Scientific Committee on Consumer Safety

SCCS

OPINION ON
the safety of cosmetic ingredients
Phenylene Bis-Diphenyltriazine (CAS No 55514-22-2)
- S86 -
Submission II

The SCCS adopted the
final Opinion by written procedure on 30 July 2018

ACKNOWLEDGMENTS

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All Declarations of Working Group members are available on the following webpage: http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

This Opinion has been subject to a commenting period of a minimum eight weeks after its initial publication (from 26 February 2018 until 30 April 2018). Comments received during this time were considered by the SCCS.
For this Opinion, comments received resulted in the following changes: section 3.3.2.2 SCCS conclusion on eye irritation, section 3.3.4 SCCS comment and general conclusion on skin penetration, section 3.3.7.1 SCCS comment. There is no change in the discussion part and in the SCCS conclusions.

1. ABSTRACT

The SCCS concludes the following:

(1) *In light of the new data provided, does the SCCS consider Phenylene Bis-Diphenyltriazine, S86 safe for use as a UV-filter in sunscreen products in a concentration up to 5.0%?*

Based on the data provided in the dossier, the SCCS considers Phenylene Bis-Diphenyltriazine, S86, safe for use as a UV-filter in sunscreen products at a concentration up to 5%.

Because of the insoluble nature of S86 and as no data were provided on safety *via* inhalation exposure, the SCCS considers its use safe only in dermally applied products and not in products that would lead to inhalation exposure.

(2) *Does the SCCS have any further scientific concerns with regard to the use of Phenylene Bis-Diphenyltriazine, S86 as a UV-filter in sunscreen and/or other cosmetic products*

Phenylene Bis-Diphenyltriazine (S86) may contain impurities (NMP and hydrazine), which are classified as CMR 1B and identified in the EU as SVHC. Therefore, the level of NMP and hydrazine should be kept at trace levels.

Potential effects of Phenylene Bis-Diphenyltriazine (S86) on the environment have not been assessed by SCCS.

Keywords: SCCS, scientific opinion, cosmetic ingredient Phenylene Bis-Diphenyltriazine (CAS No 55514-22-2) - S86 - Submission II, CAS 55514-22-2, EC 700-823-1, Regulation 1223/2009

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About the Scientific Committees

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

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

SCCS

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

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Following submission I on Phenylene Bis-Diphenyltriazine to assess its safety for use in cosmetic products, the SCCS concluded in its opinion in July 2015 (SCCS/1556/15) that:

The following conclusions apply to Phenylene bis-diphenyltriazine with median particle size distribution (number-sized) around 130-170 nm or larger.

1. Does the SCCS consider Phenylene bis-diphenyltriazine, S86, safe for use as a UV-filter in sunscreen products in a concentration up to 10.0% taking into account the scientific data provided?

The SCCS considers Phenylene bis-diphenyltriazine, S86, not safe for use as a UV-filter in sunscreen products in a concentration up to 10.0% taking into account the scientific data provided.
SCCS cannot exclude that Phenylene bis-diphenyltriazine may have a genotoxic potential.

2. Does the SCCS have any further scientific concerns with regard to the use of Phenylene bis-diphenyltriazine, S86, as a UV-filter in sunscreen and/or other cosmetic products?

An adequate physico-chemical characterisation should be provided.
The tests conducted on eye irritation and skin sensitisation are considered inconclusive.
The phototoxicity potential can as yet not be excluded.
This Opinion does not apply to inhalation exposure of Phenylene bis-diphenyltriazine since no adequate information on chronic or sub-chronic toxicity after inhalation was provided.
The SCCS noted that due to the poor biodegradation potential and the very high octanol-water partition coefficient, long-term effects or bioaccumulation of Phenylene bis-diphenyltriazine, S86, in the environment cannot be excluded. The use of Phenylene bis-diphenyltriazine as an ingredient in sunscreen products might lead to environmental exposure.

In March 2017, in light of the opinion SCCS/1556/15, the cosmetics company Pierre Fabre transmitted a new safety dossier (submission II) on Phenylene Bis-Diphenyltriazine that addresses the major issues raised by the SCCS notably i) additional physico-chemical characterization studies, ii) additional toxicity studies in line with the required guidelines (studies were performed according GLP) and iii) finally the file was rewritten focusing on the active ingredient to be more in line with normal practice.

Terms of reference

1. In light of the new data provided, does the SCCS consider Phenylene Bis-Diphenyltriazine, S86 safe for use as a UV-filter in sunscreen products in a concentration up to 5.0%?

2. Does the SCCS have any further scientific concerns with regard to the use of Phenylene Bis-Diphenyltriazine, S86 as a UV-filter in sunscreen and/or other 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

Chemical Name: 5,6,5',6'-tetraphenyl-3,3'-(1,4-phenylene)bis[1,2,4-Triazine]

INCI Name: Phenylene Bis-Diphenyltriazine

COLIPA No.: S86

Ref. 8 submission II

3.1.1.2 Chemical names

IUPAC name: 3,3'-(1,4-Phenylene)bis(5,6-diphenyl-1,2,4-triazine)

1,4-bis(5,6-diphenyl-1,2,4-triazin-3-yl)benzene

Ref: https://echa.europa.eu/registration-dossier/-/registered-dossier/6106/1

3.1.1.3 Trade names and abbreviations

Triasorb (cosmetic ingredient, S02771, Noyau, R000317, 025814)

WP30
S02374
CH0222-WP30
025589

Table 1

COSMETIC INGREDIENT	ACTIVE SUBSTANCE
S02771	**S02374**
NOYAU WP30	WP30
"X"	CH0222 – WP30
R000317	025589
025814	Phenylene bis-diphenyltriazine
	S86

SCCS comment
According to the background [provided by the Applicant], the cosmetic ingredient SO2771 is prepared by wet grinding of the active substance S02374. S02771 is formulated with an emulsifier (Eumulgin L), a preservative (Benzoic acid) and water. Additional supporting data are provided on the active substance S02374 (5,6,5',6'-tetraphenyl-3,3'-(1,4-Phenylene)bis(1,2,4-Triazine or Phenylene bis-diphenyltriazine). The ToR only refers to [the active substance] Phenylene bis-diphenyltriazine.

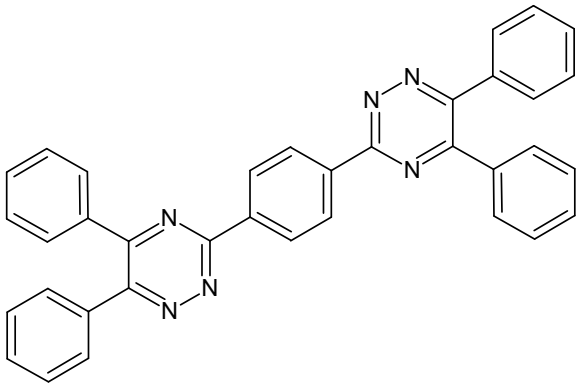
The composition of the cosmetic ingredients "X" and NOYAU WP30 has been shown to be similar to S02771. The code 025814 (no data provided) designates the same cosmetic ingredient S02771 according to a letter from the applicant.
The code R000317 designates the non-ground active substance and not the cosmetic ingredient as indicated in the Table 1 taken from the applicant (ref. 31 submission I).

3.1.1.4 CAS / EC number

CAS: 55514-22-2
EC: 700-823-1

Ref. 8 submission II

3.1.1.5 Structural formula



Ref. 8 submission II

3.1.1.6 Empirical formula

Formula: $C_{36}H_{24}N_6$

Ref. 8 submission II

3.1.2 Physical form

Physical form: solid (yellow powder)

Ref. 8 submission II

3.1.3 Molecular weight

Molecular weight: 540.62 g/mol

Ref. 8 submission II

3.1.4 Purity, composition and substance codes

A) Active substance

The chemical characterisation was performed by NMR, MS, IR and UV spectroscopy.

Purity was determined by HPLC (UV- detection) on two Bench batches ES130 and ES140 and five Pilot batches LP110, LP120, LP130, LP140 and LT30 (see Table 2).

Table 2 HPLC assay and purity of seven different batches of WP30

Batch	**Assay by HPLC (% W/W) 320 nm**	**HPLC purity [% area] 260 nm**
ES130	99.76* and 99.20**	Benzyl < 0.05
ES140	99.5***	Benzyl Not detected< 0.1
LP110	99.6	Benzyl < 0.10
LP120	99.7	Benzyl < 0.10
LP130	100.3	Benzyl < 0.10
LP140	98.9	Benzyl < 0.10
LT30	99.4	Benzyl < 0.10

*First analysis against WP30 reference standard batch DMI 08217B considered as 100% pure
** Reanalysis against WP30 reference standard batch DMI 08217B considered as 100% pure
*** Against WP30 batch ES130 considered as 99.2% pure

Infra-Red and UV spectroscopy were performed on two bench batches, ES130 and ES140 and five pilot batches, LP110, LP120, LP130, LP140 and LT30 (see Table 3).

Table 3 Infra-Red and UV spectroscopy of seven different batches of WP30

Batch	**ES130**	**ES140**	**LP110**	**LP120**	**LP130**	**LP140**	**LT30**
N° CA	**D0030V2-08**	**D0122-08**	**D0006-09**	**D0172-09**	**D0053-10**	**D0230V1-10**	**-**
IR spectrum	Correlated with RW/PF023A* 0.87	Correlated with ES130 0.99	Similar to spectrum of reference	Similar to spectrum of reference	Similar to spectrum of reference	Similar to spectrum of reference	Similar to spectrum of reference
UV spectrum	Similar to spectrum of RW/PF023A* (λmax = 332 nm)	Similar to spectrum of ES130 (λmax = 331 nm)	Similar to spectrum of reference	Similar to spectrum of reference	Similar to spectrum of reference	Similar to spectrum of reference	Similar to spectrum of reference

* Laboratory reference according to internal S02374 monograph

Elemental analysis and NMR spectroscopy were performed on two bench batches, ES130 and ES140, and four pilot batches, LP110, LP120, LP130 and LP140 (see Table 4).

Table 4 Elemental analysis and NMR spectroscopy of six different batches of WP30

Batch	**ES130**	**ES140**	**LP110**	**LP120**	**LP130**	**LP140**
N° CA	**D0030V2-08**	**D0122-08**	**D0006-09**	**D0172-09**	**D0053-10**	**D0230V1-10**
Structure						
• C	79.92*%	79.93*%	80.15	80.23	80.27	79.71
• H	4.45*%	4.49*%	4.42	4.44	4.33	4.52
• N	15.59*%	15.62*%	15.52	15.52	15.64	15.46
NMR						
• ^{1}H						

Table 4 Elemental analysis and NMR spectroscopy of six different batches of WP30						
Batch	**ES130**	**ES140**	**LP110**	**LP120**	**LP130**	**LP140**
N° CA	**D0030V2-08**	**D0122-08**	**D0006-09**	**D0172-09**	**D0053-10**	**D0230V1-10**
• ^{13}C	Conform Conform	Conform Conform	Conform Conform	Conform Conform	Conform Conform	Conform Conform
* Values not corrected for solvents and water						

Ref. 16, 22, 86 (submission II)

B) Cosmetic Ingredient S02771

The cosmetic ingredient S02771 intended to be used as a UV-filter is a 40 - 50% aqueous suspension obtained after a wet grinding step of the active substance S02374. The wet grinding step of the active substance S02374 (powder with a d(50) = 38 µm) leads to particles with a d(50) = 170-270 nm.
S02771 is formulated with an emulsifier (Eumulgin L), a preservative (Benzoic acid) and water. Its pH is between 3.5 and 4.5. The mixture of ingredients is detailed in Table 5:

Table 5

INCI Name	Trade Name	CAS number	%
Water	-	7732-18-5	45.0 – 55.0
Phenylene Bis-Diphenyltriazine	TRIASORB	55514-22-2	40-50
PPG-1-PEG-9 Lauryl Glycol Ether	Eumulgin L	154248-98-3	approx. 4.5
Benzoic acid	Benzoic Acid	65-85-0	0.2-0.3

Ref. 28, 33, 78 (submission I)

SCCS comment
Purity of the active substance (non-ground or ground) based on HPLC peak area can only be reliable when 1) it is documented that all of the active substance and impurities loaded on the column were eluted and 2) the same results are obtained by HPLC performed using HPLC columns with two different stationary phases, for example reverse phase and normal phase HPLC columns.
Peak purity of the test compound presented in Table 2 was not evaluated at λ_{max} 332nm.

3.1.5 Impurities / accompanying contaminants

A) Active substance

Impurities were determined by HPLC (UV-detection) and Ionic Chromatography on two bench batches, ES130 and ES140 and five pilot batches, LP110, LP120, LP130, LP140 and LT30 (see Table 6).

Regarding the chemical process, the most important technologically unavoidable impurities were the following:

- Residual solvents: Ethanol and N-methyl-pyrrolidone (NMP)
- Residual Dihydrazone (chemical intermediate)
- Residual Hydrazine (starting material).

For the determination of these impurities, the standard materials were used.

Table 6 HPLC (UV-detection) and Ionic Chromatography on impurities of the seven different batches of WP30

Batch	**ES130**	**ES140**	**LP110**	**LP120**	**LP130**	**LP140**	**LT30**
N° CoA	**D0030V2-08**	**D0122-08**	**D0006-09**	**D0172-09**	**D0053-10**	**D0230V1-10**	**-**
Residual solvents(ppm) HPLC • Ethanol • NMP*	< 500 2715	< 500 3130	< 500 2840	< 500 853	< 500 1782	< 500 2535	< 500 1824
Residual Dihydrazone HPLC (% w/w)	< 0,1	< 0,1	< 0,1	< 0,5	< 0,5	< 0,1	< 0,1
Residual Hydrazine Ionic Chromatography (ppm)	< 2	< 2	< 2	< 2	< 2	< 2	< 2

*: N-methyl-pyrrolidone

The sum of most important impurities such as solvents were found to be less than 0.7% (w/w) in WP30.

According to the Applicant, the identification of residual impurities shows the presence of two technically unavoidable CMR impurities, which are considered as SVHC by ECHA: NMP is classified as toxic for reproduction category 1B and Hydrazine is classified as carcinogenic category 1B (Regulation EC No 1272/2008).

However, in the toxicological studies, there was no indication for an endocrine modulating effect, clearly no mutagenic/genotoxic potential and no adverse maternal or fetal effects, nor any adverse effect on male fertility.

As these technically unavoidable impurities were present in all test materials in the same concentrations as in the intended marketed product, they were covered by the safety assessment.

These impurities are chemically well identified and the manufacturing process manages the level of these substances.

Heavy metal quantity was determined by ICP Mass spectrometry on two Bench batches ES130 and ES140 and five Pilot batches LP110, LP120, LP130, LP140 and LT30 using standard reference materials (see Table 7).

Table 7 ICP Mass spectrometry on impurities of the seven different batches of WP30

Batch	**ES130**	**ES140**	**LP110**	**LP120**	**LP130**	**LP140**	**LT30**

N° CoA	D0030V2-08	D0122-08	D0006-09	D0172-09	D0053-10	D0230V1-10	-
Heavy metals (ICP/MS) (ppm)	As,Ag,Al,Au, Ba,Bi,Cd,Cu, Co,Ce,Cr,Cs, Dy, Er,Ga,Gd,Hg ,Ho,La,Lu,M g,Mn,Mo,Nb, Ni, Pb,Pd,Pt,Pr, Rb,Ru,Sb,Sn ,Sr,Tb,Th,Ti, Tl, Tm,U,V,W,Y, Yb,Zr< 3 Li,Na,Se,Zn < 10 Ca,Fe,K,P,Si, < 150	As,Ag,Al,A u,Ba,Bi,C d,Cu,Co,C e,Cr,Cs,D y,Er,Ga,G d,Hg,Ho,L a,Lu,Mg,M n,Mo,Nb, Ni,Pb,Pd,P t,Pr,Rb,Ru ,Sb,Sn,Sr, Tb,Th,Ti,T l,Tm,U,V, W,Y,Yb, Zn ,Zr < 3 Li,Na,Se < 10 Ca,Fe,K,Si , <50 P < 150	As, Au, Pd, Ru < 0.05 Ag, Bi, Cd, Pb, Pt < 0.02 Cu, Sb, Sn, V < 0.5 Mo < 0.1 Hg < 0.2	As, Au, Pd, Ru < 0.05 Ag, Bi, Cd, Pb, Pt < 0.02 Cu, Sb, Sn, V < 0.5 Mo, Hg < 0.1	As, Au, Pd, Ru < 0.05 Ag, Bi, Cd, Pb, Pt < 0.02 Cu, Sb, Sn, V < 0.5 Mo, Hg < 0.1	As, Au, Pd, Pb < 0.05 Ag, Bi, Cd < 0.02 Cu, Mo, Sb, Sn, V < 0.5 Pt, Ru, Hg < 0.1	Pt <0.02 Ag, As, Au, Pd, Ru < 0.05 Bi, Cd, Pb < 0.1 Hg < 0.2 Cu, Mo, Sb, Sn, V < 0.5

According to the Applicant, it was demonstrated that the heavy metal quantity is under the usual threshold values for cosmetic ingredients.

