Scientific Committee on Consumer Safety

SCCS

OPINION ON

UV filter S86
Phenylene bis-diphenyltriazine

The SCCS adopted this opinion by written procedure on

13 July 2015

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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SCCS

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

Scientific Committee members

Ulrike Bernauer, Qasim Chaudhry, Pieter Coenraads, Gisela Degen, Maria Dusinska, Werner Lilienblum, Elsa Nielsen, Thomas Platzek, Christophe Rousselle, Jan van Benthem

Contact

European Commission
Health & Food Safety
Directorate C: Public Health
Unit C2 – Health Information/ Secretariat of the Scientific Committee
Office: HTC 03/073
L-2920 Luxembourg
SANTE-C2-SCCS@ec.europa.eu

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SCCS Members
Dr. U. Bernauer
Dr. Q. Chaudhry
Prof. P.J. Coenraads
Prof. G. Degen (Chairperson)
Dr. M. Dusinska
Dr. W. Lilienblum (Rapporteur)
Dr. E. Nielsen
Prof. T. Platzek
Dr. Ch. Rousselle
Dr. J. van Benthem

Former SCCS Member
Dr. S. Ch. Rastogi

External experts
Prof. A. Bernard
Prof. J. Duus-Johansen
Dr. J. Ezendam
Prof. A. M. Giménez-Arnau
Dr. E. Mirkova
Dr. E. Panteri
Prof. T. Vanhaecke

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1. BACKGROUND

In December 2012 the Commission has received a dossier by Cosmetics Europe [1] on the safety assessment of 5,6,5',6'-tetraphenyl-3,3'-(1,4-Phenylene)bis(1,2,4-Triazine), INCI name Phenylene bis-diphenyltriazine (CAS n. 55514-22-2) S86 , identified also as S02771, a new cosmetic ingredient intended to be used as a UV filter in sunscreen products.

According to Cosmetics Europe, data are provided on the commercial ingredient S02771, that is a 40 - 50% aqueous suspension obtained after a wet grinding step 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.

This submission is intended to demonstrate the safety of the ingredient for use as UV filter in sunscreen products. The submission applies for inclusion of Phenylene bis-diphenyltriazine S86 in Annex VI of the Cosmetic Regulation (EC) n. 1223/2009.

2. TERMS OF REFERENCE

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?

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?

[1] Cosmetics Europe, ex- COLIPA-: European Cosmetics Toiletry and Perfumery Association)

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Phenylene bis-diphenyltriazine

Colipa No. S 86

3.1.1.2 Chemical names

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

3.1.1.3 Trade names and abbreviations

Colipa No. S 86

COSMETIC INGREDIENT	ACTIVE SUBSTANCE
S02771	**S02374**
NOYAU WP30	WP30
"X"	CH0222 – WP30
R000317	025589
025814	

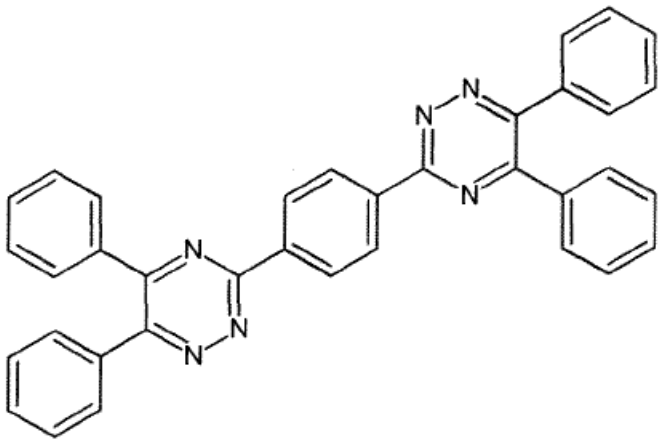
SCCS comment
According to background, cosmetic ingredient SO2771 is prepared by wet grinding of the active substance S02374. S02771 is formulated with an emulsifier (Eumulgin L), a preservative (Benzoic acid) and water. Additional supporting data are provided on the active substance S02374 (5,6,5',6'-tetraphenyl-3,3'-(1,4-Phenylene)bis(1,2,4-Triazine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

The composition of the cosmetic ingredients "X" and NOYAU WP30 has been shown to be similar to SO2771. The code 025814 (no data provided) designates the same cosmetic ingredient SO2771 according to a letter of the applicant.
The code R000317 designates the non-ground active substance and not the cosmetic ingredient as indicated in the table above, taken from the applicant (ref. 31).

3.1.1.4 CAS / EC number

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

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{36}H_{24}N_6$

3.1.2 Physical form

A) Active substance S02374: yellow powder

B) Cosmetic ingredient S02771: aqueous suspension, yellow to pale yellow

3.1.3 Molecular weight

Molecular weight of the active substance **S02374**: 540.62 g/mol Ref. 11

3.1.4 Purity, composition and substance codes

A) Active Substance S02374
WP30
CHO222, WP30
025589

Batch/Lot:

ES130	Ref. 21, 31
ES140	Ref. 21, 31
LP110	Ref. 13, 21, 31
LP120	Ref. 15, 16, 21, 31
LP130	Ref. 10, 11, 12, 14, 21, 31
LP140	Ref. 21, 31
LPFX09-1	Ref. 15
LPFX09-2	Ref. 16

Chemical characterisation was performed by NMR, MS, IR and UV spectroscopy (ref. 21). Purity was determined by HPLC (UV- detection) on two bench batches ES130 and ES140, and four pilot batches LP110, LP120, LP130 and LP140 (see table below).

Table: HPLC assay and purity of six 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

*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

Ref. 21

Elemental analysis and NMR spectroscopy were performed on two bench batches, ES130 and ES140, and four pilot batches, LPllO, LP120, LP130 and LP140 (RMN = NMR).

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
RMN						
• ^{1}H	Conform	Conform	Conform	Conform	Conform	Conform
• ^{13}C	Conform	Conform	Conform	Conform	Conform	Conform

* Values not corrected of solvents and water

Ref. 21

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 below:

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

LP110 Ref. 15, 16, 17, 31, 79
LP120 Ref. 15, 16, 31
LP130 Ref. 12, 14, 17, 31

LP150 Ref. 79
LP160 Ref. 79
LP170 Ref. 79
LP180 Ref. 79
LPFX09-1 Ref. 15, 16, 17
LPFX09-2 Ref. 15, 16, 17
07BLY032 (radiolabelled batch, dermal absorption)

Purity:
Titration of S02374 in Pilot batch LP110 of S02771 was performed by HPLC at 320nm: 40 to 50% (w/w). Analysis of benzoic acid was performed by HPLC at 234 nm: 0.20 to 0.30% (w/w). Analysis and reanalysis of batch LP110 were performed as following and the results conformed to the specifications:

	First Analysis	Reanalysis After 18 months at RT/dark
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

Ref 28, 33, 34, 78

SCCS comment
Data on NMR and MS were not provided.
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.

3.1.5 Impurities / accompanying contaminants

A) Active substance S02374

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

Regarding the chemical process, the sum of the most important expected chemical impurities such as solvents were found to be less than 0.7% (w/w).

Ref. 21, 31

Heavy metal quantity was determined by ICP-Mass spectrometry on two bench batches ES130 and ES140, and four pilot batches LP110, LP120, LP130 and LP140.
A detailed analysis was performed on the six batches of S02374.

Batch	ES130	ES140	LP110	LP120	LP130	LP140
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,Mg,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,Au,Ba,Bi,Cd,Cu,Co,Ce,Cr,Cs,Dy,Er,Ga,Gd,Hg,Ho,La,Lu,Mg,Mn,Mo,Nb,Ni,Pb,Pd,Pt,Pr,Rb,Ru,Sb,Sn,Sr,Tb,Th,Ti,Tl,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

Ref. 21, 31

Water and sulphuric ash content was <0.1% in all six batches, respectively.

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

SCCS Comment
Impurities in Phenylene bis-diphenyltriazine have not been characterised adequately by HPLC. The impurities should be characterised and quantified by HPLC-PDA detection and/or LC/MS.

Metal element quantities were determined on S02771 by ICP Mass spectrometry on Pilot batch LP 110.

Analytical Method	*Sample preparation*	*Sample mineralisation*	*Analytical technique*
A	-	HNO_3 digestion	ICP-MS Based on ISO 17294

Element	concentration ($mg.kg^{-1}$)	WLQ ($mg.kg^{-1}$)	Element	concentration ($mg.kg^{-1}$)	WLQ ($mg.kg^{-1}$)
Ag	D	0,05	Mo	D	0,50
Al	D	0,50	Na	55,53	5,00
As	ND	0,50	Nb	ND	0,20
Ba	ND	0,10	Ni	D	0,50
Bi	D	0,10	P	ND	150,00
Ca	D	50,00	Pb	D	0,05
Cd	ND	0,05	Pr	ND	0,05
Ce	D	0,05	Rb	ND	0,05
Co	D	0,05	Sb	D	0,50
Cr	0,84	0,50	Se	ND	10,00
Cs	D	0,05	Si	ND	50,00
Cu	0,69	0,50	Sm	ND	0,05
Dy	ND	0,05	Sr	D	0,05
Er	ND	0,05	Tb	ND	0,05
Eu	ND	0,05	Th	ND	0,01
Fe	D	30,00	Ti	ND	0,50
Ga	ND	0,05	Tl	D	0,05
Gd	ND	0,05	Tm	ND	0,05
Ho	ND	0,05	U	ND	0,05
K	D	50,00	V	D	0,50
La	ND	0,05	W	0,12	0,05
Li	D	5,00	Y	0,09	0,05
Lu	ND	0,05	Yb	ND	0,05
Mg	ND	0,50	Zn	D	2,00
Mn	D	0,20	Zr	1,17	0,05

WLQ : Working Limit of Quantitation
D: Detected (analyte detected - concentration lower than the WLQ).
ND: Not Detected (analyte not detected).

Ref. 29

Analytical Method	*Sample preparation*	*Sample mineralisation*	*Analytical technique*
A	-	HNO_3 digestion	ICP-MS Based on ISO 17294
B	-	HNO_3 HCl digestion	ICP-MS Based on ISO 17294

Element	Method used	concentration ($mg.kg^{-1}$)	Uncertainty 2s ($mg.kg^{-1}$)
Ag	A	< 0.05	-
As	A	< 0,05	-
Ba	A	< 0.15	-
Cd	A	< 0,10	-
Co	A	< 0.10	-
Cr	A	0,87	0,05
Cu	A	0,70	0,20
Fe	A	3,87	1,09
Ni	A	0,21	0,06
Pb	A	< 0,10	-
Zn	A	< 2,0	-
Se	A	< 1,0	-
Sb	A	< 0,50	-
Hg	B	< 0,05	-

Ref. 30

3.1.6 Solubility

A) Active substance S02374:

WP30 (no batch reported):
Water: < 0.02 µg/L (OECD 105 / EEC A.6) Ref. 12
WP30, CH0222 (batch LP130):
Water: < 0.05 mg/L (LoD 0.04 mg/L; OECD 105 / EC A.6) Ref. 10
Estimated water solubility based on Log P_{ow} <0.01 µg/L Ref. 10
WP30 (batch LP110) at 25 ^{0}C after 15 h in organic solvents:
Chloroform <20 mg/ml
DMF : 0.60 mg/ml
DMSO: 0.10mg/ml
N-methyl-2-pyrrolidone
(NMP): 4.20 mg/ml

Solubility in lipophilic cosmetic solvents:
PEG400: 0.035 mg/ml
Isopropyl Palmitate : 0.010 mg/ml

Tests on batch LP110 were performed internally with a similar protocol to OECD 105 method.

Ref. 13

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 dermal absorption). The report was not provided.

Ref. 38

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).
As the described solubility of Phenylene bis-diphenyltriazine in various solvents is very low, it should be documented that the solubilised material indeed is the active substance (Phenylene bis-diphenyltriazine), but not the impurities.
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 solubility report was not provided.

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

Ref. 36

SCCS Comment
Except for DMSO, no data on solubility was provided for the ground active substance in water and other solvents.

3.1.7 Partition coefficient (Log P_{ow})

A) Active substance S02374:
Log P_{ow}: Tests performed on WP30, batch LP130
Log Pow = 8.29 (calculated)
Log Pow= 10.5 (measured, OECD 117 / EC A.8) Ref. 10, Ref. 11

3.1.8 Additional physical and chemical specifications

A) Active substance S02374 (WP30):

Melting point:	321^{0}C (measured on batch LP130)
Boiling point:	calculated value : 758 ± 70 ^0C
Flash point:	calculated value : 320 ± 28 ^{0}C
Vapour pressure:	/
Density:	calculated value : 1.23 ± 0.06 g/cm^3
	measured on batch LP130 : 1.30 ± 0.015 g/cm^3
Viscosity:	/
pKa:	calculated value: pKa 1 : -0.57 ± 0.63
	calculated value: pKa 2 : -2.02 ± 0.63
Refractive index:	/
pH:	7.0

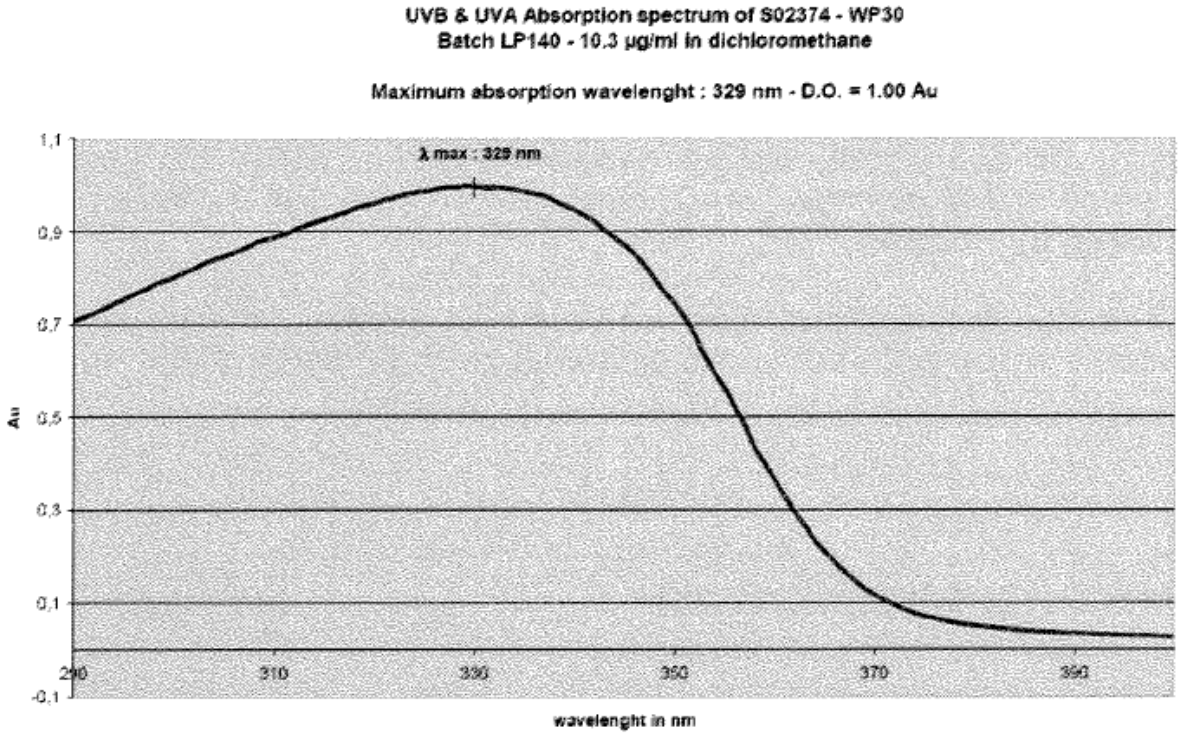
Ref. 10, Ref. 11

Particle size distribution of batch LP130:
Measured with Mastersizer 2000 (Malvern Instruments Ltd) based on laser diffraction technology.

