (day 2), and the third being assigned to pathology at 14 days post exposure (day 15). Clinical signs, mortality and body weights were assessed periodically during the study period. The BALF examinations comprised total and differential cell counts and the determination of total protein, tumour necrosis factor α (TNFα) and interleukin 6 (IL-6) levels. Pathology examinations comprised complete macroscopic pathology, lung weight determination 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 toxicologically relevant adverse effects on body weight noted in this study. At the end of the observation period (day 15), no organ weight changes, macroscopic and/or microscopic findings attributable to treatment with the placebo or test item dilutions were noted.

Entirely reversible test item-related findings were noted on day 2 of the observation period. These consisted of increases of total cell count (neutrophil numbers) and total protein in BALF and of absolute and relative lung weight, and diffuse alveolar histiocytosis and alveolar lining cell activation in the treatment group as compared with the placebo control group. These findings were consistent with marginally higher TNFα and IL-6 levels in BALF seen in test item exposed females relative to placebo control females.

Conclusion
Under the conditions of this study, the lethal concentration 50 (LC50) in rats was greater than the highest technically achievable aerosol concentration of the formulated micronised UV filter Tinosorb® M, which was generated from a 20 % aqueous dilution. The LC50 of the UV-absorbing ingredient MBBT in micronised form was therefore > 0.488 mg/L.

Transient pulmonary effects noted on day 2 of the observation period were considered to be test item-related and to be indicative of an acute pulmonary clearance reaction associated with an inflammatory response.

Ref.: RCC (2008)

Comment
The observed effects are indeed similar to those observed upon inhalation of particulate materials in general including some nano-particles. However, the massive influx of neutrophils and the decrease of macrophages, cannot be considered to be merely a mild inflammation after inhalation of particles, as is stated by the authors.
Reversibility of the BALF increase as present at day 1 was not demonstrated as BALF data of later time points were not included in the report.
It was not specified whether the particle size was expressed as number based or mass based.

Additional information:

BALF and/or plasma results 14h post end of inhalation exposure

Summary (mean ± std dev.)				
Parameter	**Males** (N= 5)		**Females** (N= 5)	
	Placebo	**Test Item**	**Placebo**	**Test Item**
Total Cells (millions)	9.76 (1.62)	14.37 (1.91) **	8.32 (1.12)	21.35 (11.27)*
% of Total cells				
Macrophages	95.9 (1.3)	46.4 (8.6)**	94.7 (1.2)	37.4 (11.8)**
Eosinophils	0.0 (0.0)	0.1 (0.1)	0.2 (0.3)	0.0 (0.1)
Lymphocytes	0.6 (0.6)	0.4 (0.4)	0.4 (0.2)	0.8 (0.7)
Neutrophils	0.2 (0.3)	50.1 (8.3)**	0.8 (0.6)	59.9 (11.7)**
Other cells	0.3 (0.2)	0.8 (0.7)	0.6 (0.2)	0.4 (0.3)
Epithelial cells	3.0 (0.7)	2.2 (0.7)	3.4 (1.0)	1.5 (0.5)**
TNFα (pg/ml)	<22.4	28 (9)	<22.4	45 (31.52)
IL6 (pg/ml)	165; 88	150; 111[a]	179; 104	236:45
Total Protein (g/l)	102 (30)	138 (32)	96 (19)	154 (49)
Plasma Protein (g/l)	52 (17)	61 (0.5)	63 (3.0)	63 (1.8)

* / ** t-test based on pooled variance, significant at 5% or 1% level.
[a] 3 animals >200, 2 at or just above LLQ

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

No studies provided with nanosized material.
For studies with MBBT: see annex 2

3.3.2.2. Eye irritation

In vitro

Guideline:	OECD n° 437 (BCOP test)
Test system:	isolated bovine cornea
Route:	eye
Substance:	Tinosorb® M, test substance nr. 10/0381-1
Batch:	P380; pH 10.5
Purity:	not provided
Dose:	750ul of the undiluted substance
GLP:	in compliance
Study period:	October 2010

Ref.: BASF SE (2010a)

and

Guideline:	OECD n° 437 (BCOP test)
Test system:	isolated bovine cornea
Route:	eye
Substance:	Tinosorb® M, test substance nr. 10/0381-1
Batch:	P380; pH 11.6
Purity:	not provided
Dose:	750ul of the undiluted substance
GLP:	in compliance
Study period:	October 2010

Ref: BASF SE (2010b)

The pH specification of the formulated micronised UV filter Tinosorb® M is pH 10.5-12 at 25 °C. Two *in vitro* studies were conducted to cover this pH specification range and to ensure that no treatment-related effects occur at either extremes. The test items “Tinosorb® M pH = 10.5” and “Tinosorb® M pH = 11.6” were used for these investigations.

A single topical dose of 750 µL of the undiluted formulated micronised UV filter Tinosorb® M was administered to the epithelial surface of three isolated bovine corneas. The corneas were exposed to the test item for 10 minutes followed by a 2-hours post incubation period.

Corneal opacity was measured qualitatively as the amount of light transmission through the cornea. Permeability was measured quantitatively as the amount of Fluorescein dye that passed across the full thickness of the cornea. Deionized water served as negative control item and 1 % (w/v) Sodium hydroxide served as positive control item.

Evaluation criteria
IVIS > 55 (risk of serious damage to eyes)
IVIS ≤ 55 (no risk of serious damage to eyes)

Results
In vitro irritancy score for Tinosorb® M pH = 10.5

Group	Mean Opacity	Mean Permeability	Irritancy Score (IVIS)
Tinosorb® M pH = 10.5	4.5	0.208	7.6
Negative control	2.6	0.001	2.6
Positive control	148.0	3.830	205.4

vitro irritancy score for Tinosorb® M pH = 11.6

Group	Mean Opacity	Mean Permeability	Irritancy Score (IVIS)
Tinosorb® M pH = 11.6	3.0	0.002	3.0
Negative control	2.6	0.001	2.6
Positive control	148.0	3.830	205.4

Under the conditions of this *in vitro* assay, the formulated micronised UV filter Tinosorb® M pH= 10.5 and pH = 11.6 did not cause serious eye damage.

Comment
The particle size distribution of the material was not listed in the report, also the purity was not reported ('available on request'). Based on historical data, the d(0.5) value was estimated by the applicant as 110-130 nm (no further details provided).

In vivo

Guideline: OECD n° 405
Species: rabbit (New Zealand white)
Route: eye
Group size: 3 females
Substance: Tinosorb® M, test substance nr. 10/0381-1
Batch: P380; pH 11.6
Purity: not reported
Dose: 0.1 ml of the Tinosorb ® M formulaton
GLP: in compliance
Study period: October 2010

Three female New Zealand White rabbits [Crl:KBL(NWZ) SPF] received a single application of 0.1 mL Tinosorb® M pH = 11.6, *i.e.* of the formulated micronised UV filter Tinosorb® M (50 % MBBT), into the conjunctival sac of the right eyelid. The treated eyes were rinsed with warm tap water about 24 hours after application. The untreated left eye of each animal served as negative control. Scoring of irritation effects was performed 1, 24, 48 and 72 hours after application according to the OECD scoring system using an otoscope lamp. Viability checks were performed on a regular basis and body weights were recorded prior to test item administration and after the last reading.

Slight conjunctival redness and slight chemosis were noted in two of three animals up to 24 hours after application. Ocular discharge was observed in one animal only 1 hour after application. No corneal or iris lesions were noted in any animal at any examination timepoint. One animal was completely free of alterations. All ocular reactions were reversible within 48 hours after application. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for corneal opacity, 0.0, 0.0 and 0.0 for iris lesions, 0.0, 0.3 and 0.3 for redness of the conjunctiva and 0.0, 0.0 and 0.0 for chemosis, respectively.

conclusion
Under the conditions of this study, the formulated micronised UV filter Tinosorb® M pH = 11.6 was slightly irritating to the rabbit eye.

Ref: Seibersdorf (2010)

Comment
This *in vivo* study was carried out for the purpose of a Japanese Quasi Drug registration.
The particle size distribution of the material was not listed in the report, also the purity was not reported. Based on historical data, the d(0.5) value was estimated by the applicant as 110-130 nm (no further details provided).
The eye irritation observed may be due to the high concentration (6-10%) of decyl glucoside which was present as a surfactant in the formulation.

3.3.3. Skin sensitisation

No studies provided with nano-sized material.
For studies with MBBT: see annex 2

3.3.4. Dermal / percutaneous absorption

Dermal penetration study *in vitro* (nano sized material)

Guideline	OECD 428
Test substance*:*	A suspension of micronized ^{14}C-FAT 75714/B (purity of radio-labelled item: 99.1 %; purity of non-radiolabelled item: ≥ 99.9 %), *i.e.* of the UV-absorbing ingredient MBBT, was prepared in formulation (batch: CE60910011) and water. The suspension was further diluted with water and the pH-value adjusted to pH 6.5.
Purity:	Non-radiolabelled: 99.9% Radiolabelled: 99.1%
Dose applied:	1.4 mg/cm^2 in 13 µl for 24 hours
Skin preparation:	Full thickness skin from rats and from humans (upper leg dorsal, frozen), removed from subcutaneous fat was used. Sections of 200 µm thickness were cut off from the top by dermatome. Pieces of 1.8 x 1.8 cm were mounted in flow-through diffusion cells, with 0.64cm^2 of skin membrane exposed to the donor chamber.
Skin temperature:	ambient
Exposure period:	24 hours
Donor chamber:	non-occluded
Receptor fluid:	(6 % (w/v) polyethoxyoleate (PEG 20 oleyl ether) dissolved in physiological saline (0.9% NaCl w/v))
Control:	No control was used
Particle size:	d(0.5)= 90 and 99 nm (2 series of 10 measurements); mass based.

Skin integrity:	50 µL of tritium water was applied to the skin membrane surface in occluded donor chamber
Recovery:	99.12% (rat skin) 94.45% (human skin)
GLP:	conducted according to GLP principles but the study report was not signed.

Methods
Split thickness skin membranes of rats ([HanBrl:WIST(SPF)]; two males) and of human donors (Caucasian donors; one male, two females) were set up in flow-through diffusion cells, the formulated micronised test item was applied onto the skin membranes at a finite dose of 13 µL/cm² and the receptor fluid collected at defined time intervals over a 24 hour period. In accordance with the standard dermal application rate for sun creams, a single target dose level of 2 mg/cm² of MBBT was used reflecting a concentration of 10 % test item in the final formulation.

Prior to test item administration, membrane integrity was checked by applying 50 µL tritium water (about 200000 dpm) to skin membranes mounted in diffusion cells between the donor and receptor chamber. 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 (Kp) of each skin membrane was calculated for the 3 - 6 hours interval. Rat skin membranes with Kp > 3.5×10^{-3} cm/h and human skin membranes with Kp > 2.5×10^{-3} cm/h were excluded from the subsequent experiment.

The formulated test item was applied onto skin membranes of 200 µm thickness at a concentration of 70.3 mg/cm³ leading to an area concentration of 1428.5 µg/cm². For the rat group 7 replicates and for the human group 5 replicates from 2 donors were used for calculation (the skin of 1 donor was excluded due to a too high Kp). During the 24-hour exposure under non-occluded conditions the receptor fluid (6 % Polyethoxyoleate (w/v)) 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 experiment. At the end of the experiment the remaining test item was removed by rinsing the skin membranes three times with a mild soap solution. The skin membranes were removed from the diffusion cell and consecutively stripped until the *stratum corneum* was removed from the skin membrane.

Radioactivity was measured in liquid specimens (receptor fluid, skin membrane rinse, cell wash) and in digested / solubilized specimens (tape strips, skin membranes) by liquid scintillation counting (LSC) and quantified as quench-corrected disintegrations per minute.

Skin rinse aliquots were analyzed by HPLC with UV detection and radioactivity flow monitor in order to investigate test item stability over the exposure period. In addition, aliquots of the final suspension were analysed in a particle sizer.

Results
For both species ^{14}C-FAT 75714/B amounted to more than 98.65 % of the radioactivity present in the skin membrane rinses.

After application, an average of only 0.06 % of the applied dose penetrated through the rat skin membrane within 24 hours. The mean test item flux could not be calculated, but an estimated flux was calculated by using the values above LOQ and setting all values which were below LOQ to the corresponding LOQ and this flux was about 0.104 µg/cm²/h. At the end of exposure, 67.91 % of the applied dose could be washed off from the skin membranes. After skin membrane rinse, 28.88 % 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.* 26.86 % of the

applied dose was determined in tape strips. A minor part, *i.e.* 2.02 % of the applied dose, was found in the remaining skin membrane after tape stripping.

For human skin membranes the test item penetration resembled closely to that observed in rat skin membranes. Within 24 hours of exposure only 0.01 % of the applied dose penetrated totally through human skin membranes. The estimated flux was 0.086 µg/cm²/h. Also, 77.56 % of the applied dose could be washed off 24 hours after exposure start. In *stratum corneum* 13.15 % of the applied dose was found and only 0.01 % in the remaining skin membrane after tape stripping. The amount of test item in lower skin layers was significantly lower in human skin membranes as compared to rat skin membranes.
The mean total absorption, based on the amount penetrated through the skin membrane and the amount measured in the remaining skin membrane layers after tape stripping, was 2.08 % (n = 7) and 0.02 % (n = 5) of the applied dose for rat and human skin membranes, respectively. The calculated absorption values for human skin were considered conservative since most values were below LOQ.

Percutaneous penetration of the micronised UV-absorbing ingredient MBBT through rat and human split thickness skin membranes over a 24-hour period

	Rat skin membranes	**Human skin membranes**
Applied dose		
Total applied dose	1428.5 µg/cm²	1428.5 µg/cm²
Recovery of applied dose (mean or mean ± SD)		
Total absorbed dose - Receptor fluid - Remaining skin membrane	2.08 % 0.06 ± 0.06 % 2.02 ± 1.92 %	0.02 % 0.01 ± < 0.01 % 0.01 ± < 0.01%
Total non-absorbed dose - Skin membrane rinse - Tape strips - Cell wash	97.04 % 67.91 ± 21.77 % 26.86 ± 18.16 % 2.27 ± 2.28 %	94.42 % 77.56 ± 4.84 % 13.15 ± 6.67 % 3.71 ± 2.20 %
Total recovery of applied dose	99.12 ± 1.88 %	94.45 ± 5.15%

SD: Standard deviation of the mean values for each 5 (human) or 7 cells (rat)

Conclusion
The micronised UV-absorbing ingredient MBBT with a particle size (d(0.5)) below 100 nm penetrated at extremely low rates and to a very limited extent through rat and human split thickness skin membranes. The penetration through rat skin membranes was higher than through human skin membranes.