Identification of impurities

During release and stability studies, six impurities have been detected in batches of S02374 active substance and/or S02771 cosmetic ingredient during the analysis of the related substances HPLC-UV. Some have previously been quantified above the reported threshold (>0.1%) in historical batch analyses.

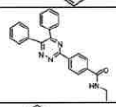
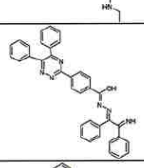
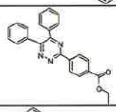
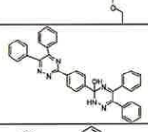
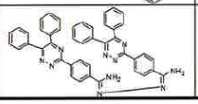
Impurities were identified using liquid chromatography hyphenated to DAD detector and high-resolution mass spectrometry (positive electrospray, sensitive mode), based on mass measurement and fragmentation pattern analysis. For each impurity, DAD spectra at λmax, accurate mass, MS/MS fragmentation and proposition of tentative structure were determined. Mature batches of WP30 active substance S02374 and cosmetic ingredient S02771 were analysed for this study.

The impurities to identify have relative retention times (RRT) versus WP30 of 0.40, 0.69, 0.72, 0.76, 0.82 and 0.90. All six impurities have been analysed and gave UV, MS and MS/MS spectra. The λmax of each impurity are different, indicating the presence of six different structural impurities.
The six impurities have been successfully identified and characterised (Table 8) with one confirmed by standard injection.

Impurities (identity and concentrations): Benzil < 0.1 %, Dihydrazone < 0.5 %, Hydrazine < 2 ppm, unidentified impurity with MW 380 = 0.10 %, unidentified impurity with MW 550 = 0.14 %

Ref.16, 22, 80, 86 (submission II)

Table 8. Identification of impurities

RRT ratio (vs WP30)	λ_{max}	*Molecular ion (m/z)*	Elemental composition	Error (ppm)	Main fragments (*m/z*) (MS/MS data)	Proposed structure	Remark
0.40	260 nm	211.0757	$C_{14}H_{10}O_2$	1.4	133.0, 105.0, 77.0		Benzil - confirmed by standard injection
0.69	283 nm	381.1708	$C_{24}H_{20}N_4O$	-0.5	353.1, 310.1, 178.1, 147.1, 130.0, 104.0		-
0.72	292 nm	559.2243	$C_{36}H_{26}N_6O$	0.4	541.2, 456.2, 353.1, 335.1, 225.1, 122.1, 105.0, 77.0		Isomer of RRT 0.82
0.76	277 nm	382.1552	$C_{24}H_{19}N_3O_2$	0.5	354.1, 310.1, 178.1, 148.0, 130.0, 104.0		-
0.82	270 nm	559.2243	$C_{36}H_{26}N_6O$	0.4	541.2, 456.2, 353.1, 335.1, 225.1, 122.1, 105.0, 77.0		Isomer of RRT 0.72
0.90	274 nm	701.2887	$C_{44}H_{32}N_{10}$	0.4	701.3, 684.3, 350.1, 335.1, 178.1, 144.1		-

B) Cosmetic ingredient S02771

Impurities of the pilot batch LP 110 were found to be less than 0,21% (w/w).
Ref. 28, 33, 78 (submission I)

SCCS Comment
Impurities have been chemically characterised by the use of HPLC-PDA and LC-HRMS based on fragmentation pattern analysis.
NMP and hydrazine have been identified as SVHC by ECHA and were included on 20 June 2011 in the Candidate List of substances for eventual inclusion in Annex XIV of REACH (ED/31/2011). Both NMP and hydrazine are, according to the Applicant, unavoidable manufacturing impurities. NMP concentration varies from 830 to 3153 ppm in the 7 batches tested, while the concentration hydrazine was found to be less than 2 ppm. These two impurities should be accurately quantified in each batch.

3.1.6 Solubility

A) Active substance

Solubility (at 25°C after 24 h if not otherwise stated) in water and organic solvents

Water (at 20°C):	< 0.02 µg/L (Pilot batch LP130, OECD 105 / EEC A.6)
DMF:	0.60 mg/mL (Pilot batch LP110, similar protocol to OECD 105)
DMSO:	0.10mg/mL (Pilot batch LP110, similar protocol to OECD 105)
NMP:	4.20 mg/mL (Pilot batch LP110, similar protocol to OECD 105)
PEG400:	0.035 mg/mL (Pilot batch LP110, similar protocol to OECD 105)
Isopropyl Palmitate:	0.010 mg/mL (Pilot batch LP110, similar protocol to OECD 105)
n-octanol:	0.0048 mg/L (batch LP110 Test not performed according to GLP or to a guideline, but in a well-trained internal research laboratory, according to internal procedures).

In principle, the solubility of WP30 in several solvents was measured in saturated solutions by HPLC. The chromatograms of the test solutions presented one main peak of WP30 (> 95% area). Thus, WP30 was shown to be largely insoluble in each of the tested solvents.
In addition, the solubility of WP30 (batch LP110) in aqueous 0.9% NaCl containing 3% bovine serum albumin was determined. However, it should be noted that the assay was performed on radio-labelled raw material and results derived from extrapolation of total radioactivity. The test was unable to precise the free fraction of WP30 versus the bound fraction. Thus, the derived value of 4.56 µg/mL cannot be used as a reference for all other *in vitro* studies.
Solubility in Acetone / olive oil mixture (3/1) and Paraffin oil was also determined: 0,00017% w/w and 0,013% w/w respectively (at 25°C after 24 h).

Equilibrium solubility of S02374 active substance was studied in 19 solvents (Table 9).

Table 9. S02374 active substance equilibrium solubility in tested solvents

Solvent	S02374 Equilibrium Solubility (mg/mL)	Comment
Chloroform	11.3	Sparingly soluble
NMP	4.00	Slightly soluble
[1 % DMA + 3 % (DMEM with Glutamax supplemented with 10 % FCS)] in HBSSc buffer	< 0.0004 (< LOQ)	Practically insoluble
[1 % DMSO + 3 % (DMEM with Glutamax supplemented with 10 % FCS)] in HBSSc buffer	< 0.0004 (< LOQ)	Practically insoluble
1 % DMSO in (McAvoy 5A supplemented with 10 % FCS)	< 0.0004 (< LOQ)	Practically insoluble
3 % BSA in 0.9 % NaCl solution	< 0.0004 (< LOQ)	Practically insoluble
0.5% CMC	< 0.0004 (< LOQ)	Practically insoluble
0.5 % CMC in 0.9 % NaCl solution	< 0.0004 (< LOQ)	Practically insoluble
1 % DMSO in (RPMI supplemented with 10 % FCS)	< 0.00004 (ND)	Practically insoluble
H_2O	< 0.00004 (ND)	Practically insoluble
0.9 % NaCl	< 0.00004 (ND)	Practically insoluble
1% DMA in HBSSc buffer	< 0.00004 (ND)	Practically insoluble
1% DMSO in HBSSc buffer	< 0.00004 (ND)	Practically insoluble
DMA	0.863	Very slightly soluble
DMF	0.511	Very slightly soluble
DMSO	0.0958	Practically insoluble
3/1 v/v acetone/olive oil	0.0915	Practically insoluble
Corn oil	0.00720	Practically insoluble
Paraffin oil	0.0109	Practically insoluble

S02374 active substance is sparingly soluble in chloroform and slightly soluble in NMP.
S02374 active substance is very slightly soluble in DMA and in DMF; and practically insoluble in DMSO, corn oil, paraffin oil and 3/1 v/v acetone/olive oil.
S02374 active substance is practically insoluble in the following aqueous solvents:

- [1% DMA +3% (DMEM with Glutamax supplemented with 10% FCS)] in HBSSc buffer
- [1% DMSO +3% (DMEM with Glutamax supplemented with 10% FCS)] in HBSSc buffer
- 1% DMSO in (RPMI supplemented with 10% FCS)
- 1% DMSO in (McAvoy 5A supplemented with 10% FCS)

- 3% BSA in 0.9% NaCl solution
- H_2O
- 0.9% NaCl
- 0.5% CMC
- 0.5% CMC in 0.9% NaCl solution
- 1% DMA in HBSSc buffer
- 1% DMSO in HBSSc buffer

For the 11 aqueous solvents tested, WP30 was either not detected (ND) or its concentration was less than LOQ. **Therefore, it can be concluded that WP30 is practically insoluble in all aqueous solvents tested in the presence or absence of proteins.**

Stability was studied in 16 solvents (see details in Table 10). The solvents were divided into 3 groups:
Group 1: Solvents where WP30 is soluble
Group 2: Protein-containing solvents
Group 3: All other solvents

Table 10 Equilibrium solubility and stability summary

Solvent	Group	Equilibrium solubility	Stability	S02374 concentration	Stability time-points
NMP	1	X	n/a	n/a	n/a
Chloroform		X	n/a	n/a	n/a
[1 % DMA + 3 % (DMEM with Glutamax supplemented with 10 % FCS)] in HBSSc buffer	2	X	X	max equilibrium conc.	24 hours
[1 % DMSO + 3 % (DMEM with Glutamax supplemented with 10 % FCS)] in HBSSc buffer		X	X	max equilibrium conc.	24 hours
1 % DMSO in (RPMI supplemented with 10 % FCS)		X	X	max equilibrium conc. and 2 % w/v suspension	24 hours
1 % DMSO in (McAvoy 5A supplemented with 10 % FCS)		X	X	max equilibrium conc.	24 hours
3 % BSA in 0.9 % NaCl solution		X	X	max equilibrium conc.	24 hours
H_2O	3	X	X	10% w/v and 50 % w/v suspensions	24 hours / 5 days
0.9 % NaCl		X	X	10% w/v suspension	24 hours / 5 days
DMSO		X	X	max equilibrium conc. and 2 % w/v suspension	24 hours / 5 days
DMA		X	X	max equilibrium conc.	24 hours
0.5 % CMC		X	X	10 % w/v suspension	24 hours
0.5 % CMC in 0.9 % NaCl solution		X	X	10 % w/v suspension	24 hours
Corn oil		X	X	10 % w/v suspension	24 hours
Paraffin oil		X	X	10 % w/v suspension	24 hours
3/1 v/v acetone/olive oil		X	X	10 % w/v suspension	24 hours
1% DMA in HBSSc buffer		X	X	max equilibrium conc.	24 hours
1% DMSO in HBSSc buffer		X	X	max equilibrium conc.	24 hours
DMF		X	n/a	n/a	n/a

S02374 active substance is stable in DMSO at the equilibrium solubility concentration for at least 5 days and in DMA at the equilibrium solubility concentration for at least 24 hours.
2% w/v suspensions of S02374 active substance in DMSO are stable for at least 5 days and 2% w/v suspensions of S02374 active substance in 1% DMSO in (RPMI supplemented with 10% FCS) are stable for at least 24 hours. The stability of S02374 active substance in other

solvents tested could not be evaluated because of the large variability in results due to the formation of aggregates upon addition of the solvent.

Ref. 10, 11, 65, 81 (submission II)

SCCS comment
It appears that 5,6,5',6'-tetraphenyl-3,3'-(1,4-Phenylene)bis(1,2,4-Triazine) is insoluble in water (solubility <0.02 µg/L).
A solubility of 4.56 µg/ml of WP30, batch LP110 in aqueous 0.9% NaCl containing 3% bovine serum albumin was reported (see section 3.1.4).

B) Cosmetic ingredient S02771
Solubility in DMSO 0.2 mg/ml

Ref. 36 (submission I)

SCCS Comment
Compared to the active substance (S02374) which is sparingly insoluble in DMSO (0.10 mg/ml) (Ref 81), the cosmetic ingredient S0771 is formulated with an emulsifier (Eumulgin L), a preservative (Benzoic acid) and water and presumably has a slightly different solubility.

3.1.7 Partition coefficient (Log P_{ow})

Test performed on Pilot batch LP130
Log P_{ow} = 8.29 – calculated
Log P_{ow}= 10.5 - Measured (OECD 117 / EC A.8)

Ref. 8, 9 (submission II)

3.1.8 Additional physicochemical specifications

A) Active substance

Melting point: 321°C (measured on Pilot batch LP130) Ref. 10

Boiling point: calculated value: 758 ± 70 °C

Flash point: calculated value: 320 ± 28 °C

Density (mass/volume):

- Calculated: 1.23 ± 0.06 g/cm³
- Measured: 1.30 ± 0.015 g/cm³ (Pilot batch LP130, EU Method A.3)

Explosive properties: Not explosive

Oxidizing properties: Absence of oxidizing properties

pKa:

- calculated value: pKa 1: -0.57 ± 0.63
- calculated value: pKa 2: -2.02 ± 0.63

Particle size distribution before grinding was measured on Pilot batch LP130 with Mastersizer 2000 (Malvern Instruments Ltd) based on Laser Diffraction technology (see figure A). This was a non-GLP study and no guideline was followed.
The results were as follows:

- d10: 15.22 µm (volume)
- d50: 38.19 µm (volume)
- d90: 86.06 µm (volume)

- d100-d0: 152.52 µm (volume)
- dmean: 45.04 µm (volume)

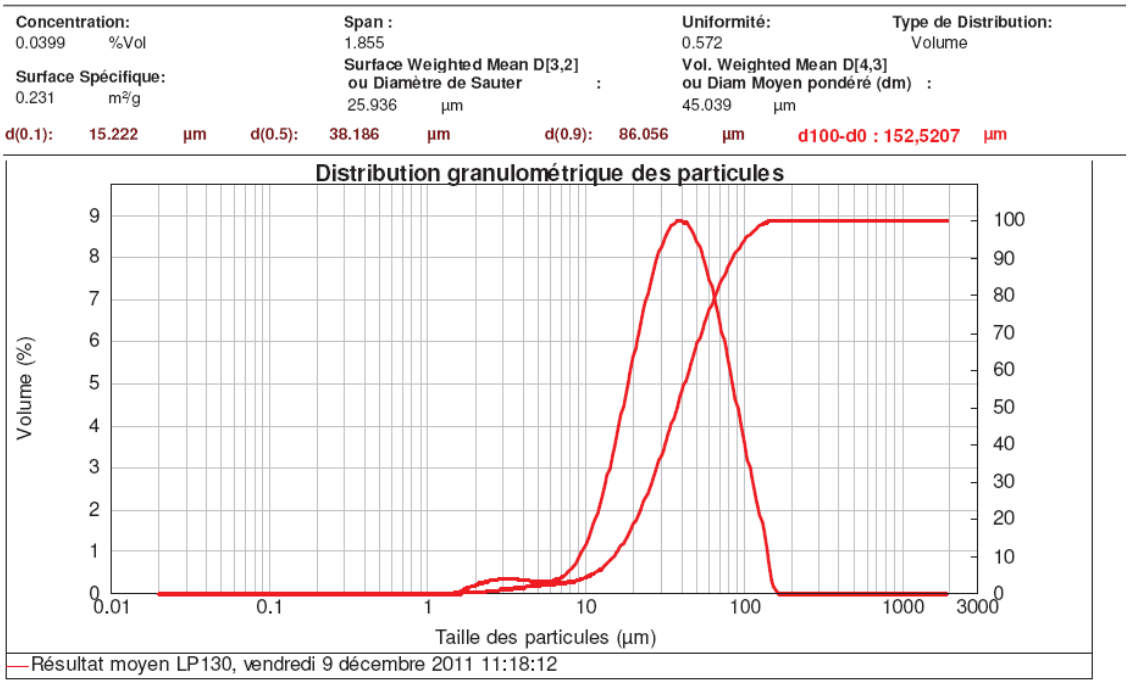


Figure A. Particle size distribution

UV-vis spectrum

The test was performed on Pilot batch LP140 (290 – 400 nm)
Sample: 10 µg/ml in dichloromethane.
The maximum absorption wavelength was at λ_{max} 329 nm

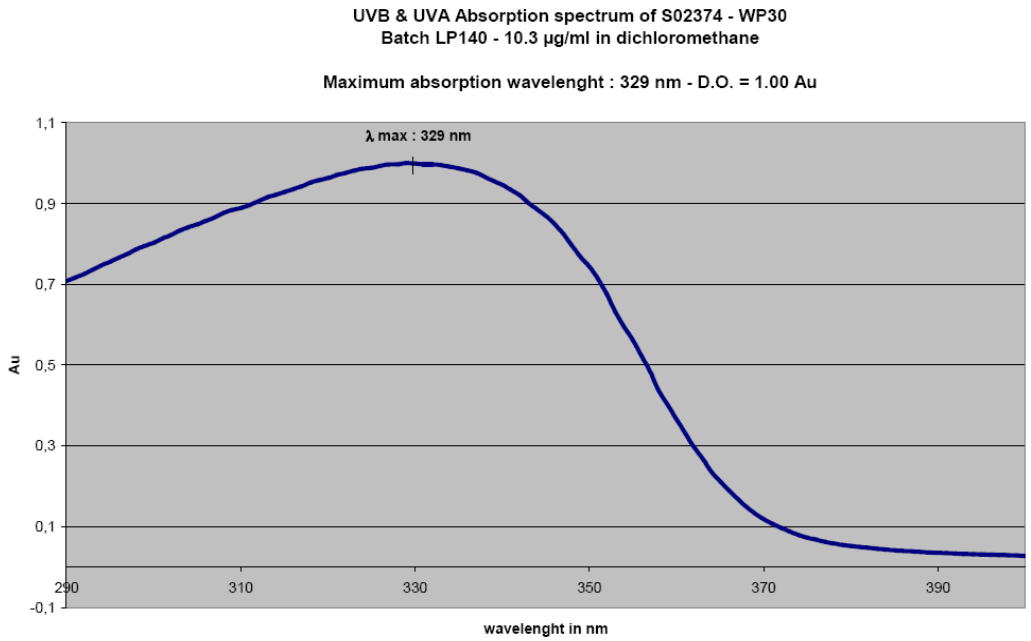


Figure B

Ref. 9, 10 (submission II)

SCCS comment
Based on the bi-modal granulometric distribution (i.e. one first small peak centered at about 3 -4 micrometers and a second large one centered at about 40 micrometers), the d_{mean} has clearly no physical meaning. The granulometric distribution exhibits 2 (two) particle groups.