- 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)

Ref. 14

UV-Vis spectrum: λ_{max} 329 nm



Ref. 23

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 and 78

Particle size distribution of typical batches:
Particle size 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.

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

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.

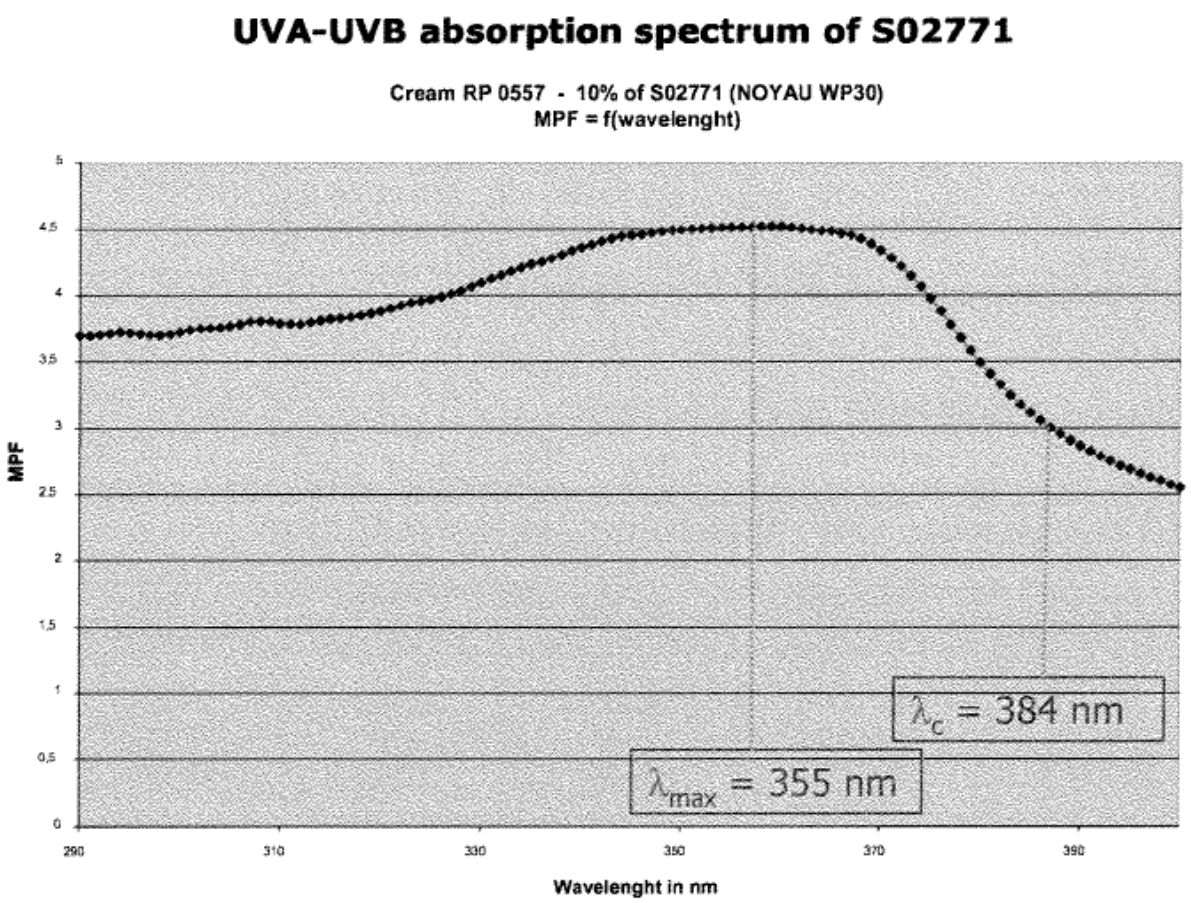
S02771 Batch	**LP150**		**LP160**		**LP170**		**LP180**	
Particle size analyzer	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS	Malvern Nanosizer ZS	Malvern NanoSight NS300 HS
Measurement	Intensity*	**Number**	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*

Ref. 79

Conclusion
The analysis of the size distribution of the particles shows that S02771 is outside the scope of the definition of nanomaterials given by the European Commission (50% measured by number; ref. 19) and outside the scope of the definition of nanomaterials given by Colipa (10% measured by volume; ref. 20).

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



Ref. 24

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.

	WP30 saturating concentration (mg/ml) at 25℃		WP30 concentration (mg/ml)
Solvents / excipients	Stirring time T 0,5h	Stirring time T 15h*	Stability time RT dark - 1 month
DMF	0,59	0,60	0,58
DMSO	0,11	0,10	0,11
PEG400	0,034	0,035	0,035
NMP	3,99	4,20	3,92
Myritol 318	n.d	n.d	n.d
Isopropyl palmitate	0,010	0,010	0,011

Ref. 13

Photostability data:

The photo-stability of the cosmetic ingredient S02771 was investigated. The test was performed under GLP conditions on batch LP110 according to ICH Topic Q1B (ref. 80): The purity was controlled with HPLC-UV after irradiation of the sample. Results are described in the table below:

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. 22

B) Cosmetic ingredient S02771

The stability of S02771 was investigated when stored under different conditions (+4 °C, +250 °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:

	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

Ref 33, 34, 35

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 (ref. 80):
The purity was controlled with HPLC-UV after irradiation of the sample. Results are described in the table below:

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

Stability of SO2771 in solvents and suspensions (batch LP110, ground active substance):
Solvents used for toxicological studies were DMSO, NaCI 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

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

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).

Overall 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 (recovery >99.9%), 2) the UV detection of the active substance was not performed at a specific wavelength (λmax 355nm), and 3) the peak purity of the active substance was not documented to be >99.9%.

Impurities in Phenylene bis-diphenyltriazine have not been characterised adequately by HPLC/UV alone. The impurities should be characterised and quantified by HPLC-PDA detection and/or LC/MS.

For the cosmetic ingredient S02771, no solubility data of the ground active substance was provided except for DMSO. Solubility data of the ground active substance should be

provided as it is not clear to which extent the particle size may change the solubility of the active substance.

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, Dermal Absorption). The report was not provided. As the described solubility of Phenylene bis-diphenyltriazine is very low, it should be documented that the solubilised material is indeed the active substance (Phenylene bis-diphenyltriazine), and not the impurities.

3.2 Function and uses

The cosmetic ingredient S02771 is intended to be used as a UV filter in sunscreen products.

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

A) Active substance S02374

Guideline:	OECD TG 423 (2001), Acute Toxic Class Method
Species/strain:	Rat, Wistar RjHan: WI (age: between 8 and 12 weeks)
Group size:	23 female animals in total
Test substance:	CH0222-WP30
Batch:	LP110
Purity:	99.6% (HPLC)
Vehicle:	Corn oil
Dose levels:	300 and 2000 mg/kg bw
Dose volume:	10 ml/kg bw
Administration:	Gavage
GLP:	/
Study period:	August to September 2009

The objective of this study was to evaluate the potential acute toxicity of the test item when administered once orally, by gavage to female Wistar rats followed by 14 days of observation. 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 that were recorded twice daily.
Acute toxicity of S02374 was first investigated using a stepwise procedure with a maximum of 2 steps per dose-level, each step using 3 animals. 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.
Bodyweights were measured on days 1, 3, 8 and 15, and necropsy findings noted for all animals on day 15.

Results
No mortality occurred and no abnormal clinical sign was observed at either dose-level. Bodyweight gain was not affected by treatment. No abnormal findings were noted in the macroscopic examination.

Conclusion
Since no dose resulted in mortality, the LD-50 was estimated to be higher than 2500 mg/kg.

Ref. 49

3.3.1.2 Acute dermal toxicity

Active substance **SO2374**

Guideline:	OECD TG 404 and 402
Species/strain:	Rat, Wistar RjHan: WI
Group size:	5 males and 5 females
Test substance:	CH0222-WP30
Batch:	LP140
Purity:	99.6%
Vehicle:	/
Dose levels:	2000 mg/kg bw
Administration:	Pure powder was applied.
GLP:	Yes
Study period:	October 2011

24 hours before test item application, fur was removed by close-clipping the dorsal part of the trunk of the animals in order to get an application site of approximately 8 x 5 cm which represented at least 10% of the total body surface area. On day 1, the test item was evenly spread directly over the application site of approximately 6 x 5 cm and covered with a gauze patch. The gauze patch was held in close contact with the skin by means of a non-occlusive tape for a 24h (±1h) 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. After clinical investigation, the animals were observed for 14 days after application.

Results
No death occurred during the course of the study and no abnormal clinical sign was observed.
For males, bodyweight gain was not affected by the treatment.
For females, the treatment induced a slight bodyweight loss (-2 grams in average) observed in all females between day 1 and day 3 but weight increase was restored thereafter. This transient observation of bodyweight gain was most probably related to the non-occlusive tape.
Neither erythema nor oedema was observed so that the Individual Irritation Index was 0 for all animals. Consequently, the Primary Irritation Index of the group was also 0. Desquamation was present on day 5 (72h reading time) in one female and on day 8 in 2 females. S02374 (CH0222 WP30) was non-irritating to skin.

As no mortality was observed, S02374 (CH0222 WP30) is not classified and the LD-50 by dermal application was estimated to be higher than 2000 mg/kg. Furthermore, S02374 was not irritating to skin.

Ref. 52

3.3.1.3 Acute inhalation toxicity

Cosmetic ingredient **SO2771**

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

SCCS comment
The study results suggest low acute inhalation toxicity of the test item.

3.3.1.4 Acute intraperitoneal toxicity

No data submitted.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Study 1:
Active substance **SO2374**

Guideline:	Annex IB.40, Directive 2000/33/CE from the Commission, April 25th 2000.
Test system:	0.5 cm^2 Reconstituted Human Epidermis (RHE) made from Human Normal Keratinocytes (Skinethic™). Abdominal skin from a 48-year-old woman.
Replicates:	1
Test substance:	WP30
Test batch:	LP110
Purity:	99.6% (HPLC, 320 nm)
Concentrations:	10%
Vehicle:	Paraffin oil
Treatment :	3 repeated applications
Treatment period:	20 h
Post-treatment incubation time:	not indicated
Positive control:	5% SDS in water
Negative control:	Crème reparatrice cicalfate, Avéne Laboratory, batch F2268, pure
Direct interaction of test item with MTT:	Negative
Colouring of epidermis:	not tested
GLP:	Yes
Study period:	May 2009

Method details
The MTT method was used for *in vitro* cytotoxicity testing. An MTT direct-interaction test was made before the main test on reconstructed epidermis. The Test Article did not interact with MTT.

Results

Product	concentration	Viability (%)	Expected viability	Acceptability
SDS	4,5 mg/ml (0,45%)	26% +/-5% (n=3)	<50%	yes
CRÈME REPARATRICE CICALFATE	Pur	86% +/-2% (n=3)	>80%	yes
S02374	Diluted at 10%	78% +/-7% (n=3)		

Conclusion
The active substance S02374, batch LP110, does not reduce MTT in a specific way. At the concentration of 10%, the cell viability is 100%. S02374, Batch LP110, is not a skin irritant.
Ref. 53

SCCS comment
Only a summary study report is available. The study plan or SOPs describing details of the study conduct are not available.
Only 1 tissue batch has been used and no replicates were performed.
The solubility of SO2374 in paraffin oil has not been reported and might be similar to that in cosmetic lipophilic solvents (<0.04 mg/ml).
On the basis of these results, a skin irritation potential of the test item cannot be excluded.

Study 2:
Cosmetic ingredient **SO2771**

Guideline:	/
Test system:	0.5 cm^2 Reconstituted Human Epidermis (RHE) made from Human Normal Keratinocytes (Skinethic™). Origin foreskin
Replicates:	3 epidermis samples for each condition tested: Test item, controls and vehicle.
Test substance:	Noyou WP30, SO2771
Test batch:	LP110 (yellow powder, not the suspension)
Purity:	99.6% (HPLC, 320 nm)
Concentrations:	10%
Vehicle:	0.5% Carboxymethylcellulose (CMC) in water
Dose levels:	
Treatment period:	42 ± 1 min
Post-treatment incubation time:	42 ± 1 h
Positive control:	5% SDS in water
Negative control:	Ultra-pure water.
Direct interaction of test item with MTT:	Negative
Colouring of epidermis:	not tested
GLP:	No
Study period:	February 2011

The MTT method was used for *in vitro* cytotoxicity testing.

Results

Test Article	concentration	OD	Mean OD	%viability	Mean% viability	Standart deviation (%)	Classification
S02771 Pilot Batch: LP110	10%	2.242 2.086 2.167	2.165	112.8 105.0 109.1	100.0	3.9 Conforms	**Non irritant**

Conclusion
The cosmetic ingredient S02771, batch LP110, does not reduce MTT in a specific way. At the concentration of 10%, the cell viability is 100%. S02771, batch LP110, is not a skin irritant.
Ref. 54

SCCS comment
Only a summary study report is available. The study plan or SOPs describing details of the study conduct are not available.

Only 1 tissue batch has been used and no information is given on the integrity of the tissues used.
On the basis of these results, a skin irritation potential of the test item cannot be excluded.

3.3.2.2 Mucous membrane irritation / Eye irritation

HET CAM Test

Study 1:
Active substance **SO2374**

Guideline:	JORF of 26/12/96, French decree of November 29th, 1996
Test system:	HET CAM test, chorionallantoic membrane of the hen's egg,
Endpoints:	Vascular damage (hyperaemia, haemorrhage and coagulation)
Number of samples:	Four for the test item (number not given for positive and negative controls)
Treatment period:	20 seconds
Test substance:	CHO222-WP30
Batch:	LP110
Purity:	99.6% (HPLC, 320 nm)
Concentrations:	10%
Vehicle:	Paraffin oil
Positive control:	3.2% Lauryl sulfobetaine (solvent not given in the report)
Negative control:	0.9% NaCl in water
GLP:	Yes
Study period:	July 2009

The test item was placed directly onto the chorionallantoic membrane of the hen's egg and left in contact for 20 seconds. The membrane was examined for vascular damage. Irritancy was scored according to the severity.

Results
With the test item (10%), zero scores were obtained for hyperaemia, haemorrhage and coagulation. The positive control showed a total score of 12 and the negative control a total score of zero, respectively.

Conclusion
The test item, S02374, batch LP110, is not an irritant to the eye or to mucous membranes at a concentration of 10%.

Ref: 55

SCCS comment
Only a summary report is available. The study plan or SOPs describing details of the study conduct are not available.
The solubility of SO2374 in paraffin oil has not been reported and might be similar to that in cosmetic lipophilic solvents (<0.04 mg/ml).
The report indicates a very short exposure time of only 20 seconds. Usually the exposure/observation time is 5 minutes.
The HET CAM test can only provide supportive evidence to identify serious eye damage.
On the basis of these results, an eye irritation potential of the test item cannot be excluded.

Study 2:
Cosmetic ingredient **SO2711**

Guideline:	JORF of 26/12/96, French decree of November 29th, 1996
Test system:	HET CAM test, chorionallantoic membrane of the hen's egg,
Endpoints:	Vascular damage (hyperaemia, haemorrhage and coagulation)
Number of samples:	Four for the test item (number not given for positive and negative controls)
Treatment period:	20 seconds
Test substance:	SO2711
Batch:	LP110
Purity:	99.6% (HPLC, 320 nm)
Concentrations:	10%
Vehicle:	0.9% NaCl in water
Positive control:	3.2% Lauryl sulfobetaine (solvent?)
Negative control:	0.9% NaCl in water
GLP:	Yes/No
Study period:	June – September 2011

The test item was placed directly onto the chorionallantoic membrane of the hen's egg and left in contact for 20 seconds. The membrane was examined for vascular damage. Irritancy was scored according to the severity.