Ref: RCC (2007)

Given the fact that 5 replicates of human skin (2 donors) and 7 replicates of rat skin (2 donors) were assessed, the mean value + 2 SD will be used for the calculation of the Margin of Safety (MoS). This results in a total absorption of 0.048 % (= $0.02 + 2 \times \sqrt{(0.01^2 + 0.01^2)}$ for human skin and 5.922 % (= $2.08 + 2 \times \sqrt{(0.06^2 + 1.92^2)}$ for rat skin.

Comment

- It is noted that the recovery for the human samples is lower than for the rat samples
- Instead of a target dose of 2mg/ cm², only 1.4mg/ cm² was applied.

Dermal penetration study with damaged skin *in vitro* (nano sized material)

Guideline	OECD 428
Test substance:	A suspension of micronized Tinosorb® M, ^{14}C-FAT 75714/B i.e. of the UV-absorbing ingredient MBBT, was prepared in formulation (batch: CE83400003) and water. The suspension was further diluted with water and the pH-value adjusted to pH 6.5, to a final concentration of 92.5 mg test item per ml suspension
Purity:	Non-radiolabelled: 100% Radiolabelled: 99.1%
Dose applied:	1.9 mg/cm^2 in 13 µl for 24 hours
Skin preparation:	Full thickness skin from humans (upper leg dorsal or abdominal area, frozen), removed from subcutaneous fat was used. Before sectioning, 5 tape strips were performed. Sections of 200 µm thickness were cut off from the top by dermatome. Pieces of 1.8 x 1.8 cm were mounted in flow-through diffusion cells, with 0.64cm^2 of skin membrane exposed to the donor chamber.
Skin temperature:	ambient
Exposure period:	24 hours
Donor chamber:	non-occluded
Receptor fluid:	(6 % (w/v) polyethoxyoleate (PEG 20 oleyl ether) dissolved in physiological saline (0.9% NaCl w/v))
Control:	No control was used
Particle size:	d(0.5)= 87 and 91 nm (2 series of 10 measurements)
Skin integrity:	50 µL of tritium water was applied to the skin membrane surface in occluded donor chamber
Recovery:	100.67%
GLP:	in compliance

Method

The percutaneous penetration of FAT 75714 (purity: 99.1 %), *i.e.* of the UV-absorbing ingredient MBBT, formulated as micronised UV filter Tinosorb® M and suspended in formulation 2000 UP was determined *in vitro* using split-thickness skin membranes of 200 µm thickness from pre-damaged human cadaver skin. Pre-damaged skin membranes were produced by stripping the membranes 5 times with an adhesive tape.

The skin membranes (7 replicates) were set up in flow-through diffusion cells, the formulated test item (94.186 mg/mL) was applied onto the skin membranes at a single dose level of 1913 µg/cm² and the perfusates were collected at defined time intervals for a period of 24 hours.

Results

Within 24 hours the portion of radio-labelled MBBT penetrating through pre-damaged human skin membranes accounted for 0.01 % of the applied dose. 97.82 % of the applied dose was removed by skin rinse and additional 2.00 % by tape stripping. In the lower skin layers about 0.06 % of the dose was found. Together with the amount measured in the remaining skin membrane after tape stripping, the amount totally penetrated in/through pre-damaged human skin membrane accounted for 0.06 % of the applied dose. The mean test item flux calculated with LOQ values as worst case calculation was < 0.058 µg/cm²/h through pre-damaged human skin membranes. Recovery was 100.67 %.

Conclusion

The UV-absorbing ingredient MBBT formulated as micronised UV filter Tinosorb® M penetrated at an extremely low rate through pre-damaged human skin membranes.

Ref: Harlan (2009)

Comment of SCCS
As a worst case approach the SCCS calculates the total absorbed dose as the receptor fluid + the remaining skin membrane. Given the fact that 7 replicates of 2 donors were used, the mean value + 2 SD will be used for the calculation of the Margin of Safety (MoS), resulting in an absorption of 0.01+0.06 + 2x. This results in a conservative value of the total absorption of 0.35 % (= 0.07 + 2 × $\sqrt{(0.01^2 + 0.14^2)}$ for pre-damaged human skin.
It is not clear whether the particle size is number based or mass based.

3.3.5. Repeated dose toxicity

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

Guideline/method	Exploratory study
Species:	Rat, Wistar Han
Group size:	5 males and 5 females per dose group (2 animals per sex in satellite group).
Test substance:	Tinosorb® M, FAT 75'634/B
Batch:	02711CL3
Conc a.i.:	50.2%
Particle size:	no data provided in the study report, the applicant indicated that the d(05) = 128 nm presented as additional information in Submission II
Dose applied:	0, 1000, 1500 and 2000 mg/kg bw/day, later reduced to 100, 400 and 800 mg/kg bw/day, see study description below. Skin area: ~45 (males)-30 (females) cm^2).
Route:	topical application on skin of which fur was removed with animal clipper.
Exposure time:	6 hours per day
GLP:	in compliance
Date of report:	dec 2005 (study completed sept 2003)
Published:	no

Methods
Repeated dermal treatments of 5 rats [Crl:WI (GLX/BRL/Han) IGS B0] per sex per dose level with the test item FAT 75'634/B, i.e. with the formulated micronised UV filter Tinosorb® M, suspended in water (first treatment period: day 1 – 17; the pH was adjusted to 8.0) or in base ointment (Batch numbers SO0616 and SP0657; composition not further specified; second treatment period: day 50 – 58; no pH adjustment) at dose levels of 1000, 1500 or 2000 mg/kg bw/d (dose volume: 2.5 mL/kg bw) resulted each in local dermal intolerance and in clinical signs of pain.

Based on this outcome, the dermal treatment was stopped for a period of 5 days (day 59 – 63). Thereafter, the same animals received the test item suspended in base ointment (hydrated, hydrophilic) at reduced dose levels of 100, 400 or 800 mg/kg bw/d (dose volume 2.5 mL/kg bw) for 29 consecutive days (third treatment period: day 64 – 92). A concurrent control group of 5 rats per sex was treated with Tinosorb® M Placebo in base ointment under the same experimental conditions. Viability, clinical signs, food consumption, body weights, parameters of clinical laboratory investigations (haematology and clinical chemistry), test item plasma levels, selected organ weights and macroscopic and microscopic

changes (skin samples and other organs) were assessed and evaluated. The test substance was measured in plasma samples on day 8, 57 and 93. Dose formulations were analysed for content, homogeneity and stability.

Results

During the first and the second treatment period, dermal administration of the test item induced signs of poor local tolerance and pain (vocalization, hyperactivity to stimuli or aggressiveness, and/or abnormal posture) with dose-related incidence and severity at 1000, 1500 and 2000 mg/kg bw/d in both water and base ointment.

During the third treatment period, when dermally applied for 29 consecutive days at 100, 400 or 800 mg/kg bw/d in base ointment, the test item did not induce any local or systemic clinical signs of toxicity. Treatment with the test item did not result in increased mortality, any change in body weight or food consumption, or any modification of haematology and blood chemistry parameters in any group during the three treatment periods. At necropsy, no test item-related effects on organ weights and on macroscopical endpoints were noted in any group. There were no histopathological findings upon examination of the skin sites and other organs related to the treatment with the test item. Thus, the dose level of 800 mg/kg bw/d was established as the highest achievable dose level for repeated dermal treatment.

Plasma levels of the test substance were below or occasionally slightly above the limit of quantification (5ng/ml). There was no dose relation in the plasma levels.

Chemical analysis revealed satisfactory homogeneity of the test item in base ointment and demonstrated good agreement between nominal and analytically determined test item concentrations up to and including 320 mg/L, *i.e.* up to the dose concentration used for the high dose group. The stability of the test item in base ointment was satisfactory for at least 9 days at 4 °C.

Table 1: Incidence of clinical lesions recorded at the application site

Lesion	**Dose level (mg/kg bw/d)**							
	Control		Low dose		Mid dose		High dose	
	M	F	M	F	M	F	M	F
First treatment period (vehicle: water)								
	0		1000		1500		2000	
Desquamation	0	0	0	4/5	1/4	3/5	0	2/5
Scabs	0	0	0	0	0	0	0	1/5
Erythema	0	0	0	0	1/4	1/5	0	1/5
Second treatment period (vehicle: ointment)								
	0		1000		1500		2000	
Desquamation	0	0	0	0	0	0	0	0
Scabs	0	0	0	1/5	0	0	2/5	1/5
Erythema	0	0	0	0	0	1/5	1/5	1/5
Third treatment period (vehicle: ointment)								
	0		100		400		800	
Desquamation	0	0	0	0	0	0	0	0
Scabs	1/5	0	0	0	1/5	0	0	1/5
Erythema	0	0	0	0	0	3/5	0	1/5

M: male; F: female

Conclusion

Under the conditions of this dose range-finding study, the dermal no-observed-effect-level (NOEL) of the formulated micronised UV filter Tinosorb® M was determined to be 800 mg/kg bw/d in rats, when applied topically on the skin for 29 consecutive days in base

ointment. The observed signs of poor local tolerance and pain might be vehicle related since they were not confirmed in a 90-day dermal toxicity study in rats using corn oil as vehicle (RCC Ltd., 2002) up to the high dose level of 1000 mg/kg bw/d.

Ref.: CIT (2005)

Comment
At 400 and 800 mg/kg body weight minimal local skin reactions were observed, that were not considered in the determination of the NOAEL. Therefore the NOAEL of 800 mg/kg bw (equivalent to 400 mg a.i./kg bw) is based on lack of clinical signs when compared to the higher doses that induced pain and discomfort in the animals during the first and second treatment cycle. Given the changes in the study protocol during the study, the results can only be used as supportive for the risk assessment. An adequate NOAEL cannot be derived. It is noted that this dose range-finding toxicity was performed to provide a scientific basis for dose level setting in a carcinogenicity study by dermal route in rats. Moreover, a 39 week dermal study in mini-pigs was provided.
It is not convincing that the clinical signs are vehicle related, since the 90 day dermal study that is referred to above is not carried out using nano-sized material.

The composition of the base ointment is not specified in the study report.
It is not clear whether the particle size is number based or mass based

Dermal dose range finding toxicity study in mini-pigs

Guideline/method	Exploratory study
Species:	Göttingen mini-pigs
Group size:	one male and one female for each of the studies performed
Test substance:	Tinosorb® M, FAT 75'634/B
Batch:	02711CL3
Conc a.i.:	50.2%
Particle size:	no data provided in the study report, the applicant indicated that the d(05) = 128 nm presented as additional information in Submission II
Dose applied:	First study: increasing doses starting at 100, 400, 800, 1000 and 2000 mg/kg bw/day, for three consecutive days, respectively Second study: dose of 2000 mg/kg bw for 14 days 2.5 ml/kg was applied on the skin
Skin area:	at least 10% of the total body area on the dorsum ranging from 371 to 441 cm^2 was clipped free of hair, of which 90% (334 to 397 cm^2) was treated depending on the weight of the animal.
Route:	topical application on skin
Exposure time:	First series: 6 hours per day on three consecutive days Second series: 6 hours per day for 14 consecutive days
GLP:	no
Date of report:	7 July 2004 (study completed 1 March 2004)
Published:	no

Methods
A 14-day dermal range-finding study using one female and one male Göttingen mini-pig (Dalmose, Denmark) per study phase was performed with the test item FAT 75'634/B, *i.e.* with the formulated micronised UV filter Tinosorb® M, suspended in base ointment. In study phase 1, progressively increasing dermal doses (100, 400, 800, 1000 or 2000 mg/kg bw; dose volume: 2.5 mL/kg bw) were administered for 6 hours per day on 3 consecutive days,

respectively. In study phase 2, the animals received 2000 mg/kg bw at the same dose volume 2.5 mL/kg bw for 6 hours per day on 14 consecutive days based on the outcome of study phase 1. The test item was applied unoccluded to each animal to about 90 % of a prepared dosing area and compared to the remaining 10 % untreated control area.
Treatment schedule phase I.

	Dose-level (mg/kg/day)	Days	Animal numbers
Group I (one male and one female)	100	1 to 3	E70001 and E70051
	400	4 to 6	
	800	7 to 9	
	1000	10 to 12	
	2000	13 to 15	

Viability, clinical signs, food consumption, body weights and microscopic changes (skin samples) were assessed and evaluated.

Results
There were no test item-related effects on viability, clinical signs, food consumption and body weights. No microscopic findings were noted on the skin samples from the application sites.

Conclusion
Under the experimental conditions of this study, the maximum tolerated dose (MTD) for repeated dermal treatment with the formulated micronised UV filter Tinosorb® M was above the high dose level of 2000 mg/kg bw/d in Göttingen minipigs. The dose levels selected for the main study were 500, 1000 and 2000 mg/kg bw/d.

Ref: CIT (2004)

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

Dermal repeated dose toxicity study in mini-pigs

Guideline/method	Exploratory study
Species:	Göttingen mini-pigs
Group size:	four male and four female animals per dose level
Test substance:	Tinosorb® M, FAT 75'634/B
Batch:	0069CL3, and 001722CL4
Conc a.i.:	50.2%
Particle size:	for batch 00696CL3 presented as additional information in Submission II the d(05) = 115 nm Batch 001722CL4 d(0.5) 117nm mass based d(0.5) 61nm number based.
Dose applied:	500, 1000, or 2000 mg/kg bw. 2.5 ml/kg was applied on the skin
Skin area:	at least 10% of the total body area on the dorsum ranging from 371 to 441 cm² was clipped free of hair, of which 90% (334 to 397 cm^2) was treated depending on the weight of the animal.
Route:	topical application on skin
Exposure time:	6 hours per day for a period of 39 weeks

GLP: in compliance
Date of report: 10 February 2006 (study completed 24 January 2005)
Published: no

Methods
Three groups of 4 Göttingen minipigs (Dalmose, Denmark) per sex per dose level received dermal applications of FAT 75'634, *i.e.* of the formulated micronised UV filter Tinosorb® M, suspended in a hydrophilic ointment at 500, 1000 or 2000 mg/kg bw/d (dose volume: 2.5 mL/kg bw) for 39 weeks on closely clipped dermal skin (approx. 10 % of the body surface area). The corresponding dose levels expressed in topical dosage equivalents are depicted in Table 5.7.