B) Cosmetic ingredient S02771 (tests performed on ground batch LP110):

Density: 0.9 - 1.1
pH: 3.5-4.5
Dry extract: 48.9 % (w/w)

Ref. 28, 33, 78 (submission I)

Particle size distribution of typical batches:
Particle size distribution was measured on two ground test batches, LPX09-1 and LPX09-2 and batch No. LP110 with Mastersizer 2000 (Malvern Instruments Ltd) and with Horiba LA950 (Horiba Scientific) based on Laser Diffraction technology. Based on this technology, the results expressed either in particle volume or number showed a particle size distribution beyond the threshold of 100nm.

Table 11

S02771 Batch	**LPFX09-1**		**LPFX09-2**		**LP110**		
Particle size analyzer	Malvern Mastersizer 2000	Horiba LA950	Malvern Mastersizer 2000	Horiba LA950	Malvern Mastersizer 2000	Malvern Mastersizer 2000	Horiba LA950
Measurement	volume		volume		volume	**Number**	volume
d5	150 nm	-	150 nm	-	140 nm	130 nm	-
d10	160 nm	-	160 nm	-	150 nm	140 nm	-
d50	**270 nm**	**176 nm**	**260 nm**	**181 nm**	**250 nm**	**170 nm**	**184 nm**
d75	590 nm	-	440 nm	-	450 nm	200 nm	-
d90	**1850 nm**	**253 nm**	**1170 nm**	**258 nm**	**1320 nm**	**240 nm**	**255 nm**
d95	2810 nm	-	1570 nm	-	1940 nm	280 nm	-
d99	-	347 nm	-	352 nm	-	-	334 nm

Ref. 15, 16, 17, 79 (submission I)

In addition to the batch LP110 manufactured in June 2010 and used for the toxicological studies, several ground batches were manufactured in June and July 2014. These batches (LP150, LP160, LP170, LP180; semi-industrial batch size) were manufactured for industrial development purposes. The physical and chemical analysis results are within the specifications and similar to the results obtained for batch LP110.

Table 12

S02771 Batch	LP150		LP160		LP170		LP180	
Particle size analyzer	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS
Measurement	Intensity*	**Number**	Intensity*	**Number**	Intensity*	**Number**	Intensity*	**Number**
d10		90 nm		111 nm		84 nm		115 nm
d50	263 nm	**130 nm**	289 nm	**165 nm**	268 nm	**129 nm**	252 nm	**153 nm**
d90	420 nm	**212 nm**	620 nm	**308 nm**	420 nm	**235 nm**	420 nm	**257 nm**

** Average of several measurements*

SCCS conclusion
The analysis of the size distribution of the particles shows that S02771 is outside the definition given by EU Regulation on cosmetic products (EC) No 1223/2009.

UV-Vis spectrum:
The spectrum was performed on a cream (w/o emulsion) containing 10% of S02771 (batch LP110)

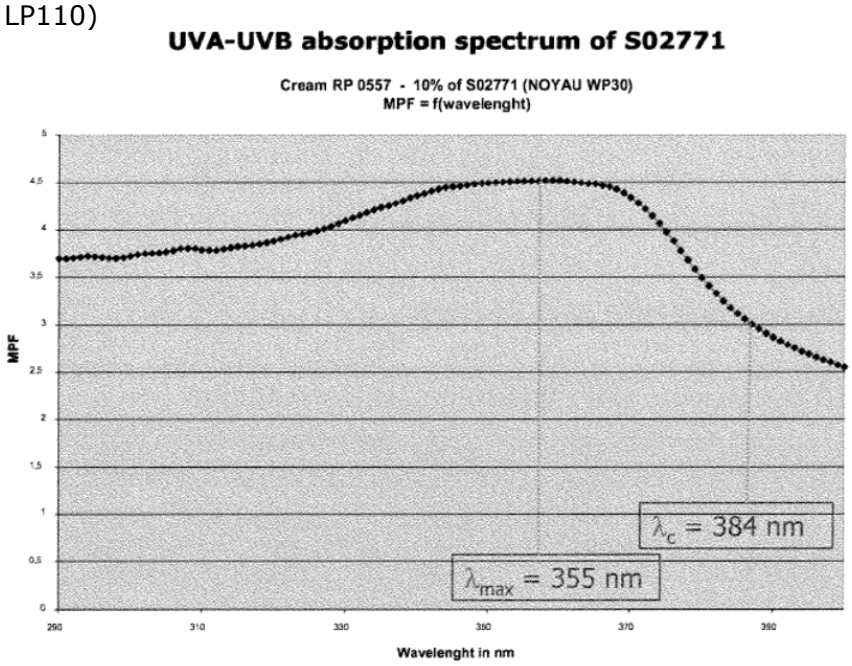


Figure C

The specific extinction coefficient ε is equal to 52492 L mol-1 cm-1.
The variation of the maximum wavelength between powder form (active substance S02374, solubilised in an organic solvent) and aqueous suspension (cosmetic ingredient S02771, formulated at 10% w/w in an emulsion cream) is mainly due to the finely dispersed solid

form of the active substance and secondarily to the other ingredients of the cosmetic formulation.

3.1.9 Homogeneity and Stability

A) Active substance S02374 (WP30):

Saturating concentration of WP30 in various solvents and excipients; and stability after 1 month at room temperature in the dark.

Table 13

	WP30 saturating concentration (mg/mL) at 25 °C		**WP30 concentration (mg/mL)**
Solvents/excipients	**Stirring time T 0.5 h**	**Stirring time T 15 h***	**Stability time RT dark – 1 month**
DMF	0.59	0.60	0.58
DMSO	0.11	0.10	0.11
PEG 400	0.034	0.035	0.035
NMP	3.99	4.20	3.92
Myritol 318	n.d.	n.d.	n.d.
Isopropyl palmitate	0.01	0.01	0.011

Ref. 11 (submission II)

Stability data of WP30 in the sample solutions stored in the injector tray at 5±3°C, protected from light, are presented in the Table 14:

Table 14

Sample solution	# Prep.	WP30 concentration (µg/mL)			% Mean recovery vs T_0	
		T_0	T_{24h}	T_{49h}	T_{24h}	T_{49h}
1% $CHCl_3$ in NMP (50.0 µg/mL)	1	50.2	50.2	50.0	99	100
	2	49.7	49.0	49.5		
H_2O/NMP (1/1, v/v) (1.00 µg/mL)	1	1.09	1.11	1.06	101	98
	2	1.06	1.05	1.04		
DMA/NMP (1/1, v/v) (1.00 µg/mL)	1	1.04	1.02	1.03	99	99
	2	1.03	1.02	1.03		
DMSO/NMP (1/1, v/v) (1.00 µg/mL)	1	1.04	1.03	1.02	100	99
	2	1.04	1.04	1.03		
NMP phase sample from NMP/paraffin oil (1/1, v/v) (50.0 µg/mL)	1	52.1	52.2	51.6	100	99
	2	51.9	52.3	51.5		

Ref. 82 (submission II)

Photo-stability data:

The photo-stability of the active substance S02374 was investigated. The test was performed under GLP conditions on batch LP110 according to ICH Topic Q1B.

The purity was controlled with HPLC-UV after irradiation of the sample. Results are described in the Table 15:

Table 15

Analysis	Specification	S02374 (Active substance)
Appearance	Comparable to non stressed sample	Comparable to non stressed sample
Assay of S02374	Recovery % [90% - 110%] compared to non stressed sample	99%

The active substance S02374 is not light sensitive under the test conditions.

Ref. 17 (submission II)

B) Cosmetic ingredient S02771

The stability of S02771 was investigated when stored under different conditions (+4°C, +25°C, +40°C, in the dark, exposed to daylight). Analysis and reanalysis of batch LP110 (ground) were performed as follows and the results indicate that the cosmetic ingredient is stable at room temperature for at least 18 months:

Table 16

	First Analysis	Reanalysis 12 December 2011
Appearance	Suspension	Suspension
Color	Yellow	Yellow
pH		3.13
Dry extract p.cent (w/w)	48.9	49.1
Water content p.cent (w/w)	49.3	48
Sum of impurities p.cent (w/w)	0.21	0.21
Benzoic acid content By HPLC p.cent (w/w)	0.2	0.2
Microbial contamination Total aerobic microbial count (UFC/g) Total Yeast and moulds count (UFC/g)	 <10 <10	 <10 <10
Assay by HPLC at 320nm p.cent (w/w) Dosage of S02374	44.6	45.0

It was concluded that the pH of S02771 when stored for one year at +4 °C is stable. It decreased over time at room temperature (in the dark and exposed to daylight) and at 40°C.

Ref: 33, 34, 35 (submission I)

Photo-stability data:

The photo-stability of the cosmetic ingredient S02771 was investigated. The test was performed under GLP conditions on ground batch LP110 according to ICH Topic Q1B.

The purity was controlled with HPLC-UV after irradiation of the sample. Results are described in the table Table 17:

Table 17

Analysis	Specification	S02771 (Cosmetic ingredient)
Appearance	Comparable to non stressed sample	Comparable to non stressed sample
Assay of S02374	Recovery % [90% - 110%] compared to non stressed sample	98%

The cosmetic ingredient S02771 is not light -sensitive under the test conditions.

Ref. 22 (submission I)

Stability of S02771 in solvents and suspensions (batch LP110, ground active substance): Solvents used for toxicological studies were DMSO, NaCl solution, Carboxymethylcellulose (CMC) gel and water, and the test results were summarised:

The following test items:

- LP110 at 0.2 mg/ml in DMSO remained stable at 72 h, at ambient temperature (AT) and at 37°C,
- LP110 at 10 % (w/w) in NaCI at 0.9 % remained stable at 24h, at AT and at 37^0C,
- LP110 at 6.25 % (w/w) in water remained stable at 24h at AT,
- LP110 at 15 % (w/w) in water remained stable at 24h at AT,
- LP110 at 25 % (w/w) in water remained stable at 24h at AT,
- LP110 at 50 % (w/w) in water remained stable at 24h at AT.

Ref. 36 (submission I)

Furthermore, the test item "10% of S02771 dispersed in a 0.5% CMC gel" remained stable and homogeneous for at least 5 days stored at ambient temperature (in the dark) and at 4°C.

Ref. 37 (submission I)

Due to the very low solubility of the active substance, the test item remained a suspension in the solvent (ground active substance with a d(50)= 170 - 270 nm), except in one case with DMSO at a very low solubility (0.2 mg/ml).

SCCS comment
The SCCS notes a considerable difference in retention times between the first HPLC chromatography and those after one year (ref. 34).
Peak purity of the test compound should have been evaluated at λmax 332nm for the stability studies. NMP, which is a manufacturing impurity of the test compound, was used as a dilution solvent prior to the HPLC analysis in the photostability studies.

Overall SCCS Comments to physico-chemical characterisation
Purity of Phenylene bis-diphenyltriazine described on the basis of HPLC-UV detection cannot be accepted because 1) it was not documented that all of the test substance loaded on the HPLC column was eluted (2) the UV detection of the active substance was not performed at a specific wavelength (λmax).

Impurities have been chemically characterised by the use of HPLC-PDA and LC-HRMS based on fragmentation pattern analysis. NMP and hydrazine have been identified as SVHC by ECHA and were included on 20 June 2011 in the Candidate List of substances for eventual inclusion in Annex XIV of REACH (ED/31/2011). NMP and hydrazine are, according to the applicant, unavoidable manufacturing impurities of NMP concentration, which varied from 830 to 3153 ppm in the 7 batches tested, while the concentration of hydrazine was found to be less than 2 ppm. These two impurities should be accurately quantified in each batch.

For the cosmetic ingredient S02771, no solubility data of the ground active substance was provided except for DMSO. Compared to the active substance (S02374) which is sparingly insoluble in DMSO (0.10 mg/ml) (Ref 81), the cosmetic ingredient S02771 is formulated with an emulsifier (Eumulgin L), a preservative (Benzoic acid) and water and presumably has a slightly different solubility.

For the 11 aqueous solvents tested, WP30 was either not detected (ND) or its concentration was less than LOQ. Therefore, it can be concluded that WP30 is practically insoluble in all aqueous solvents tested in the presence or absence of proteins. It appears that 5,6,5',6'-tetraphenyl-3,3'-(1,4-Phenylene)bis(1,2,4-Triazine) is insoluble in water (solubility <0.02 µg/L). A solubility of 4.56 µg/ml of WP30, batch LP110 in aqueous 0.9% NaCl containing 3% bovine serum albumin was reported (see section 3.1.4). The report was not provided.

The active ingredient is considered stable when stored protected from light and moisture (Ref. https://echa.europa.eu/registration-dossier/-/registered-dossier/6106/4/8).

3.2 Function and uses

The active ingredient, Phenylene bis-diphenyltriazine (WP30, S02374) will be wet grinded with water and will be formulated to a commercial cosmetic ingredient (NOYAU WP30, S02771). This resulting commercial ingredient NOYAU WP30 (S02771) is a 40 - 50% aqueous suspension of the active substance WP30 (S02374). In addition, the cosmetic ingredient (NOYAU WP30 S02771) is further formulated with an emulsifier (Eumulgin L) and a preservative (Benzoic acid).

The cosmetic ingredient (NOYAU WP30, S02771) is intended to be used as a UV filter in sunscreen products in a concentration of up to a maximum content of 10%. Consequently, the active ingredient, Phenylene bis-diphenyltriazine (WP30, S02374) will be a constituent in final sunscreen products at a concentration of up to a maximum of 5%.

3.3 Toxicological evaluation

The Table 18 provides an overview of the guidelines and/or GLP followed by each study reviewed in this submission. The testing guidelines cited are those in force at the time the studies were conducted. In addition, scientifically reliable studies are also listed for sake of completeness.