Results
With the test item (10%), zero scores were obtained for hyperaemia, haemorrhage and coagulation, respectively. The positive control showed a total score of 12 and the negative control a total score of zero, respectively.

Conclusion
The test item, S02771, batch LP110, is not irritant to the eye or to mucous membranes at the concentration of 10%.

Ref: 56

SCCS comment
Only a summary study report is available. The study plan or SOPs describing details of the study conduct are not available.
The number of eggs used for the positive and negative controls is unclear.
The water solubility of the active substance of SO2771 is very low (<0.02 µg/L).
The report indicates a very short exposure time of only 20 seconds. Usually the exposure/observation time is 5 minutes.
The HET CAM test can only provide supportive evidence to identify serious eye damage. On the basis of these results, an eye irritation potential of the test item cannot be excluded.

Neutral Red Uptake Test

Study 1:
Active substance **SO2374**

Guideline:	JORF of 30/12/99, French decree of December 27th, 1999
Test system:	Rabbit corneal fibroblasts (SIRC)
Replicates:	not indicated
Test substance:	CHO222-WP30
Batch:	LP110
Purity:	99.6% (HPLC, 320 nm)
Concentrations:	10% (- 25% - 50% - 100%)
Vehicle:	Paraffin oil

Treatment period:	60 seconds
Post-treatment incubation time:	not indicated
Positive control:	SDS, 0.01 – 0.05 – 0.2%
Negative control:	not indicated
GLP:	Yes
Study period:	September 2009

Results
The percentage of cell death observed at a 50% concentration of test item is 3.5%.

Conclusion
S02374, batch LP110, is practically not irritant to the mucous membranes (in particular the eye) at the concentration of 10%.

Ref: 57

SCCS comment
Only a summary study report is available. The study plan or SOPs describing details of the study conduct are not available.
The solubility of SO2374 in paraffin oil has not been reported and might be similar to that in cosmetic lipophilic solvents (<0.04 mg/ml).
No negative controls were included.
No results are given for the positive control.
On the basis of these results, an eye irritation potential of the test item cannot be excluded.

Study 2:
Cosmetic ingredient **SO2711**

Guideline:	JORF of 30/12/99, French decree of December 27th, 1999
Test system:	Rabbit corneal fibroblasts (SIRC)
Replicates:	not indicated
Test substance:	SO2711
Batch:	LP110
Purity:	99.6% (HPLC, 320 nm)
Concentrations:	10% (- 25% - 50% - 100%)
Vehicle:	0.9% NaCl in water
Treatment period:	60 seconds
Post-treatment incubation time:	not indicated
Positive control:	SDS, 0.01 – 0.05 – 0.2%
Negative control:	not indicated
GLP:	Yes
Study period:	March-April 2011

Results
The percentage of cell death observed at a 50% concentration of the test item is 4%.

Conclusion
S02771, batch LP110, is practically not irritant to the mucous membranes (in particular the eye) at the concentration of 10%.

Ref: 58

SCCS comment
Only a summary report is available. The study plan or SOPs describing details of the study conduct are not available.
No negative control included.

No results are given for the positive control.
On the basis of these results, an eye irritation potential of the test item cannot be excluded.

Overall SCCS conclusion on the skin and eye irritation
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 SO2374 and the cosmetic ingredient SO2711 (ground form). However, no signs of skin irritation were noted in the acute dermal toxicity study in rats on the active substance SO2374.
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.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Study 1
A) Active substance **SO2374**

Guideline:	OECD TG 429 / EC B.42; US-EPA TG OPPTS 870.2600, EPA 712-C03-197
Species/strain:	Female mice, CBA/CaOlaHsd, 8-9 weeks old
Group size:	3 (preliminary test) 5 per dose group (main test)
Test substance:	CHO222-WP30 (S02374)
Batch:	LP110
Purity:	99.6% (HPLC)
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)
GLP status:	Yes (main test only)
Study period:	September 2009

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 ^{3}H-methyl thymidine by intravenous injection (tail vein) of 250 µl 0.9% saline. About 5 hours after the injection, the animals were sacrificed, the draining auricular lymph nodes excised and weighed for each individual animal. After a standard work-up procedure, the radioactivity was determined.

Results
All animals survived throughout the test period without showing any clinical signs.
SI values in each test group:

The stimulation index at a concentration of	6.25%	was	**1.2**
The stimulation index at a concentration of	12.5%	was	**0.9**
The stimulation index at a concentration of	25%	was	**1.1**

Mean values of the Stimulation Indices were ≤ 1.2 for all three concentrations and no dose response relationship was seen. Hence, none of the three tested concentrations of the test item reached the stimulation index of 3.
All animals showed the accepted weight development, which includes a weight loss of up to 2 grams throughout the study.

EC3 values (derived by linear interpolation) could not be calculated because the stimulation indices of all concentrations were below 3.

Conclusion
Under the experimental conditions adopted, S02374 (batch No. LP110) at the doses tested is expected to have no sensitising properties and therefore should not be regarded as a dermal sensitiser.

Ref: 59

SCCS comment
No data on the solubility of the test item in acetone/olive oil (AOO), 3+1 (v/v) has been provided. Thus, the relevance of the test cannot be assessed.

Study 2
B) Cosmetic ingredient **SO2771**

Guideline:	OECD TG 442B Skin Sensitization: Murine Local Lymph Node Assay: BrdU-ELISA
Species/strain:	Mice, CBA/J Rj strain
Group size:	4 (preliminary test) 4 per dose group and control group (main test)
Test substance:	SO2771 (yellow suspension, pH 4.11, content of the ground active substance 46.5%)
Batch:	LP110
Purity:	>99%
Vehicle:	Water for injection
Concentration:	6.25%, 12.5%, and 25% (w/w)
Negative control:	Vehicle alone (water)
Positive control:	2,4-dinitrochlorobenzene (DNCB) at the concentration of 0.5% acetone/olive oil (AOO, 4:1, v/v) compared with a vehicle control group (AOO)
GLP status:	Yes (main test only)
Study period:	February 2011 (according to the applicant; see SCCS comment)

The concentrations were based on the results of a preliminary study in which no effect on ear thickness was seen at any dose and no local reaction was seen after application of S02771 at 6.25%, 12.5% and 25%, however rigid ears were observed in animals treated at 50% on day 3 and day 4.
At the Sponsor's request, three groups were treated with S02771 at 6.25%, 12.5% and 25% in water. Positive and negative control groups were also included. In addition to the treatment procedure described for the preliminary study, any lymphoproliferative response was measured by incorporation of 5-bromo-deoxyuridine (BrdU) on day 6: All animals of all groups received a single intraperitoneal injection of 250 µL of 0.9% sodium chloride containing 2.5 mg of BrdU. Approximately 5 hours later, the animals were euthanised. Bilateral draining auricular lymph nodes were excised. After a standard work-up procedure, the results were elucidated using an ELISA system on day 7. Stimulation Index (SI) was expressed as the ratio of mean values of optical density of the test nodes relative to that recorded for control (vehicle) nodes and Cellularity Index expressed as the ratio of mean

amount of cells (x 10^6 cells) in the treated group and the mean amount of cells (x 10^6 cells) in the vehicle group.

Results
No mortality, clinical or local signs and no effect on bodyweight gain were observed.
An increase in ear thickness was seen with S02771 at 6.25% (+13% on day 3), at 12.5% (+20% on day 3) and at 25% (+5% and +35%, respectively on day 2 and day 3) when compared with the vehicle (water).
Amount of cells, cellularity index and stimulation index of S02771 at 6.25%, 12.5% and mainly at 25% were higher than the values found for the vehicle. Stimulation index of S02771 6.25%, 12.5% and 25% were in the range of the values found for the vehicle.
Whatever the dose tested, S02771 did not produce a stimulation index (SI) equal to or greater than 3. Consequently, the EC3 value, defined as the theoretical concentration resulting in a SI value of 3, cannot be calculated for S02771.
DNCB at 0.5% showed a statistically significant stimulation index of 3.3. DNCB at 0.5% induced a cellularity index of 5.3 with a total amount of viable cells per node of 17.9 x 10^6 compared to 3.4 x 10^6 with the vehicle control group. A statistically significant increase of 35% in ear thickness was observed on day 3 compared with the vehicle control group.

Conclusion
Under the experimental conditions adopted, S02771 (batch No. LP110) at the doses tested did not induce delayed contact hypersensitivity in the murine Local Lymph Node Assay using CBA/J Rj female mice after three consecutive days of treatment.

SCCS comment
Unusual for a GLP study, the date of the experimental phase of the study is not given in the study report. The study was conducted according to the OECD TG 442B using BrdU ELISA to measure lymphocyte proliferation (July 2010). Therefore, an SI of 1.6 instead of 3 should have been used as criterion for a sensitisation potential.
According to OECD TG 442B, wholly aqueous vehicles should be avoided in the LLNA, because those vehicles are likely to run off the skin. Thus the relevance of the test is questionable. In principal, in such a case, a vehicle that contains a solubiliser (e.g. 1% Pluronic®L29) may be used.

Overall SCCS comment on sensitisation
For the above reasons, the relevance of the studies provided is unclear. Therefore, a skin sensitising potential cannot be excluded.

3.3.4 Dermal / percutaneous absorption

Study 1
Active substance SO2374 on intact skin

Guideline:	OECD 428
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 (non-labelled):	CHO222 - WP30 (non-ground active substance)
Batch:	LP110
Purity:	99.6% (HPLC at 320 nm)
Test substance (labelled):	^{14}C WP30
Substance code:	07BLY032
Batch:	/
Specific activity:	244 µCi/g
Purity:	99.1%
Doses applied:	10 mg of sun screen formulation / cell (0.543 µCi/cell)
containing	1 mg WP30

Dose volume/amount:	5 mg/cm^2
Receptor fluid:	3% bovine serum albumin in 0.9% NaCl solution
Exposure time:	24 h
Method of analysis:	Liquid scintillation counting
GLP:	Yes
Study period:	August-September 2010

Method details
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 cm^2) were included in the study if the TEWL was ≤ 13 ± 3 g/m^2/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 radiolabelled batch No. 07BLY032 used for the dermal absorption of S02771 was performed before this study. Stability of the formulation was tested over 24.67 hours

Results
TEWL values were between 0.7 and 4.6 g/m^2/h.
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.

Trace amounts of radioactivity in receptor fluid were reported for 2 donors, whereas for one donor, all receptor fluid values were below the LoQ of 0.029 µg eq/cm^2.
High variability in absorbed fractions (sum of radioactivity in epidermis, dermis and receptor fluid) were observed and ranged between
- 0.33% and 0.04% (mean 0.17 % ± 0.16 (SD)) for donor 1
- 0.12% and 0.02% (mean 0.05 % ± 0.04 (SD)) for donor 2
- 0.16% and 0.03% (mean 0.10 % ± 0.06 (SD)) for 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 1.7 µg eq/cm² (mean ± 2SD: 0.58 ± 0.6) according to SCCP/0970/06 guidance.

Ref. 38, 39

SCCS comment
In contrast to the claim in the applicant's dossier, the study was most probably performed with the active (non-ground) substance Phenylene bis-diphenyltriazine (SO2374) in a sunscreen formulation (see Certificate of Analysis in the report).
The study did not comply with the SCCS Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10) as only 3 donors were used. The study authors recognised the high variability in dermal absorption and therefore they suggested adding 2 standard deviations to the mean value. According to the applicant, the absorption (mean ± 2 SD) from 3 donors was 0.11 % ± 0.22 % = 0.33 % corresponding to 1.7 µg eq/cm². A re-calculation from ref. 39 revealed a value 1.8 µg eq/cm² (mean ± 2SD: 0.58 ± 1.20).

The study demonstrating that WP30 in the receptor fluid is soluble at a concentration of 4.56 µg/ml is not available and should be provided. The experimental conditions of the

solubility experiment should be reported. In addition, as the described solubility of Phenylene bis-diphenyltriazine is very low, it should be documented that the solubilised material indeed is the active substance (Phenylene bis-diphenyltriazine) and not the impurities or other possible artefacts.

Study 2
Active substance SO2374 on intact and irradiated skin

Guideline	OECD TG 428
Test system:	Human dermatomed skin (0.3 ± 0.1 µm)
Membrane integrity:	TEWL
Sample number:	4 donors, 2 cells per donor in each condition
Conditions:	(healthy skin, stripped, irradiated and stripped/irradiated
Test substance (non-labelled):	WP30 (non-ground active substance)
Batch:	LP110
Purity:	99.6 % 8HPLC)
Test substance (labelled):	[^{14}C] WP30
Radiolabelled batch:	07BLY032
Specific activity:	244 µCi/g
Purity:	99.1% (HPLC)
Doses applied:	10 mg of sunscreen formulation / cell (ca. 0.5 µCi/cell)
Dose volume/amount:	5 mg/cm^2
Receptor fluid:	3% bovine serum albumin in 0.9% NaCl solution
Exposure time:	24 h
Method of analysis:	Liquid scintillation counting
GLP:	Yes
Study period:	October 2011 – January 2012

Methods
Human skin samples were obtained during abdominal surgery from four donors and frozen. After thawing and depending on test conditions skin was processed 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 cm^2. Healthy human skin was included in the study if the TEWL was ≤ 13 ± 3 g/m^2/h. Radiochemical purity was checked before application of the formulation, however, stability of the formulation was not investigated. 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 hr exposure time, tape stripping was performed and radioactivity in the different compartments was determined by liquid scintillation counting.

Results:
Different pre-treatment conditions influenced TEWL differently: whereas stripping had an influence on TEWL, TEWL was not influenced by irradiation. Apart from initially stripped skin, 1-8 tape strips were used to remove stratum corneum after test substance exposure.
Further results as provided by the applicant are given in the following table:

Skin treatment	Mass balance [%]; n=8	Mean ± 1 SD	Mean ± 2 SD	Absorption (sum of mean + 2SD)
		[Based on amount present in epidermis, dermis and receptor fluid]		
Healthy skin	94 – 138 %	0.28% ± 22 % (n=8)	0.28% ± 44 % (n=8)	0.72 % (n=8)

		0.27 ± 0.28 % (n=4)	0.27 ± 0.56 % (n=4)	0.83 % (n=4)
Irradiated skin	95 – 105 %	0.10 ± 0.04 % (n=8)	0.10 ± 0.08 % (n=8)	0.18 % (n=8)
Stripped skin	94 – 114 %	0.66 ± 0.84 %	0.66 ± 1.68 %	2.34 %
Irradiated and stripped skin	94 – 101 %	0.53 ± 0.42 %	0.53 ± 0.84 %	1.37 %

These results showed that no impact of the irradiation was observed on the skin penetration. However, the penetration increased after stripping or irradiation and stripping from 0.72% to 2.34% and 1.37%, respectively.

Ref.: 61

SCCS comment

In contrast to the claim in the applicant's dossier, the study was most probably performed with the labelled active (non-ground) substance Phenylene bis-diphenyltriazine (SO2374).
Mass balance in healthy human skin was outside acceptance criteria in 6 of 8 cells; however, study authors calculated absorption by either using results from all cells (n=8) or by excluding 4 cells with highest deviations (n = 4). A discussion on probable causes was included in the report. Most probably, due to inhomogeneity, higher amounts than calculated have been applied.
High variability in the amounts detected in the various compartments was observed. Thus, the addition of 2 SD to the obtained means is justified. In many cells investigated, radioactivity in receptor fluid was below the LoQ of 0.029 µg eq/cm^2. With respect to healthy skin, only 2 from 8 cells were within the acceptance criteria based on recovery. Thus, no conclusion can be drawn on healthy skin. Absorption in (1) irradiated, (2) stripped and (3) irradiated and stripped skin (mean ± 2 SD) was 0.18, 2.34 and 1.37 % of the applied dose, respectively.
As irradiation did not change TEWL, the value obtained from irradiated skin might also be taken for healthy skin.
Calculation of µg eq/cm^2 for irradiated skin using data of the applicant: 0.92 µg/cm^2 (mean ± 2SD: 0.52 ± 0.4).