Table 2: Topical daily dosage equivalents

Tinosorb® M	**MBBT[a]**		
(mg/kg bw)	**(mg/kg bw)**	**(%)[b]**	**mg/cm²**
500	250	10	7
1000	500	20	14
2000	1000	40	28

a: UV-absorbing ingredient in micronised form
b: based on a dose volume of 2.5 mL/kg bw active ingredient

A concurrent control group of 4 animals per sex received Tinosorb® M Placebo in hydrophilic ointment at a concentration equivalent to the concentration of this component in the high dose group.

Viability, clinical signs, food consumption, body weights, ophthalmoscopic endpoints, parameters of clinical laboratory investigations (haematology, clinical chemistry and urinalysis), test item plasma levels (week 13, 26 and end of study before daily dosing), selected organ weights and macroscopic and microscopic changes (skin samples) were assessed and evaluated. Dose formulations were analysed for content, homogeneity and stability.

Results
Chemical analysis of dose formulations met the acceptance criteria for stability, homogeneity and concentrations. No test item-related effects were noted on viability, clinical signs, food consumption, body weights and on haematology, clinical chemistry and urinalysis parameters as well as organ weights. There were no test item-related findings at ophthalmological examination. No test item-related macroscopic and microscopic findings were noted in organs and tissues including treated skin samples.

In high dose animals treated at 2000 mg/kg bw/d, detectable plasma concentrations below 30 ng/mL of MBBT (micronized form) were observed in one male and one female only. Test item concentrations in plasma were below the limit of quantitation (< 5 ng/mL) in all other samples taken during the study.

Conclusion
Under the experimental conditions of this study, no target organ toxicity was identified. Topical application of the test item did not induce a significant systemic exposure.
The no-observed-adverse-effect-level (NOAEL) for repeated dermal treatment of minipigs with the UV-absorbing ingredient MBBT and the formulated micronised UV filter Tinosorb® M was 1000 mg/kg bw/d and 2000 mg/kg bw/d, respectively.

MBBT is not subject to classification for specific target organ toxicity according to Regulation (EC) No. 1272/2008.

Ref: CIT (2006a)

Comment
Some absorption was noted in two animals (30ng/mL), while in all other animals (n=22) levels were below detection limit of 5 ng/mL.

3.3.5.3. Chronic (> 12 months) toxicity

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

No studies provided with nano-sized material.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No studies provided with nano-sized material.
For studies with MBBT: see Annex II

3.3.7. Carcinogenicity

Rat skin carcinogenicity

Guideline/method:	OECD 451 (1981)
Species/strain:	Wistar Han rats
Group size:	50 animals per sex and dose
Test substance:	FAT 75'634/B (Tinosorb M; microfine material; d(0.5) = 128 nm. Contains approximately 50% active ingredient Tinosorb MBBT (FAT 75'714).
Batch:	02711CL3
Concentrations:	100, 400 or 800 mg/kg/day under a constant dosage-volume of 2.5 ml/kg bw/day (Correspond to 50, 200, or 400 mg/kg bw/day Tinosorb MBBT (active ingredient)
Exposure:	Daily by cutaneous application for at least 104 weeks.
Vehicle:	Base ointment, hydrated, hydrophilic
Negative control:	No treatment group and one placebo group (Containing all the substances of the formulation without the active component MBBT)
Positive Controls:	None
GLP:	In compliance
Study period:	2003 -2006.

Method
Groups of 50 male and 50 female Wistar Han rats (7 weeks old) received the test item, FAT 75'634/B, daily by cutaneous application at the dose-level of 100, 400 or 800 mg/kg bw/day (corresponding to 50, 200 or 400 mg/kg bw/day of the active ingredient Tinosorb MBBT (FAT 75'714)) for at least 104 weeks. The quantity of dosage form applied to each animal was adjusted according to the most recently recorded body weight. A constant dosage-volume of

2.5 ml/kg bw/day was used. An additional group of 50 males and 50 females received the placebo diluted in the vehicle under the same experimental conditions and acted as negative control group. A further group, also of 50 males and 50 females, was not treated and acted as an untreated group.

The animals were checked daily for mortality, clinical signs and possible signs of irritation. A detailed clinical examination was performed weekly. The animals were palpated every 2 weeks after 6 months of treatment in order to monitor the onset and progression of any palpable masses. Body weight and food consumption were recorded at designed intervals (weekly during the first 13 weeks of treatment and then monthly).

Results
The study authors stated that there were no test item-related effects on survival rate during the study. The body weight changes and food consumption were unaffected by treatment with the test item at any dose-level.

Scabs were seen at the application site at a higher incidence and severity in males treated at 100 and 400 mg/kg bw/day and in both males and females given 800 mg/kg bw/day with dose-relationship.

The clinical signs observed mainly occurred late during the treatment period and were associated with age and general condition of the animals. The incidence, nature and time of onset of the clinical signs seen in treated group animals were similar to those observed in control animals.

The frequency of palpable masses (0 to 12% in males; 22 to 38% in females) and their time of onset (weeks 27 to 104) were similar in control and treated groups. For females, a greater number of dose-related palpable masses were observed. Since no treatment-related neoplastic findings were noted, these incidences were considered as fortuitous.

There were no hematological or blood biochemical differences which could be attributed to the treatment with the test item.

The test item was not detectable in vehicle control animals. In test-treated groups, the test item was detectable in a few occasions without sex- or dose-relationship and at minimal levels (6.0 to 47.9 ng/ml). Consequently, it was considered that the systemic exposure via the cutaneous route was minimal.

No treatment-related macroscopic changes were noted. The incidence and distribution of the neoplastic and non-neoplastic findings corresponded to the usual background pathology observed in aging Wistar rats kept under laboratory conditions and did not suggest any relationship to treatment.

Conclusion
Under the conditions of this study, the formulated micronised UV filter Tinosorb® M was not carcinogenic when applied by the dermal route at 100, 400 or 800 mg/kg bw/day. Accordingly, the UV-absorbing test item ingredient MBBT in its micronised form did not reveal carcinogenic properties at 50, 200 or 400 mg/kg bw/day.

Ref.: CIT (2006b)

Comment

An increased frequency of malignant tumours with metastasis was found in the low dose and high dose male rats, but not in the middle dose or in the female rats in a two year dermal painting study with FAT 75'634/B; microfine material (d(0.5) = 128 nm) containing 50% MBBT. However, since little information is available in relation to the response of carcinogenic substances after dermal application of Wistar rats in the performing Laboratory and no positive control are available. Moreover, due to the abnormal dose response results and effects observed only in males it is difficult to draw any conclusion from the study with regard to potential carcinogenic effects.
The SCCS considers the study of no value.

3.3.8. Reproductive toxicity

No studies provided with nanosized material.
For studies with MBBT: see Annex II

3.3.8.1. Reproduction toxicity

No studies provided with nanosized material.
For studies with MBBT: see Annex II

3.3.8.2. Teratogenicity

No studies provided with nanosized material.
For studies with MBBT: see Annex II

3.3.9. Toxicokinetics and metabolism

3.3.9.1; Toxicokinetics *in vivo*

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No studies provided with nanosized material.
For studies with MBBT: see Annex II

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

For studies with MBBT: see Annex II

Photo-co-carcinogenicity (microfine material)

Title:	Thirteen-week topical range-finding study of Tinosorb® M, Tinosorb® MBBT and Tinosorb® S2 in hairless mice, with or without simulated sunlight
Guideline:	/
GLP:	In compliance

[2] Information on Tinosorb S is not provided in the present document.

Test item: Tinosorb® M; microfine material; d(0.5) = 127 nm or 115 nm
Tinosorb® MBBT (non-micronised)
Batch/lot n°: 05122CL2M, 00696CL3 (Tinosorb® M)
02347CN2, 15420CL2 (Tinosorb® MBBT)

Methods
Twenty-two groups of 6 albino hairless mice [Crl:SKH1-hrBR] per sex were assigned to groups receiving no treatment, treatments of only UV irradiation at 2 levels (600 and 1200 RBU/week), vehicle (base ointment) without or with UV irradiation at 2 levels (600 and 1200 RBU/week), or the UV absorbing ingredient MBBT or the formulated micronised UV filter Tinosorb® M in base ointment both without or with UV irradiation at 1 level (1200 RBU/week). The treatments with vehicle and test items were administered topically (100 µL/mouse) once daily and then the appropriate groups of mice were irradiated once daily, 5 days per week, for 13 weeks. Formulations were applied to the back and sides (approximately 25 cm²) of the mice before daily UV irradiation exposures on Monday, Wednesday and Friday and after UV irradiation exposures on Tuesday and Thursday.

The test item purity was 99.8 % for MBBT (non-micronised) and 50.7 % for Tinosorb® M. (microfine).

Dosage equivalents of MBBT (considered as 100 % pure) and Tinosorb® M (considered as 50 % pure)

Test item concentration		**Dermal dose level**	
(mg/g)	(%)	(mg/cm²)	(mg/kg bw/d)
25	2.5	0.1	80
50	5.0	0.2	160
100	10.0	0.4	325
200	20.0	0.8	650

The lower UV irradiation level was 600 RBU (Robertson-Berger units) per week and the higher UV irradiation level was 1200 RBU per week[3].

All mice were observed for viability and clinical signs. Each mouse was graded for skin reactions on a weekly basis. Skinfold thickness was measured periodically at three sites per mouse. Body weights were recorded weekly. At the end of the treatment period all study animals were sacrificed and examined macroscopically. Dose formulations used in this study were analysed by HPLC with UV detection in order to verify adequate concentration, homogeneity and stability.

Results
In male and female mice no adverse cutaneous or other clinical responses occurred that were related to administration of any of the formulations of the test items MBBT or Tinosorb® M. Erythema was reduced in some groups of male mice administered test article formulations and exposed to UV irradiation, as compared with the groups administered the vehicle formulation and exposed to UV irradiation. A similar pattern of findings of mean skinfold thickness also occurred, and significant reductions in mean skinfold thickness occurred in some groups of

[3] The RBU (Robertson-Berger unit) is a measure of biological effectiveness of UV irradiation. A dose of 400 RBU approximates one minimal erythemal dose in previously untanned human skin.

mice administered the test item formulations and exposed to UV irradiation, as compared with the groups only administered the vehicle formulation and exposed to UV irradiation.

The few incidences of mortality (1 male in the vehicle control group and 1 male treated with MBBT at 50 mg/g and 1200 RBU/week) observed during the course of the study were unrelated to the treatment with the test items MBBT and Tinosorb® M. There were no test item-related effects on body weights. Chemical analyses confirmed acceptable concentration, homogeneity and stability of MBBT in the vehicle.

Conclusion
Under the given experimental conditions, clinical observations including cutaneous observations, skinfold thickness and body weight did not preclude the use of any of the concentrations of the UV-absorbing ingredient MBBT or the formulated micronised UV filter Tinosorb® M (2.5 to 20 %) in a long-term study. Adverse effects of these formulations did not occur on observed parameters over those seen in the groups of mice administered the vehicle formulation and either not exposed or exposed to UVR.

Ref.: CR-DDS (2003)

Title:	Twelve-month topical study to determine the influence of Tinosorb® M (FAT 75'634) on photocarcinogenesis in hairless mice
Reference:	Study report number 203-007
Guideline:	/
GLP:	In compliance
Test item:	Tinosorb® M (FAT 75'634); microfine material; d(0.5) = 115 nm
Batch/lot n°:	00696CL3

Methods
The potential of the formulated micronised UV filter Tinosorb® M, containing 50 % of the UV-absorbing ingredient MBBT, to influence the development or growth of skin tumours in hairless mice [Crl:SKH1-hrBR] exposed to UV irradiation was determined. A total of 5 experimental groups with each 36 mice per sex were treated on 5 days/week for 40 consecutive weeks. The experimental design included 2 calibration groups receiving only UV irradiation at 600 or 1200 RBU (Robertson-Berger units) per week, 1 vehicle control group receiving base ointment and 600 RBU/week and 2 groups receiving MBBT (applied as Tinosorb® M) in base ointment at 50 or 200 mg/g and 1200 RBU/week. The dosage equivalents are shown below:

Dosage equivalents of Tinosorb® M

Test item concentration		**Dermal dose level**	
(mg/g)	(%)	(mg/cm²)	(mg/kg bw/d)
50	5	0.2	160
200	20	0.8	650

Formulations (100 µL/mouse) were applied topically to the back and sides (approximately 25 cm²) of the mice before daily UV irradiation exposures on Monday, Wednesday and Friday and 1 hour after UV irradiation exposures on Tuesday and Thursday.

The study design specified termination 12 weeks after the last UV irradiation dose, or when animal tumour burden criteria required an earlier termination of a study group. The study

determined the increase in UV irradiation-induced skin tumours, assessed as the prevalence and the time to onset of tumours. The animals were observed for external changes in skin and their tumours charted, measured, and tabulated weekly. Body weights were recorded on a weekly basis. A record of gross changes at necropsy, and tissue or lesion histopathology were not conducted for this study.

Results

Non-tumour endpoints

There were no test item-related adverse effects on survival, clinical signs including local skin reactions, body weights and necropsy observations. A protective effect of Tinosorb® M and its UV-absorbing ingredient MBBT was indicated by a reduced mortality in the exposed groups compared to the high UV irradiation control group (1200 RBU/week).