Table 18

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
ACUTE TOXICITY				
Oral				
Acute oral toxicity in rats, 2010	WP30 (batch: LP 110 (purity: 99.6%))	OECD TG 423	Yes	IRFP 2010
Dermal				
Acute dermal toxicity	WP30	OECD TG 404 and	Yes	IRFP 2012,

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
in rats, 2011	(batch: LP 110 (purity: 99.6%))	402		35
SKIN IRRITATION				
MTT Direct interaction test, 2009a	WP30 (batch: LP 110 (purity: 99.6%))	Annex 1B.40, Directive 2000/33/CE	Yes	Eurosafe 2009 36
Human reconstructed epidermis (tissues), 2016*	WP30 (batch: LT 30 (purity: 99.4%))	OECD 439	Yes	CiToxLab 2016a 53
EYE IRRITATION				
Mucous membrane irritation, *in vitro* HET-CAM test, 2009b	WP30 (batch: LP 110 (purity: 99.6%))	JORF[1] of 26/12/96, French decree of November 29th, 1996	Yes	Eurosafe 2009 37
Mucous membrane irritation, *in vitro* neutral red release (NRR) assay, 2009c	WP30 (batch: LP 110 (purity: 99.6%))	JORF[1] of 30/12/99, French decree of December 27th, 1999	Yes	Eurosafe 2009 38
Bovine Corneal Opacity and Permeability (BCOP), *in vitro**	WP30 (batch: LT 30 (purity: 99.4%))	OECD 437	Yes	CiToxLab 2016b 54
EpiOcular® cornea epithelial model*	WP30 (batch: LT 30 (purity: 99.4%))	Based on OECD 492	Yes	CiToxLab 2016c 55
SKIN SENSITISATION				
Local lymph node assay (LLNA), 2009	WP30 (batch: LP 110 (purity: 99.6%))	OECD TG 429 / EC B.42	Yes	Bioservice 2009 39
REPEATED DOSE TOXICITY				
Combined repeated dose toxicity study with the reproduction/developmental screening test, oral, rats, 2011	WP30 (batch: LP 140 (purity: 99.8))	OECD TG 422	Yes	CiToxLab 2012 34
13-week Oral Toxicity (Gavage) Study in the Wistar Rat Followed by a 4-week Treatment-free Period, 2012	NOYAU WP30 (batch: LP 110, purity: 100%)	OECD 408 Directive 96/54/EC, B. 26 Regulation (EC) No 440/2008	Yes	Harlan 2012 43
REPRODUCTIVE TOXICITY				
Combined repeated dose toxicity study with the reproduction/developmental screening test, oral, rats, 2011	WP30 (batch: LP 140 (purity: 99.8))	OECD TG 422	Yes	CiToxLab 2012 34,

[1] JORF: Journal Officiel de la République Française

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
Prenatal Developmental Toxicity Study; ORAL; RAT;	NOYAU WP30 (batch: LP 110, purity: 100%)	OECD 414	Yes	Harlan 2012 47
MUTAGENICITY/GENOTOXICITY: *IN VITRO*				
Bacterial gene mutation assay, *in vitro* 2009a	WP30 (batch: LP 110 (purity: 99.6%))	OECD TG 471 / EC B.13/14	Yes	Lemi 2009a 445
In vitro Mammalian Cell Gene Mutation Test, 2009b	WP30 (batch: LP 110 (purity: 99.6%))	OECD TG 476	Yes	Lemi 2009b 45
In vitro Mammalian Micronucleus test, 2009c	WP30 (batch: LP 110 (purity: 99.6%))	OECD TG 476	Yes	Lemi 2009b 45
PHOTO-INDUCED TOXICITY				
In vitro phototoxicity, 3T3, NRU, 2009	WP30 (batch: LP 110 (purity: 99.6%))	OECD 432	Yes	Eurosafe 2009 48
In vitro phototoxicity, MTT, 2010	WP30 (batch: LP 110 (purity: 99.6%))	According to Annex 1B.40, Directive 2000/33/CE	No	Eurosafe 2010 49
In vitro phototoxicity, 3T3, NRU, 2016*	WP30 (batch: LT 30 (purity: 99.4%))	OECD 432	Yes	CiToxLAB 2016d 56
In vitro phototoxicity, MTT, 2010*	WP30 (batch: LT 30 (purity: 99.4%))	Published methods: EPISKIN Phototoxicity Assay (EPA);	Yes	CiToxLAB 2016e 57
Photoirritation, Guinea pigs, 2012	NOYAU WP30 (batch: LP 110, purity: 100%)	According to an approved protocol	Yes	NOYAU WP30
Photosensitisation, Guinea pigs, 2011	NOYAU WP30 (batch: LP 110, purity: 100%)	According to an approved protocol	Yes	CERB 2011 52
Photomutagenicity *in vitro,* Bacterial gene mutation assay, 2012	NOYAU WP30 (batch: LP 110, purity: 100%)	CPMP/SWP/398/01 OECD 471	Yes	RTC 2012 84

* new submission

Introductory remarks:

This new submission II dossier is based on data of the active ingredient (Phenylene bis-diphenyltriazine, S86, WP30, S02374) including new experimental and analytical data. Only in cases where no or not sufficient data regarding a specific toxicological endpoint were available, this submission II dossier was supplemented with data of the cosmetic ingredient (NOYAU WP30, S02771) for bridging and read-across purposes.

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline: OECD 423

Species/strain:	Rat/Wistar RjHan: WI
Group size:	23 female animals in total
Test substance:	WP30
Batch:	LP110
Purity:	99.6% (HPLC)
Vehicle:	Corn oil (Sigma-Aldrich, Ref.C8267, batch 098K0008, expiry date: 28/05/2014)
Dose levels:	300 and 2000 mg/kg
Administration:	Oral (gavage)
GLP:	Yes
Study period:	13 August 2009 to 15 September 2009

Material and methods:

The objective of this study was to evaluate the potential acute toxicity of the test item when administered once orally, by gavage in female Wistar rats followed by 14 days of observation. The test item WP30, a fine yellow powder, was tested using a stepwise procedure with a maximum of 2 steps per dose-level; each step using 3 animals aged 8 to 12 weeks at the time of dosing. The starting dose was fixed at 300 mg/kg; 2000 mg/kg was then tested in the absence of mortality at 300 mg/kg. For both dose levels, two steps were performed. The test item was mixed with corn oil and administered at a dosing volume of 10 mL/kg. Parameters evaluated included mortality and clinical signs which were recorded twice daily. Body weights were measured on days 1, 3, 8 and 15 and necropsy findings noted for all animals on day15.

Results:

No mortality occurred and no abnormal clinical signs were observed at either dose level.

Body weight gain was not affected by treatment. No abnormal findings were noted in the macroscopic examination.

Conclusion:

Within the experimental conditions of the study, WP30 led to no mortality: the LD_{50} was estimated to be higher than 2500 mg/kg bw for female Wistar rats.

Ref. 32 (submission II)

3.3.1.2 Acute dermal toxicity

Guideline:	OECD TG 404 and 402
Species/strain:	Rat/Wistar RjHan: WI
Group size:	5 rats/ex
Test substance:	WP30
Batch:	LP110
Purity:	99.6% (HPLC)
Vehicle:	
Dose levels:	2000 mg/kg
Administration:	Single dermal application
GLP:	Yes
Study period:	06 October 2011 to 25 October 2011

Material and methods:

The objective of this study was to evaluate the potential toxicity of WP30 after a single topical application for a 24-hour exposure period on skin of Wistar rats. Five male and five female Wistar rats (RjHan: WI, SPF albino rats from Janvier) were treated at 2000 mg/kg corresponding to the limit test. On the day of application (D1), a weight-based quantity of

test item was moistened with purified water and applied directly onto the dorsal part of the trunk of each animal which had been clipped the day before. The test item was evenly spread over the application site which represented at least 10% of the body surface. The application site was then covered with a gauze patch held in close contact with the skin by means of a non-occlusive tape for a 24-h exposure period. At the end of the exposure period, the patch was removed and the test site was wiped with a cotton pad soaked with purified water. Animals were observed at least once daily for mortality and general clinical signs for 15 days and were weighed on D1, D3, D8 and D15. Skin reactions were evaluated for all animals approximately 1 hour (D2), 24h (D3), 48h (D4) and 72h (D5) after patch removal at the end of the contact period and on D8 and D11. The responses, erythema and edema, were scored according to a table graded from 0 (no skin reaction) up to 4 (severe reaction).

Results:

No death occurred during the course of the study and no abnormal clinical signs were observed.

Body weight:

For males, body weight evolution was not affected by the treatment.

For females, the treatment induced a slight body weight loss (-2 grams in average) observed in all females between D1 and D3 but weight increase was restored thereafter. This transient observation on body weight evolution was most probably related to the non-occlusive tape.

Skin reaction:

Neither erythema nor edema was observed so that the Individual Irritation Index was equal to 0 for all animals. Consequently, the Primary Irritation Index of the group was also equal to 0.

Desquamation was present on D5 (72 h reading time) in one female and on D8 in 2 females.

Conclusion:

Within the experimental conditions of the study, the potential acute dermal toxicity of WP30 was evaluated after a single topical application on Wistar rat skin. 5 males and 5 females were treated at 2000 mg/kg bw in a limit test for a 24-hour exposure period. No mortality was observed and the LD_{50} by dermal application was estimated to be higher than 2000 mg/kg bw. Furthermore, WP30 was not irritating to the skin of rats.

Ref. 35 (submission II)

3.3.1.3 Acute inhalation toxicity

***Taken from* SCCS/1556/15**

Cosmetic ingredient **S02771**

Designed as a dose range-finding study for a 14-day inhalation study, an acute nose-only inhalation study was performed. The study was not conducted under GLP. The fixed target aerosol concentration was 2.0 mg of the suspension, corresponding to about 0.92 mg of the ground active substance per litre of aerosol. Exposure times were 1, 2 and 4 hours per group. One rat of each gender was used for the 3 exposure groups (no control group was reported). No overt signs of toxicity were observed under these study conditions. For a more detailed description of the study, see 3.3.5.4.

Ref.: 85 (submission I)

SCCS comment (SCCS/1556/15)
The study results suggest low acute inhalation toxicity of the test item.

3.3.1.4 Acute intraperitoneal toxicity

No data submitted.

SCCS overall conclusion on acute toxicity
The acute toxicity of WP30 can be considered as very low.

The acute oral LD_{50} in rats > 2500 mg/kg bw.

In the acute dermal toxicity study in rats, no relevant systemic effects were noted and there was no sign of skin irritation. The dermal LD_{50} in rats was > 2000 mg/kg bw.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

In vivo

No standard skin irritation study in experimental animals is available.

However, in the acute dermal toxicity study in rats there was no sign of skin irritation (Ref. 35 (submission II))

In vitro

Taken from SCCS/1556/15

Skin irritation tests *in vitro* were performed with the active substance S02374 and the cosmetic ingredient S02771 by use of Reconstituted Human Epidermis (RHE) (SkinethicTM). For both studies, only a summary study report is available. Apart from insufficient documentation, there were shortcomings in study conduct.

SCCS comment (SCCS/1556/15)
On the basis of the *in vitro* studies provided on skin irritation, a skin irritation potential cannot be excluded for both the active (non-ground) substance S02374 and the cosmetic ingredient S02711 (ground form). However, no signs of skin irritation were noted in the acute dermal toxicity study in rats on the active substance S02374.

New submission

Guideline:	OECD 439 (July 2015), ECVAM Standard Operating Procedure version 1.2: Episkin™ Skin irritation test 42 hours Determination of IL-1α concentration in the culture medium
Test system:	Human reconstructed epidermis (Episkin™ tissues)
Replicates:	Triplicate tissues for each item
Test substance:	WP30

Batch:	LT30
Purity:	99.4%
Vehicle:	Paraffin oil
Concentration:	10% (w/v) in paraffin oil
Route:	topical
Exposure:	Single application (10 µl)
Exposure duration:	15 minutes
Post-treatment incubation time:	42 hours
Negative control:	Dulbecco's Phosphate-Buffered Saline (D-PBS)
Positive control:	Sodium Dodecyl Sulphate (SDS) at a 5% (w/v) aqueous solution
Direct interaction with MTT:	Negative
Colouring potential test item:	Negative
GLP:	Yes
Study period:	30 May 2016 – 2 September 2016

Material and methods:

A solubility test was performed prior to the preliminary tests in order to evaluate the solubility of the test item in paraffin oil at the preselected concentration (i.e. 10% (w/v)). The solubility of the test item in formulation was evaluated by visual inspection.

The skin irritation potential of the test item (WP30), diluted at 10% (w/v) in paraffin oil was investigated using the Episkin® reconstructed human epidermis model. Preliminary tests were performed to detect the ability of the test item formulation to directly reduce MTT as well as its colouring potential. Following the preliminary tests, the skin irritation potential of the test item at 10% (w/v) in paraffin oil was tested in the main test. The test item formulation and both the negative and positive controls were applied topically on triplicate tissues and incubated at room temperature for 15 minutes. At the end of the treatment period, each tissue was rinsed with D-PBS and incubated for 42 hours at +37°C, 5% CO_2 in a humidified incubator. The cell viability was then assessed by means of the colorimetric MTT reduction assay.

Relative viability values were calculated for each tissue and expressed as a percentage of the mean viability of the negative control tissues which was set at 100% (reference viability).

In addition, the concentration of the inflammatory mediator IL-1α was evaluated in the culture medium retained following the 42-hour recovery period. This quantification, based on an ELISA assay, was performed since the mean relative viability of the test item formulation-treated tissues was > 50% following the MTT reduction assay.

Results:

Preliminary tests

In the preliminary tests, the test item formulation was found not to have direct MTT reducing properties or colouring potential.

Main test

All acceptance criteria for the negative and positive controls were fulfilled. The study was therefore considered to be valid.

Following a 15-minute exposure and a 42-hour recovery period, the relative mean viability of the tissues treated with the test item formulation was 105% with a standard deviation of 10% as assessed by the MTT assay.

Table 19

Group	cOD		Viability (%)	
	Mean	SD	Mean	SD
Negative control	0.751	0.034	100	4
Positive control	0.068	0.017	9	2
W30	0.787	0.77	105	10

cOD = blank corrected optical density

SD = standard deviation

As the mean viability was > 50% after the MTT reduction, the IL-1α concentrations in culture media samples retained from the three negative controls and test item formulation-treated tissues were analysed by ELISA.

The IL-1α concentration value of one tissue was found below the limit of quantification (< 5.00 pg/mL). Consequently, the mean IL-1α concentration from the three test item formulation-treated tissues was not calculated. The IL-1α concentration values of the two other test item formulation-treated tissues were found < 60 pg/mL (16.0 and 17.0 pg/mL). Therefore, the results met the criteria for an *in vitro* classification as non-irritant to skin.

The study was performed according to the OECD test guideline of 28 July 2015, with the following exceptions:

- the pre-incubation of tissues was performed for at least 24 hours instead of between 18 and 24h,

- the colouring potential was evaluated in water by the naked eye instead of by spectral analysis in water and/or isopropanol.

The above exceptions are however part of the EpiskinTM test method validated SOP (i.e. ECVAM DB-ALM Protocol No. 131: EpiskinTM Skin irritation test method 15 min-42 hours) which was used in this study; and were therefore considered not to have any impact on the validity of this present study.

Conclusion:

Under the experimental conditions of this study, the test item, diluted at 10% (w/v) in paraffin oil, is considered to be non-irritant to skin, when tested within the Episkin® reconstructed human epidermis model.

This result is in line with a former *in vitro* study of lower reliability on WP30 (batch LP100, purity: 99.6%) performed on reconstituted human epidermis (RHE) derived normal human keratinocytes. In this study, WP 30 at a concentration of 10% dissolved in paraffin oil did not reduce MTT and was also shown to reveal no skin irritant potential under the test conditions.

Ref. 53,65,66,67 (submission II)

SCCS comment

Information on the barrier function of the epidermis tissue is not provided.
References to historical data of the model with regard to acceptability ranges of the positive and negative controls are not mentioned in the report.
IL-1α release has been used as an adjunct to the OECD guideline EpiSkin viability test to increase the sensitivity of the assay. SCCS notes that the cut-off limit for mean IL-1α stated in the SOP published by ECVAM is 50 pg/ml and not 60 pg/ml.

Overall SCCS conclusion on skin irritation
On the basis of the results obtained in the new Episkin™ study, it can be concluded that WP30 diluted at 10% (w/v) in paraffin oil is not a skin irritant. Also, no signs of skin irritation were noted in the acute dermal toxicity study in rats on the active substance SO2374. In addition, new BCOP and EpiOcular *in vitro* tests indicate that S02374 is not irritant to the eye and therefore it is very unlikely to be a skin irritant.

3.3.2.2 Mucous membrane irritation / Eye irritation

Taken from SCCS/1556/15

Eye irritation tests *in vitro* were performed with the active substance **S02374** and the cosmetic ingredient **S02771** by use of both the HET-CAM Test and the Neutral Red Uptake test. For both studies, only a summary study report is available. Apart from insufficient documentation, there were shortcomings in study conduct.

SCCS comment (SCCS/1556/15)
As no conclusions can be drawn based on the eye irritation studies, an eye irritation potential of the test items (active substance SO2374 and the cosmetic ingredient SO2711) cannot be excluded.

New submission

Guideline/method:	OECD 437 (July 2013)
GLP:	Yes
Test system:	Corneas obtained from freshly slaughtered calves
Replicates:	Triplicate tissues
Test substance:	WP30
Batch:	LT30 (purity: 99.4%)
Concentration:	10% (w/v) in paraffin oil
Vehicle:	Paraffin oil
Positive control:	20% imidazole solution in 0.9% NaCl (w/v)
Route:	topically to the cornea
Exposure:	Single application
Exposure duration:	4 hours
Study period:	08 June 2016 – 09 June 2016

Material and methods:

The eye irritation potential of the test item (WP30), diluted at 10% (w/v) in paraffin oil was investigated using the Bovine Corneal Opacity and Permeability (BCOP) test method. Corneas obtained from freshly slaughtered calves were mounted in corneal holders. Both chambers of the corneal holder were filled with complemented MEM culture media (cMEM) and pre-incubated for 1 hour (± 5 minutes) at +32°C. A single experiment was performed using three corneas for each treated series (test item, positive and vehicle controls). Before the treatment, a first opacity measurement was performed on each cornea using an opacitometer. The test item was applied at the concentration of 10% (w/v) in paraffin oil in a single experiment using a treatment time of 4 hours and using the open-chamber method. Vehicle and positive controls were applied using the same treatment time and the closed-chamber method. At the completion of the treatment period, all items were removed from the front opening of the anterior chamber and the epithelia were rinsed. A second opacity measurement was then performed. After the second opacity measurement, the medium of

the anterior chamber was removed and filled with a fluorescein solution. The holders were then incubated vertically for 90 minutes (± 5 minutes) at +32°C. At the end of the incubation period, the Optical Density of the solution from the posterior chamber of each holder was measured in order to determine the permeability of the cornea. Each cornea was then observed for opaque spots and other irregularities.