Overall SCCS comment on dermal absorption

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. 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 SO2374, not with the ground cosmetic ingredient SO2771. 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.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (10 days) oral toxicity

Dose range finding study for the repeated dose toxicity study.

Cosmetic ingredient SO2771

In an oral dose range finding study in rats (gavage) with the cosmetic ingredient SO2771 (NOYAU WP30) at doses of 0, 100, 300 or 1000 mg of the suspension /kg bw/day, all animals survived until the scheduled study period. No clinical signs were noted in any animal during the course of the study. The mean bodyweights and the mean food consumption were unaffected by treatment with the test item. No test item related changes in parameters of haematology and clinical biochemistry were noted. All macroscopic and microscopic findings recorded were considered to be background findings.

In conclusion, based on the results of this oral gavage 10-day dose range finding study in Wistar rats, the doses of the suspension of 100 mg/kg bw/day, 300 mg/kg bw/day and 1000 mg/kg bw/day of S02771 (suspension containing 48.5% of the ground active substance) corresponding to around 50, 150 and 500 mg of the ground active substance/kg bw/day, could be administered also in repeated dose toxicity studies.

Ref. 50

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

Cosmetic ingredient SO2771

Guideline:	OECD TG 408 (1998); Directive 96/54/EC, B. 26. "Subchronic Oral Toxicity", 30 September 1996, including Additional Testing for Neurotoxicity.
Species/strain:	Rat, RccHan™: WIST(SPF)
Group size:	Controls: 16 males and 16 females (8 males and 8 females as recovery group) Low and mid dose groups: 10 males and 10 females each High dose group: 16 males and 16 females (8 males and 8 females as recovery group) One additional group: 2 males and 2 females
Test substance:	NOYAU WP30 (yellow suspension containing 48.5% of the ground active substance).
Batch:	LP110
Purity:	99.6% (HPLC at 320 nm)
Vehicle:	0.5% carboxymethyl cellulose (CMC) in water
Dose levels:	0, 100, 300, 1000 mg of the suspension/kg bw/day corresponding to 0, 48.5, 146 and 485 mg of the ground active substance /kg bw/day
Dose volume:	10 ml/kg bw
Route:	Oral
Administration:	Gavage once daily
GLP:	Yes
Study period:	June 2011 - April 2012

The animals were about 6 weeks old at delivery. They were treated by oral gavage for a period of 13 weeks followed by a four-week treatment-free recovery period.
Clinical signs, outside cage observation, food consumption and bodyweights were recorded periodically during pre-test, the treatment and recovery periods. Functional observational

battery, locomotor activity and grip strength were performed during week 13 and 17. Ophthalmoscopy investigations were performed during acclimation (all animals) and at the end of treatment (control and high-dose animals only).
At the end of the dosing and the treatment-free recovery period, blood samples were withdrawn for haematology and plasma chemistry analyses. Urine samples were collected for urinalyses.
Thyroid hormones (T3, T4 and TSH) were analysed in plasma samples of week four and 13.
Vaginal smears for oestrus cycle were investigated in all females of control and treated groups over a 14-day period during the two last weeks of the treatment period.
All animals were sacrificed, necropsied and examined post mortem. Samples of the tissues and organs were collected from all animals at necropsy and specified organs were weighed. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals. Due to test item-related morphologic changes that were detected in kidneys of high-dose males, those same organs from the mid- and low-dose group animals were examined to establish a no-effect level.

Stability of the test item in the vehicle CMC was proven for up to 9 days and fresh preparations were made within this time interval (see ref. 51). Concentration and homogeneity of dose formulations were determined in samples taken at least once before the first administration and then on three occasions after experimental start. The dose formulations were analysed using a HPLC method provided by the sponsor.

Results

The application formulations investigated during the study were found to comprise NOYAU WP30 in the range of 86.1% to 104.0% and, thus, the required content limit of ±20% with reference to the nominal content was met. The homogeneous distribution of NOYAU WP30 in the preparations was approved because single results found did not deviate more than 6.1% (<15%) from the corresponding mean.

Viability/mortality:
One male (no. 2) of the control group was found spontaneously dead on treatment day 22. One male (no. 29) and one female (no. 81) treated with 300 mg/kg/day of the test item were found spontaneously dead on treatment days 45 and 22, respectively. A clear cause of death could not be established. Findings of alveolar haemorrhage in animals 2 and alveolar oedema in animal 29 may have contributed to mortality. As the unscheduled deaths were in the intermediate dose level (300 mg/kg/day), not found in the highest dose level, and occurring also in a control animal, the relation with the test item could not established. Further, the presence of lung observations such as haemorrhage in two of the animals is more suggestive to be a consequence of the gavage procedure than related to the test item. All other animals survived the scheduled treatment and recovery periods.

Clinical signs:
No clinical signs were noted in males during the treatment and recovery period. Females treated with 100 mg/kg/day or 300 mg/kg/day of the test item also showed no clinical signs during the treatment period.
Hair loss and scabs on the cervical neck were noted in one female treated with 1000 mg/kg/day of the test item from treatment week four to seven (hair loss until week nine). Slight reddish sore at this site was noted on one day in treatment week four. Slight breathing noises were noted in one female treated with 1000 mg/kg/day on two days in week four. One female had hair loss on different sites in week 13. These findings could be considered to be related to the treatment but not to the test item.
No clinical signs were noted in females formerly treated with 1000 mg/kg/day during the recovery period.

Bodyweights and food consumption:
The mean bodyweights and the mean bodyweight gain of test item treated males and females were comparable with those of the control animals at the end of the treatment and at the end of the recovery period.
No test item related changes of absolute and relative food consumption were noted in test item treated animals when compared with control animals at the end of the treatment and at the end of the recovery period.

Ophthalmoscopic examinations:
Corneal opacity of both eyes and persistent hyaloids vessels of both eyes were noted during the acclimation period in a few males and females of all groups. In a few females, a persistent pupillar membrane was additionally noted in one or both eyes. These findings were considered to be typical background findings of animals of this strain and age.
Very similar findings were noted in animals at the end of treatment and the incidence of the corneal opacity of test item treated animals was comparable with that of the control animals.

Functional observational battery:
Grip strength:
The mean fore hand grip strength of females treated with 1000 mg/kg/day was slightly increased ($p<0.05$) when compared with the controls during week 13. This was considered to be incidental. No other changes in mean fore hand grip strength and hind limb grip strength were noted in any test item treated male or female when compared with controls at the end of treatment and recovery period.
Locomotor activity:
No changes of mean locomotor activity of test item treated animals were noted when compared with controls during the treatment period.
The mean locomotor activity (0 to 10 minutes interval) of females treated with 1000 mg/kg/day was increased ($p<0.01$) in week 13. This increased activity during the first minutes is not unusual and therefore considered not to be test item related.
The mean locomotor activity (50 to 60 minutes interval) of males formerly treated with 1000 mg/kg/day was slightly increased ($p<0.05$) and the mean locomotor activity (30 to 40 minutes interval) of females of the same dose group was slightly decreased when compared with controls during the recovery period. These findings were considered to be incidental.

Clinical laboratory investigations:
Haematology:
No test item related changes of parameters of haematology were noted. The following statistically significant values were noted when comparing test item treated animals with controls.
The mean relative and absolute reticulocyte counts were increased ($p<0.05$, 19 and 75%, respectively) in males treated with 100 mg/kg/day of the test item when compared with controls at the end of the treatment period. The mean values of maturity indices were increased (medium and high reticulocytes; $p<0.01$ and $p<0.05$, respectively, 25 and 100%, respectively) and the mean value of the low reticulocytes index was slightly decreased ($p<0.01$, 10%) accordingly in males of this dose group when compared with controls at the end of the treatment period. At the end of the recovery period, the mean value of absolute reticulocyte count of females formerly treated with 1000 mg/kg/day of the test item was slightly decreased ($p<0.05$, 41%) and the mean relative and absolute value of neutrophiles was slightly decreased ($p<0.05$, $p<0.01$, respectively) in females of this group when compared with controls. At the end of the recovery period, the mean relative value of lymphocytes was slightly increased ($p<0.05$) and the mean absolute value of monocytes was slightly decreased ($p<0.01$) in females of this group when compared with controls. These findings were considered to be incidental.

Clinical biochemistry:
No test item related changes of parameters of clinical biochemistry were noted. The following statistically significant values were noted when compared with test item treated animals with controls.
The mean value of globulin was slightly decreased ($p<0.01$) and the mean *albumin to globulin ratio* was slightly *increased* ($p<0.01$) in males treated with 100 mg/kg/day of the test item when compared with controls at the end of the treatment. This was considered not to be test item related because no effect was noted in both groups with higher dosage. The mean value of total bilirubin was slightly increased ($p<0.05$) in females treated with 1000 mg/kg/day, the mean value of sodium of females of this group was slightly increased ($p<0.05$), and the mean value of triglycerides was slightly increased ($p<0.05$) in females of this group when compared with controls at the end of treatment. These findings were considered to be incidental because they could not be associated with histopathological findings. Additionally, these findings were not noted at the end of the recovery period. The mean value of albumin was slightly decreased ($p<0.01$) and the mean albumin to globulin ratio was slightly decreased ($p<0.05$) in females treated with 300 mg/kg/day of the test item when compared with controls at the end of the treatment. This was not considered to be test item related because no dose response relationship could be observed.

Urinalysis:
No test item related changes of parameters of urinalysis were noted in test item treated males and females when compared with controls at the end of the treatment and the recovery period, respectively.

Oestrous Cycle:
Most of test item treated females showed regular oestrous cycles like the control females during the treatment period. Therefore no investigations were performed during the recovery period.

Thyroid hormone analysis:
Levels of TSH, Total T3 and Total T4 in rat serum were measured and after 3 and 13 (1^{st} day of week 14) weeks of treatment with the test item at 100 mg/kg/day, 300 mg/g/day and 1000mg/kg/day:
No test item related differences concerning TSH, total T3 and total T4 levels were observed in all treated groups when compared to the respective control values. Therefore the samples of week 18 were not investigated.

Pathology:
Macroscopic findings
There were no gross lesions that could be attributed to treatment with the test item. All gross lesions recorded were considered to be within the range of normal background alterations.
Organ weights:
No changes in mean organ weights, mean organ to bodyweight ratios and organ to brain weight ratios were noted in test item treated animals when compared with control animals at the end of the treatment period and at the end of the recovery period.
Microscopic Findings:
Kidney:
A dose-dependent increase of mean severity of hyaline droplets was recorded in exposed groups of males. This finding resolved after the recovery period. Variable severity scores were observed in the control groups of the exposure and the recovery period.

All PAS-stained testes of the study were qualitatively staged. There were no abnormal lesions encountered during sperm staging regarding completeness of stages and maturation of cell populations. Individual lesions recorded were within the range of background alterations that may be recorded in this type of study, in rats of this strain and age.

Other Findings:
The remaining findings recorded were within the range of normal background lesions which may be recorded in animals of this strain and age.

Conclusion
Oral administration for 13 weeks of the cosmetic ingredient containing the ground active substance in a suspension (S02771) to Wistar rats at doses of the suspension of 0, 100, 300 and 1000 mg/kg/day, corresponding to 0, 48.5, 146 and 485 mg of the ground active substance/kg bw/day, resulted in no test item related clinical signs, no test item related changes in mean fore hand grip strength and hind limb grip strength, no test item related changes of mean locomotor activity, no test item related changes of absolute and relative food consumption, comparable mean body weights and mean body weight gain of test item treated males and females and control animals, no changes in mean organ weights, mean organ to bodyweight ratios and organ to brain weight ratios compared with controls and no gross lesions that could be attributed to treatment with the test item.

Test item-related findings were generally restricted to hyaline droplets in the kidneys of males treated with 1000 mg/kg/day of the test item S02771. Hyaline droplets were found at increased mean severity in males of this dose group. Hyaline droplets are a normal background finding in male rats. This finding in groups 1-3 was considered to be within the historical background range and the range observed in control groups of this study. Under the conditions of this study, the finding resolved after the treatment free period and is therefore considered to be of reversible nature. The occurrence of hyaline droplets (males only) is deemed to be related to treatment. Since hyaline droplets in the male rat relate to accumulation of alpha2-microglobulin, and little or none of this protein is present in man, a nephropathy in man that involves the same mechanism is unlikely to occur with the test item.

In addition, it can be noted that 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.

After oral administration of S02771 to Wistar rats at doses of the suspension of 100, 300 and 1000 mg/kg/day, for 13 weeks and a four-week treatment-free recovery period, based on histopathology a NOEL (No Observed Effect Level) could be established for the ground active substance (content 48.5% in the suspension)
- at 146 mg/kg/day for males,
- at 485 mg/kg/day for females.
A NOAEL (No Observed Adverse Effect Level) for males could be established at 485 mg/kg/day for the ground active substance.

Ref. 64

3.3.5.3 OECD 422 Screening Test

A Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test conducted with the non-ground active substance (**S02374**) according to OECD TG 422 is described in section 3.3.8.2.
The No Observed Effect Level (NOEL) for parental toxicity was considered to be 1000 mg/kg bw/day.

3.3.5.4 Repeated Dose (14 days) Inhalation toxicity

Cosmetic ingredient S02771

Acute inhalation study designed as a dose range-finding study for the following 14-day inhalation study:

Guideline:	/
Species/strain:	No data given (probably Sprague Dawley)
Group size:	1 male and 1 female per concentration
Test substance:	Noyau WP30 (suspension containing 45.9% of the ground active substance)
Batch:	batch LP110
Purity:	99.6% (HPLC, 320 nm)
Vehicle:	water
Dose levels:	see table below
Administration:	Nose only inhalation; for exposure times, see table below
GLP:	/
Study period:	25-27 February 2013 (in-life phase including day of last necropsy)

The animals were exposed to test item atmospheres as shown below:

Group	Target Dose Level (mg/kg/day)*#	Target Aerosol Conc. (mg/L)#	Exposure Duration (hours)	No. of Animals Main Study	
				Male	Female
1. Noyau WP30-Low Dose	90	2.0	1	1	1
2. Noyau WP30-Mid Dose	180	2.0	2	1	1
3. Noyau WP30-High Dose	360	2.0	4	1	1

* Calculations were based on a theoretical body weight of 250g and a deposition of 100%
#: Target dose levels were calculated using the gravimetric results of the filter analysis.

The study was not conducted under GLP. The 45.9% formulated test item (Noyau WP30, Batch No. LP110) was stirred for at least 30 minutes and was diluted down to 12% using sterile water for injection (on the day of dosing). The formulations were stored at room temperature and stirred for at least 30 minutes prior to use. The animals were exposed, via nose-only inhalation, to a fixed aerosol concentration of Noyau WP30 for 1, 2 and 4 hours, for Groups 1, 2 and 3, respectively. The atmosphere was generated using a Hudson UP Draft II Opti Neb clinical nebulizer. Fresh test item formulation was continuously pumped into the nebulizer using an infusion pump and feeding rate of 0.43mL/min. Syringes filled with fresh formulation (being continuously stirred) were replaced approximately every hour.

Results
The estimated achieved doses were as follows:

Group	Mean Aerosol Conc. (mg/L)#	Exposure Duration (hours)	Estimated Achieved Dose (mg/kg)*			Particle Size	
			Male	Female	Mean	MMAD (µm)	GSD
1. Noyau WP30-Low Dose	2.47	1	114	107	111	2.1	2.01
2. Noyau WP30-Mid Dose	2.52	2	235	215	225		
3. Noyau WP30-High Dose	2.35	4	430	403	417	2.1	-

#: Expressed as total solids.
*: Assuming a 100% deposition.