Skin reactions occurred in both male and female mice and included erythema, oedema, flaking, thickening, wrinkling, residue, scab(s) and white raised area(s). None of these findings were considered adverse responses to administration of the Tinosorb® M formulations. In male mice, the incidence and severity of skin reactions were comparable in all mice exposed to the high UV irradiation dose (mice administered the 50 or 200 mg/g formulation and the high UV irradiation calibration group) except that the occurrences of flaking grade 2 (distinct scales) were increased in mice administered the Tinosorb® M formulations, as compared with the UV irradiation calibration group. In female mice, the incidence and severity of skin reactions were comparable in all groups of mice exposed to the high UV irradiation dose except that the occurrences of oedema grade 1, flaking grade 1 and flaking grade 2 were increased in mice administered the 50 mg/g Tinosorb® M formulation, as compared with the vehicle formulation group.

Time to tumour

A dose-dependent protective effect of Tinosorb® M on the time for development of the first tumour (>1 mm) was detected. Unbiased median time data are summarized below:

Unbiased median week to tumours with a size of ≥ 1 mm

Tinosorb® M (mg/g)	**Vehicle**	**50**	**200**	**n.a.**	**n.a**
UV (RBU/week)	**600**	**1200**	**1200**	**600**	**1200**
Time to tumour (weeks)					
Males	34.5	26.0	28.0	37.0	22.5
Females	33.0	27.0	28.0	35.5	22.5
Sexes combined	33.5	26.0	28.0	37.0	22.5

n.a.: not applicable, no treatment with test item or vehicle, exposure to UV irradiation only.

For the onsets of tumours with a size above 1 mm in comparison with the high UV irradiation calibration group, administration of 50 or 200 mg/g Tinosorb® M delayed the development of skin tumours.

Sacrifice of groups
Sacrifice of groups, based on tumour burden criteria, occurred in week 36 for the 1200 RBU/week calibration group, in week 40 for the Tinosorb® M low dose group, in week 45 for the Tinosorb® M high dose, in week 49 for the vehicle control group and in week 52 for the 600 RBU/week calibration group.

Tumour yield
The average number of tumours per surviving mouse (tumour yield) was highest in the 1200 RBU/week calibration group followed by the vehicle control group, the low dose group receiving Tinosorb® M at 50 mg/g and 1200 RBU/week, the 600 RBU/week calibration group, and the high dose group receiving Tinosorb® M at 200 mg/g and 1200 RBU/week. The vehicle enhanced the tumour yield compared to the low UV irradiation calibration group. Furthermore, Tinosorb® M decreased the tumour yield compared to the high UV irradiation calibration group (1200 RBU/week).

Tumour yield of tumours with a size of ≥ 1 mm

Tinosorb® M (mg/g)	**Vehicle**	**50**	**200**	**n.a.[a]**	**n.a[a]**
UV (RBU/week)	**600**	**1200**	**1200**	**600**	**1200**
Treatment week (#)	**49**	**36**	**36**	**49**	**36**
Average number of tumours per surviving mouse					
Males	5.59	5.12	3.47	4.48	6.06
Females	5.89	4.42	2.91	4.00	6.32
Sexes combined	5.74	4.73	3.19	4.26	6.20

a: n.a.; not applicable, no treatment with test item or vehicle, exposure to UV irradiation only.

Group comparisons of tumour onset (Peto analysis)
The comparison of tumour relative risk by Peto analysis revealed that 50 or 200 mg/g Tinosorb® M in base ointment significantly delayed ($p < 0.001$) the development of skin tumours in sexes combined and male and female mice as compared to the 1200 RBU calibration group. Additionally, the dose-related effectiveness of Tinosorb® M was seen in the significantly delayed ($p < 0.01$ or $p < 0.001$) skin tumour onset for 200 mg/g as compared with the 50 mg/g formulation.

Tumour potency factors (TPFs)
The estimated tumour potency factors (TPFs) are summarised below. The TPF results show that the vehicle slightly enhanced a photocarcinogenic response, that MBBT / Tinosorb® M formulations effected a dose-related reduction of TFP compared to the 1200 RBU calibration group, that dermal treatment with the test item did not adversely affect the photocarcinogenic response and reduced the potential of the high UV irradiation dose to induce tumours in a dose-dependent manner.

Tumour potency factors (TPFs) for tumours with a size of ≥ 1 mm

MBBT (mg/g)	**Vehicle**	**50**	**200**	**n.a.**	**n.a**
UV (RBU/week)	**600**	**1200**	**1200**	**600**	**1200**
TPFs					
Males	1.10	**1.63**	**1.47**	1	**2**

Females	1.12	**1.52**	**1.43**	1	**2**
Sexes combined	1.14	**1.64**	**1.47**	1	**2**

Conclusion
MBBT applied as Tinosorb® M (microfine material) in base ointment formulations showed a dose-dependent reduction in UV irradiation-induced skin tumour development compared to UV irradiation alone as indicated by the increased time to first tumour, delayed mortality, lower tumour yield and reduced tumour potency factor. Based on these results, the UV-absorbing ingredient MBBT applied in the formulated micronised UV filter Tinosorb® M shows a protective effect for photo-co-carcinogenesis.

Ref.: CR-DDS (2005e)

3.3.11. Human data

3.3.11.1. Human skin distribution and penetration *in vitro*

3.3.11.2. Phototoxicity and photoallergenicity *in vivo*

Title: Evaluation of phototoxicity in humans
Reference: HTR project number 100239A
Guideline: /
GCP: In compliance
Test item: CGF-C 2089 (microfine material)
Batch/lot n°: Not indicated

Methods
A total of 30 subjects were enrolled for the *in vivo* phototoxicity patch test on humans, of which 28 subjects (4 males, 24 females) completed all study phases.

The test item CGF-C 2089 (5 %, purity not indicated), *i.e.* the UV-absorbing ingredient MBBT in micronised form, formulated as micronised UV filter Tinosorb® M (10 %) in white cream base common to cosmetic lotions was topically applied to 28 human volunteers. Each 200 µL of the test item, vehicle control (white cream base with placebo), and saline were topically applied to separate sites on each volunteer on one side of the spine. Duplicate applications were made on the opposite side of the spine. The treatment sites were covered with an occlusive dressing. After 24 hours of exposure, the patches and excess test material from the left paraspinal region were removed. The test sites were then exposed to 16 J/cm² UVA irradiation followed by exposure to 0.75 times the volunteer's MED (minimal erythemal dose) of UVB irradiation. The patches from the non-irradiated test sites on the right paraspinal region were then removed and served as controls to assess non-phototoxic reactions. Skin reactions were assessed 1, 24, 48, and 72 hours following irradiation and patch removal.

Results
The irradiated test item-treated sites exhibited lower skin reactions than the irradiated vehicle control and saline treatment sites. Only very few dermal responses with a score of grade 1 or higher were found during the study, leading to the conclusion that the test articles are non-irritating.

Conclusion
Under the test conditions put forth in this *in vivo* study, the UV-absorbing ingredient MBBT (CGF-C 2089; 5%) formulated as micronised UV filter in Tinosorb® M (10 %) did not show evidence of eliciting a phototoxic response in human subjects. MBBT in micronised form was not an irritant to human skin.

Ref.: Hill Top Research (1998a)

Title:	Evaluation of human photoallergy by repeated insult patch test
Reference:	HTR Project No. 100239B
Guideline:	/
GCP:	In compliance
Test item:	CGF-C 2089 (microfine material)
Batch/lot n°:	Not indicated

Methods
A total of 35 subjects were enrolled for the *in vivo* human repeated insult patch test (HRIPT) adapted according to Kaidbey and Kligman (1980), of which 26 subjects (3 males, 23 females) completed all study phases.

Induction period (weeks 1 – 3)

For induction, 200 µL of the test item CGF-C 2089 (5 %, purity not indicated), *i.e.* the UV-absorbing ingredient MBBT in micronised form, formulated as micronised UV filter Tinosorb® M (10 %) in white creme base, vehicle control (white cream base with placebo) and saline were applied six times (twice per week) to separate skin application sites of the volunteers. The application sites were covered with an occlusive dressing for 24 hours. Within 10 minutes after removal of the patches, the application sites were exposed to UVB/UVA irradiation at twice the MED (minimal erythemal dose).

Challenge period (week 6)

After a rest period (weeks 4 and 5), a challenge experiment was carried out. Duplicate topical applications of 200 µL of the test item, vehicle control, and saline were made to naive sites on both sides of each volunteer's spine. The test sites were covered with an occlusive dressing. After 24 hours of exposure, the patches and excess test material from one side of the spine were removed. The test sites were then exposed to 16 J/cm² UVA irradiation followed by exposure to 0.75 times the volunteer's MED of UVB irradiation.

Skin reactions were assessed 1, 24, 48 and 72 hours following irradiation and patch removal using erythema scores from 0 (no visible reaction) to 3 (strong reaction).

Results
The irradiated test item–treated sites exhibited lower skin reactions than the irradiated vehicle control and saline treatment sites.

Conclusion
Under the test conditions, both the UV-absorbing ingredient MBBT (5 %) and the formulated micronised UV filter Tinosorb® M (10 %) were no photosensitisers or sensitisers to human skin.

Ref.: Hill Top Research (1998b)

3.3.11.3. Human patch test

Title: Human patch tests of Tinosorb® M (substance)
Reference: /
Guideline: /
GCP: /
Test item: Tinosorb® M (20% aqueous solution), microfine material
Batch/lot n°: 000620B1

Methods
A total of 40 subjects (13 males and 27 females) were enrolled for an *in vivo* human patch test. 0.015 mL of a 20 % aqueous solution of the formulated micronised UV filter Tinosorb® M (controls: purified water and 20 % Tinosorb® M with water instead of MBBT) was used in 8-mm Finchamber for 24 hours occlusively. Skin reactions were observed 1 and 24 hours after patch removal and scored and evaluated according to Japanese Criteria[4].

Results
Slight erythema were observed in 3 subjects and evident erythema in 1 subject at 1 hour after patch removal, but the reaction disappeared within 1, 2 and 4 days after patch removal. For the control treatments, slight erythema were observed in 5 subjects and evident erythema in 1 subject. All skin reactions were fully reversible at the dose and control patch sites.

The calculated skin stimulation index of Tinosorb® M was 6.25. The indices for the base solution and purified water were 6.25 and 8.75, respectively.

Conclusion
Under the conditions of this study and considering the underlying evaluation scale, the formulated micronised UV filter Tinosorb® M in water showed no potency of causing reactions on human skin.

Ref.: Kawai Institute of Dermatology (2001a)

Comment
Neither guidelines nor GLP have been reported. These tests can serve as supporting evidence, however they are of limited valued.

Title: Human patch tests of Tinosorb® M containing sunscreen (product)
Reference: /
Guideline: /
GCP: /
Test item: 20 % Tinosorb® M containing sunscreen, microfine material
Batch/lot n°: /

Methods
A total of 40 subjects (13 males and 27 females) were enrolled for an *in vivo* human patch test. 0.015 mL of a sunscreen formulation containing 20 % of the formulated micronised UV filter Tinosorb® M (control: Tinosorb® M-free sunscreen formulation) was used in 8-mm Finn

[4] Kawamura *et al.* (1970) Nichihi-kaishi 80 No.5, 301; Nasu (1985). Hifu (skin) 27 No.4, 793-803

Chamber for 24 hours occlusively. Skin reactions were observed 1 and 24 hours after patch removal and scored and evaluated according to Japanese Criteria[5].

Results
Slight erythema was observed in 1 subject at 1 hour after patch removal, but the reaction disappeared within 24 hours after patch removal. For the control treatments, slight erythema was observed in 2 subjects. The skin reactions were fully reversible at the dose and control patch sites.

The calculated skin stimulation index of the test item formulation was 1.25. The index for the control sunscreen formulation was 2.5.

Conclusion
Under the conditions of this study and considering the underlying evaluation scale, the formulated micronised UV filter Tinosorb® M in sunscreen showed no potency of causing reactions on human skin.

Ref.: Kawai Institute of Dermatology (2001b)

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

Margin of Safety (MoS) as provided in the dossier

While microfine MBBT (d(0.5) = 95 nm) is not considered to be absorbed across human skin or absorbed systemically to a relevant extent, the calculation of a Margin of Safety (MoS) for the dermal route of exposure is provided in the table below. Taking the standardized approach to risk characterization, the systemic estimated human exposure that could be assumed to result from 10 % microfine MBBT in cosmetic products applied to normal skin is used. The resulting systemic exposure dose in humans (SED Human) is then compared with the systemic exposure dose in rats (SED Rat) estimated from the subchronic dermal repeated dose toxicity study in rats using the *in vivo* NOAEL and the dermal absorption rate determined in the *in vitro* penetration study with rat skin.

MoS calculations for microfine MBBT based on human skin *in vitro* study results on normal skin, as provided by the applicant.

Parameter	Microfine MBBT d(0.5) = 95 nm
Adult body weight	60 kg
Body surface area	17500 cm²
Sunscreen applied (if at 1 mg/cm²)	18 g
Microfine MBBT applied (10 %)	1800 mg

[5] Kawamura *et al.* (1970) Nichihi-kaishi 80 No.5, 301; Nasu (1985). Hifu (skin) 27 No. 4, 793-803

Skin absorption (human), (RCC Ltd., 2007)[a]	0.048 % of applied dose
SED Human	0.0144 mg/kg bw/d
NOAEL (Rat 13-week dermal study; RCC Ltd., 2002)[b]	1000 mg/kg bw/d
Skin absorption (rat), (RCC Ltd., 2007)[a]	5.922 % of applied dose
SED Rat	59.2 mg/kg bw/d
MoS (SED Rat / SED Human)	4112

SED: Systemic exposure dose
NOAEL: No-observed-adverse-effect-level
MoS: Margin of safety
a: 2 SD were added to the mean absorption value
b: in this study MBBT was not in nanosized form.

Based on the calculations presented above, the MoS for MBBT with a d(0.5) = 95 nm is 4112 based on a comparison of the internal dose between rat and man.

It should be noted, that the above calculations are highly conservative, with regard to the human skin absorption value used. Dermal absorption values were mainly below the limit of quantification and the majority of the dose was recovered from the skin compartment, *i.e. stratum corneum*, rather than the receptor fluid.