Results:

Macroscopic examination

No notable opaque spots or irregularities were observed on test item-treated corneas.

In vitro Irritancy Score

With one exception (mean OD490 nm of the vehicle control), all acceptance criteria were fulfilled. The study was therefore considered as valid.

The mean *In vitro* Irritancy Score (IVIS) of the test item-treated corneas was 0. As the mean IVIS was < 3, the test item was considered as a test chemical not requiring classification for eye irritation or serious eye damage (UN GHS No Category).

Ref. 54 (submission II)

SCCS comment
On the basis of the results obtained in the BCOP study, it can be concluded that WP30 diluted to 10% (w/v) in paraffin oil is not a strong eye irritant. This, however, does not exclude a mild or moderate eye irritancy potential. Under the conditions of this study, an eye irritation potential of WP30 at 10% (w/v) cannot be excluded.

Guideline/method:	Based on OECD 492 (July 2015)
GLP:	Yes
Test system:	Reconstructed human Cornea-like Epithelium
Replicates:	Duplicate tissues
Test substance:	WP30
Batch:	LT30 (S02374, purity: 99.4%)
Concentration:	10% (w/v) in paraffin oil
Vehicle:	Paraffin oil
Positive control:	20% imidazole solution in 0.9% NaCl (w/v)
Negative control:	Sterile deionized water
Route:	topically to the cornea
Exposure:	Single application
Exposure duration:	30 min.
Study period:	09 June 2016 – 16 June 2016

Material and methods:

The acute eye irritation potential of the test item (WP30), diluted at 10% (w/v) in paraffin oil was investigated by measurement of its cytotoxic effect on the EpiOcular® cornea epithelial model. Preliminary tests were performed to detect the ability of the test item formulation to directly reduce MTT as well as its colouring potential. Following the preliminary tests, the eye irritation potential of the test item formulation was assessed in the main test. The test item formulation and both negative and positive controls were applied topically on duplicate tissues and incubated at +37°C for 30 minutes. At the end of the treatment period, each tissue was rinsed with D-PBS, incubated for 12 minutes at room temperature to remove any remaining test item formulation absorbed into the tissue, blotted on absorbent material, and then incubated for another 2 hours at 37°C, 5% CO2 in a humidified incubator. The cell viability was then assessed by means of the colorimetric MTT reduction assay. Mean viability values were calculated for each tissue and expressed as

a percentage of the mean viability of the negative control tissues which was set at 100% (reference viability).

Results:

Preliminary tests

In the preliminary tests, the test item formulation was found not to have direct MTT reducing properties or colouring potential.

Main test

With one exception (% difference between the viabilities of the two positive control-treated tissues), all acceptance criteria were fulfilled. The study was therefore considered to be valid.

The relative mean viability of the tissues treated with the test item formulation was 129% with a difference of 11% between duplicate tissues. As the mean viability was > 60% after the MTT reduction, the results met the criteria for a non-irritant response.

Conclusion:

Under the experimental conditions of this study, WP30 diluted at 10% (w/v) in paraffin oil is considered to be non-irritant to Reconstructed human Cornea-like Epithelium.

Ref. 55 (submission II)

The results reported above are in line with numerous former *in vitro* studies of lower reliability on WP30 (batch LP100, purity: 99.6%).

WP30 tested at a concentration of 10% in paraffin oil on the chorionallantoic membrane of the hen's egg (HET-CAM test revealed no irritant potential to the eye/mucous membranes.

Ref. 37, 68, 69, 70, 71 (submission II)

When WP30 was tested in the neutral red uptake (NRU test on rabbit corneal fibroblasts (SIRC) dissolved in 10% paraffin oil, it was shown to be practically not irritant to the mucous membranes.

Ref. 38 (submission II)

SCCS conclusion on eye irritation
Based on the newly submitted data, SCCS considers that the active ingredient WP30 at 10% w/v in paraffin oil is not irritating to the eye

3.3.3 Skin sensitisation

Taken from SCCS/1556/15

In the LLNA, the active (non-ground) substance **S02374** was tested at concentrations of 6.25%, 12,5% and 25% in Acetone/Olive Oil (AOO) and did not induce skin sensitisation. However, no data on the solubility of the test item in the vehicle AOO was provided and therefore the relevance of this study is unclear.

In another LLNA, the cosmetic ingredient **S02771** (containing 46.5% of the ground active substance in the suspension) was tested at concentrations of 6.25%, 12.5% and 25% and did not induce skin sensitisation. The test was performed with water as the vehicle. Wholly aqueous vehicles should be avoided in the LLNA, because those vehicles are likely to run off

the skin and the substance may not become bioavailable at all. Thus, the relevance of this test is questionable.

SCCS comment (SCCS/1556/15)
In conclusion, both tests conducted on skin sensitisation are considered inconclusive and a skin sensitising potential of the active substance S02374 and the cosmetic ingredient S02771 cannot be excluded.

New submission

No new skin sensitisation data were submitted, but the Applicant provided novel information on the solubility of the non-ground active substance S02374 in AOO. This information was used to re-evaluate the LLNA study provided in Submission I.

Local Lymph Node Assay (LLNA)

Guideline/method:	OECD 429, EC B.42, OPPTS 870.2600, EPA 712-C03-197
GLP:	Yes
Species/strain:	Female mice, CBA/CaOlaHsd, 8-9 weeks old
Group size:	3 (preliminary test) 5 per dose group (main test)
Test substance:	WP30
Batch:	LP110 (Purity: 99.6%)
Vehicle:	Acetone/olive oil (AOO), 3+1 (v/v)
Concentration:	Preliminary test: 25% (w/w) in AOO Main test: 6.25%, 12.5%, 25% (w/w) in AOO
Route:	Application to the dorsal surface of both ears on days 1, 2 and 3
Positive control:	Independent experiment with 1% p-Phenylenediamine in AOO 3+1 (v/v) conducted in September 2009
Negative control:	Vehicle alone (acetone/olive oil)
Study period:	06 October 2009 – 5 November 2009

Material and methods:

The sensitising potential of WP30 was tested in a Local Lymph Node Assay (LLNA) in mice using *in vivo* 5-bromo-20-deoxyuridine incorporation according to OECD 429, EC B.42, OPPTS 870.2600, EPA 712-C03-197 under GLP conditions. Evaluation of local irritation was carried out in parallel. The concentrations used were based on a preliminary study using a concentration of 25% (w/w) in AOO, in which no signs of toxicity or skin irritation were observed. Three dose groups and one negative control group (vehicle alone) were tested. Topical applications were performed once daily for 3 consecutive days. Five days after the first topical application, all animals received 20 µCi 3H-methyl thymidine by intravenous injection (tail vein) of 250 µl 0.9% saline. About 5 hours after the injection, the animals were sacrificed and the draining auricular lymph nodes were excised and weighed for each individual animal. After a standard work-up procedure, the radioactivity was determined.

Results:

The solubility of WP30 as suspension in AOO (3+1) was verified up to the investigated concentration of 10%.

None of the three tested concentrations of the test item reached the stimulation index of 3:

Concentration (%)	Stimulation index
6.25	1.2

12.5	0.9
25	1.1

All animals survived throughout the test period without showing any clinical signs.

All animals showed the excepted body weight gain, which includes a body weight reduction of up to 2 g throughout the study.

Results of radioactivity determination were supported by the means of the lymph node weights per group, which showed no significant difference compared to the negative control.

Conclusion:

Under the experimental conditions, WP30 was shown to have no skin sensitising properties and should therefore not be considered as a dermal sensitiser.

Ref. 39, 65 (submission II)

SCCS comments
The sensitising potential of the active substance S02374 was tested in the LLNA using *in vivo* ^{3}H-methyl thymidine incorporation and not 5-bromo-20-deoxyuridine. The solubility of the active substance S02374 in AOO is poor. The test substance probably forms a stable dispersion in this vehicle and was prepared freshly on each day of application. Due to the oily nature of the vehicle, SCCS assumes that the test substance, although not fully solubilised, will stick to the ears in the LLNA. S86 tested up to 25% is not considered as a skin sensitiser.

3.3.4 Dermal / percutaneous absorption

Taken from SCCS/1556/15

SCCS comment (SCCS/1556/15)
The SCCS notes that the study report indicating a high increase of solubility of the active substance in the receptor fluid in presence of bovine serum albumin is not available and should be provided (see 1.6).
The first study (not fully in line with SCCS basic criteria) yields 0.33 % dermal absorption (mean ± 2SD). In the second study, data on healthy skin cannot be used, whereas the results from irradiated skin could be taken into account (0.18 %).
For calculation of the SED, **0.33 %** dermal absorption, corresponding to 1.8 µg/cm^2 as worst case, can be used.
Both studies were most likely conducted with the non-ground active substance S02374, not with the ground cosmetic ingredient S02771. Based on the experience with other organic UV filters of high molecular weight containing nanomaterials, a low dermal absorption may also be expected for the ground active substance.

New submission

No new skin penetration data were submitted, but the Applicant provided novel information on the solubility of the non-ground active substance S02374 in AOO. This information was used to re-evaluate the studies provided in Submission I.

Guideline/method: OECD 428

GLP:	Yes
Test system:	Human dermatomed skin (0.3 ± 0.1 µm)
Membrane integrity:	TEWL test
Sample number:	3 human donors, 6 samples per donor
Test substance:	a) non-labelled: WP30 b) labelled: [^{14}C]-WP30
Batch:	a) LP110 (Purity: 99.6%) b) 07BLY032 (specific activity: 244 µCi/g, purity: 99.1%)
Doses applied:	10 mg of sunscreen formulation/cell (0.543 µCi/cell) containing 1 mg WP30
Dose:	5 mg/cm²
Receptor fluid:	3% bovine serum albumin in 0.9% NaCl solution
Exposure time:	24 h
Method of analysis:	Liquid scintillation counting
Study period:	31 August – 10 September 2010

Material and methods:

The aim of this study was to investigate the rate and extent of the *in vitro* dermal absorption of WP30 following topical application of the emulsion to the surface of human skin. Frozen stored skin samples (abdominal site) from 3 individual female donors were used in static diffusion cells. The dermatomed skin samples (mean thickness 0.27 ± 0.05 µm; application area 2 cm²) were included in the study if the Transepidermal Water Loss (TEWL) was ≤ 13 ± 3 g/m²/h for abdomen skin. The formulation tested contained 10% WP30 (w/w) corresponding to 10 mg of WP30 for 100 mg of formulation. Around 0.5 µCi was applied on each cell. Solubility of the test substance in the receptor fluid was 4.56 µg/mL. The receptor fluid was sampled at approximately 30 min, 2, 4, 6, 8 and 24 hours after application. One to six tape strips (average 3 - 4 strips) were used per sample to remove stratum corneum. The characterisation of the radio-labelled batch No. 07BLY032 was performed before this study. Stability of the formulation was tested over approximately 24 hours.

Results:

Results are expressed as % relative to the amount applied.

The individual total recovery of the radioactivity was between:

- 88% and 100% for donor 1.

- 87% and 98% for donor 2.

- 94% and 101% for donor 3.

All recoveries were within the acceptance criteria defined in the study plan.

All receptor compartment fluid analyses were below the limit of quantification (<0.07 ngeq/cm^2) with the exception of cell A at 30 min and cell C at 4 hours indicating that ^{14}C WP30 did not readily penetrate all the way through the skin samples.

According to the SCCP/0970/06 guidance, the absorbed fraction is considered to be the sum of the radioactivity measured in epidermis, dermis and receptor fluid.

The absorbed fractions were between:

- 0.33% and 0.04% for the donor 1

- 0.12% and 0.02% for the donor 2

- 0.16% and 0.03% for the donor 3

All evaluable cells combined from the three donors yielded a mean total recovery of the radioactivity of 94.38 ± 4.23 %. The absorbed fraction of the applied WP30 for the formulation was 0.33% (mean ± 2SD: 0.11 ± 0.22 %) of the applied dose corresponding to 1.8 ng eq/cm² (mean ± 2SD: 0.58 ± 1.20).

Discussion and conclusion:

The study was carried out with WP30 in the sunscreen formulation. The certificate of analysis of the radioactive non-ground active substance was enclosed in the report for information. The certificate of analysis of the ground active substance and the preparation of the test item are provided. The term "pulpe WP30" mentioned corresponds to the ground substance. A specific study was performed to determine whether the WP30 was the only compound solubilised in the receptor fluid or not (Reference: Add 17 PFDC 2015 77). This study confirmed that WP30 was the only material solubilised in the receptor fluid chosen for the skin absorption study, with no potential impurities. Therefore, this additional study validated the previously obtained results. It should be noted that there was a binding of WP30 to the proteins of the BSA as WP30 was only detected in the precipitate.

With regards to the reliability it should be considered that the drafting of the study plan was initiated when guidance SCCP/0970/06 was applicable (before June 2010). Therefore the study does not comply with the current guidance SCCS/1358/10, but nevertheless complies with the previous one. Six samples from 3 donors were used, corresponding to a total of 18 samples.

The mean total recovery of the radioactivity was 94% validating the results obtained with human skins. The absorbed fraction of the applied WP30 formulation was 0.33% of the applied dose corresponding to 1.8 ng eq/cm². These values will be used for risk assessment and the margin of safety calculation.

Ref. 24, 25, 41, 77 (submission II)

A further *in vitro* dermal penetration study on human dermatomed skin was performed to mimic the conditions for altered skin barrier and/or for solar irradiation compared with healthy skin.

Guideline/method:	OECD 428
GLP:	Yes
Test system:	Human dermatomed skin (0.3 ± 0.1 µm)
Membrane integrity:	TEWL test
Sample number:	4 human donors, 2 cells per donor in each condition
Conditions:	healthy skin, stripped, irradiated and stripped/irradiated
Test substance:	a) non-labelled: WP30 b) labelled: [^{14}C]-WP30
Batch:	a) LP110 (Purity: 99.6%) b) 07BLY032 (specific activity: 244 µCi/g, purity: 99.1%)
Doses applied:	10 mg of sunscreen formulation/cell (0.543 µCi/cell) containing 1 mg WP30
Dose:	5 mg/cm²
Receptor fluid:	3% bovine serum albumin in 0.9% NaCl solution
Exposure time:	24 h
Method of analysis:	Liquid scintillation counting
Study period:	14 November – 02 December 2011

Material and methods:

The aim of this study was to investigate the rate and extent of the *in vitro* dermal absorption of WP30 following topical application the surface of human skin to mimic the conditions for altered skin barrier and/or for solar irradiation. Human skin samples were obtained during abdominal surgery from four donors and then frozen. After thawing the skin was processed depending on test conditions by either stripping and/or irradiation (39 min a 3 MED (minimal erythema dose)), dermatomed to 300 ± 100 µm thickness and mounted into static diffusion cells, the application area was 2 cm². Healthy human skin was included in the study if the TEWL was ≤ 13 ± 3 g/m²/h. Radiochemical purity was checked before application of the formulation. Receptor fluid was sampled at approximately 1, 4 and 24 hours after application; solubility of the test substance in the receptor fluid was 4.56 µg/mL. At the end of the 24-h exposure time, tape stripping was performed and radioactivity in the different compartments was determined by liquid scintillation counting.

Results and conclusion:

Healthy skin

For the four donors, the mean of the amount bioavailable after 24 hours on healthy skin corresponding to the total amount recovered in the receiving compartment, the dermis and epidermis (excluding *stratum corneum*), is equal to 0.28% ± 0.22% of the amount deposited. As a consequence and considering the current SCCS procedure to use 2 standard deviations, a portion of 0.72% is considered as bioavailable and to be used for comparison with skin altered and/or subjected to solar irradiation.

Skin subjected to solar irradiation:

For the four donors, the mean of the bioavailable amount from irradiated skin, after 24 hours, corresponding to the total amount recovered in the receptor fluid, the dermis and epidermis (excluding *stratum corneum*) is equal to 0.10% ± 0.04% of the amount deposited. As a consequence and considering the current SCCS procedure to use 2 standard deviations, 0.18% is considered as bioavailable in the conditions mimicking irradiated skin.

Altered skin subjected to solar irradiation

For the four donors, the mean of the bioavailable amount from stripped skin subjected to solar irradiation after 24 hours corresponding to the total amount recovered in the receptor fluid, the dermis and epidermis (excluding *stratum corneum*) is equal to 0.53% ± 0.42% of the amount deposited. As a consequence and considering the current SCCS procedure to use 2 standard deviations, 1.37%, is considered as bioavailable to calculate the margin of safety in the conditions mimicking altered skin barrier and irradiated skin.