The higher than intended aerosol concentrations obtained were not considered to adversely impact the interpretation of the data.
There were no test item related changes in bodyweights in any groups receiving Noyau WP30 at all doses. Red fur staining was seen in all animals of all groups, but is commonly seen in inhalation studies and is attributable to tube restraint. There were no abnormal gross pathological observations in any animals. The lung weights, both absolute and relative to body weights, were slightly elevated when compared to in-house historical values, but without dose relationship and in absence of other adverse observations, these changes were not considered significant. Single nose-only inhalation exposure of Noyau WP30 to Sprague Dawley rats at estimated achieved doses of up to 417 mg/kg did not result in any observed overt signs of toxicity. Slightly higher lung weights were noted in all animals of all groups without any dose-dependent relationship and were therefore not considered significant.

Conclusion
Since this aerosol concentration did not result in any signs of overt toxicity, it was chosen for the high dose group in the subsequent 14 day repeat dose inhalation study.

Ref. 85

SCCS comment
See 3.3.1.3

Cosmetic ingredient SO2771

Guideline: ICH M3(R2), OECD 412 (14 instead of 28 days)
Species/strain: Rat, Sprague Dawley
Group size: 10 males and 10 females per control or dose group; recovery groups with 5 males or females, respectively.
Test substance: Noyau WP30 (yellow suspension containing 45.9% of the ground active substance)
Batch: LP110
Purity: >99% (HPLC at 320 nm)
Vehicle: Water
Controls: Group 1 as air controls; group 2 (aerosol controls) receiving the vehicle (water)
Target conc. aerosol: 0, 0, 0.6, 1.2, 2.4 mg of the suspension/L aerosol corresponding to 0, 0, 0.28, 0.55 and 1.10 mg of the ground active substance/L aerosol
Target dose levels: 0, 0, 107, 215, 430 mg of the suspension/kg bw/day corresponding to 0, 0, 50, 100 and 200 mg of the ground active substance/kg bw/day
Administration: Nose-only inhalation
GLP status: Yes
Study period: February to April 2013 (in-life phase including day of last necropsy)
Final report March 13, 2015 (see SCCS comment)

Methods
For aerosol generation, the test item (suspension) was diluted with water so that the dose formulation strength of the test item for low, mid and high dose groups was 3, 6 and 12%, respectively. Sprague Dawley rats were nosed-only exposed for 4 hours per day to target aerosol concentrations of 0 (air control), 0 (vehicle control), 0.6, 1.2 and 2.4 mg/L corresponding to targeted doses of 0 (air control), 0 (vehicle control), 107, 215 and 430 mg/kg/day Noyau WP30 for 14 days and allowed to recover over a 14 day period.
The animals were exposed to test item atmospheres as shown below:

Group	Target Dose Level (mg/kg/day)*#	Target Aerosol Conc. (mg/L)#	Exposure Duration (hours)	No. of Animals			
				Main Study		Recovery	
				Male	Female	Male	Female
1. Air Control	0	0	4	10	10	5	5
2. Control**	0	0	4	10	10	5	5
3. Test Item-Low Dose	107	0.6	4	10	10	5	5
4. Test Item-Mid Dose	215	1.2	4	10	10	5	5
5. Test Item-High Dose	430	2.4	4	10	10	5	5

* Calculations based on a body weight of 250g and a deposition of 100%
**: Group 2 animals were exposed to the vehicle item (Sterile water for injection, USP) only.
#: The target dose levels and aerosol concentration were calculated using the gravimetric result of the filter analysis during technical trials.

Results
The aerosol particle size (MMAD) was 1.6, 1.7 and 2.1 µm for low, mid and high dose, respectively and considered to be respirable. Test item concentrations determined daily in the aerosols were all in the acceptable range of ± 15% from nominal concentrations.
The estimated achieved doses were as follows:

Group	Overall Aerosol Concentration (mg/L)	Exposure Duration (hours)	Estimated Achieved Dose (mg/kg)***$			Particle Size (µm)
			Male	Female	Mean	
1. Air Control * (0 mg/kg/day)	0	4	0	0	0	-
2. Control** (0 mg/kg/day)	0	4	0	0	0	-
3. Noyau WP30-Low Dose (107 mg/kg/day)	0.63	4	112	117	115	1.6
4. Noyau WP30-Mid Dose (215 mg/kg/day)	1.21	4	215	224	220	1.6
5. Noyau WP30-High Dose (430 mg/kg/day)	2.13	4	377	394	386	1.9

*: Group 1 animals were exposed to air only
** Group 2 were exposed to the vehicle item (Sterile water for injection, USP) only.
***: Assuming a 100% deposition
$:Achieved dose calculated using average body (kg) weights during the dosing period on Days 1, 7 and 14.

The mean estimated achieved aerosol concentrations of the test item were 0 (air control), 0 (vehicle control), 0.63, 1.21 and 2.13 mg/L corresponding to 0, 0, 0.29, 0.55 and 0.98 mg of the ground active substance/L aerosol. The mean estimated achieved dose levels of the test item were 0, 0, 115, 220 and 386 mg/kg/day corresponding to 0, 0, 54, 102, 179 mg/kg bw /day of the ground active substance.

One high dose male dosed with 430 mg/kg/day of Noyau WP30 was euthanised early on day 9 due to poor and deteriorating condition which included laboured and gasping respiration.

Macroscopic and microscopic findings in the respiratory tract were considered responsible for the early demise of the animal.

Recurring clinical signs during treatment included wheezing respiration noted primarily post dosing in all treatment groups and at a much lower incidence in the control groups. Following 7 days of exposure, Noyau WP30-related decreases in bodyweight were noted for high dose males when compared to controls (Group 2). This change correlated with a decreased food consumption noted during the same period. The high dose males showed weight gain until the end of the study, but notably the weight gain was less than in the other treated groups.

At the end of the treatment and recovery period, there were no Noyau WP30-related effects on haematology parameters with the exception of a Noyau WP30-related change which included increased neutrophil counts (NEUT) in all treated groups. The changes in neutrophil counts were within normal expected ranges but the increase was likely associated with the observed inflammation in the lungs. There were increases in globulin (GLOB) and a general decrease in albumin (ALB) in high dose males with a subsequent decrease in the albumin/globulin ratio (A/G), when compared to controls. The changes in the parameters were within normal expected ranges for normal rats, but the increase in globulin was likely associated with the observed inflammation in the lungs.

Organ weight changes and macroscopic and microscopic findings were noted in the respiratory tract of rats exposed to the test item.

Group mean lung weights (absolute and relative to bodyweight) were statistically significantly higher at dose levels of 107, 215, and 430 mg/kg/day of Noyau WP30 when compared to the group mean weights of controls (increases of about 30% up to around 70%). The higher weights were, generally, proportional to the dose, correlating with macroscopic and/or microscopic finding with no evidence of reversal following a 14-day recovery.

Macroscopic observations related to the administration of Noyau WP30 were noted in the lungs and tracheobronchial lymph nodes of rats at dose levels of 107, 215, and 430 mg/kg/day of Noyau WP30. Prominent lobular architecture, pale discoloration, and heaviness were noted in the lungs and enlargement, dark discoloration, and firmness were noted in the tracheobronchial lymph nodes. Generally, there was no clear difference in the incidence of macroscopic findings between the different treatment groups. Noyau WP30-related enlargement and dark discoloration were noted rarely in the mediastinal lymph nodes of male rats at doses of 107 and 215 mg/kg/day of Noyau WP30. Macroscopic findings in the lungs correlated with organ weight and microscopic findings and macroscopic findings in lymph nodes correlated with microscopic findings. There was no evidence of reversal of macroscopic findings following a 14-day recovery.

Microscopic observations related to the administration of Noyau WP30 were noted in the respiratory tract (lungs, larynx, trachea, tracheobronchial lymph node, and nasal cavities) of rats at dose levels of 107, 215, and 430 mg/kg/day of Noyau WP30. Microscopic findings in the lungs included inflammation, increased alveolar macrophages, mixed cell perivascular infiltrate and lymphoid hypercellularity of bronchiolar associated lymphoid tissue (BALT). These changes were associated with concurrent accumulation of abnormal material in bronchioloalveolar lumina. Microscopic findings in the lungs correlated with increased lung weights and macroscopic findings. Microscopic findings in the larynx included respiratory epithelium degeneration/necrosis, inflammation, squamous metaplasia, and/or hyperplasia with or without concurrent lumen exudate and squamous epithelium hyperplasia and inflammation. Microscopic findings in the trachea included respiratory epithelium degeneration/necrosis, erosion/ulceration, inflammation, and/or hyperplasia. Microscopic findings in the tracheobronchial lymph node (rarely in the mediastinal lymph node) included lymphoid hypercellularity and macrophage aggregation. Microscopic findings in the

tracheobronchial and mediastinal lymph nodes correlated with macroscopic findings. Microscopic findings in the nasal cavities were noted at all levels involving respiratory, olfactory, squamous, and transitional epithelium. Microscopic changes included: respiratory epithelium degeneration/necrosis, erosion/ulceration, squamous metaplasia, hyperplasia, goblet cell hyperplasia, and/or inflammation with or without concurrent lumen exudates; olfactory epithelium degeneration/necrosis, erosion/ulceration, respiratory metaplasia, and/or inflammation; squamous epithelium erosion/ulceration and/or inflammation, and; transitional epithelium hyperplasia and/or inflammation. The incidence and severity of microscopic changes in the respiratory tract were, generally, proportional to the dose. There was no evidence of reversal of microscopic findings in the lungs and tracheobronchial lymph node, progressive ongoing reversal of microscopic findings in the nasal cavities and larynx, and almost complete reversal of microscopic findings in the trachea following a 14-day recovery.

Ref. 86

SCCS comment
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 RDT 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 4th of 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.

3.3.5.5 Chronic (> 12 months) toxicity

No data submitted.

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2(uvrA$^-$) (pKM101).
Replicates:	triplicates in 2 individual experiments
Test substance:	WP 30 (**SO2374**, non-ground active substance)
Solvent:	DMSO
Batch nr.:	LP110
Purity:	>99% (HPLC at 320 nm)
Concentrations:	50, 150, 500, 1500 and 5000 µg/plate without and with S9-mix
Treatment:	Experiment I without and with S9-mix and experiment 2 without S9-mix: direct plate incorporation method with at least 48-72 h incubation.

	Experiment 2 with S9-mix: pre-incubation method with 20 minutes pre-incubation and at least 48-72 h incubation with S9-mix.
GLP:	In compliance
Date:	26 May 2009 – 18 June 2009

WP 30 was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a test measuring the bacteriostatic activity of WP 30. Bacteriostatic activity was evaluated for 5 concentrations up to the prescribed maximum concentration of 5000 µg/plate.
Experiment 1 and experiment 2 without S9-mix were performed with the direct plate incorporation method; in experiment 2 with S9-mix the pre-incubation method with at least 20 minutes pre-incubation was used. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation was not reported. Toxicity was reported as percentage bacteriostatic activity relative to the bacteriostatic activity found in the negative controls. Up to 1500 µg/plate bacteriostatic activity was between 3 and 9%. Up to 43% bacteriostatic activity was found for the highest concentration of 5000 µg/plate.
WP 30 treatment did not result in either experiment in a biologically relevant increase in the number of revertant colonies in any of the four tester strains of *Salmonella typhimurium* nor in *Escherichia coli* neither in the absence nor in the presence of S9-mix.

Conclusion
Under the experimental conditions used, WP 30 was not genotoxic (mutagenic) in the gene mutation tests in bacteria.

Ref. 65

SCCS comment
Bacteriostatic activity or other parameters for cytotoxicity were not reported in the Ames test. As solubility of the non-ground active substance SO2374 in DMSO is 0.10 mg/ml (100 µg/ml), some of the higher concentrations may be above the solubility limit of S02374 in DMSO. Strangely, precipitation was not reported.
Based on the solubility in receptor fluid measured in the dermal absorption study (ref. 38), for all genotoxicity tests *in vitro*, one can expect a solubility of about 4.56 µg/ml in a protein containing aqueous medium. Consequently, all concentrations used in this test are above the solubility limit of SO2374.

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2(uvrA$^-$) (pKM101).
Replicates:	triplicates in a single experiment
Test substance:	Cosmetic ingredient **S02771.** The content of the ground active
substance	is 46.4%, indicating that the final concentration of the ground active substance is about half of the concentration applied.
Solvent:	DMSO
Batch nr.:	LP110
Purity:	>99% (HPLC at 320 nm)
Concentrations:	All concentrations given were made by use of the suspension. 0.02, 0.06, 0.19, 0.56 and 1.67 µg/plate without and with S9-mix
Treatment:	pre-incubation method was used with 20 minutes pre-incubation and 48 h incubation without and with S9-mix. Direct plate incorporation with 48 h incubation without and with S9-mix
GLP:	In compliance

Date: 28 February 2011 – 10 March 2011

S02771 was investigated for the induction of gene mutations in *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a cytotoxicity assay with 5 concentrations up to the prescribed maximum concentration of 5000 µg/plate and as low as 60 µg/plate based on the solubility profile of the ground active substance. Cytotoxicity was evaluated on the basis of a reduction in the number of revertant colonies and/or thinning of the bacterial background lawn. Every concentration was tested with both the direct plate incorporation method and the pre-incubation method, with 20 minutes pre-incubation. Negative and positive controls were in accordance with the OECD guideline.

Results
In the cytotoxicity assay a decrease in the number of revertant colonies > 50% compared to the negative control value was observed at all concentrations tested indicating cytotoxicity of S02771. The lowest cytotoxic concentration of 1.67 µg/plate was used as the highest concentration in the Ames test.
S02771 treatment did not result in a biologically relevant increase in revertant colonies in any of the five tester strains either in the absence or in the presence of S9.

Conclusion
Under the experimental conditions used, S02771 was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref. 66

SCCS comment
Data on cytotoxicity were not reported in the main test. The content of the ground active substance is 46.4% indicating that the final concentration of the ground active substance is about half of the concentration of the test item SO2771 applied. Concentrations in the tests were below the solubility limit of the active substance in a protein containing aqueous medium reported to be 4.56 µg/ml (ref. 38)

***In vitro* gene mutation test in mammalian cells using the *tk* locus**

Guideline:	OECD 476 (1997)	
Cells:	L5178Y $tk^{+/-}$ mouse lymphoma cells	
Replicates:	duplicates in 2 independent experiments	
Test substance:	WP30 (**SO2374**, non-ground active substance)	
Solvent:	DMSO	
Batch no.:	LP110	
Purity:	>99% (HPLC at 320 nm)	
Concentrations:	0.015, 0.05, 0.15 and 0.5 µg/ml without and with S9-mix.	
Treatment:	Assay 1:	4 h treatment without S9-mix or 3 h treatment with S9-mix; expression period 44 h and selection period of 11-14 days
	Assay 2:	20 h treatment without S9-mix or 3 h treatment with S9-mix; expression period 44 h and selection period of 11-14 days
GLP:	in compliance	
Date:	6 July 2009 – 6 November 2009	

WP30 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on cytotoxicity measuring relative suspension and relative total growth.

In the main tests, cells were treated for 4 h (without S9-mix), 3 h (with S9-mix) or 20 h (without S9-mix) followed by an expression period of 44 h to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as a percentage of relative total growth of the treated cultures compared to the total growth of the solvent control cultures. To discriminate between large (with normal growth kinetics, indicative for mutagenic effects) and small colonies (with slow growth kinetics, indicative for a clastogenic effect) colony seizing was performed. Negative and positive controls were in accordance with the OECD guideline.