SCCS Comment
The MOS calculation is based on read-across: no repeated dose toxicity study with the nano-sized material is available in rats.
There is a 39 week dermal toxicity study in the mini-pig available carried out with the nano MBBT, from which it can be concluded that no effects are observed until the highest dose level tested (1000 mg a.i./kgbw/day). Since there are no dermal penetration data available for mini-pig skin a MoS cannot be calculated. However, in a weight of evidence approach, it can be concluded that based on the low absorption in humans (dermal penetration values derived are conservative because of the low levels), and the absence of effects in a 39-week dermal study in the mini-pig up to the highest dose tested (1000mg a.i./kg bw/day), there seems to be no concern for the dermal application of nano-sized MBBT (up to 10% in sunscreen formulations) with regard to systemic effects.

3.3.14. Discussion

It should be noted that the risk assessment for S79 is based on mass- dose metrics, and not on particle number or surface area. At this moment the most adequate dose metric for hazard and exposure characterisation for nanoparticles is still under discussion. However, as cosmetic ingredients are usually assessed and regulated based on the concentration in the finished product, this dose metric is maintained in this opinion.

Physicochemical properties
This assessment only refers to the materials described in the present opinion, including the specific preparation procedure reported for the nano-sized material. MBBT 'as manufactured' has been characterized by a considerable number of physicochemical techniques. The solubility of MBBT, an organic UV absorber in water is very low, while there is a considerable solubility

reported in the lipid phase. The manufacturing of nano-MBBT by ball milling leads to a pre-final solid-in-liquid emulsion which has been characterized for the size distribution by Dynamic Light Scattering (DLS), Fiber-Optic Quasi-Elastic Light Scattering (FOQELS), CLS disc centrifugation and Transmission Electron Microscopy (TEM). Despite this, a weakness of the present submission is that the data raise questions in regard to the potential cut-off in the study being at particle sizes of less than 50 nm. Evidence has been provided that the radioactive batch needed for toxicological studies shows the same physicochemical characteristics as the non-radioactive material. This, as such characterized, nano-MBBT has been used in the consequent toxicological studies, and has been documented to be constant in its batch to batch variation on the basis of two datasets taken for materials manufactured according to the same recipes some years apart.

Irritation /sensitisation

In a BCOP study nanosized MBBT (in the Tinosorb formulation, pH 11.6 and pH 10.5; particle size not reported in study report, but according to the applicant (d(0.5) = 110-130nm) did not cause serious eye damage.

In an eye irritation study in rabbits, the formulated micronised UV filter Tinosorb® M pH = 11.6 was slightly irritating to the eye.

No skin irritation study was performed with nanosized MBBT. Non-nano-sized MBBT was not irritating in the rabbit skin irritation study. In a 14-day cumulative primary irritation study in guinea pigs, MBBT in non-nano-sized form was not a skin irritant at concentrations of 15 or 40%. MBBT was considered to be slightly irritant at 65% with a Primary Irritation Index of 0.04.

No sensitization study was carried out using nano-sized MBBT.

In a maximization study in guinea pigs, none of the 20 animals exposed to non-nanosized MBBT, showed a dermal reaction after the challenge.FA few case reports indicate possible allergy against MBBT (Gonzalez-Perez,Contact DERM 2007; . However, it was also suggested that this might be due to the composition of the preparation with decyl glucoside as allergen(ref Andrade Contact Dermatitis 2010; O Connell et al Contact Dermatitis 2011;) ..

Dermal absorption:

In an *in vitro* dermal penetration study with nano-sized MBBT particles (d(0.5)= 90nm), the mean absorption for rat skin was 2.08 % and for human skin the value was 0.02%. Since 5 replicates of human skin (2 donors) and 7 replicates of rat skin (2 donors) were assessed, the mean + 2 SD were used for the calculation of the MoS. This results in a total absorption of 5.92% for the rat and 0.048% for human skin (it should be realized that these are very conservative values, given the low dose (levels around the limit of quantitation) that could be recovered in the perfusate).

An additional study was performed, in which nanosized MBBT (d(0.5)= 90 nm) was applied to predamaged human skin. Again, the concentrations in the perfusate that could be measured were very low (below the limit of detection). The mean *in vitro* dermal absorption under these conditions was 0.07%, resulting in a total absorption of 0.35% when the mean +2SDs was calculated. For the conventional assessment of UV filters dermal absorption is tested on intact skin only. Given the conservative estimate of the dermal absorption, the uncertainty in the penetration values, the severe damage to the human skin in the in vitro experiment and the fact that in the exposure assessment it is assumed that the total body surface area is exposed, the results of the damaged skin are not taken into account for the calculation of an MoS.

Following a 6-hour topical exposure, the *in vivo* dermal absorption of the non-nanosized UV-absorbing ingredient MBBT from a 0.2 and a 10 % formulation was very low and accounted for not more than 0.8 % and 0.4 % of the dose, respectively, over 5 days. No systemically measurable concentrations were detected.

Inhalation

The submitted data on exposure to spray applications is not sufficient. Appropriate data are needed on the physics of the spray generation and the atmospheric conditions during use (including behaviour of the aerosols in time).

In an acute inhalation study the massive influx of neutrophils and the decrease of macrophages cannot be considered to be merely a mild inflammation after inhalation of particles.

No information is available on the effects of S79 after repeated exposure to lower doses via inhalation. From other inhalation studies using nano-materials, it is known that the nano-material is not cleared from the lung in the same way as is the case for 'conventional' substances. In addition, the effect of the formulation on the behaviour of the S79 particles in the human lung is not known.

Therefore, at the moment, it cannot be concluded that the use of 10% S79 in spray formulations is safe, because of the uncertainties associated with the repeated exposure of the lung to low doses of S79 and the limited information on actual exposure during use of spray applications.

In view of the noted effects in the lung, to ensure the safe use in spray application the following information is needed: a 28-day repeated dose inhalation study, including tissue distribution and systemic toxicity, determination of an LOAEL and NOAEL and possible recovery.

Repeated dose toxicity

In a dermal repeated dose range-finding study in rats, some clinical effects (vocalization and pain) were noted at dose levels of 400 mg a.i./kg bw/day and higher when exposed to nano-sized MBBT. These, or other effects were not seen in the 39 week mini pig study, where the animals were exposed to dose levels up to 1000 mg a.i./kg bw/day.

No effects were noted in rats in dermal or oral repeated dose toxicity studies with non-nanosized MBBT.

Genotoxicity

No genotoxicity studies have been performed with the nano-sized UV-absorbing ingredient MBBT.

MBBT in non-nanosized form did not induce DNA repair in liver cells of treated rats.

MBBT in non-nanosized form did not induce damage to the chromosomes or the mitotic apparatus of mouse bone marrow cells. However, there is no evidence that the test substance has reached the target cells and/or was taken up by these cells.

Therefore, these studies do not allow drawing conclusions on a potential genotoxic hazard of the nanosized form of the compound. The validity of the assays is questionable when performed with the particulate test material unless it is demonstrated that the testing compound would have reached the target organs/cells. Genotoxicity studies with the nano-sized material, in which exposure of the target organ/cells is demonstrated, are needed for the safety assessment of S79.

Toxicokinetics

Although dermal uptake is very low, as shown in the skin penetration studies, potential bioaccumulation after repeated skin applications should be considered.

Carcinogenicity
An increased frequency of malignant tumours with metastasis was found in the low dose and high dose in male rats, but not in the middle dose or in the female rats in a two year dermal painting study with FAT 75'634/B; nanosized MBBT (doses: 50, 200, 400 mg a.i./kg bw/day). However, since little information is available in relation to the response of carcinogenic substances after dermal application of Wistar rats in the performing Laboratory and no positive control is available it is difficult to draw any conclusion from the study with regard to potential carcinogenic effects.

4. CONCLUSION

Since no appropriate data on genotoxicity of nano form of MBBT were provided, no conclusion on the safety of this substance can be drawn. However regarding systemic effects there seems no concern for the dermal application of nano-sized MBBT.

In addition there are some aspects that need further attention:
- In the study in rats, clinical effects (pain and vocalization) after dermal application were noted at concentrations of 20% (500mg a.i. /kg bw/d and higher). In the carcinogenicity study, scabs were seen at a dose level of 100 mg a.i./kg/bw/day and higher. It is worthwhile to monitor possible irritation effects via the existing cosmetovigilance programs.
- Attention needs to be paid to identification/presence in selected tissues to obtain information on potential bioaccumulation given the physicochemical properties (lipophilicity) of the substance.

The SCCS noted that due to the poor biodegradation potential and the very high octanol-water partition coefficient long term effects or bioaccumulation of MBBT in the environment cannot be excluded, therefore the MBBT is currently classified with R 53/Chronic 4 ("may cause long term effects on the aquatic environment"). The use of MBBT as ingredient in sunscreen products might lead to environmental exposure.

5. MINORITY OPINION

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Annex I

Overview on particle size distributions (mass based) of the different batches:

Date of analysis	Batch	Particle size (µm)		Surface area (m^2/g)
		d(0.5)	d(0.9)	
Tinosorb® M				
24-May-2002	005839D2	0.127	0.265	54
06-Oct-2002	003736D2	0.128	0.297	53
	05122CL2	0.127	0.241	54
10-Jan-2003	00007CL3	0.118	0.218	58
	00696CL3	0.115	0.214	59
	02711CL3	0.128	0.233	54
02-Jul-2003	08706CL3	0.125	0.230	55
12-Sep-2003	11327CL3	0.118	0.219	58
12-Jan-2004	00023CL4	0.125	0.257	57
14-Jun-2004	07132CL4	0.118	0.239	56
03-Nov-2004	13115CL4	0.120	0.231	58
06-Jan-2005	00069CL5	0.117	0.217	58
19-Apr-2005	04673CL5	0.127	0.241	58
22-Jan-2006	14628CL5	0.120	0.229	57
14-Feb-2006	01408CL6	0.119	0.221	57
19-May-2006	05806CL6	0.121	0.228	67
05-Dec-2006	12623CL6	0.129	0.245	53
03-Jan-2007	13636CL6	0.119	0.225	57
	00292CL7	0.128	0.247	53
11-Jul-2007	11145CL7	0.124	0.243	54
08-Jan-2008	00071CL8	0.129	0.243	53
04-Jul-2008	06908CL8	0.124	0.227	55
21-Dec-2008	13536CL8	0.118	0.213	57
03-Jan-2009	13557CL8	0.119	0.215	57
05-Jul-2009	03844CL9	0.117	0.217	58

Date of analysis	Batch	Particle size (µm)		Surface area (m^2/g)
		d(0.5)	d(0.9)	
Tinosorb® M				
04-Dec-2009	0003961345	0.124	0.231	55
13-Jan-2010	0004044844	0.117	0.215	52
09-Jul-2010	0004499156	0.117	0.219	58
19-Dec-2010	0004901430	0.128	0.241	53
03-Jan-2011	0004901438	0.127	0.238	54
29-Jun-2011	0005307759	0.128	0.242	53
30-Aug-2011	0005423704	0.118	0.223	58
MBBT				
21-Nov-2006	02174CN6	142.44	484.30	approx. 0.192[a]
21-Nov-2006	04296CN6	195.82	511.94	approx. 0.192[a]
21-Nov-2006	00140CN6	281.34	683.71	approx. 0.248[a]

Annex II:

Studies performed with non-nanosized MBBT

The summaries of these studies have been copied from submission II. For submission I, only a short summary of the end-point is presented. The study reports were assessed in more detail when necessary for the safety assessment of the nanosized material (in that case a comment from the SCCS was added)

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

For Tinosorb as test article TKA 40027 (Batch No: EN 302690.12, purity 97.7%) an acute oral toxicity study was performed according to OECD 401 in male (n=5) and female (n=5) albino rats. One single oral dose of 2000 mg/kg was administered orally by gavage. No deaths were observed and for both sexes the LD50 was considered greater than 2000 mg/kg body weight. At the dose of 2000 mg/kg body weight some indications for toxicity were observed i.e. piloerection, hunched posture and dyspnoea.

Ref.: 2 (subm I)

3.3.1.2. Acute dermal toxicity

No studies were provided with Tinosorb M. For Tinosorb as test article TKA 40027 (Batch No: EN 302690.12, purity 97.7%) an acute dermal toxicity study was performed according to OECD 402 in male (n=5) and female (n=5) albino rats. One single dose of 2000 mg/kg body weight was administered on the back of the animals on a shaved area. No deaths were observed and for both sexes the LD50 after dermal administration was considered greater than 2000 mg/kg body weight.

Ref.: 3 (subm I)

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Skin irritation in rabbits

For Tinosorb as test article TKA 40027 (Batch No: EN 302690.12, purity 97.7%) a skin irritation test was performed according to OECD 404 in male New Zealand white rabbits (n=3). A gauze patch of approximately 12-16 cm^2 was applied to shaved skin (36 cm^2 of skin was shaved). The gauze patch contained 0.5 g of the test article. Skin reactions were evaluated at 1, 24, 48, and 72 hours after removal of the skin patch.
Grade 1 erythema and oedema were observed in all animals one hour after treatment. Grade 1 erythema was observed in one animal up to 48 hours after treatment.

Ref.: 5 (subm I)

Skin irritation in guinea pigs after repeated exposure

Title:	A 14-day cumulative primary skin irritation study in guinea pigs with CGF-C2089
Guideline:	/
GLP:	In compliance
Test item:	CGF-C2089 (non-micronized)
Batch/lot n°:	001719B0

Methods
The test item CGF-C2089, i.e. the UV absorbing ingredient MBBT in non-micronised form, was administered for 14 consecutive days on the skin of Hartley-derived albino guinea pigs. Six animals per dose level received a 0.1 or 0.2 mL dermal application of the test item suspended on 0.5% (w/v) CMC in 0.1% (w/v) Tween 80 at 15, 40, or 65% (w/w). After approximately 24 hours, residual test item was removed. This procedure was repeated daily for a total of 14 exposure periods. Application sites were scored for signs of dermal irritation for up to 72 hours following the final test item removal on day 14. Viability, clinical signs of systemic toxicity and body weights were assessed periodically during the study period. Dose formulations were analysed for concentration verification during the study period. Dose formulations were analysed for concentration by means of a HPLC method.

Results
There were no test item related effects on viability, clinical signs of systemic toxicity and body weights.
Exposure to 15% formulations of MBBT resulted very slight erythema (1/6 test sites on two occasions, day 3 and day 8). No other signs of dermal irritation were noted at both dose levels after the last exposure on day 14.