The amount absorbed is demonstrated in the figure D:

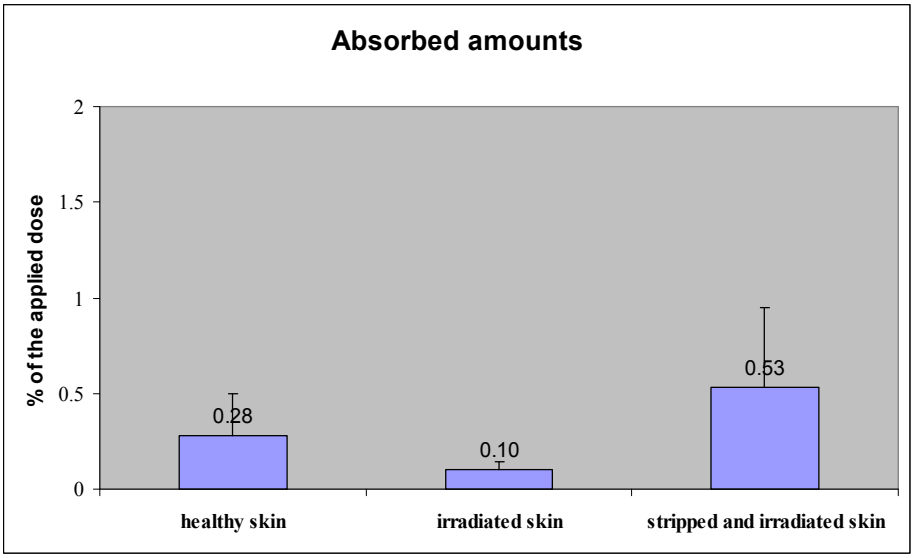


Figure D

The results show a low bioavailability of WP30 on healthy skin after application of 5 mg/cm² of a formulation such as "solar emulsion" containing 10% of WP30 for 24 hours.

Analysis of variance (ANOVA) with two factors (treatment and donor) complemented by a comparison of average pairwise shows no significant difference between healthy skin and irradiated skin but a significant difference between healthy skin and impaired or irradiated skin.

Finally, the skin bioavailability of WP30 remained very low, even for skin with altered barrier function and subjected to solar irradiation.

Ref. 40, 41 (submission II)

SCCS comment
The SCCS notes that there is no standard protocol to investigate skin penetration through damaged skin. The study using UV-irradiated skin showed no significant change in skin absorption compared to healthy skin. In the study using healthy skin, the mass balance criterium (between 85-115%) was not met for 6 out of 8 samples. The study authors say that "the investigation performed has not clearly identified the unexpected event but the Applicant hypothesised that the formulation could have been unhomogenous and that the exact amount of radioactivity applied was higher than expected." Therefore this study cannot be used to determine skin penetration value for the MoS calculation.

General conclusion on skin penetration
For the MoS calculation, SCCS would rely on the most appropriate study. The first study provided in the new submission and performed on healthy skin was considered by SCCS the more robust. However in this study only 3 donors were used. Based on the recommendation from the SCCS Notes of Guidance (2016), at least 8 evaluable samples from 4 different donors should be used. Therefore SCCS decided to use the mean +2SD, which the Applicant themselves also used in their study report Therefore 0.33% skin penetration will be used for the safety assessment of S86.

3.3.5 Repeated dose toxicity

Taken from SCCS/1556/15

Repeated dose toxicity

A 13-week oral toxicity test followed by a 4-week treatment-free period according to OECD 408 was performed on rats with the cosmetic ingredient S02771 containing 48.5% of the ground active substance in the suspension. After oral administration of S02771 to Wistar rats at doses of the suspension of 100, 300 and 1000 mg/kg/day, and based on histopathology, a NOEL (No Observed Effect Level) could be established at 300 mg/kg/day for males, at 1000 mg/kg/day for females. The NOAEL of the suspension could be established at 1000 mg/kg/day corresponding to a **NOAEL of the ground active substance at 485 mg/kg bw/day**, i.e. the highest dose level in the study.

One of the objectives of this study was to include parameters to assess possible endocrine disruptor properties of the test item. In the males, absence of effects on thyroid hormones, on sexual organ weights, on sexual and endocrine organ histopathology and on testicular staging allow to conclude the absence of visible endocrine disruptor effects of S02771 with regards to the endocrine parameters tested. In females, absence of effects on thyroid hormones, the absence of effects on the 2-week vaginal oestrus cycles, on sexual organ weights and on sexual and endocrine organ histopathology allow to conclude the absence of

visible endocrine disruptor effects of S02771 with regards to the endocrine parameters tested.

Additional supporting repeated dose toxicity data on the non-ground active substance S02374 were obtained from an OECD 422 study (i.e. a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test by oral route (gavage) in rats): Based on the experimental conditions of this study, the No Observed Effect Level (NOEL) was considered to be 1000 mg/kg/day.

A subacute inhalation toxicity study in rats (14-day exposure, nose-only, 4 hours per day) with the cosmetic ingredient S02771 was performed. The achieved aerosol concentrations of the suspension were 0, 0.63, 1.21 and 2.13 mg/L corresponding to 0, 0.29, 0.55 and 0.98 mg/L for the ground active substance.

The LOAEC is 0.63 mg/L and the LOAEL calculated from the inhalation exposure is 115 mg/kg bw/day for the test item, corresponding to 0.29 mg/L and 54 mg/kg bw/day for the ground active substance. A NOAEC or NOAEL value cannot be derived from this study due to the serious effects observed in the respiratory tract, possibly due to a particle overload effect of the test item particles that are barely soluble and persistent. No safe concentration for the use in spray applications can be derived.

The concentrations/doses for the repeated-dose inhalation study derived from an acute inhalation toxicity study (used as a pilot study) were too high.

The dates of the in-life phase of the study were partly after the date of the animal testing ban of 13 March 2013. In a letter dated 4 March 2015, the applicant argues that the study was performed to comply not only with European but also with other international regulations. Furthermore it was claimed that the substance was considered to be used as a new pharmaceutical ingredient and that the study also had to be conducted for deriving concentration limits of the substance at work places.

3.3.6 Reproductive toxicity

3.3.6.1 Fertility and reproduction toxicity

Taken from SCCS/1556/15

An OECD 422 Screening test, i.e., a combined repeated dose toxicity study with the reproduction/developmental toxicity in Sprague-Dawley rats, was conducted with the non-ground active substance S02374 at doses of 0, 100, 300 or 1000 mg/kg bw/day. In a satellite study, toxicokinetics after oral exposure were investigated (see below). Based on this study, the No Observed Effect Level (NOEL) for parental toxicity and for reproductive performance (mating and fertility) was considered to be 1000 mg/kg/day. The NOEL for toxic effects on progeny was considered to be 1000 mg/kg/day.

No new data submitted

3.3.6.2 Developmental Toxicity

Taken from SCCS/1556/15

The cosmetic ingredient S02771 was investigated in a developmental toxicity study (OECD 414) at dose levels of 0, 100, 300, 1000 mg/kg bw/day for the test item, corresponding to 0, 46, 139, 464 mg/kg bw/day for the ground active substance. No adverse maternal and foetal effects related to the test item were observed and a NOEL of 464 mg/kg bw/d was derived for maternal and foetal effects.

No new data submitted.

SCCS conclusion on reproductive toxicity

In the combined repeated dose toxicity study with the reproduction/developmental toxicity, which was already mentioned above, rats received WP30 at doses of 0, 100, 300 or 1000 mg/kg bw/day. In a satellite study, toxicokinetics after oral exposure were investigated (see below). From this study, the No Observed Effect Level (NOEL) for parental toxicity, for reproductive performance (mating and fertility) as well as for toxic effects on the progeny was derived at 1000 mg/kg bw/day, the highest and limit dose tested.

The cosmetic ingredient, NOYAU WP30 (containing 46.4% of WP30) was administered orally by gavage once daily to pregnant females from day 6 to day 20 *post coitum* at dose levels of 100, 300 and 1000 mg/kg bw/day (corresponding to 0, 46, 139, 464 mg/kg bw/day for WP30). There was no sign of systemic toxicity in pregnant females up to and including the dose level of 1000 mg/kg bw/day. NOYAU WP30 had no influence on the relevant reproduction data (post-implantation loss and the mean number of fetuses per dam) up to and including the dose level of 1000 mg/kg bw/day. Based on these results, the NOEL (No Observed Effect Level) for both maternal general toxicity and prenatal developmental toxicity was considered to be 1000 mg/kg bw/day (corresponding to 464 mg/kg bw/day of WP30, the highest dose used).

3.3.7 Mutagenicity / Genotoxicity

3.3.7.1 Mutagenicity / genotoxicity *in vitro*

Taken from SCCS/1556/15

The active substance and the cosmetic ingredient have been investigated in *in vitro* genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations. Phenylene bis-diphenyltriazine did not induce mutations in bacteria nor in mammalian cells. Exposure to Phenylene bis-diphenyltriazine did not result in an increase in cells with micronuclei.

Although the results of these tests may point to an easy conclusion, many shortcomings were noticed in the exposure of the cells during testing that hinder the conclusion. The solubility of Phenylene bis-diphenyltriazine in water is very low, <0.02 µg/l. Therefore, in the genotoxicity tests, DMSO was used as solvent. The solubility in DMSO was reported to be 0.1 mg/ml for the non-ground active substance (S02374) and 0.2 mg/ml for the ground active substance. Under the test conditions where Phenylene bis-diphenyltriazine in DMSO is

diluted in aqueous media, the solubility will decrease. The solubility in a protein containing aqueous medium was reported to be 4.56 µg/ml (ref. 38 submission I).

In one of the Ames tests (ref. 65 submission I), concentrations were used above the solubility of Phenylene bis-diphenyltriazine in DMSO. Strangely, a lack of expected precipitation was reported. In the second Ames test (ref. 66 submission I), lower concentrations, below the solubility limit of Phenylene bis-diphenyltriazine, were used. Consequently, the results of both tests have limited value.

In the oldest gene mutation test in mammalian cells (ref. 67 submission I) and in the oldest micronucleus test (ref. 69 submission I), low concentrations were used below the solubility limit of Phenylene bis-diphenyltriazine. As no indications of cellular exposure were observed, the results of these tests have limited value.

However, in the second gene mutation test in mammalian cells (ref. 68 submission I) and more or less in the second micronucleus test as well (ref. 70 submission I), more relevant concentrations in the range of the solubility limit of Phenylene bis-diphenyltriazine were used. Both tests were negative and no indications for cellular exposure were observed in either of them.

SCCS comment (SCCS/1556/15)
The results of the available reports do not point to a genotoxic potential of Phenylene bis-diphenyltriazine. However, Phenylene bis-diphenyltriazine was predominantly tested at too low concentrations and the shortcomings in cellular exposure observed in these tests may conflict with the conclusion that Phenylene bis-diphenyltriazine can be considered to have no genotoxic potential. Consequently, SCCS cannot exclude that Phenylene bis-diphenyltriazine may have a genotoxic potential when used at higher concentrations in the tests.

New submission

No new data were provided by the applicant but due to intermittently expressed concerns within the evaluation process by SCCS, an independent expert reviewed all existing mutagenicity/genotoxicity data of WP30 as well as of the cosmetic ingredient NOYAU WP30 (Ref. 78 submission II). He pointed out that the mutagenesis and the genotoxicity of WP30 were assessed through a battery of *in vitro* tests covering all genetic events possibly leading to genotoxicity, namely gene point mutation (Ames test and MLA/Tk), numerical and structural chromosomal aberrations (MLA/Tk and micronucleus test). These endpoints were measured either in prokaryotic cells (bacteria) and/or in eukaryotic cells (mammalian cells). All the experimental conditions are thus totally complementary. The main challenge was inherent to the poor solubility of the test item, whatever the solvent and the need of choosing a vehicle compatible with the test systems.

In that view, the best compromise was to select dimethylsulfoxide. Interestingly, depending on the test, either toxicity and/or presence of precipitate were also observed, meaning that the highest achievable concentration was actually tested despite the initial low solubility.

Indeed, the highest concentration often matched the criteria for selection of the maximum dose following the corresponding OECD guidelines that recommend testing at only one concentration, producing turbidity or visible precipitate. On the other hand, the quality of the first set of tests (Ames test, MLA/Tk and micronucleus test) carried out on WP30 per se were considered as not optimal. Therefore, all these 3 tests were reiterated in other labs using the cosmetic ingredient NOYAU WP30 (containing 46.4% WP30). A slight difference in the selection of the highest concentration tested was noted but in each case, the initial

maximum dose tested as expressed in active substance was equal or higher than the one retained by using WP30.

The expert came to the overall conclusion that both sets of 3 studies on NOYAU WP 30 and WP30 clearly indicated the absence of mutagenic potential of WP30 in bacteria, in mammalian cells and of genotoxic potential in mammalian cells. Therefore, no further complementary tests are considered as necessary.

SCCS comments
During the consultation period, SCCS was made aware that these 3 studies on WP 30 were not GLP studies but as deviations from GLP stated relate to minor issues (e.g. to the certificate provided by sponsor that it is not done under GLP conditions and also the address of the sponsor is changed). Therefore, the SCCS has used these studies.

SCCS Conclusion on mutagenicity/genotoxicity
It has been noted by the SCCS that:

- No new experimental studies on genotoxicity were provided by the Applicant in submission II. The studies already assessed by the SCCS in the previous Opinion (2015) included a battery of *in vitro* tests for both the active compound WP30/S86/S02374 and the cosmetic ingredient S02771 and covered the key genetic events that could lead to genotoxicity, i.e. gene point mutations (Ames test and MLA/Tk), numerical and structural chromosomal aberrations (MLA/Tk and micronucleus test). However, in some of the studies the description provided for the stock solutions of S86 in DMSO and dilutions in agar or culture medium was inadequate and did not provide details, e.g. whether or not S86 precipitated on dilution in aqueous media. Also, a quite wide range of concentrations of S86 stock suspensions/solutions in DMSO were used in the studies, from 50-200 mg/mL for Ames tests and 100, 200, 3930 or 10000 µg/mL for tests on mammalian cells. It is not clear whether this could have influenced properties of the precipitates that may have been ?generated on dilution in aqueous media.
- Although the results of the *in vitro* tests were negative, the very low solubility of S86 in the test media cast a degree of uncertainty over interpretation of the results. This is because whilst negative results can be interpreted as proof of a lack of (geno)toxicity, they may also be regarded as being due to the very low (or potentially no) exposure of the test systems to the test substance.
- Further supporting evidence from *in silico* assessment was therefore sought by the SCCS. In response, the Applicant provided limited *in silico* assessment, which indicated S86 to be non-genotoxic. In view of the shortcomings, the SCCS carried out a more detailed internal *in silico* assessment, which also indicated that S86 is unlikely to be genotoxic.

In conclusion, whilst appreciating the general difficulties in relation to testing of very poorly soluble substances, the SCCS considers that the negative *in vitro* test results, supported by the results of *in silico* assessment, have provided sufficient weight of evidence to regard S86 as not likely to be genotoxic.

3.3.7.2 Mutagenicity / genotoxicity *in vivo*

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3.3.8 Carcinogenicity

/

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

Taken from SCCS/1556/15

The low concentrations of the active substance in blood alone are not sufficient to indicate a very low oral bioavailability because the substance is highly lipophilic and when orally absorbed will be mainly distributed in fat and other lipophilic tissues. Therefore, the toxicokinetic data provided do not enable a conclusion on the amount/percentage of the substance orally absorbed.
However, the high molecular weight (541), high lipophilicity ($logP_{o/w}$ > 4), low solubility, and high melting point (321 ºC) of the active substance suggest a low oral bioavailability.

Because of insufficient toxicokinetic data, the default value of 10% for oral bioavailability may be used for the MOS calculation (according to SCCS Notes of Guidance).

No new data submitted.

3.3.9.2 Toxicokinetics in humans

New submission

No data submitted.

SCCS conclusion on toxicokinetics
The very low water solubility (< 0.02 µg/L), the high molecular weight of 540.616 g/mol and the high log Pow value of 8.29 (calculated) point to low bioavailability of WP30 regarding the relevant oral and dermal uptake routes.
From the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422) by oral route (gavage) in rats, regarding the determination of blood plasma concentration for toxicokinetic calculation, none of the satellite male and female rats had significant blood plasma levels on day 1 or at the end of the treatment period (blood plasma level < 0.500 ng/mL, the limit of quantification), with the exception of two satellite males and four satellite females which had blood plasma levels slightly higher than the limit of quantification on study day 1 or at the end of the treatment period. Therefore, the toxicokinetic parameters were not calculated.

Finally, as the high molecular weight, high lipophilicity ($logP_{ow}$ > 4), low solubility, and high melting point of WP30 suggest a low oral bioavailability and due to only limited toxicokinetic data, the default value of 10% for oral bioavailability will be used for the MOS calculation (according to SCCS Notes of Guidance).

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

Taken from SCCS/1556/15

In the neutral red uptake phototoxicity test, both the non-ground active substance (SO2374) and the ground active substance (S02771) are considered as potentially phototoxic at low concentrations.

In phototoxicity tests with the non-ground active substance (S02374) in reconstructed human epidermis with MTT *in vitro* and with the ground active substance (in S02771) in fresh human skin discs with MTT *in vitro*, no firm conclusion on the phototoxic potential of both test items can be drawn from the results obtained in these studies.