Results
The preliminary cytotoxicity test did not show cytotoxic effects for concentrations between 0.005 and 0.5 µg/ml. The values for relative total growth ranged from 70.6 up to 103.2% without metabolic activation and from 63.8 up to 108% with metabolic activation. Both in the preliminary cytotoxicity test as in the main tests the recommended toxic range of approximately 10-20 % survival compared to the concurrent negative controls was not covered.
In both experiments a biologically relevant increase in the mutant frequency compared to the negative control was not observed. For the long-term treatment of 20 h a statistically significant increase in mutant frequency was found at 0.5 µg/ml. This increase was considered not biologically relevant since it was less than 2 times the value of the solvent control.

Conclusion
Under the experimental conditions used, WP30 (SO2374) was not mutagenic in this gene mutation test in mouse lymphoma cells.

Ref. 67

SCCS comment
The recommended toxic range of approximately 10-20 % survival compared to the concurrent negative control was not reached. The authors consider the statistically significant increase in mutant frequency at 24 h treatment without S9-mix as not biologically relevant because it was less than 2 times the value of the solvent control. SCCS agrees because the increase was lower than the global evaluation factor for the microwell method of 126 x 10^{-6}.
Concentrations in the tests were far below the solubility limit of WP30 (SO2374) in a protein containing aqueous medium reported to be 4.56 µg/ml (ref. 38).

***In vitro* gene mutation test in mammalian cells using the *tk* locus, fluctuation test**

Guideline:	OECD 476 (1997)
Cells:	L5178Y $tk^{+/-}$ mouse lymphoma cells
Replicates:	duplicate cultures in two independent experiments
Test substance:	**S02771** (cosmetic ingredient) The content of the ground active substance is 46.4%, indicating that the final concentration of the ground active substance is about half of the concentration applied)
Solvent:	DMSO
Batch no.:	LP110
Purity:	>99% (HPLC at 320 nm)
Concentrations:	All concentrations given were made by use of the **suspension**. Experiment 1: 0.500, 1.00, 2.00, 4.00 and 8.00 µg/ml without and with S9-mix. Experiment 2: 0.500, 1.00, 2.00, 4.00, 8.00 and 16.00 µg/ml without S9-mix. 3.28, 4.10, 5.12, 6.40, 8.00 and 16.00 µg/ml with S9-mix
Treatment:	Experiment 1: 3 h treatment without and with S9-mix; expression period 48 h and selection period of 13 days Experiment 2: 24 h treatment without S9-mix and 3 h treatment with

S9-mix; expression period 48 h and selection period of 13 days

GLP: in compliance
Date: 30 March 2011 – 19 May 2011

S02771 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from phenobarbital/ 5,6-benzoflavone-induced rats was used as exogenous metabolic activation system. A preliminary solubility trail was performed to obtain the maximal practicable concentration in DMSO. Test concentrations were based on the results of a preliminary cytotoxicity test measuring relative survival with the maximal practicable concentration in DMSO as highest concentration.
In the main tests, cells were treated for 3 h without and with S9-mix or for 24 h without S9-mix followed by an expression period of 24 to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as total suspension growth and relative total growth of the treated cultures relative to the total growth of the solvent control cultures. The suspension growth factor measured over 2 days is a parameter to evaluate the number of generations during the phenotypic expression time. Negative and positive controls were in accordance with the OECD guideline.

Results
On the basis of the preliminary solubility trail, performed to obtain the maximal practicable concentration in DMSO, 32.0 µg/ml was chosen as the highest concentration to be used in the preliminary cytotoxicity test. In the preliminary cytotoxicity test, at the end of the 3 h treatment period both without and with S9-mix, no relevant cytotoxicity was observed up to 32.0 µg/ml. Precipitation was, however, observed at 8.00 µg/ml and above. At the end of the 24 h treatment, mild cytotoxicity was observed at 16.00 µg/ml, reducing the survival to 47% of the concurrent negative control while slight cytotoxicity was observed over the remaining concentrations tested. Precipitation was observed at 8.00 µg/ml (3 h treatment) or 4.00 µg/ml (24 h treatment) and above.
In experiment 1, no precipitation was noted either at the beginning or at the end of the treatment at any concentration tested. In experiment 2, precipitation was observed at the end of 3 h treatment at 5.12 µg/ml and above, whereas at the 24 h treatment precipitation occurred at 8.00 µg/ml and above.
The recommended toxic range of approximately 10-20 % survival compared to the concurrent negative control was not covered in experiments 1 where slight cytotoxicity was seen at 4.00 µg/ml and above, reducing RTG to 70%. In experiment 2, for the 3 h treatment, no cytotoxicity was noted whereas after 24 h treatment, cytotoxicity was seen at 4.00 µg/ml and above, reducing relative total growth to 50-60%.
No biologically relevant increase in the mutant frequency compared to the negative control was observed in either experiment. The induced mutant frequencies found for treated colonies were lower than the global evaluation factor (GEF: an increase of the mutant frequency of the concurrent negative control with 126 x 10^{-6}).

Conclusion
Under the experimental conditions used, S02771 was not mutagenic in this gene mutation test in mouse lymphoma cells.

Ref. 68

SCCS comment
The recommended toxic range of approximately 10-20 % survival compared to the concurrent negative control was not reached. As precipitation occurred at the highest concentrations tested, the use of higher concentrations is not meaningful.
The global evaluation factor (GEF) was used to determine a positive result: the induced mutant frequency is higher than the GEF which is 126 x 10^{-6} for the microwell method.
The purity was not mentioned in the report. However, the batch was used in other genotoxicity tests where the purity was reported as (about) 100%.

The content of the ground active substance is 46.4% indicating that the final concentration of the ground active substance is about half of the concentration applied.

***In vitro* micronucleus test**

Guideline:	draft OECD 487	
Cells:	Chinese Hamster Ovary (CHO) cells	
Replicates:	duplicate cultures	
Test substance:	WP30 (**SO2374**, non-ground active substance)	
Solvent:	DMSO	
Batch:	LP110	
Purity:	>99% (HPLC at 320 nm)	
Concentrations:	0.05, 0.15 and 0.5 µg/ml without and with S9-mix.	
Treatment:	Assay 1:	4 h treatment; harvest time 28 hours after the end of treatment
		4 h treatment; harvest time 44 hours after the end of treatment without S9-mix
		4 h treatment; harvest time 28 hours after the end of treatment with S9-mix
	Assay 2:	48 h treatment; harvest immediately after the end of treatment without S9-mix
		4 h treatment; harvest time 44 hours after the end of treatment with S9-mix
GLP:	In compliance	
Date:	27 May 2009 – 30 June 2009	

WP30 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in CHO cells. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. A preliminary cytotoxicity test in Balb/c 3T3 cells was performed with 4 h exposure and concentrations up to 500 µg/ml measuring trypan blue exclusion in order to determine the cytotoxicity of WP30. Thereafter, the cytostatic and cytotoxic effects of WP30 are evaluated towards CHO cells (survival index, mitotic index) in order to determine the mitotic index and to choose 3 test concentrations for the main micronucleus test. The treatment period in the main test was 4 h without and with S9-mix or 48 h without S9-mix. Harvest time was 0, 28 or 44 hours after the end of treatment. The mitotic indices were calculated to evaluate cytotoxicity. Negative and positive controls were in accordance with the draft guideline.

Results
Up to 1.5 µg/ml WP30 is not cytotoxic to Balb/c 3T3 cells, whereas at 500 µg/ml cytotoxicity results in about 45% reduction of viability. Up to 0.5 µg/ml, no significant decrease of living CHO cells was observed. Treatment with WP30 without S9-mix resulted in a survival index between 92 and 111%; with S9-mix between 93 and 104%. Finally, also the mitotic indices (MI) indicated that WP30 at the concentrations used was not cytotoxic. Without S9-mix in experiment 1, 4 h treatment, harvest 24 h after the end of treatment and the highest MI reduction compared to the MI in the negative control was 9.5%; 4 h treatment, harvest 44 h after the end of treatment 29.8% and in experiment 2 with 48 h treatment 16.1%. With S9-mix, in experiment 1 with harvest at 24 h after the end of treatment, the reduction in MI was 7.1 % and in experiment 2 with the harvest 44 h after the end of treatment 18.7%.
No biologically relevant increase in CHO cells with micronuclei was observed in either experiment at any concentration tested, either with or without metabolic activation.

Conclusion
Under the experimental conditions used, WP30 was not genotoxic (clastogenic and/or aneugenic) in this micronucleus test in CHO cells.

Ref: 69

SCCS comment
Under the conditions used in this micronucleus test WP30, exposure did not result in cytotoxicity. Concentrations in the tests were below the solubility limit of WP30 (SO2374) in a protein containing aqueous medium reported to be 4.56 µg/ml (ref. 38). This may indicate insufficient or no cellular exposure.

***In vitro* micronucleus test**

Guideline:	OECD 487 (2010)
Cells:	L5178Y $tk^{+/-}$ mouse lymphoma cells
Replicates:	duplicate cultures in a single experiment
Test substance:	SO2771 (cosmetic ingredient) The content of the ground active substance is 46.4% indicating that the final concentration of the ground active substance is about half of the concentration applied.
Solvent:	DMSO
Batch:	LP110
Purity:	>99% (HPLC at 320 nm)
Concentrations:	All concentrations given were made by use of the **suspension.** 0.22, 0.67 and 2.0 µg/ml without and with S9-mix.
Treatment:	4 h treatment; harvest time 24 hours after the end of treatment without and with S9-mix 24 h treatment; harvest time 24 hours after the end of treatment without S9-mix
GLP:	Yes
Date:	14 March 2011 – 18 May 2011

SO2771 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in CHO cells. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. A preliminary cytotoxicity test was performed according to the conditions of the micronucleus test with concentrations up to 2.0 µg/ml of the test item, measuring cell proliferation and cell mortality using the trypan blue exclusion method. The evaluation of the relative increase of viable cell count (RICC) and the relative population doubling (RPD) were performed for each concentration.
The treatment period in the main test was 4 h without and with S9-mix or 24 h without S9-mix. Harvest time was 24 hours after the end of treatment. Also in the main test, cytotoxicity was determined by RICC and RPD. Negative and positive controls were in accordance with the draft guideline.

Results
In the preliminary study on cytotoxicity, after 24 h exposure without S9-mix as well as after 4 h exposure with S9-mix, RICC and RPD did not indicate cytotoxicity. Based on these results, the highest concentration of the test item selected for the main study was 2.0 µg/ml. The same concentration (2.0 µg/ml) was also the lowest concentration at which minimal precipitation was visible in DMSO.
In the micronucleus test, no or slight cytotoxicity was induced both with short (4 hour) or long (24 hours) exposure to SO2771 at 2.0 µg/ml.
A biologically relevant increase in cells with micronuclei was not observed at any concentration whatever the exposure conditions.

Conclusion
Under the experimental conditions used, SO2771 was not genotoxic (clastogenic and/or aneugenic) in this micronucleus test in mouse lymphoma cells.

Ref. 70

SCCS comment
Under the conditions used in this micronucleus test SO2771 exposure did not result in cytotoxicity. This may indicate insufficient or no cellular exposure.
The purity was not mentioned in the report. However, the batch was used in other genotoxicity tests where the purity was reported as (about) 100%.
The content of the ground active substance is 46.4% indicating that the final concentration of the ground active substance is about half of the concentration applied.
Concentrations in the test were below the solubility limit of the ground active substance in protein containing aqueous media reported to be 4.56 µg/ml (ref. 38).

Overall conclusion on mutagenicity/genotoxicity
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 or 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 hindering 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 (SO2374) 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).

In one of the Ames tests (ref. 65), concentrations were used above the solubility limit of Phenylene bis-diphenyltriazine in DMSO. Strangely, a lack of expected precipitation was reported. In the second Ames test (ref. 66), 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) and in the oldest micronucleus test (ref. 69), 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) and more or less also in the second micronucleus test (ref. 70), more relevant concentrations in the range of the solubility limit of Phenylene bis-diphenyltriazine, were used. Both tests were negative. Again in both tests no indications for cellular exposure were observed.

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, the SCCS cannot exclude that Phenylene bis-diphenyltriazine may have a genotoxic portential at higher concentrations.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

No data submitted.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

No data submitted.

3.3.7 Carcinogenicity

No data submitted.

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

No data submitted.

3.3.8.2 Other data on fertility and reproduction toxicity

S02374 (active substance)

Guideline:	OECD 422, Screening test, combined repeated dose toxicity study with the reproduction/developmental toxicity
Species/strain:	Sprague-Dawley rats
Group size:	Three dose groups of 10 male and 10 females, one control group with 10 males and 10 females Three dose groups of 8 males and 8 females for the toxicokinetic satellite study
Test substance:	WP30 (powder, non-ground active substance)
Batch:	LP140
Purity:	99.8%
Application:	oral (gavage)
Vehicle:	0.5% (w/w) aqueous carboxymethylcellulose
Dose levels:	0, 100, 300 or 1000 mg/kg bw/day.
Dose volume:	10 ml/kg bw.
Observations:	Before mating (2 weeks), during mating (3 weeks at least for the males and up to 3 weeks for the females) and, for the females, throughout gestation until day 5 *post partum.*
GLP:	Yes
Study period:	September 2011 - December 2011

Three groups of 10 male and 10 female Sprague-Dawley rats received the test item, S02374, daily, by oral (gavage) administration, before mating (2 weeks), during mating (3 weeks at least for the males and up to 3 weeks for the females) and, for the females, throughout gestation until day 5 *post partum (p.p.),* at dose-levels of 100, 300 or 1000 mg/kg/day. A group of 10 males and 10 females received the vehicle control, 0.5% (w/w) aqueous methylcellulose, under the same experimental conditions.
In addition, three groups of eight males and eight females also received the test item for 2 weeks at dose-levels of 100, 300 or 1000 mg/kg/day for blood plasma concentration measurements.

Animals were checked daily for clinical signs, mortality, and detailed clinical observations were conducted weekly. Bodyweights and food consumption were recorded weekly until mating and then at designated intervals throughout gestation and lactation. The animals were paired for mating after 2 weeks of treatment and the dams were allowed to litter and rear their progeny until day 5 *p.p.* The total litter sizes and numbers of pups of each sex were recorded after birth. The pups were observed daily for clinical signs of toxicity and pup bodyweights were recorded on days 1 and 5 *p.p.*
A Functional Observation Battery including touch response, forelimb grip strength, pupillary reflex, visual stimulus response, auditory startle reflex, tail pinch response, righting reflex, landing foot splay, rectal temperature and motor activity was performed on five males and females per group at the end of the study. Prior to sacrifice, blood samples were also taken from these animals for analysis of haematology and blood biochemistry parameters.

The males were sacrificed after completion of the mating period. Bodyweights and selected organs weights were recorded and a complete macroscopic *post-mortem* examination performed, with particular attention paid to the reproductive organs. A microscopic examination was also conducted on selected organs from the first five males in the control

group and the high-dose group. Microscopic examination was conducted on all macroscopic lesions from all groups.
Dams were sacrificed on day 6 *p.p.* Bodyweights and selected organs weights were recorded and a complete macroscopic examination was performed, with particular attention paid to the reproductive organs. A microscopic examination was then conducted on selected organs from the first five females to deliver in the control group and the high-dose group and on any macroscopic lesions from all groups.
Pups, including those found dead before study termination, were also submitted for a macroscopic *post-mortem* examination.

Results
The S02374 concentrations in the administered dosage forms were within an acceptable range of variation (± 15%). S02374 was not detected in control samples.

Toxicokinetics
On determination of blood plasma concentration for toxicokinetic calculation, none of the satellite male and female rats had measurable 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. Overall, it was considered by the applicant that there was no significant systemic exposure to the test item.

Mortality or clinical signs
There were no treatment-related deaths and no treatment-related clinical signs.

Bodyweight and food consumption
There were no treatment-related effects or significant toxicological effects on mean bodyweight, mean bodyweight change and mean food consumption either in males or in females.

Macroscopic *post-mortem* examination
There were no treatment-related macroscopic findings.
Microscopic examination
There were no treatment-related microscopic findings.