Exposure to 40% formulations of MBBT resulted in very slight erythema on 4/6 test sites sporadically during the day 1-14 day exposure period. No dermal irritation was observed after day 11 of the exposure period of 14 days.

Exposure to 65% formulation of MBBT produced very slight and intermittent erythema on 3/6 test sites during the exposure period. During the 1-72 hours scoring interval after the final test item removal, very slight erythema was noted in 1/6 animals.

The very slight irritation was sporadic across groups and may have been associated with the physical removal of the test item daily as there was no irritation after the final test article removal with the exception of one test site which was 1 hour after the final test article removal.

The achieved test item concentrations in the dose formulations were acceptable for the study purpose.

Conclusion
Under the conditions of this study, the UV-absorbing ingredient MBBT in non-micronised form was no skin irritant at concentrations of 15 or 40%. MBBT was considered to be a slight irritant at 65% with a Primary Irritation Index of 0.04 by the study director, however, the sporadic signs of very slight erythema noted in this study were not unambiguously test item related. It is likely that findings were more associated with the daily physical removal of the test substance.

Ref.: 41 (subm II)

Comment
In an ISO standard a Primary Irritation Index or PII score between 0 - 0.4 in rabbits is considered to be in a negligible response category. The PII score is rather low and may be considered negligible. As indicated repeated test item removal may have added to the low irritation observed.

3.3.2.2 Sensitization

In a Maximisation test, Tinosorb (non-nanosized, TKA 40027, batch no EN 302690.12, purity 97.9%) was tested in 10 male and 10 female guinea pigs. The induction consisted of three pairs of intradermal injections (0.1ml adjuvant, 5% test article in arachide oil and 5% test article in an adjuvant saline mixture) and an epidermal application one week later (30% test article) under occlusion for 48h). In week 5, the animals were challenged via a dermal application to 10% of the test substance. None of the animals of the test group showed skin reactions 24 and 48 hours after removing the challenge application.
Ref Subm I (Hageman 1991)

3.3.4. Dermal / percutaneous absorption

Title:	Methylene-bis-benzotriazolyl tetramethylbutyl phenol (CGF-C2089): Metabolic fate following oral administration or *in vivo* dermal application in the rat
Guideline:	OECD 417 and OECD 427[6]
GLP:	In compliance
Test item:	Methylene-bis-benzotriazolyl tetramethylbutyl phenol (CGF-C2089)), non-micronized material
Batch/lot n°:	001719BO

Methods
For the assessment of percutaneous absorption *in vivo*, two doses were prepared in the commercial formulation, which were equivalent to concentrations of 0.2 % and 10 % of CGF-C2089, *i.e.* of the UV-absorbing ingredient MBBT. The formulation comprised unlabelled and ^{14}C-radiolabelled test item homogeneously dispersed in the vehicle (formulation, Xanthan gum, Propylene glycol and water) such that a dose of a set volume (100 µL/rat) was equivalent to the nominal dose level of 0.2 or 10 mg/rat.

In each case, unlabelled test item (purity: 99.6 %) and ^{14}C-radiolabelled test item (radiochemical purity: 99.1 %) were mixed and milled to a particle size comparable to that of the commercial formulation, nominally 200 nm. The particle size of the milled test substance was determined by scanning electron microscopy (SEM) to be in the range 300 and 2000 nm, with a typical particle size of approximately 1000 nm.

The dermal absorption in 32 male rats [Alpk:AP$_f$SD, Wistar derived] was investigated following a single application of nominally 0.2 or 10 mg of the formulated active ingredient to 10 cm² of skin. After dosing, the application sites were protected, but not occluded, using O-rings incorporating a nylon gauze cover. A strip of non-occlusive elasticized bandage was wrapped around the rat and over the application devices to help to hold them in place. Rats were housed

[6] Only the study part according to OECD 427 is presented in this section. For the study part performed according to OECD 417 please refer to section 5.9.2.

individually in metabolism cages for the collection of urine and faeces. After a 6-hour exposure, the first two groups were terminated and the application sites of all the remaining rats were washed to remove the unabsorbed dose. Urine, faeces and cage wash were collected from each cage after the 6-hour skin wash, and then at daily intervals after dosing for the duration of each experiment. Groups of 4 rats were terminated at 6, 24, 72 and 120 hours after dosing. Under anaesthesia, the skin was washed to remove unabsorbed residual test item before exsanguination. The application site skin was then tape-stripped to remove the *stratum corneum*. The dose formulations and all samples, including selected tissues and residual carcasses were analyzed for radioactivity by means of liquid scintillation counting. Disintegration per minute (dpm) values were calculated using the appropriate quench correction data.

Results
The homogeneity of the radiolabelled test item in both dose formulations was satisfactory throughout the periods of dosing. The test item was stable in both dose formulations for longer than their period of use in the study.

Following dermal exposure to the 0.2 % formulation for 6 hours, approximately 97 % of the applied radioactivity was removed from the skin surface by aqueous washing. Approximately 0.7 % (0.4 % was found in the *stratum corneum*) of the dose remained associated with the application site and some of this was available for absorption. However, the area under the curve (AUC) could not be calculated because of the not detectable radiolabel in the blood. The residue associated with the application site remained low, and declined at later timepoints. The amount of dose absorbed remained similar at 0.2 - 0.8 % after 6, 24, 72 and 120 hours.

Following dermal exposure to the 10 % formulation for 6 hours, approximately 98 % of the applied radioactivity was washed from the skin surface. Approximately 0.2 % (0.1 % was found in the *stratum corneum*) of the dose remained associated with the application site following the 6-hour skin-wash and some of this was available for absorption. The residue associated with the application site remained similar at later time-points. The amount of dose absorbed remained similar at 0.2 - 0.4 % after 6, 24, 72 and 120 hours.

Percutaneous penetration of the UV-absorbing ingredient MBBT through rat skin in vivo over a 5-day period

	Time after application			
	6 hours	24 hours	72 hours	120 hours
Recovery of applied dose for the 0.2 % formulation (% or % ± SD)				
Total absorbed dose[a]	< 0.34	0.80 ± 1.20	0.27 ± 0.05	< 0.53
Total non-absorbed dose[b]	97.98 ± 1.59	98.62 ± 3.12	97.70 ± 1.95	99.07 ± 2.38
Total recovery	98.32 ± 1.72	99.42 ± 2.02	97.97 ± 1.99	99.60 ± 2.29
Recovery of applied dose for the 10 % formulation (% or % ± SD)				
Total absorbed dose[a]	< 0.21	< 0.41	< 0.18	0.34 ± 0.17
Total non-absorbed dose[b]	97.63 ± 4.63	98.06 ± 4.25	99.86 ± 4.24	101.08 ± 0.63
Total recovery	97.84 ± 4.68	98.46 ± 3.77	100.03 ± 4.22	101.42 ± 0.55

a: Sum of radioactivity recovered in urine, faeces cage wash, bandage, tissues, GI tract with contents and carcass; given as percentage of applied dose
b: Sum of radioactivity recovered in 6-hour skin wash and/or terminal skin wash and *stratum corneum*, skin application site, covers and O-rings; given as percentage of applied dose
SD: Standard deviation of the mean value for 3 or 4 animals

Conclusion

Following a 6-hour topical exposure, the *in vivo* dermal absorption of the micronised UV-absorbing ingredient MBBT from a 0.2 and a 10 % formulation was very low and accounted for not more than 0.8 % and 0.4 % of the dose, respectively, over 5 days. The topically applied micronised UV-absorbing ingredient MBBT did not achieve systemically measurable concentrations and was thus not bioavailable.

Ref.: CTL (2002a)

3.3.5. Repeated dose toxicity

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

Title:	14-Day dose range-finding dermal toxicity (semi-occlusive) study in the Wistar rat
Guideline:	Comparable to OECD 410
GLP:	In compliance
Test item:	CGF-C2089 (non-micronised)
Batch/lot n°:	001719B0

Methods

The test item CGF-C2089 (purity: >99 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80 was administered daily (semi-occlusive) to the skin of 14 rats [HanIbm:WIST (SPF)] per sex per dose level at 100, 300 or 1000 mg/kg bw for at least 14 days. A concurrent untreated control group and a concurrent vehicle control group of each 14 rats per sex were included in this study.

Clinical signs, food consumption and body weights were recorded periodically. After treatment on days 1 and 14, blood samples were withdrawn for plasma analyses. At the end of the treatment period, all animals were sacrificed and examined for gross lesions. Organ weights of selected organs were recorded. Concentration, homogeneity and stability of dose formulations were determined.

Results

The dermal administration did not yield evidence for test item-related effects upon viability, clinical signs of systemic toxicity, food consumption, body weights, organ weights and incidence or severity of gross lesions. Local effects were seen in 3 male rats only (very slight skin flaking in 1 animal of the 300 mg/kg bw/d group and in 2 animals of the 1000 mg/kg bw/d group (transient)) and were considered to be treatment- but not test item-related. Mean plasma levels of the test item were below the limit of quantitation (1 ng/mL). Analysis of dose formulations revealed decreasing recoveries with increasing test item concentration. Based on this outcome and in order to improve test item suspension, it was decided to use corn oil as vehicle for the subsequent 90-day dermal study in rats (RCC Ltd., 2002).

Conclusion

Under the conditions of this study, 1000 mg/kg bw/d of the UV-absorbing ingredient MBBT in non-micronised form was established as the no-observed-effect-level (NOEL). Thus, the dermal no-observed-adverse-effect-level (NOAEL) was > 1000 mg/kg bw/d.

Ref.: RCC (2001)

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

Title:	CGF-C2089: 90-Day dermal toxicity (semi-occlusive) study in the Wistar rat
Guideline:	OECD 411
GLP:	In compliance
Test item:	CGF-C2089 (non-micronised)
Batch/lot n°/	0017198B0

Methods

The test item CGF C2089 (purity: >99 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in corn oil was administered for 6 hours per day (semi-occlusive) to five groups of 10 rats per sex per dose level at 0 (untreated), 0 (vehicle), 150, 450 or 1000 mg/kg bw for 91/92 days. The untreated control, the vehicle control and the high dose groups each contained an additional 10 rats per sex that were allowed to recover for a 28-week period after the last exposure.

Clinical signs, food consumption, body weights, ophthalmoscopic endpoints, haematology, clinical chemistry and urinalysis parameters, test item and hormone plasma levels, estrous cycles, selected organ weights and macroscopic as well as histopathologic findings were assessed and evaluated. Dose formulations were analysed for content, homogeneity and stability. For plasma level determinations on days 1, 15 and 28 (all allocated animals) and day 57 (control animals only) as well as day 64 (treated animals only), blood was collected from 3 satellite animals/sex/dose level for each timepoint once prior to dosing and at 0.5, 1, 2, 6 and 24 hours after treatment. Plasma levels were then quantified by means of a LC/MS method.

Results

Chemical analysis of dose formulations confirmed appropriate dosing.

Test item-related deaths (1 animal each in the 0 (control), 150 and 1000 mg/kg bw/d groups were killed *in extremis*) and signs of clinical toxicity did not occur. Signs of local irritation (transient flaking / erythema) reported were comparable between vehicle control and dose groups and considered to be related to the mechanical effect of the daily removal of the wrapping. No test item-related effects were noted on food consumption, body weights, ophthalmoscopic endpoints, body weight, haematology, clinical biochemistry and urinalysis, oestrus cyclicity, hormone levels (evaluated for testosterone, oestradiol, thyroid stimulating hormone, follicle stimulating hormone and luteinising hormone), macroscopic findings and organ weights. Microscopic changes were limited to the skin of the dose site and included hyperkeratosis and acanthosis at incidences similar across the study groups.

Evaluation of test item plasma levels on day 1 showed no evidence for dermal absorption. On treatment days 15 and 28, sample contamination was noted in animals at all dose levels. Values were reported but not considered valid for assessment. On treatment day 57, minor traces of the test item were detected in three control males and one vehicle control male, whereas all control females were without findings. Analytical quantification of plasma samples collected after 64 days from test item-treated animals revealed varying levels in plasma, neither dose-dependent nor consistent, and this finding was considered as contamination-related.

Conclusion

Under the conditions of this study, no target organ was identified following repeated dermal treatment of rats with the UV-absorbing ingredient MBBT in non-micronised form. The dermal no-observed-effect-level (NOEL) was established at the high dose level of 1000 mg/kg bw/d. The corresponding dermal no-observed-adverse-effect-level (NOAEL) was > 1000 mg/kg bw/d.

Ref.: RCC (2002)

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

3.3.6.2.1. Mutagenicity / Genotoxicity in vivo

Title: Bone marrow micronucleus test by intraperitoneal route in mice
Guideline: OECD 474
GLP: In compliance
Test item: CGF-C2089 (non-micronised)
Batch/lot n°: 001719B0

Methods

Three groups of 5 mice per sex [Swiss Ico:OF1(IOPS Caw)] received a first and, 24 hours later, a second intraperitoneal treatment with CGF-C2089 (purity: 99.6 %), *i.e.* with the UV-absorbing ingredient MBBT in non-micronised form, suspended in corn oil at dose levels of 500, 1000 or 2000 mg/kg bw (dose volume: 10 mL/kg bw). A concurrent control group of 5 mice per sex were treated in the same manner with the vehicle only. A positive control group of 5 mice per sex received a single oral gavage of Cyclophosphamide at a dose level of 50 mg/kg bw.

All treatment and negative control group animals were killed 24 hours after the last treatment and the positive control group animals were killed 24 hours after the single treatment. Following preparation of bone marrow smears, the number of micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes (PE) for each animal. The PE and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

Results

The mean values of MPE and the PE/NE ratio in the test item-treated groups were equivalent to those of the vehicle control group and no statistically significant difference was noted. The mean values of MPE and the PE/NE ratio for the vehicle and positive controls were consistent with the laboratory's historical data. The positive control item Cyclophosphamide induced a highly significant increase ($p < 0.001$) in the frequency of MPE, indicating the sensitivity of the test system.

No clinical signs, mortalities have been observed.