From both a phototoxicity (photoirritation) and photosensitisation study in guinea pigs, no firm conclusion on the phototoxicity and photosensitisation potential of the ground active substance (in S02771) can be drawn due to insufficient exposure time to the test item.

New submission

Phototoxicity *in vitro*

Guideline/method:	OECD 432
GLP:	Yes
Test system:	Mouse fibroblasts (Balb/c 3T3, clone A31)
Replicates:	six replicates/concentrations x 2 plates treated, 2x 12 replicates of negative control, 2x 12 replicates of positive control
Test substance:	WP30
Batch:	LT30 (purity: 99.4%)
Concentration:	6.73, 9.90, 14.56, 21.41, 31.48, 46.28, 68.03 and 100 µg/mL
Vehicle:	DMSO
Vehicle control:	HBSS containing 1% DMSO
Positive control:	Chlorpromazine hydrochloride (CPZ)
Exposure:	Single application of test item, 1h incubation
Source of light:	UVACUBE 400, SOL-500 (Hönle UV Technology, Germany) with filter H1 Intensity of irradiation: 5J/cm^2
Exposure duration:	50 min
Study period:	03 June 2016 – 22 June 2016

The *in vitro* phototoxic potential of the test item (WP30) was investigated using the 3T3 NRU phototoxicity test.

The assay compares the cytotoxicity of chemicals applied to mouse fibroblasts (Balb/c 3T3, clone A31) in the presence or absence of exposure to a non-cytotoxic level of UVA light (5 J/cm²). Cytotoxicity is measured as the inhibition of the capacity to take up the vital dye, Neutral Red (NR), one day after UVA treatment.

Results:

Solubility test

The test item resulted in a homogenous suspension when prepared in DMSO at 10 mg/mL and also when this formulation was 100-fold diluted in HBSS.

Preliminary test

According to the solubility results, a preliminary test was performed with the following test item concentrations: 0.032, 0.101, 0.317, 1.003, 3.169, 10.01, 31.65 and 100 µg/mL in HBSS containing 1% DMSO (serial dilution factor of 3.16). The following results were obtained:

- in the non-irradiated plate: no change in cell morphology was observed and no decrease in NR uptake was noted at any tested concentration,

- in the irradiated plate: a change in cell morphology and a decrease in NR uptake was noted at concentrations ≥ 31.65 µg/mL, with associated cell viability < 50%. The calculated IC50 was 28.56 µg/mL.

Main test

The acceptance criteria were fulfilled and the study was therefore considered to be valid.

According to the results obtained in the preliminary test, the following concentrations were used for the main test: 6.73, 9.90, 14.56, 21.41, 31.48, 46.28, 68.03 and 100 µg/mL (serial dilution factor of 1.47). The following results were obtained:

- in the non-irradiated plate: no change in cell morphology was observed and no decrease in NR uptake was noted at any tested concentrations,
- in the irradiated plate: a change in cell morphology was observed at concentrations ≥ 31.48 µg/mL, associated with rounded cells and a decrease in NR uptake was noted at concentrations ≥ 68.03 µg/mL, with associated cell viability < 50%. The calculated IC50 was 47.99 µg/mL.

The main phototoxicity findings for NR uptake following analysis with the PHOTOTOX software are presented in the Table 20:

Table 20

Parameter	Value	Conclusion
Test item CH0222 - WP30 MPB-025589/S02374	IC50 +UVA =47.99 µg/mL IC50 -UVA = not reached > PIF = 2.085 MPE = 0.134	Probably phototoxic

Conclusion:

Under the experimental conditions of this study, WP30 tested as a homogenous suspension in DMSO/HBSS up to 100 µg/mL was considered as probably phototoxic, according to the classifications presented in the OECD guideline 432.

Ref. 56 (submission II)

Guideline/method: published references:
a) the EPISKIN Phototoxicity Assay (EPA); Development of an *in vitro* tiered strategy using 17 reference chemicals to predict phototoxic potency. Lelièvre *et al*. 2007. Toxicology *In vitro*, 21, pg 977-995,
b) DB-ALM Protocol N°121: EPIDERMTM Phototoxicity assay.

GLP: Yes

Test system: Human reconstructed epidermis EpiSkin™ model

Replicates:	2 plates per test item and each of controls; 4 tissues/test item and controls
Test substance:	WP30
Batch:	LT30 (purity: 99.4%)
Concentration:	10% w/v in paraffin oil (corresponding to 100 mg/mL)
Vehicle:	Paraffin oil
Negative control:	Dulbecco's Phosphate-Buffered Saline (D-PBS).
Positive control:	Chlorpromazine (CPZ) at 0.5 mg/mL in water for injections
Exposure:	Single application, 2 hours incubation with test item
Direct interaction with MTT:	No
Colouring of epidermis:	No
Duration of exposure:	18-24 hours
Source of light:	/
Intensity of irradiation:	UVA 7J/cm^2
Study period:	20 June 2016 – 17 November 2016

Material and methods:

The *in vitro* epidermal phototoxic potential of the test item (WP30) diluted at 10% (w/v) in paraffin oil was investigated, using the human reconstructed epidermis EpiSkin™ model. Preliminary tests were performed to detect the ability of the test item to directly reduce MTT as well as its colouring potential.

Following the preliminary tests, the epidermal phototoxicity potential of the test item was tested in the main test. The test item formulation, the vehicle, the negative and positive controls were applied topically on four tissues (two exposed to UVA and two maintained in the dark) and incubated at 37°C, 5% CO_2 for 2 hours (± 5 minutes). At the end of the treatment period, any excess of test and control items was eliminated, and the tissues were exposed to UVA or placed in the dark (for the non-irradiated treated tissues). At the end of the UVA (or dark) exposure period, the plates were incubated for a 2-hour (± 5 minutes) post-exposure period at room temperature and protected from light. At the end of this period, tissues were incubated overnight at 37°C, 5% CO_2.

The cell viability was then assessed by means of the colorimetric MTT reduction assay.

Relative viability values were calculated for each tissue and expressed as a percentage of the mean viability of the negative control non-UVA irradiated tissues. Then, the decrease in viability due to UVA exposure (CδV) was calculated.

In addition, the concentration of the inflammatory mediator IL-1α was evaluated based on an ELISA assay in the culture medium retained following the overnight incubation period. The increase in IL-1α release due to UVA exposure (CδIL) was then calculated.

Results:

Preliminary tests

In the preliminary tests, the test item formulation was found not to have direct MTT reducing properties or colouring potential.

Main test

All acceptance criteria were fulfilled. The study was therefore considered to be valid.
Following UVA exposure and recovery period, the decrease in viability due to UVA exposure (CδV) of the tissues treated with the test item formulation was 4%, and the increase in IL-1α release due to UVA exposure (CδIL) was 13.9 pg/mL.
For the vehicle control, the decrease in viability due to UVA exposure (CδV) was 14%, and the increase in IL-1α release due to UVA exposure (CδIL) was 23.5 pg/mL.
As the CδV values were < 25% and the CδIL value were < 40 pg/mL, the results met the criteria for a non-phototoxic response.

Conclusion:

Under the experimental conditions of this study, WP30 diluted at 10% (w/v) in paraffin oil is considered to be non-phototoxic.

In addition, former studies are available, which are considered to be of low reliability due to deficiencies in methods, reporting and assessment and because these studies did not completely comply with current test and assessment guidelines.

Ref. 48, 49, 57 (submission II)

Phototoxicity *in vivo*

Guideline/method:	According to an approved protocol
GLP:	Yes
Species/strain:	Guinea pig/Hartley Crl:HA
Group size:	Preliminary study on irritancy: 2 males, 2 females Main study: 5 males and 5 females
Test substance:	NOYAU WP30 (yellow suspension containing 48.5% of WP30)
Batch:	LP110 (purity: 99.6%)
Concentration:	50% (in water) and 100% (preliminary study) and 100% (main study)
Volume:	0.75 mL
Route:	Open epicutaneous application on back; area about 24 cm²
Negative control:	Test substance without UV, and UV without test substance
Positive control:	8-Methoxy-psoralene (8-MOP) 0.5 mg/mL
Source of light:	Vilber-Lourmat: VL-215.L 365 nm peak and VL-214.M 312 nm peak
Irradiation:	Irradiation dose: 3 J/cm² UVA and 0.1 J/cm² UVB, approx 30 minutes after application of test items
Observations:	Scoring of skin reactions at 24 and 48 hrs after application: no visible change = 0 discrete or patchy erythema = 1 moderate and confluent erythema = 2 intense erythema and swelling = 3
Study period:	02 February 2011 – 11 February 2011

The aim of the study was to determine the possible phototoxic potential of NOYAU WP30 (yellow suspension containing 48.5% of WP30, batch: LP110, purity: 99.6%) following a single cutaneous application in the guinea pig. The study involved 2 males and 2 females for a preliminary test and 5 males and 5 females for the main study. The study consisted of an initial application of the test item to the skin, followed by exposure of the treated skin surface to UVA-type ultraviolet rays, covering an emission spectrum ranging from 320 to 380 nm, with a peak ray at 365 nm, or the UVB type covering an emission spectrum ranging from 280 nm to 320 nm, with a peak ray at 312 nm. If the test item has a phototoxic potential, an erythematous and/or oedematous reaction should have been seen 24 hours and/or 48 hours after exposure. The experiment included study of the phototoxic potential of 8-methoxy-psoralen (8-MOP) as method control test item. 48 hours before applications, all guinea pigs were clipped and epilated over the dorsal region from the neck to the lumbar region over an area of approximately 70 cm² (7 cm x 10 cm). Only healthy animals, free of any trace of cutaneous lesions, were selected.

For the determination of MNIC (Maximum Non-Irritant Concentration) by cutaneous application, two males and two females received a cutaneous application of 0.75 mL of the undiluted test item and the test item diluted at 50% over an area measuring 24 cm² (3 cm x 8 cm), using one concentration on each flank, *i.e.* 2 concentrations per animal. After application, the area concerned was massaged in order to encourage transcutaneous

penetration of the applied test item. Any lesions were evaluated for each concentration 24 hours and 48 hours after application using the scale shown above.
The main study involved 10 animals (5 females, 5 males). The dorsal region was divided into 6 zones and the test item was applied to the retroscapular region at the MNIC (about 24 cm²) in a volume of 0.75 mL. Following application, the area concerned was massaged in order to encourage transcutaneous penetration of the applied test item. For control, 30 µL of 8-MOP (8-methoxy-psoralen) solution was applied to zone 6 over an area measuring approximately 3 cm². Animals were placed in restraint cages after application. Thereafter, the irradiation procedure followed, consisting of 3 J/cm² UVA and 0.1 J/cm² UVB, approximately 30 minutes after application of the test items. No dressing was applied to the application area.

Results:

The application of the test item did not induce colouration of the application site and did not interfere with grading of any skin lesions.
The Maximum Non-Irritant Concentration (MNIC) determined by cutaneous application was the undiluted test item.
The Maximum Non Erythematous Dose (MNED) of irradiation was 3.0 J/cm² for UVA type rays and 0.10 J/cm2 for UVB type rays.
No cutaneous reaction was observed 24 hours and 48 hours after application of the undiluted test item and exposure to UVA and UVB rays.
All animals treated with 8-methoxy-psoralen showed an erythematous and/or edematous reaction at time 24 and 48 hours after exposure and demonstrated the sensitivity and suitability of the study procedure.

Conclusion and discussion:

Under the experimental conditions, the undiluted NOYAU WP30 (containing 48.5% of WP30) was found to be non-phototoxic in the guinea pig. With regards to period between application and start of irradiation, it should be noted that the duration of application can be considered as sufficient to determine the phototoxic potential of a test item. This was proven by the results obtained with the positive control. Another study with a longer application time prior to irradiation, even under occlusion, is assumed to bring no additional information regarding the phototoxic potential. Thus, the study is considered both scientific reliable and relevant for this endpoint.

Ref. 51 (submission II)

SCCS comment on the *in vitro* photoxicity tests
The poor solubility of the test item (in fact a suspension) limits the interpretation of the 3T3 NRU test. In such cases, the reconstructed human skin phototoxicity test may represent a more appropriate first-line assay.

Photosensitisation *in vivo*

Guideline/method:	According to an approved protocol
GLP:	Yes
Species/strain:	Guinea pig/Hartley Crl:HA
Group size:	In total 15 (10 males and 5 females, i.e., negative control group 5, treated (irradiated) group 10 animals)
Test substance:	NOYAU WP30 (yellow suspension containing 48.5% of WP30)
Batch:	LP110 (purity: 99.6%)
Concentration:	Undiluted
Volume:	0.25 mL on each test area

Route:	Induction of sensitisation by concomitant injection of Freund's adjuvant at the time of first application of test article (day 1), followed by application of test article on day 3 and 5. After 3 weeks open epicutaneous application on different areas on the back; each area of about 8 cm2 was dosed with 0.25 mL
Negative control:	Test substance without UV, and UV without test substance
Positive control:	none
Source of light:	Vilber-Lourmat: VL-215.L 365 nm peak and VL-214.M 312 nm peak
Irradiation:	Irradiation dose: 3 J/cm2 UVA and 0.1 J/cm2 UVB, approximately 30 minutes after application of test items.
Observations:	Scoring of skin reactions at 24 and 48 hrs after application as cited above.
Study period:	14 February 2011 – 11 March 2011

Ref. 52 (submission II)

Material and methods:

The aim of the study was to determine the possible photosensitising potential of NOYAU WP30 (yellow suspension containing 48.5% of WP30, batch: LP110, purity: 99.6%) in the guinea pig. The study involved 15 animals, i.e. 10 males and 5 females allocated in 2 groups: Group 1 as a negative control group of 5 male animals and Group 2 as a group dosed with the test item of 5 animals of each sex.

The induction phase was performed on days D1, D3 and D5. On D1, all animals received 4 intradermal injections in the cervical region (zone 1) of 0.1 mL of complete Freunds adjuvant diluted 50% in isotonic saline. The test item at MNIC determined in the phototoxicity study was applied to the same zone in all animals (i.e. undiluted test item). Application of the test item involved a volume of 0.25 mL over an area of approximately 8 cm². After application, the treatment area was massaged in order to enhance transcutaneous penetration of the applied test item. Animals were placed in restraint cages in order to avoid licking of the treatment zone.

For irradiation lasting approximately 30 minutes after application, animals were exposed to UVB type rays, then to UVA type (zone1). An opaque mask was placed on the back (zones 2 and 3) of the animals during irradiation. Animals in the negative control group were exposed to no UV irradiation on zone 1. For each type of irradiation, animals were exposed to the Minimum Erythematous Dose (MED) of irradiation. These doses were determined about every six months. They were 3.5 J/cm² for UVA type rays and 0.15 J/cm² for UVB type rays. At each exposure, the exact amount delivered in J/cm² was monitored using a VLX 3W radiometer. Irradiation was stopped when the MED. was reached by constant monitoring.

The procedure on D3 and D5 was identical to that of D1 without intradermal injections of complete Freunds adjuvant. The resting period lasted from D6 to D21 and the animals were rested from D6 to D21, i.e. for 16 days.

Approximately 48 hours before challenge of the photosensitising reaction, all previously trimmed guinea pigs were depilated over the dorsal region with the exception of the induction site. Trimming and depilation involved an area of approximately 50 cm². On D24, 0.25 mL of the test item at the MNIC was applied topically to the right half of the lumbar region (zone 2) of all animals, over an area of approximately 8 cm² never previously in contact with the test item. After application, the treatment area was massaged in order to enhance transcutaneous penetration of the applied test item. Animals were placed in restraint cages in order to avoid licking of the treatment zone. This was followed by irradiation approximately 30 minutes after cutaneous application: animals of treated group were exposed to UVA type rays to the Maximum Non-Erythematous Dose (MNED). This irradiation concerned zones 2 and 3, zone 3 being an irradiation control zone. The cervical region treated with the test item on D1, D3 and D5 (zone 1) was covered with an opaque

mask during irradiation. Animals in the negative control group were not exposed to UV irradiation on zones 2 and 3. The animals were exposed to the MNED of irradiation at 3.0 J/cm² for UVA. At each exposure, the exact amount delivered in J/cm2 was monitored using a VLX 3 W radiometer and irradiation was stopped when the MNED was reached.

Results, discussion and conclusion:

At 24 hours and 48 hours, the negative control animals and animals treated with NOYAU WP30 (containing 48.5% of WP30) did not show any cutaneous reaction. Under the experimental conditions, NOYAU WP30 was found to be non-photosensitising in the guinea pig. With regards to the period between application and the start of irradiation, it should be noted that the duration of application can be considered as sufficient to determine the phototoxic potential of a test item as this study was carried out according to an approved protocol that was sufficient and reliable for detecting photosensitising potential. Therefore, there is no need for an additional study with a longer application time prior to irradiation, even under occlusion. Thus, the study is considered both scientific reliable and relevant for this endpoint.