Pairing, mating and fertility
There were no treatment-related effects on pairing, mating and fertility parameters.
There were no indications of abnormal estrous cycles in any treated females.

Pup observations
There were no effects on live birth, viability and lactation indexes, Functional Observation Battery and motor activity. Amongst the findings noted during the study, none were indicative of a treatment-related effect.

Haematology
There were no effects on mean haematology parameters.

Blood biochemistry and urinalysis
There were no toxicological significant effects on mean blood chemistry parameters and urinalysis.

Conclusion
Based on the experimental conditions of this study,
- the No Observed Effect Level (NOEL) of the non-ground active substance for parental toxicity was considered to be 1000 mg/kg/day,

- the NOEL of the non-ground, active substance for reproductive performance (mating and fertility) was considered to be 1000 mg/kg/day,
- the NOEL of the non-ground, active substance for toxic effects on progeny was considered to be 1000 mg/kg/day.

Blood plasma levels were around or < 0.500 ng/mL, the limit of quantification. Overall, it was considered that there was no significant systemic exposure to the test item.

Ref. 51

3.3.8.3 Developmental Toxicity

Cosmetic ingredient S02771

Guideline:	OECD 414
Species/strain:	Rat, RccHanTM: WIST(SPF), 11 weeks old
Group size:	22 mated females per dose group and controls
Test substance:	NOYAU WP30 (yellow suspension containing 46.4% of the ground active substance)
Batch:	LP110
Purity:	99.6% (HPLC)
Vehicle:	0.5% Carboxymethyl Cellulose (CMC) in water
Dose level:	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
Dose volume:	10 ml/kg bw
Administration:	oral, gavage, once daily
GLP:	Yes
Study period:	December 2010 - January 2011

The females were treated from day 6 through day 20 *post coitum*. Control animals were dosed with the vehicle alone. All females were sacrificed on day 21 *post coitum* and the foetuses were removed by Caesarean section.

Results:
Application formulations were found to be homogeneously prepared and sufficient formulation stability under storage conditions was approved.
Maternal Data:
Mortality and general tolerability: All females survived the scheduled study period. No clinical signs related to the treatment were observed at any dose level.
Food consumption, bodyweights, bodyweight gain and corrected bodyweight gain (corrected for the gravid uterus weight) were not affected by the treatment with the test item at any dose level.
Reproduction Data: The relevant reproduction data (post-implantation loss and the mean number of foetuses per dam) were not affected by treatment with the test item at any dose level.
Macroscopic findings: No macroscopic findings were found during necropsy of females at any dose level.

Foetal Data:
External examination: No test item-related abnormal findings were noted during external examination of foetuses at any dose level. No test item-related effects on foetal sex ratios were noted at any dose level.
Bodyweights: No test item-related effects on foetal bodyweights were noted at any dose level.
Visceral examination (microdissection technique): No test item-related findings were noted during visceral examinations of foetuses at any dose level.
Skeletal and cartilage examinations: Results of foetal bones and cartilage examinations gave no indication of a test item-related effect.

Conclusion
The test item did not show any toxic potential on pregnant females up to and including the dose level of 1000 mg/kg bw/day.
S02771 had no influence on the relevant reproduction data (post-implantation loss and the mean number of foetuses 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, the highest dose used for the test item (suspension).

Ref. 71

SCCS comment
The NOEL for both maternal general toxicity and prenatal developmental toxicity was 464 mg/kg bw/day for the ground active substance, i.e. the highest dose level in the study.

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

In a satellite study of a screening test according to OECD TG 422 (see section 3.3.8.2), toxicokinetics were investigated with three dose groups of 8 male and 8 female rats per group with the active substance (S02374) applied daily for 2 weeks at oral doses of 100, 300 and 1000 mg/kg bw/day given by gavage.

Results
On determination of blood plasma concentration for toxicokinetic calculation, none of the satellite male and female rats had measurable 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 (mostly on the range 0.5 – 0.85 ng/ml and maximally 1.64 ng/ml) 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.

Conclusion
It was concluded that there was no significant systemic exposure to the test item.

Ref. 51

SCCS comment
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).

3.3.9.2 Toxicokinetics in humans

No data.

3.3.10 Photo-induced toxicity

3.3.10.1	**Phototoxicity / photo-irritation / photosensitisation**

Neutral Red Uptake Phototoxicity Test

Study 1
Active substance SO2374

Guideline/method:	OECD 432
Test system:	Balb/cT3T (clone A31) mouse fibroblasts c(clone A31)
Replicates:	1 cell batch per condition
Test substance:	WP 30 (non-ground active substance)
Batch:	LP110
Purity	/
Test concentrations:	8 concentrations (n=6 each) 0.15 – 0.22 – 0.32 – 0.47 – 0.69 – 1.02 – 1.50 and 2.00 µg/ml
Vehicle:	dimethylacetamide (DMA) (1% v/v final)
Duration of exposure:	Test 1: 60 minutes incubation, followed by 50 minutes UVA irradiation Test 2: 60 minutes incubation, plus 50 minutes without irradiation
Source of light:	Honle UV France SOL-500, with filter H1
Intensity of irradiation:	UV-A dose 5 J/cm^2
Negative Control:	HBSSc with 1% DMA
Positive control:	Chlorpromazine in 8 concentrations (n=6 each): For Test 1 (test with UVA): 0.05 – 0.2 – 0.5 – 1 – 2 – 3 – 4 – 5 µg/ml in HBSSc For Test 2 (cytotoxicity test without UVA): 40 – 50 – 60 – 70 – 80 – 90 – 100 – 120 µg/ml in HBSSc
GLP:	Yes
Study period:	September 2009

A monolayer of cultured Balb/c 3T3 cells was treated with the test substance as well as with a positive control for 60 ± 10 minutes and then incubated in the presence or absence of UVA irradiation (5 J/cm^2). The percentage of cell viability as a function of the test concentration was measured by neutral red uptake as optical density (OD) at 540 nm. The determination of the EC_{50} (with or without UVA), the calculations of the photo irritation factor (PIF) and the mean photo effect (MPE) values were performed.

Results
For the positive reference chlorpromazine, the EC_{50} (without UVA) was 80.0 µg/ml (according to the predictive model, version 1) and 81.1 µg/ml (according to predictive model version 2), the EC_{50} (with UVA) was 0.714 µg/ml (according to predictive model version 1) and 0.774 µg/ml (according to predictive model 2). These results make it possible to derive for the positive reference a PIF of 112 (according to predictive model 1), a PIF of 105 (according to predictive model 2) and a MPE of 0.684. The results of the positive control were in accordance with the acceptance criteria (PIF>6 and MPE>0.15).

Under the same conditions, the cytotoxicity graphs obtained for the test article WP30 showed, over the range of test concentrations, an absence of cytotoxicity in the absence of UVA irradiation, but showed cytotoxicity in the presence of UVA irradiation. The EC50 (with UVA) was 0.288 µg/ml (according to the predictive model version 1), and 0.276 µg/ml (according to predictive model 2). These results make it possible to derive for the test article WP30 (non-ground active substance) a PIF of 6.94 (according to the predictive model version 1) and 7.26 (according to predictive model 2), and a MPE of 0.672.

Conclusion:
The test article WP30 has been tested at a maximum concentration of 2.00 µg/ml. Based on the cytoxicity curves, an absence of cytotoxicity was observed without UVA irradiation but cytotoxicity in the presence of UVA irradiation was present.

Ref. 72

Study 2
Active ingredient SO2771

Guideline/method:	OECD 432
Test system:	Balb/cT3T (clone A31) mouse fibroblasts
Replicates:	1 cell batch per condition
Test substance:	S02771 (yellow suspension, content 46.4% of the ground active substance)
Batch:	LP110
Test concentrations:	8 concentrations (n=6 each): 0.135 – 0.198 – 0.291 – 0.428 – 0.630 – 0.926 – 1.36 and 2.00 µg/ml for the test item (suspension), corresponding to concentrations of the ground active substance of 0.063 – 0.092 – 0.135 – 0.199 – 0.292 – 0.430 - 0.631 – 0.928 µg/ml
Vehicle:	DMSO (1% v/v final)
Duration of exposure:	Test 1: 60 minutes incubation, followed by 50 minutes UVA irradiation Test 2: 60 minutes incubation, plus 50 minutes without irradiation
Source of light:	Honle UV France SOL-500, with filter H1
Intensity of irradiation:	UV-A dose 5 J/cm^2
Duration of irradiation:	50 min
Untreated Control:	HBSSc with 1% DMSO
Positive control:	Chlorpromazine in 8 concentrations (n=6 each): For Test 1 (test with UVA): 0.05 – 0.2 – 0.5 – 1 – 2 – 3 – 4 – 5 µg/ml of the test item (suspension) in HBSSc For Test 2 (cytotoxicity test without UVA): 40 – 50 – 60 – 70 – 80 – 90 – 100 – 120 µg/ml of the test item (suspension) in HBSSc
GLP:	Yes
Study period:	March- June 2011

A monolayer of cultured Balb/c 3T3 cells was treated with the test substance as well as with a positive control for 60 ± 10 minutes and then incubated in the presence or absence of UVA irradiation (5 J/cm^2). The percentage of cell viability as a function of the test concentration is measured via the neutral red uptake assay as optical density (OD) at 540 nm. The determination of the EC_{50} (with or without UVA), the calculation of the photo irritation factor (PIF) and the mean photo effect (MPE) were performed.

Results
For the positive reference chlorpromazine, the EC_{50} (without UVA) was 92.0 µg/ml (according to the predictive model, version 1) and 93.2 µg/ml (according to the predictive model version 2), the EC_{50} (with UVA) was 0.520 µg/ml (according to predictive model version 1) and 0.533 µg/ml (according to predictive model 2). These results allow to derive for the positive reference a PIF of 177 (according to predictive model 1) and 175 (according to predictive model 2), and a MPE of 0.730. The results of the positive control were in accordance with the acceptance criteria (PIF>6 and MPE>0.15).

Under the same conditions, the graphs obtained for the test substance S02771 showed, over the range of test concentrations, an absence of cytotoxicity in the absence of UVA irradiation, but showed cytotoxicity in the presence of UVA irradiation. The EC_{50} (with UVA) was 0.430 µg/ml for the test item suspension corresponding to 0.200 µg/ml of ground active substance (according to the predictive model version 1), and 0.431 µg/ml for the test item suspension corresponding to 0.200 µg/ml of ground active substance (according to predictive model 2). These results allow to derive for the test article S02771 a PIF of 4.65 (according to the predictive model version 1) and 4.67 (according to predictive model 2), and a MPE of 0.551.

Conclusion
Test article S02771 has been tested at a maximum concentration of 2.00 µg/ml corresponding to a maximum test concentration of the ground active substance of 0.928 µg/ml. Based on the cytotoxicity curves, an absence of cytotoxicity was observed without UVA irradiation but cytotoxicity in the presence of UVA irradiation was present.

Ref. 73

SCCS comment on the neutral red uptake phototoxicity tests
The applicant performed two 3T3 studies: one in 2009 and a second one in 2011. In the first study, HBSS with 1% dimethylacetamide (DMA) (not guideline-compliant) was used and in the second one HBSS and 1% DMSO (compliant with the guideline). Although the solvent composition was changed, the final result of the study was positive.

The range of concentrations of the ground active substance in the test (0.063–0.928 µg/ml) was far below the optimal concentration range: 1-100 µg/ml for the most reliable performance of the 3T3 test (Spielmann et al. 1998) and also below the solubility limit of the ground active substance in protein containing aqueous media reported to be 4.56 µg/ml (ref. 38).
Consequently, although a low maximum concentration of 0.928 µg/ml of the ground active substance was tested, phototoxicity was induced even below this concentration (EC_{50}= 0.134 and 0.200 µg/ml). Slight opacity was already reported for the maximum concentration of 0.928 µg/ml of the ground active substance (poor solubility).

Based on these results, both the non-ground active substance (SO2374) and the ground active substance in the cosmetic ingredient SO2771 are considered as potentially phototoxic.

Phototoxicity *in vitro* in reconstructed human epidermis with MTT

Active ingredient SO2771

Guideline/method:	/
Test system:	Reconstructed human epidermis: SkinEthic (0.5 cm^2)
Replicates:	1 tissue batch per condition
Test substance:	S02771 (Noyau WP 30 AVENE) (suspension of the ground active substance, content not provided, assumed to be 50%)
Batch:	LP110
Purity:	/
Test concentrations:	5% and 10% of the suspension corresponding to about 2.5 and 5% of the ground active substance
Vehicle:	0.5% Carboxymethylcellulose (CMC) in 0.9% NaCl
Duration of exposure:	18-24 hrs.
Source of light:	BioSun Vilber Lourmat
Intensity of irradiation:	UVA dose 6 J/cm^2
Negative Control:	0.5% CMC for test article, ultrapure water for the positive control

Positive control: Chlorpromazine hydrochloride 0.04 , 0.11 and 0.33 mg/ml in water
Direct interaction with MTT: Negative
Colouring of epidermis: not tested
GLP: Yes
Study period: January 2011

The test item (suspension) was mixed with CMC and concentrations of 5 and 10% of the test item corresponding to about 2.5 and 5% of the ground active substance were examined. Chlorpromazine hydrochloride was used as positive control, ultrapure water and CMC as negative controls. Each concentration, including controls, was tested at a volume of 100 µl per tissue in duplicate and incubated for 18-24 hours. One test group of skin equivalents treated with the test substance and the controls were irradiated with artificial sunlight to an irradiation dose of 6 J/cm^2 UVA. A second group of skin equivalents treated with the test substance concentrations and controls were kept in the dark. Tissues were then rinsed with PBS to remove the test material, transferred to six new well plates with fresh medium and incubated overnight. On the next day, the assay medium was replaced by MTT-medium and tissues were incubated for 3 hours with MTT. Tissues were then rinsed with PBS, and the formazan salt was extracted with isopropanol. Optical density (OD) was determined at 570 nm in a plate spectrophotometer and cell viability was calculated for each tissue as % of the corresponding vehicle control either irradiated or un-irradiated.

Results

The obtained results are summarised in the tables below:

Table: Results obtained in the MTT-assay for the negative controls.

Controls	**OD mean value**		**OD (+UV)/ OD (-UV)x100**	**$OD_{vehicle}$ /OD_{NT}x100**	
	-UVA	**+UVA**		**-UVA**	**+UVA**
untreated	1.702	1.652	97.1	-	-
ultrapure water	1.453	1.521	104.7	85.4	92.1
carboxy-Methyl Cellulose (CMC) 0.5 %	1.448	1.489	102.8	85.1	90.1

Table: Results obtained in the MTT-assay for the positive control (chlorpromazine hydrochloride).

Concentration	% viability		Reduction in viability (%)
	-UVA	+UVA	
0,04 mg/mL	96.6	83.3	13.3
0,11 mg/mL	84.5	17.4	67.1
0,33 mg/mL	14.7	2.9	11.8

Table: Results obtained in the MTT-assay for the test item (suspension).

Concentration	% viability		Reduction in viability (%)
	-UVA	+UVA	
5 %	23.2	32.2	- 9.0
10 %	23.0	26.4	- 3.4

Conclusion
After irradiation with 6 J/cm^2 UVA, the test item S02771 tested on reconstructed human epidermis induced a reduction in cellular viability of -9% and -3.4% at concentrations of 5% and 10% of the test item, corresponding to about 2.5 and 5% of the ground active substance, respectively.
Nevertheless, without UVA irradiation, the viability percentages are below 40% indicating that the test item is cytotoxic and that its phototoxicity cannot be assessed under the conditions of this study.

Ref. 75

SCCS comment
Only a summary study report is available.
The content of the ground active substance was not provided.
No conclusion on the phototoxic potential of the test items can be drawn from the results obtained in this test.