Cytogenetic effects of the UV-absorbing ingredient MBBT

Group	Dose level	MPE/1000PE	PE/NE
	(mg/kg bw/d)	(mean ± SD)	(mean ± SD)
Males			
Vehicle control[a]	0	0.5 ± 0.5	0.3 ± 0.2
Test item	500	0.6 ± 1.3	0.2 ± 0.1

Group	Dose level	MPE/1000PE	PE/NE
	(mg/kg bw/d)	(mean ± SD)	(mean ± SD)
	1000	0.6 ± 0.9	0.2 ± 0.1
	2000	0.2 ± 0.4	0.2 ± 0.1
Positive control[b]	50	25.2 ± 4.6*	0.4 ± 0.1
Females			
Vehicle control[a]	0	0.4 ± 0.9	0.4 ± 0.1
Test item	500	0.3 ± 0.4	0.3 ± 0.1
	1000	0.4 ± 0.7	0.3 ± 0.1
	2000	0.5 ± 0.9	0.4 ± 0.1
Positive control[b]	50	12.7 ± 1.8*	0.8 ± 0.4

a: Corn oil
b: Cyclophosphamide
MPE: Micronucleated polychromatic erythrocytes
PE: Polychromatic erythrocytes
NE: Normochromatic erythrocytes
SD: Standard deviation
*: $p < 0.001$ (based on the 2 x 2 contingency table for MPE)

Conclusion
Under the conditions of this study, the UV-absorbing ingredient MBBT in non-micronised form did not induce damage to the chromosomes or the mitotic apparatus of mouse bone marrow cells.

Ref.: CIT (2002)

Comment of the SCCS
The SCCS does not agree to the conclusion on the non-micronised form. This study does not allow us to draw conclusions on a potential genotoxic hazard of the non- micronised form of the compound as there is no indication of exposure.

Title: *In vivo* / *in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes with FAT 75'714/B
Guideline: OECD 486
GLP: In compliance
Test item: FAT 75'714/B
Batch/lot n°: 15420CL2 , non-micronised

Method
Three male Fischer rats (IFFA CREDO origin) per dose (1000 or 2000 mg/kg bw) and per expression time (12-16 hours in assay 1, 2-4 hours in assay 2) were treated by oral gavage with FAT 75'714/B (purity: 99.8 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in 0.5 % CMC for the unscheduled DNA synthesis (UDS) test (dose volume: 10 mL/kg bw). At each expression time, assigned animals were killed and liver cells prepared. Hepatocyte cultures were then radiolabelled with ^{3}H-Thymidine, prepared for autoradiography and grains counted using an image analysis system. Concurrent negative (0.5 % CMC) and positive controls (2-Acetamidofluorene at 25 mg/kg bw for assay 1; Dimethylhydrazine at 10 mg/kg bw for assay 2) were included in this study.

Results
Over the two experiments at the two doses tested, the net nuclear grain counts were below the threshold value for a positive response. Furthermore, the frequency of cells in S-phase was low. Overall, treatment with the test item did not induce a proliferative effect in rat liver and

did not reveal genotoxic activity. Treatment with the positive control items resulted in the appropriate response over background.

Unscheduled DNA synthesis in rat hepatocytes

Group	NNG Count	Cells in repair NNG Count ≥ 5	% cells in repair NNG Count ≥ 5
	(mean ± SD)	(mean ± SD)	(mean ± SD)
Assay 1: 12-16 hour expression time			
Vehicle control[a]	-1.98 ± 3.60	6.00 ± 1.06	1.19 ± 0.67
1000 mg/kg bw	-2.13 ± 3.66	6.62 ± 0.02	1.56 ± 1.05
2000 mg/kg bw	-1.84 ± 3.78	6.17 ± 0.89	2.18 ± 0.51
Positive control[b]	5.77 ± 4.87	8.90 ± 0.65	59.2 ± 3.31
Assay 2: 2-4 hour expression time			
Vehicle control[a]	-2.63 ± 4.20	5.41 ± 0.20	1.96 ± 0.89
1000 mg/kg bw	-3.16 ± 4.14	6.08 ± 0.91	1.53 ± 0.19
2000 mg/kg bw	-4.03 ± 4.42	5.85 ± 0.35	0.71 ± 0.53
Positive control[c]	14.61 ± 6.10	15.32 ± 0.88	94.26 ± 12.23

a: 0.5 % CMC
b: 2-Acetamidofluorene (2-AAF)
c: Dimethylhydrazine
NNG: Net nuclear grain

Conclusion
Under the experimental conditions employed, the UV-absorbing ingredient MBBT in non-micronised form did not induce DNA repair in liver cells of treated rats.

Ref.: Institut Pasteur de Lille (2004)

Comment of the SCCS:
The SCCS does not agree to the conclusion on the non-micronised form. This study does not allow us to draw conclusions on a potential genotoxic hazard of the non-micronised form of the compound since it is not demonstrated that the testing compound has reached the target.

3.3.8. Reproductive toxicity

3.3.8.1 Endocrine effects

Title: CGF-C2089 and CGF-C1607[7]: Androgen competitive binding assay
Guideline: Not indicated
GLP: No
Test item: CGF-C2089 (non-micronised)
Batch/lot n°: CTL test substance Y11707/001

Methods

[7] Information on CGF-C1607 is not provided in the present document

The androgen competitive binding assay was conducted according to Ashby and Lefevre (2001) using cytosol isolated from the prostates of 8 week old male rats [Alpk:APfSD] treated 24 hours previously with the GnRH-anatagonist Antarelix to mimic surgical castration. Aliquots of prostate cytosol were incubated with duplicate dose levels of the test item in 10 µL Dimethyl sulfoxide (DMSO) and ^{3}H-R1881 (17α-Methyl-^{3}H-trienolone) for 17 hours at 4 °C. The receptor-ligand complex was precipitated and the radioactivity determined by liquid scintillation counting. Ten-fold dilutions of R1881 (Methyl-trienolone) and testosterone (positive control) and the test item CGF-C2089 (5×10^{-10} to 5×10^{-4} mol/L in DMSO), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form were assayed. Two separate experiments were conducted, each with duplicate dose levels of test item and of vehicle alone to ascertain a 100 % binding value.

Results
MBBT failed to compete with R1881 in the androgen competitive binding assay. Testosterone and R1881 induced the appropriate response.

Conclusion
Under the conditions of this *in vitro* study, the UV-absorbing ingredient MBBT displayed no intrinsic androgenic activity.

Ref.: CTL (2001a)

Title:	CGF-C2089 and CGF-C1607[8]: Estrogen competitive binding assay
Guideline:	Not indicated
GLP:	No
Test item:	CGF-C2089 (non-micronised)
Batch/lot n°:	CTL test substance Y11707/001

Methods
The estrogen competitive binding assay was conducted using the cytosol isolated from the uteri of 21-25 day old female rats [Alpk:APfSD] as described by Ashby *et al.* (1999, 2000), using methods based upon Shelby *et al.* (1996). Uterine cytosol was incubated with duplicate dose levels of the test item in Dimethyl sulfoxide (DMSO) and ^{3}H-17β-Estradiol at 4 °C for 17 hours. The receptor-ligand was precipitated with Hydroxyl apatite, washed and then suspended in Optiphase for the determination of radioactivity in a liquid scintillation counter. Ten-fold dilutions of 17β-Estradiol, the anti-oestrogen Faslodex (positive control) and the test item CGF-C2089 (purity not indicated), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form (5×10^{-10} to 5×10^{-4} mol/L in DMSO) were assayed. Two experiments were conducted, each with duplicate dose levels of the test item and of vehicle alone to ascertain a 100 % binding value.

Results
MBBT failed to compete with ^{3}H-17β-Estradiol in the estrogen competitive binding assay. 17β-Estradiol and Faslodex induced the appropriate response.

Conclusion
Under the conditions of this *in vitro* study, the UV-absorbing ingredient MBBT in non-micronised form displayed no intrinsic estrogenic activity.

Ref.: CTL (2001b)

[8] Information on CGF-C1607 is not provided in the present document.

Title: CGF-C2089: Uterotrophic assay in immature rats
Guideline: Not indicated
GLP: In compliance
Test item: CGF-C2089 (non-micronised)
Batch/lot n°: 003542B0

Methods
An uterotrophic assay was performed using 19-20 days old rats [Alpk:APfSD]. The test item CGF-C2089 (purity: ≥ 98 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in Arachis oil was administrated to groups of 10 female rats at daily dose levels of 0 (vehicle control), 250, 500 or 1000 mg/kg bw by single subcutaneous injection on 3 consecutive days. An additional group of rats was treated daily under the same experimental conditions with the positive control item 17β-Estradiol benzoate at 0.4 mg/kg bw on 3 consecutive days.

Clinical signs and body weights were recorded daily during the study period. All animals were sacrificed 24 hours after the last administration. The uteri were trimmed free of fat, gently blotted and weighed. Dose formulations were analysed for concentration and homogeneity using a HPLC method with UV detection.

Results
No signs of test item-related toxicity were noted in this study. The test item was inactive in the immature rat uterotrophic assay at all dose levels tested. The positive control item induced a distinct increase in blotted uterus weights (± adjustment for body weight) thus confirming sensitivity of the test system. Based on chemical analysis results, the dose formulations were acceptable for use in this study.

Conclusion
Under the conditions of this *in vivo* study, the UV-absorbing ingredient MBBT in non-micronised form displayed no uterotrophic potency.

Ref.: CTL (2002b)

3.3.8.1. Reproduction toxicity

Reproduction toxicity

Title: Oral (gavage) fertility and general reproduction toxicity study of Tinosorb® MBBT (FAT 75'714) in rats
Guideline: ICH Harmonised Tripartite Guideline, Stages A and B
GLP: In compliance
Test item: Tinosorb® MBBT (FAT 75'714) (non-micronised)
Batch/lot n°: 15420CL2

Methods
The test item Tinosorb® MBBT (purity: 99.8 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80 was administered by daily single oral gavage to 25 rats [Crl:CD(SD)IGS BR VAF/Plus] per sex per dose level at 100, 300 or 1000 mg/kg bw (dose volume: 10 mL/kg bw). Concurrent control animals (25 rats per sex) were treated with the vehicle only under the same experimental conditions.

Male rats were treated daily beginning 28 days before cohabitation, through cohabitation (maximum of 16 days), and continuing through the day before sacrifice, which was the end of the cohabitation period. Female rats were dosed once daily beginning 15 days before cohabitation, during cohabitation and continuing through day 7 of gestation.

Viability, clinical signs, food consumption and body weights were determined periodically. Oestrous cycling was assessed during the in-life period. Males were sacrificed at the end of the cohabitation period and the reproductive organs weighed (testes, epididymides, seminal vesicles), and sperm evaluated for concentration and motility. Females were sacrificed on gestational day 13 and examined to determine the number of *corpora lutea*, implantation sites, and of viable and non-viable embryos. *Placentae* were examined for size, colour and shape.

Dose formulations were analyzed for concentration, homogeneity and stability.

Results
No test item-related effects on viability, clinical signs, body weight, body weight development and food consumption were noted in male and female rats at the dose levels tested. No necropsy observations were found to be test-article related. There were no test item-related adverse effects on mating, fertility and other reproductive parameters such as oestrous cycling or male reproductive organ weights and sperm concentration and motility. In females, no Caesarean-sectioning parameters (numbers of *corpora lutea*, preimplantation loss, implantations, appearance of *placentae* and viable versus non-viable embryos) were affected by test item dosages as high as 1000 mg/kg bw/d.

Conclusions
Under the conditions of this study, the oral no-observed-effect-level (NOEL) for parental and reproductive toxicity of the UV-absorbing ingredient MBBT (non-micronised) was set at the high dose level of 1000 mg/kg bw/d. Based on the study results, MBBT does not display adverse effects on reproduction parameters and taking all other data on reprotoxicity into account, it is thus not subject to classification as reproductive toxicant according to Regulation (EC) No. 1272/2008.

Ref.: CR-DDS (2005a)

Title:	Oral (gavage) developmental and perinatal / postnatal reproduction toxicity study of Tinosorb® MBBT (FAT 75'714) in rats, including a postnatal behavioural / functional evaluation
Guideline:	ICH Harmonised Tripartite Guideline, Stages C through F
GLP:	In compliance
Test item:	Tinosorb® MBBT (FAT 75'714) (non-micronised)
Batch/lot n°:	15420CL2

Methods
The test item Tinosorb® MBBT (purity: 99.8 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80 was administered by oral gavage to 24 pregnant female rats [Crl:CD(SD)IGS BR VAF/Plus] per dose level at 100, 300 or 1000 mg/kg bw/d (dose volume: 10 mL/kg bw) from day 6 of gestation through day 20 of lactation or day 24 of gestation (rats that did not deliver a litter). A concurrent control group of 24 pregnant female rats received the vehicle only under the same experimental conditions.

F0 generation rats were observed for viability, clinical signs, body weight development and food consumption. Maternal behaviour was assessed on days 1, 4, 7, 14 and 21 of the lactation

period. After completion of the 21-day postpartum period, the F0 generation animals were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites was recorded.

Each litter was evaluated for viability. The pups in each litter were counted and clinical observations as well as body weights were recorded. Reflex and physical developmental measures (surface righting, eye opening, acoustic startle, air righting, and pupil constriction) were also evaluated during the pre weaning period. Pups culled on day 28 *post partum* were sacrificed and a gross necropsy was performed; in addition, the brain was cross-sectioned and examined for hydrocephaly.

F1 generation rats selected for continued observation were examined for clinical signs during the post weaning period. Body weights and food consumption data except during cohabitation were determined. The rats were evaluated for sexual maturation, motor activity and for performance in a watermaze test. At approximately 90 days of age, the F1 generation rats were assigned to cohabitation. Male rats were sacrificed after completion of the cohabitation period; a gross necropsy, assessment of reproductive organ weights, microscopical examination and a sperm evaluation were performed. Female rats were sacrificed on day 21 of gestation, Caesarean-sectioned, and a gross necropsy was performed. Foetuses weight, sex and gross external alterations were evaluated. Dose formulations were analysed for concentration, homogeneity and stability.

Results
There were no indications of test item-related general toxicity or adverse effects on reproduction in the F0 and F1 generation rats or development in the F1 generation rats and the F2 generation foetuses.