Applicant's conclusion on phototoxicity:

The data on the photo-cytotoxic potential of WP30 obtained in *in vitro* assays are heterogeneous. In the most recent Neutral Red Uptake (NRU) assay in mouse fibroblasts (3T3 cells), WP30 turned out as probably phototoxic. However, in the Human reconstructed epidermis EpiSkin™ model, WP demonstrated clearly no phototoxic potential. From a weight of evidence aspect, the EpiSkin™ model is considered as the more reliable test systemic to mimic Human exposure.
NOYAU WP30, containing 48.5% of WP30 was clearly neither phototoxic nor photosensitising *in vivo* in guinea pigs, when tested undiluted up to the Maximum Non-Irritant Concentration (MNIC) and the Maximum Non Erythematous Dose (MNED) of irradiation.
Therefore, and due to the fact that WP30 was shown to be photo-stable and revealed no skin irritative potential *in vitro*, the weight of evidence of all data make it very unlikely that WP30 possess a relevant photo-toxic/photosensitising potential for Human exposed to this UV filter.

Overall SCCS comment on phototoxicity/photosensitisation
Although the 3T3 NRU phototoxicity test indicates probably phototoxic (>PIF = 2.08), the SCCS agrees that, based on the phototoxicity test on human reconstructed skin and the photosensitisation test in guinea pigs, S86 is unlikely to be phototoxic.

3.3.10.2 Photomutagenicity / photoclastogenicity

New submission

Guideline/method:	OECD 471
GLP:	Yes
Test system:	*S. typhimurium* TA 1537, TA 98 and TA 100 *E. coli* WP2 uvr A
Replicates:	Three replicate plates, three independent experiments
Test substance:	NOYAU WP30 (yellow suspension, WP30 content 46.4%)
Batch:	LP110 (purity: 100%)
Vehicle:	DMSO
UVA doses:	TA98: 0.063, 0.125 and 0.25 J/cm2 TA100: 0.04, 0.02 and 0.04 J/cm2 TA 1537: 0.2, 0.4 and 0.8 J/cm2 WP2 : 0.003, 0.006 and 0.012 J/cm2

UVB doses:	WP2: 0.001, 0.002 and 0.004 J/cm2
Concentrations:	Experiment I: 125, 250, 500, 1000 and 2000 µg/plate of test item Experiment II: 62.5, 125, 250, 500 and 1000 µg/plate of test item Experiment II: 62.5, 125, 250, 500 and 1000 µg/plate of test item (TA100 only)
Treatment:	direct plate incorporation with at least 72 h incubation
Positive Controls:	Methylene Blue, Chlorpromazine, 9,10-Dimethyl-1,2-benzanthracene and 8-Methoxypsoralen
Negative controls:	Yes (vehicle)
Study period:	09 March 2012 – 08 June 2012

NOYAU WP30 (batch: LP110, purity: 100%, containing 46.4% WP30) was investigated for photo-mutagenicity in *Salmonella typhimurium* and *Escherichia coli* (Ames test). A purpose-built irradiation system that utilizes fluorescent tubes (40W) as a source of ultraviolet light was used. The UVA and UVB irradiations were automatically monitored by sensors that are calibrated every 2 years or after approximately 50 h of use. The plates are placed at a distance from the light source in order to have a final reported irradiation dose in Joules/cm2, automatically determining the irradiation time.

Test concentrations were based on the results of a preliminary toxicity test on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. A wide range of concentrations was used up to the prescribed maximum concentration of 5000 µg/plate. Two wide-spaced UV doses were selected for each bacterial tester strain on the basis of the maximum tolerated concentration. UVB was only used for the WP2 tester strain over uncovered plates.

The mutation experiments were performed with the direct plate incorporation method. Untreated control plates, not exposed to UV light, were prepared for each tester strain to control the spontaneous level of revertants. Untreated vehicle control plates, not exposed to UV light, were also prepared for each tester strain. The maximum concentration of S02771 was also assayed without UV irradiation in order to assess its genotoxic potential without photoactivation.

Negative and positive controls were in accordance with the OECD guideline.

Results:

In the preliminary cytotoxicity test, precipitation occurred at 5000 µg/plate (interfering with the evaluation of the background lawn) and at 1580 µg/plate. At the highest UVA exposure, reduction in revertant colonies was observed with TA98 (at the 3 highest concentrations), TA1537 (at the highest concentrations) and TA100 (at the 2 highest concentrations). However, these results may be attributable to a slight toxic effect as well as to a mechanical effect induced by the precipitate. On the basis of these evaluations and due to the high level of precipitation at 5000 µg/plate, the maximum concentration for experiment I was 2000 µg/plate.

In experiment I, toxicity as indicated by thinning of the background lawn was observed for all tester strains both in the presence and absence of UV irradiation at the highest concentration. Precipitation was observed at the 2 highest concentrations; at 2000 µg/plate precipitation interfered with scoring of the background lawn. The maximum concentration for experiment II and III was set at 1000 µg/plate. In experiment II, precipitation, not interfering with scoring, was seen at the highest concentration.

In all experiments UV exposure induced increases in revertant numbers over the background values in all tester strains, with the exception of TA1537.

A biologically relevant increase over the background UV effect was not observed in any experiment, in any NOYAU WP30 (containing 46.4% WP30) concentration or in any tester strain.

The negative and strain-specific positive control values were within the laboratory historical control data ranges indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

Conclusion:

Under the experimental conditions used, NOYAU WP30 (containing 46.4% WP30) was not photo-genotoxic (photo-mutagenic) in these gene mutation tests in bacteria. Moreover, it has to be considered that WP30 per se was proven to be photostable, which also increased the reliability of this study.

Ref. 85 (submission II)

SCCS comment
A photomutagenicity study using the Ames test was clearly negative under the conditions of the study.

3.3.11 Human data

No data provided.

3.3.12 Special investigations

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3.4 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MoS)

According to the intended usage as a broad-band UV filter of WP30 in concentrations of up to 5% in sunscreens and with respect to the SCCS principles to assume an application of 18 g/day for a sunscreen lotion to be used for risk assessment purposes, the following scenario has to be taken into consideration for the calculation of the systemic exposure dose (SED) and the respective margin of safety (MoS).

CALCULATION OF THE MARGIN OF SAFETY

Amount of sunscreen applied daily	**18 g**
Typical Adult body weight	**60 kg**
Concentration of S86 in sunscreen	**C = 5%**
Estimated daily exposure per kg	**A= 15 mg/kg bw/d**
Dermal absorption per application	**DAp = 0.33%**
Systemic exposure dose	**SED = 0.0495 mg/kg bw/d**
No observed adverse effect level (90-day, oral, rat)	**NOAEL = 485 mg/kg bw/d**
Bioavailability 10%*	**$NOAEL_{syst}$ = 48.5 mg/kg bw/d**
Margin of Safety	**$NOAEL_{syst}$/SED = 980**

* Standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, 2016.

Thus, although several worst case assumptions were made, the MoS far exceeds the recognized safety limit of MoS $\geq$100.

3.5 DISCUSSION

This new submission II dossier is based on data of the active ingredient (Phenylene bis-diphenyltriazine, S86, WP30, S02374) including new experimental and analytical data. Only in cases where no or insufficient data regarding a toxicological endpoint were available, this submission II dossier was supplemented with data on the cosmetic ingredient (NOYAU WP30, S02771) for bridging and read-across purposes.

Physicochemical properties

Purity of Phenylene bis-diphenyltriazine described on the basis of HPLC-UV detection cannot be accepted because 1) it was not documented that all of the test substance loaded on the HPLC column was eluted (2) the UV detection of the active substance was not performed at a specific wavelength (λmax),

Impurities have been chemically characterised by the use of HPLC-PDA and LC-HRMS based on fragmentation pattern analysis. NMP and hydrazine have been identified as SVHC by ECHA and were included on 20 June 2011 in the Candidate List of substances for eventual inclusion in Annex XIV of REACH (ED/31/2011). NMP and hydrazine are, according to the applicant, unavoidable manufacturing impurities of NMP concentration, which varied from 830 to 3153 ppm in the 7 batches tested, while the concentration hydrazine was found to be less than 2 ppm. These two impurities should be accurately quantified in each batch.

For the cosmetic ingredient S02771, no solubility data of the ground active substance was provided except for DMSO. Compared to the active substance (S02374) which is sparingly insoluble in DMSO (0.10 mg/ml), the cosmetic ingredient S02771 is formulated with an emulsifier (Eumulgin L), a preservative (Benzoic acid) and water and presumably has a slightly different solubility.

For the 11 aqueous solvents tested, WP30 was either not detected (ND) or its concentration was less than LOQ. Therefore, it can be concluded that WP30 is practically insoluble in all aqueous solvents tested in the presence or absence of proteins. It appears that 5,6,5',6'-tetraphenyl-3,3'-(1,4-Phenylene)bis(1,2,4-Triazine) is insoluble in water (solubility <0.02 µg/L). A solubility of 4.56 µg/ml of WP30, batch LP110 in aqueous 0.9% NaCl containing 3% bovine serum albumin was reported (see section 3.1.4). The report was not provided.

The active ingredient is considered stable when stored protected from light and moisture.

Function and uses

The cosmetic ingredient (NOYAU WP30, S02771) is intended to be used as a UV filter in sunscreen products in a concentration of up to a maximum content of 10%. Consequently, the active ingredient, Phenylene bis-diphenyltriazine (WP30, S02374) will be a constituent in final sunscreen products at a concentration of up to a maximum of 5%.

General toxicity

The NOAEL of 485 mg/kg bw derived from a 4–week oral study that was already identified by SCCS in the Opinion from 2015 can be used to calculate the margin of safety. It corresponds to the highest dose of the cosmetic ingredient S02771 containing 48.5% of the ground active substance in the suspension tested in the study and no adverse effects have been observed. Specific parameters were included in this study to asses some endocrine-mediated effects (effects on thyroid hormones, on the testicular function, on oestrus cycles)

which make it possible to conclude the absence of visible endocrine disruptor effects of S02771 with regards to the endocrine parameters tested.

Additional supporting repeated dose toxicity data on the non-ground active substance S02374 were obtained from an OECD 422 study (i.e. a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test by oral route (gavage) in rats): Based on the experimental conditions of this study, the No Observed Effect Level (NOEL) was considered to be 1000 mg/kg/day.

Acute toxicity

The acute toxicity of WP30 can be considered as very low.
The acute oral LD50 in rats > 2500 mg/kg bw.
In the acute dermal toxicity study in rats, no relevant systemic effects were noted and there was no sign of skin irritation. The dermal LD50 in rats was > 2000 mg/kg bw.

Irritation and corrosivity

On the basis of the results obtained in the new Episkin™ study, it can be concluded that WP30 diluted at 10% (w/v) in paraffin oil is not a skin irritant. Also, no signs of skin irritation were noted in the acute dermal toxicity study in rats on the active substance S02374. In addition, new BCOP and EpiOcular *in vitro* tests indicate that S02374 is not irritating to the eye.

Skin Sensitisation

Due to the oily nature of the vehicle, SCCS assumes that the test substance, although not fully solubilised, will stick to the ears in the LLNA. S86 tested up to 25% is not considered to be a skin sensitiser.

Dermal absorption

No new skin penetration data were submitted, but the Applicant provided novel information on the solubility of the non-ground active substance S02374 in Acetone/olive oil. This information was used to re-evaluate the studies provided in Submission I.
For calculation of the SED, 0.33 % dermal absorption, corresponding to 1.8 µg/cm^2 as worst case, can be used.
Finally, the skin bioavailability of WP30 remained very low, even for skin with altered barrier function and subjected to solar irradiation.

Reproductive toxicity

An OECD 422 Screening test, i.e., a combined repeated dose toxicity study with the reproduction/developmental toxicity in Sprague-Dawley rats, was conducted with the non-ground active substance S02374 at doses of 0, 100, 300 or 1000 mg/kg bw/day. In a satellite study, toxicokinetics after oral exposure were investigated (see below). Based on this study, the No Observed Effect Level (NOEL) for parental toxicity and for reproductive performance (mating and fertility) was considered to be 1000 mg/kg/day. The NOEL for toxic effects on progeny was considered to be 1000 mg/kg/day.
The cosmetic ingredient S02771 was investigated in a developmental toxicity study (OECD 414) at dose levels of 0, 100, 300, 1000 mg/kg bw/day for the test item, corresponding to

0, 46, 139, 464 mg/kg bw/day for the ground active substance. No adverse maternal and foetal effects related to the test item were observed and a NOEL of 464 mg/kg bw/d was derived for maternal and foetal effects.

Mutagenicity / genotoxicity

No new experimental studies on genotoxicity were provided by the Applicant in submission II. The studies already assessed by the SCCS in the previous Opinion (2015) included a battery of *in vitro* tests for both the active compound WP30/S86/S02374 and the cosmetic ingredient S02771 and covered the key genetic events that could lead to genotoxicity, i.e. gene point mutations (Ames test and MLA/Tk), numerical and structural chromosomal aberrations (MLA/Tk and micronucleus test). However, in some of the studies the description provided for the stock solutions of S86 in DMSO and dilutions in agar or culture medium was inadequate and did not provide details, e.g. whether or not S86 precipitated on dilution in aqueous media. Also, a quite wide range of concentrations of S86 stock suspensions/solutions in DMSO were used in the studies, from 50-200 mg/mL for Ames tests and 100, 200, 3930 or 10000 µg/mL for tests on mammalian cells. It is not clear whether this could have influenced properties of the precipitates that may have generated on dilution in aqueous media.

Although the results of the *in vitro* tests were negative, the very low solubility of S86 in the test media cast a degree of uncertainty over interpretation of the results. This is because whilst negative results can be interpreted as proof of a lack of (geno)toxicity, they may also be regarded as being due to the very low (or potentially no) exposure of the test systems to the test substance.

Further supporting evidence from *in silico* assessment was therefore sought by the SCCS. In response, the Applicant provided limited *in silico* assessment, which indicated S86 to be non-genotoxic. In view of the shortcomings, the SCCS carried out a more detailed internal *in silico* assessment, which also indicated that S86 is unlikely to be genotoxic.

In conclusion, whilst appreciating the general difficulties in relation to testing of very poorly soluble substances, the SCCS considers that the negative *in vitro* test results, supported by the results of *in silico* assessment, have provided sufficient weight of evidence to regard S86 as not likely to be genotoxic.

Carcinogenicity

No data provided.

Toxicokinetics

The very low water solubility (< 0.02 µg/L), the high molecular weight of 540.616 g/mol and the high log Pow value of 8. 29 (calculated) indicated that WP30 had low bioavailability regarding the relevant oral and dermal uptake routes.
From the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422) by oral route (gavage) in rats, regarding the determination of blood plasma concentration for toxicokinetic calculation, none of the satellite male and female rats had significant blood plasma levels on day 1 or at the end of the treatment period (blood plasma level < 0.500 ng/mL, the limit of quantification), with the exception of two satellite males and four satellite females which had blood plasma levels slightly higher than the limit of quantification on study day 1 or at the end of the treatment period. Therefore, calculation of the toxicokinetic parameters was not performed.

Finally, as the high molecular weight, high lipophilicity (logPow > 4), low solubility and high melting point of WP30 suggest a low oral bioavailability and due to only limited toxicokinetic data, the default value of 10% for oral bioavailability will be used for the MOS calculation (according to SCCS Notes of Guidance, 2016).

Photo-induced toxicity

Phototoxicity/photosensitisation

Although the 3T3 NRU phototoxicity test indicates probably phototoxic (>PIF = 2.08), the SCCS agrees that, based on the phototoxicity test on human reconstructed skin and the photosensitisation test in guinea pigs, S86 is unlikely to be phototoxic and photosensitising.

Photomutagenicity / photoclastogenicity

A photomutagenicity study using the Ames test was clearly negative under the conditions of the study.

Human data

No data provided.

4. CONCLUSION

- *In light of the new data provided, does the SCCS consider Phenylene Bis-Diphenyltriazine, S86 safe for use as a UV-filter in sunscreen products in a concentration up to 5.0%?*

Based on the data provided in the dossier, the SCCS considers Phenylene Bis-Diphenyltriazine, S86, safe for use as a UV-filter in sunscreen products at a concentration up to 5%.

Because of the insoluble nature of S86 and as no data were provided on safety *via* inhalation exposure, the SCCS considers its use safe only in dermally applied products and not in products that would lead to inhalation exposure.

- *Does the SCCS have any further scientific concerns with regard to the use of Phenylene Bis-Diphenyltriazine, S86 as a UV-filter in sunscreen and/or other cosmetic products*

Phenylene Bis-Diphenyltriazine (S86) may contain impurities (NMP and hydrazine), which are classified as CMR 1B and identified in the EU as SVHC. Therefore, the level of NMP and hydrazine should be kept at trace levels.

Potential effects of Phenylene Bis-Diphenyltriazine (S86) on the environment have not been assessed by SCCS.

5. MINORITY OPINION

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7. GLOSSARY OF TERMS

See SCCS/1564/15, 9th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 144

8. LIST OF ABBREVIATIONS

See SCCS/1564/15, 9th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 144