All F0 generation female rats survived until scheduled sacrifice. None of the clinical signs or gross lesions observed at necropsy of these rats was considered test item-related. There were no dose-dependent changes in body weight, body weight development and food consumption throughout gestation and lactation. All pregnant rats delivered litters and had surviving pups when the litters were weaned on day 21 of lactation. With few exceptions considered as incidental findings within historical control ranges, *i.e.* a slight decrease in the lactation index due to mainly a single litter in the high dose group and an increased percentage of high dose male pups per litter, all natural delivery and litter observations (including evaluations of reflex and physical development) were comparable among the four dose groups.

There were no test item-related deaths among the F1 generation male and female rats. Clinical signs, body weight, body weight gain and food consumption as well as anatomical indices of sexual maturation and performance in a motor activity test and watermaze task of these rats were not affected by maternal administration of the test item. There were no statistically significant or biologically important effects on the mating and fertility parameters. All necropsy observations in the F1 generation rats were considered unrelated to maternal dosages. Treatment with the test item did not induce changes in male reproductive organ weights or sperm motility and density. Microscopic examination of reproductive organs did not reveal test item-related alterations. No Caesarean-sectioning or litter parameters were affected by the maternal dosages of the test item, and all F2 foetuses appeared normal at gross external examination.

The concentration, homogeneity and stability of the dose formulations was acceptable.

Conclusion
Under the experimental conditions employed, the oral no-observed-effect-level (NOEL) for general toxicity and for adverse effects on development and reproduction in rats of the UV-absorbing ingredient MBBT in non-micronised form was established at the high dose level of 1000 mg/kg bw/d. Therefore and taking all other data on reprotoxicity into account, MBBT is not subject to classification as reproductive toxicant according to Regulation (EC) No. 1272/2008.

Ref.: CR-DDS (2005b)

Developmental toxicity

Developmental toxicity in rats

Title:	Dose range-finding prenatal toxicity study with CGF-C002089 in the rat
Guideline:	Not indicated
GLP:	No
Test item:	CGF-C002089 (non-micronised)
Batch/lot n°:	507514.52

Methods
Groups of 5 pregnant female rats [HanIbm:WIST (SPF)] per dose level were treated with the test item CGF-C002089 (purity: 99.9 %), *i.e.* with the UV-absorbing ingredient MBBT in non-micronised form, in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80 by daily oral gavage from days 6 to 17 of gestation at 100, 300 or 1000 mg/kg bw (dose volume: 10 mL/kg bw). Additional 5 female pregnant rats received vehicle only and served as concurrent control group. All animals were sacrificed on day 21 of gestation and the foetuses removed by Caesarean section.

Viability, clinical signs, food consumption and body weights were recorded periodically during the study period. *Post mortem* examinations included macroscopic examination of dams and the assessment of the number of *corpora lutea*. Foetuses were sexed, weighed and examined for gross external abnormalities. Sex ratios, pre-implantation loss and post-implantation loss were calculated.

Results
Test item-related effects on survival, clinical appearance, food consumption, body weights or macroscopic findings did not occur in any dam. No test item-related reproductive effects (mean numbers of *corpora lutea* and implantation sites, and percent of pre- and post-implantation loss) were noted. Test item-related foetal effects (external abnormalities, sex ratios and body weights) did not occur.

Conclusion
Under the condition of this range-finding study, the maternal and developmental no-observed-effect-level (NOEL) was 1000 mg/kg bw/d. The corresponding no-observed-adverse-effect-level (NOAEL) for maternal and developmental effects was > 1000 mg/kg bw/d. Identical dose levels were selected for the main study.

Ref.: RCC (1998a)

Title: Prenatal toxicity study with CGF-C002089 in the rat
Guideline: OECD 414
GLP: In compliance
Test item: CGF-C002089 (non-micronised)
Batch/lot n°: 507514.52

Methods
The test item CGF-C002089 (purity: 99.9 %), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80 was administered by single daily oral gavage to 22 pregnant female rats [HanIbm:WIST (SPF)] per dose group from days 6 to 17 of gestation at 100, 300 or 1000 mg/kg bw (dose volume: 10 mL/kg bw). A concurrent control group of 22 female rats was treated with the vehicle only under the same experimental conditions. Animals were sacrificed on day 21 of gestation and the foetuses removed by Caesarian section.

Viability, clinical signs, food consumption and body weights were recorded periodically during the study period. *Post mortem* examinations included macroscopic examination of dams and the assessment of the number of *corpora lutea*. Foetuses were sexed, weighed and examined for gross external abnormalities. Viscera, brains and skeletons of foetuses were examined for abnormalities and variations. Sex ratios, pre-implantation loss and post-implantation loss were calculated. Dose formulations were analysed for content, homogeneity and stability.

Results
Test item-related effects on survival, clinical signs, food consumption, body weights or macroscopic findings did not occur in any dam. No test item-related reproductive effects (mean number of implantation sites, mean post-implantation loss, and mean number of foetuses per dam) were noted. Test item-related foetal effects did not occur in the parameters external, visceral, and skeletal abnormalities; sex ratios; body weights; and stage of development. The few observed significant differences in the various maternal or foetal parameters assessed were not considered test item-related since they were within the range of normal biological variability for the strain and age of rat used and/or did not exhibit a dose-response. The concentration, homogeneity and stability of the dose formulations were acceptable in this study.

Conclusion
Under the condition of this study, the maternal and developmental no-observed-effect-level (NOEL) of the UV-absorbing ingredient MBBT in non-micronised form was the high dose level of 1000 mg/kg bw/d. The corresponding no-observed-adverse-effect-level (NOAEL) for maternal and developmental effects was > 1000 mg/kg bw/d. Based on this outcome and taking all other data on repro-toxicity into account, MBBT does not display adverse effects on development in rats and is not subject to classification as reproductive toxicant according to Regulation (EC) No. 1272/2008.

Ref.: RCC (1998b)

Developmental toxicity in rabbits

Title: Oral (stomach tube) dosage-range developmental toxicity study of Tinosorb® MBBT (FAT 75'714) in rabbits
Guideline: Not indicated
GLP: In compliance
Test item: Tinosorb® MBBT (FAT 75'714) (non-micronised)

Batch/lot n°: 15420CL2

Methods
Suspensions of the test item Tinosorb® MBBT (purity: 99.8 %), *i.e.* of the UV-absorbing ingredient MBBT in non-micronised form, in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80, were administered by oral gavage once daily to 6 timed-mated female New Zealand White rabbits [Hra:NWZ(SPF)] per dose level on days 6 through 19 of gestation at 250, 500 or 1000 mg/kg bw (dose volume: 10 mL/kg bw). Concurrent control animals received the vehicle only under the same experimental conditions.

Viability, clinical signs, food consumption and body weights were recorded periodically. All surviving rabbits were sacrificed on day 29 and examined for the number and distribution of *corpora lutea*, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gravid uterine weights were recorded. Foetuses were weighed and examined for gross external alterations and sex.

Results
One mid-dose doe was sacrificed on day 23 after an abortion that was not related to the treatment with the test item. All remaining rabbits survived until scheduled sacrifice. In the dams, no test item-related changes were noted in clinical signs, food consumption, body weights and gross lesions. Caesarean sectioning, litter observations and the foetal gross observations were all within the ranges observed historically.

Conclusion
A no-observed-effect-level (NOEL) was not derived by the study author. Based on the results of this study, the oral NOEL for maternal and developmental toxicity of the UV-absorbing ingredient MBBT in non-micronised form was at the high dose level of 1000 mg/kg bw/d. Dosages of 100, 300 and 1000 mg/kg bw/d were selected for the main developmental toxicity study with MBBT in rabbits.

Ref.: CR-DDS (2005c)

Title:	Oral (stomach tube) developmental toxicity study of Tinosorb® MBBT (FAT 75'714) in rabbits
Guideline:	ICH Harmonised Tripartite Guideline: Detection of toxicity to reproduction of medical products
GLP:	In compliance
Test item:	Tinosorb® MBBT (FAT 75'714) (non-micronised))
Batch/lot n°:	15420CL2

Methods
The test item Tinosorb® MBBT (purity: 99.8%), *i.e.* the UV-absorbing ingredient MBBT in non-micronised form, suspended in 0.5 % (w/v) CMC in 0.1 % (w/v) aqueous Tween 80, was administered by oral gavage to 20 female pregnant rabbits [Hra:NWZ(SPF)] per dose group on days 6 through 19 of gestation at dosages of 100, 300 or 1000 mg/kg bw/d (dose volume: 10 mL/kg bw).

Viability, clinical observations, food consumption and body weights were assessed periodically during the study period. Rabbits were sacrificed on day 29, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of *corpora lutea* were recorded. The uterus of each rabbit was excised, weighed and

examined for pregnancy, number and distribution of implantation sites, live and dead foetuses and early and late resorptions. *Placentae* were examined for size, colour and shape. Foetuses were weighed, sexed and examined for gross lesions. Cavitated organs were evaluated in all foetuses. The brain was examined *in situ*. All foetuses were examined for skeletal alterations. Dose formulations used in this study were analysed for content, homogeneity and stability.

Results
Viability, clinical observations, food consumption and body weights in the does were unaffected by the treatment with the test item at all dose levels. No external, soft tissue or skeletal alterations (malformations or variations) in foetuses were caused by the test item up to and including the high dose level of 1000 mg/kg bw/d. All litter parameters remained unaffected and clinical observations were considered unrelated to the test item.

Conclusion
Under the conditions of this study, the oral no-observed-effect-level (NOEL) for maternal and developmental toxicity of the UV-absorbing ingredient MBBT in non-micronised form was established at 1000 mg/kg bw/d. Thus, MBBT does not display adverse effects on development in rabbits and taking all other data on reprotoxicity into account, it is not subject to classification as reproductive toxicant according to Regulation (EC) No. 1272/2008.

Ref.: CR-DDS (2005d)

In Submission 1, information on photosensitization and phototoxicity of MBBT was submitted. The text below is directly copied from submission I

Photosensitisation

Species
Albino Dunkin Hartley Guinea Pig, HsdPoc: DH, SPF, body weight range at start of acclimatization 282-407 g; age at start of acclimatization 5-7 weeks.

Number of animals
The study was performed with 30 male animals. The animals were distributed as follows: Ten animals for the control group and 20 animals for the test group.

Substance:
• Test article: CGF-C002089
• Batch No.: 507514.52
• Purity: 99.9%

Test procedure

Induction (test days 1-10)
On test day 1 the nuchal skin area of the test group was shaved and a test site of 6-8 cm2 was defined by four 0.1 ml injections intradermally of Freund's Complete Adjuvant and physiological saline 1:1 into the corners. 0.1 ml of the 75% test article dilution in PEG 400 were then epicutaneously applied to a skin area of 8 cm^2.
The test site was exposed to 1.8 J/cm2 UV-B irradiation and 1 OJ/cm2 UV-A.
The mixture of Freund's Complete Adjuvant and physiological saline was injected only once. The remaining procedure (topical application of the test article followed by irradiation) was repeated four times within 2 weeks (days 1, 3, 6, 8 and 10). At day 1 of induction the control animals received 4 injections intradermally of Freund's Complete Adjuvant and physiological

saline 1:1 (0.1 ml) into the corners of the test site of 6-8 cm2. No additional applications were performed during induction. The animals remained untreated.

Challenge
On day 22 concentrations of 75%, 50%, 25% and 15% of the test article in PEG 400 were applied to the left flank at a dose of 0.025 ml/2 cm2.• The left flank was then exposed to 1 OJ/cm2 UV-A irradiation only. After irradiation of the left flank, the right flank was treated with the test article accordingly without irradiation. The control animals were treated in the same way as described above.

Light source

Induction
UV-A 320-400 nm 10J/cm²
UV-B 280-320 nm 1.8J/cm²

Challenge
UV-A 320-400 nm 10J/cm²
Philips Actinic "TLD" Lamps (36W/08)
Philips UV-B-Sunlamp TL 20W/12
Philips Actinic "TLD" Lamps (36W/08)
The duration of the exposure was regulated by a time control device.

Observation times
Each animal was assessed for reactions at 24, 48 and 72 hours after exposure.

Main findings
Under the experimental conditions employed, none of the animals of the test group showed skin reactions 24, 48 or 72 hours after removing of the challenge application.

Sensitisation rate: 0 out of 20 treated animals

Conclusion
The test substance possesses no photoallergenic and allergenic potential in the guinea pig under the study conditions. The results presented for the control group may lead to the conclusion that the test substance showed no irritation potency nor phototoxic potency.

Phototoxicity
Method:

Species:
Albino Dunkin Hartley Guinea Pig, HsdPoc: DH, SPF, body weight range at start of acclimatisation 296-385 g; age at start of acclimatization 5-7 weeks.
Number of animals: The study was performed with 15 male animals. The animals were distributed as follows: Five animals for the control group and 1 0 animals for the test group.

Substance:
• Test article: CGF-C002089
• Batch No.: 507514.52
• Purity: 99.9%

The test article was homogeneously mixed with PEG 400 as vehicle. 0.025 ml/ 2 cm² of the test article suspension were applied at concentrations of 25% and 15%. The remaining test article concentrations of 75% and 50% were applied with a spatula in order to saturate the test sites. The test sites on all animals were pretreated approximately 30 to 50 minutes prior to the test article application with 2% DMSO diluted with ethanol (0.025 ml/cm²) to enhance the skin penetration of the test article.

Light source:
• Philips Actinic "TLD" Lamps (36W/08)
• Energy 1 x1 Oexp. 4 Ergs/cm²/sec
• Spectrum 320-400nm
• Irradiation dose 20J/cm² UV -A

The different test article concentrations were applied to the left flank of the animals. Thirty minutes after application of the test article the left flank of the animals was exposed to non-erythematogenic UV-A irradiation (20J/cm²).
After irradiation the same treatment was performed on the right flank but the sites remained unexposed to light after treatment and served as control sites. Control animals were exposed to UVA similarly, except they were treated with the solvent (PEG 400) only.

Observation times
The animals were examined 24, 48 and 72 hours after application of the test article for signs of erythema and oedema.

Main findings
No phototoxic reactions were observed after test article administration in PEG-400.

Conclusion
Due to the results determined it is concluded that under the conditions used, the test substance does not exhibit phototoxic potential in guinea pigs of this strains and age