Phototoxicity *in vitro* in fresh human skin discs with MTT

Active substance SO2374

Guideline/method:	/
Test system:	Fresh human skin discs (8 mm)
Tissue replicates:	1 tissue batch per condition (Caucasian woman 39 years old)
Test substance:	WP 30 (non-ground active substance)
Batch:	LP110
Purity:	99.6%
Test concentrations:	50 and 100 mg/ml (n=3 each)
Vehicle:	Paraffin oil
Duration of exposure:	Test 1: 20 hours incubation, followed by rinsing with PBS and UVA irradiation Test 2: 20 hours incubation, followed by rinsing with PBS (without UVA irradiation)
Source of light:	Honle UV France SOL-500, with filter H1
Intensity of irradiation:	UVA dose 6 J/cm^2
Negative Control:	Crème reparatrice Cicalfate (Laboratoires Avène, lot F2268) (n=3 each) MilliQ water for the controls with phenergan.
Positive control:	Phénergan crème 2% (UCB Pharma, lot CH141) (n=3 each)
GLP:	Yes
Study period:	October 2009 - January 2010

The test substance WP30 was dissolved in paraffin oil. Concentrations of 50 and 100 mg/ml were examined. The test solutions were applied to filter pads which were then applied to the skin. Ciclafate cream was used as a negative control, Phenergan 2% cream served as a positive control. Each concentration, including controls, was tested in triplicates. One test group of skin treated with the test substance and controls were irradiated with artificial sunlight at 1.7 mW/cm^2 UVA corresponding to an irradiation dose of 6 J/cm^2. The other group of skin discs treated with the test substance concentrations and controls were kept in the dark. Tissues were then rinsed with PBS to remove test material, transferred to new well plates with fresh medium and incubated overnight. The next day, the assay medium was replaced by MTT-medium and tissues were incubated for 3 hours with MTT. The formazan salt was extracted with isopropanol. Optical density (OD) was determined at 540 nm and cell viability was calculated for each tissue as % of the corresponding vehicle control either irradiated or non-irradiated.

Results
For the positive reference Phenergan 2%, the decrease in percentage viability observed in the absence versus the presence of UVA irradiation was 21%. The result of the positive reference (% decrease > 20%) validates the study conditions.
Under the experimental conditions, the decrease in percentage viability by the test article WP30 was 18% and 7% for the 5% and 10% concentrations of the test article, respectively. There was no cytotoxicity observed for the test article WP 30 in the absence and the presence of UVA irradiation.

Conclusion
According to the internal criteria of the study (viability decrease less than 20% for non-phototoxic substances), the results obtained indicate that WP 30, lot LP 110 can be considered as non-phototoxic in a concentration of 5% and 10% under the conditions of this study.

Ref. 74

SCCS comment
Only a summary study report is available. Only 1 tissue batch, of which the skin showed stretch marks, has been used.
No firm conclusion on the phototoxic potential of the test items can be drawn from the results obtained in this test.

Phototoxicity in vivo

Phototoxicity (photoirritation) study in guinea pigs

Reference:	CERB Report 20100368TCOP (2012)
Date of report:	Jan 18 2012
Guideline/method:	Not specified
Study period:	02 – 11 febr 2011
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 substances:	**S02771** (ground active substance, content 46.4% in the suspension)
Batch:	LP110
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
GLP:	Yes

The study consisted of an initial application of the study test item to the skin, followed by exposure of the treated skin surface to UVA type ultraviolet rays or UVB type rays. The experiment included study of the phototoxic potential of 8-methoxy-psoralen as reference test item.
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 (M.N.I.C.) determined by cutaneous application was the undiluted test item. The Maximum Non Erythematous Dose (M.N.E.D.) 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 oedematous reaction at time 24 and 48 hours after exposure.
Under the experimental conditions adopted, the undiluted test item S02771 (Batch No. LP110) was found to be non-phototoxic in the guinea pig.

SCCS comment
The duration of the application of the test items (approx. 30 min) before irradiation may have been too short. Although this is sufficient for the positive control (8-MOP) because of the poor penetration of the test article into the skin, a longer application time (even under occlusion) should have been considered.

Photosensitisation study in guinea pigs

Reference:	CERB Report 20100369TCOP (2011)
Date of report:	May 2 2011
Guideline/method:	Not specified
Study period:	27 Jan – 11 Mar 20111
Species/strain:	Guinea pig / Hartley Crl:HA
Group size:	In total 15, 10 males and 5 females. Negative control group 5, treated (irradiated) group 10 animals.
Test substances:	**S02771** (suspension, content of ground active substance 46.4%).
Batch:	LP110
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, 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

GLP: Yes

The study involved 15 animals, i.e. 10 males and 5 females allocated in 2 groups:
Group 1: a negative control group of 5 male animals. This was used to confirm that the development, under the experimental conditions adopted, of skin lesions in the treated group, in the absence of lesions in the negative control group dosed with the test item but exposed to no UV irradiation, was indeed indicative of a photosensitisation reaction.
Group 2: a group dosed with the test item of 5 animals of each sex. This was used to determine whether the test item has photosensitising properties under the experimental conditions adopted.

Induction phase was realised on days D1, D3 and D5.
Modality of administration 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 M.N.I.C 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.
Irradiation procedure:
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 irradiations. Animals of negative control group were exposed to no UV irradiation on zone 1.
Irradiation doses:
For each type of irradiation, animals were exposed to the Minimum Erythematous Dose (M.E.D.) 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 M.E.D. was reached (constant monitoring).
Other times of induction phase:
The procedure on D3 and D5 was identical to that of D1 without intradermal injections of complete Freunds adjuvant.
Resting phase: D6 to D21. Animals were rested from D6 to D21, i.e. for 16 days.

Challenge phase
Preparation of animals: 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 cm^2.
Details of administration: On D24, 0.25 mL of the test item at the M.N.I.C. determined during the phototoxicity study (i.e. undiluted test item) 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.
Irradiation procedure: Approximately 30 minutes after cutaneous application, animals of treated group were exposed to UVA type rays to the Maximum Non-Erythematous Dose (M.N.E.D.). 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 of negative control group were without exposure to UV irradiation on zones 2 and 3.
Irradiation dose: Animals were exposed to the Maximum Non-Erythematous Dose (M.N.E.D.) of irradiation. This dose was determined about every six months. It was 3.0 J/cm2 for UVA type rays. At each exposure, the exact amount delivered in J/cm2 was

monitored using a VLX 3 W radiometer. Irradiation was stopped when the M.N.E.D. was reached (constant monitoring).

Summary of results and conclusions
At times 24 hours and 48 hours, the negative control animals and animals treated with S02771 did not show any cutaneous reaction. Under the experimental conditions adopted, S02771 (Batch No. LP110) was found to be non-photo sensitising in the guinea pig.

SCCS comment
The duration of the application of the test items (approx. 30 min) before irradiation may have been too short. Because of the poor penetration of the test article into the skin, a longer exposure time (even under occlusion) should have been considered.
No firm conclusions can be drawn from the results of this study.

Overall SCCS comment on phototoxicity/photosensitisation
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 (SO2374) in reconstructed human epidermis with MTT in vitro and with the ground active substance (in SO2771) 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 SO2771) can be drawn due to too short exposure time to the test item.

3.3.10.2 Photo-mutagenicity / photo-clastogenicity

Bacterial gene mutation assay

Guideline:	OECD 471 (1997) and CPMP/SWP/398/01 Note for Guidance of the Mutagenicity Testing
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA1537 and *Escherichia coli* WP2.
Replicates:	triplicates in 3 individual experiments
Test substance:	S02771 (yellow suspension, ground active substance, content 46.4%)
Solvent:	DMSO
Batch nr.:	LP110
Purity:	>99% (HPLC)
UVA doses:	TA98: 0.063, 0.125 and 0.25 J/cm^2 TA100: 0.04, 0.02 and 0.04 J/cm^2 TA 1537: 0.2, 0.4 and 0.8 J/cm^2 WP2 : 0.003, 0.006 and 0.012 J/cm^2
UVB doses:	WP2: 0.001, 0.002 and 0.004 J/cm^2
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.
GLP:	In compliance
Date:	16 May 2012 – 8 June 2012

S02771 was investigated for photo-mutagenicity in *Salmonella typhimurium* and *Escherichia coli* (Ames test). A purpose-built irradiation system, which utilises 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/cm^2 determining automatically 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 S02771 concentration or in any tester strain.

Conclusion
Under the experimental conditions used, S02771 was not photo-genotoxic (photo-mutagenic) in these gene mutation tests in bacteria.

Ref. 76

SCCS comment
The purity was not mentioned in the report. However, the batch was used in other genotoxicity tests where the purity was reported as (about) 100%.
Solubility of the ground active substance in DMSO is 0.20 mg/ml (200 µg/ml). Apparently, nominal concentrations of 220 mg/plate and above are above the solubility limit of the ground active substance in DMSO. The highest nominal concentration in the preliminary cytotoxicity test was 5000 µg/plate (5000 µg/2.2 ml) and in the mutation experiments 2000 µg/plate (2000 µg/2.2 ml). Precipitation was found at nominal exposure to 1000 µg/plate.

3.3.11 Human data

No data provided.

3.3.12 Special investigations

No data provided.

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

/

3.3.14 Discussion

Physico-chemical properties

General aspects

The molecular structure of the non-ground **active substance S02374** contains multiple chromophores and accordingly the substance has a broad UV absorption spectrum. The very low water solubility of the active non-ground substance (< 0.02 µg/L), its high molecular weight of 540.62 g/mol and its high log Pow value of 8.29 (calculated) indicate that the active substance may have low bioavailability through the relevant uptake routes (oral, dermal, inhalation).

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. The analysis of the size distribution of the particles shows that S02771 is outside the scope of the definition of nanomaterial given by the European Commission (50% nanoparticles (< 100 nm) measured by number).
S02771 is formulated with an emulsifying agent (**Eumulgin L**), a preservative (**Benzoic acid**) and water. The pH of the formulation is between 3.5 and 4.5. Identification of residual impurities shows the presence of two CMR impurities:

The applicant's denominations of the batches do not distinguish between ground and non-ground batches. The kind of a batch used (non-ground or ground) can only be discerned in the respective context.

Purity and composition

The composition of the cosmetic ingredients "X" and NOYAU WP30 has been shown to be similar to SO2771. The code 025814 (no data provided) designates the same cosmetic ingredient SO2771 according to the applicant's letter.
The code R000317 designates the non-ground active substance and not the cosmetic ingredient as indicated in 3.1.1.3 (table).

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.

Impurities / accompanying contaminants

B) Cosmetic Ingredient S02771

Impurities in Phenylene bis-diphenyltriazine have not adequately been characterised by HPLC. The impurities should be characterised and quantified by HPLC-PDA detection and/or LC/MS.

Solubility
A) Active Substance S02374

The SCCS considers Phenylene bis-diphenyltriazine insoluble in water (solubility <0.02 µg/L). As the described solubility of Phenylene bis-diphenyltriazine in various solvents is very low, it should be documented that the solubilised material is indeed the active substance (Phenylene bis-diphenyltriazine), but not the impurities.

B) Cosmetic Ingredient S02771

Except in DMSO, no data on solubility was provided for the ground active substance in water and other solvents. Solubility data of the ground material should be provided as it is not clear to which extent differences in particle size distributions may cause changes of the solubility of Phenylene bis-diphenyltriazine.

Toxicological Evaluation
The toxicological studies were conducted on the commercial cosmetic ingredient S02771 and additional supporting data have also been provided on the active substance S02374.

As far as reported, the by-products of the cosmetic ingredient S02771 are chemically identified and their toxicological profiles are well known. These by-products are present in all test materials at the same concentrations as in the intended marketed product and where therefore included in toxicity testing and safety assessment.

Acute toxicity
Acute oral and dermal toxicity were tested in rats with the active substance **S02374**. In an oral study with 300 and 2000 mg/kg bw/day, no mortality occurred and no abnormal clinical sign was observed at either dose-level. Bodyweight gain was not affected by treatment. No abnormal findings were noted in the macroscopic examination.

In a dermal study performed with 2000 mg/kg bw/day, no relevant systemic effects were observed. Furthermore, no signs of skin irritation were noted.

A dose range-finding study for subacute inhalation (single nose) was conducted with the cosmetic ingredient **S02771** by a single exposure of rats for 1, 2 or 4 hours. The aerosol used contained about 2.5 mg of the suspension per litre corresponding to a concentration of the ground substance of about 1.25 g/L. Slightly higher lung weights were noted in all animals of all groups without any exposure-dependent relationship and were therefore not considered significant.

Local toxicity
Skin irritation tests *in vitro* were performed with the active substance **S02374** and the cosmetic ingredient **S02771** by use of Reconstituted Human Epidermis (RHE) (Skinethic™).
For both studies, only a summary study report is available. Apart from insufficient documentation, there were shortcomings in study conduct.
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 SO2374 and the cosmetic ingredient SO2711 (ground form). However, no signs of skin irritation were noted in the acute dermal toxicity study in rats on the active substance SO2374.

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.

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.

In the LLNA, the active (non-ground) substance **S02374** was tested at concentrations of 6.25%, 12,5% and 25% in 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.
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.

Dermal absorption
In contrast to the claim in the applicant's dossier, the studies on dermal absorption were most probably performed with the labelled (non-ground) substance Phenylene bis-diphenyltriazine and using the formulation of the cosmetic ingredient.

The solubility study demonstrating that Phenylene bis-diphenyltriazine in the receptor fluid is soluble at a concentration of 4.56 µg/ml is not available and should be provided. The experimental conditions of the solubility experiment should be reported. In addition, as the described solubility of Phenylene bis-diphenyltriazine is very low, it should be documented that the solubilised material is indeed the active substance Phenylene bis-diphenyltriazine and not the impurities or other possible artefacts.

The study on **healthy skin** did not comply with the SCCS Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10) as only 3 donors were used. High variability in the amounts detected in the various compartments was observed. Thus, the addition of 2 SD to the obtained means is justified. For calculation of the SED, **0.33 %** dermal absorption corresponding to 1.8 µg/cm^2 as worst case can be used. In many cells investigated, radioactivity in receptor fluid was below the LoQ of 0.029 µg eq/cm^2. With respect to healthy skin, only 2 from 8 cells were within the acceptance criteria based on recovery. Thus, no firm conclusion can be made regarding healthy skin.

The study on **impaired/irradiated skin** was performed in line with the SCCS Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10). High variability in the amounts detected in the various compartments was observed. Thus, the addition of 2 SD to the obtained means is justified. In many cells investigated, radioactivity in receptor fluid was below the LoQ of 0.029 µg eq/cm^2. With respect to healthy skin, only 2 from 8 cells were within the acceptance criteria based on recovery. Thus, no firm conclusion can be drawn from data on healthy skin. Absorption in (1) irradiated, (2) stripped and (3) irradiated and stripped skin (mean ± 2 SD) was 0.18, 2.34 and 1.37 % of the applied dose, respectively.

Both studies were most probably conducted with the active substance SO2374, not with the ground cosmetic ingredient SO2771.
The study on intact human skin yielded 0.33 % dermal absorption but was not fully in line with the SCCS Basic Criteria. As irradiation did not change TEWL, the value obtained from irradiated skin might also be taken for healthy skin. Hence, the results of the study on impaired/irradiated skin support the data from healthy skin (0.18 % dermal absorption).

The value of **0.33 % dermal absorption (1.8 µg/cm^2)** from the study on intact human skin can be taken as a worst case for the MoS calculation.

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 supension. 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 days exposure, nose-only, 4 hours per day) with the cosmetic ingredient **SO2771** 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 4th of 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.

Mutagenicity

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 hindering 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 (SO2374) 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).

In one of the Ames tests (ref. 65), 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), 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) and in the oldest micronucleus test (ref. 69), 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) and more or less in the second micronucleus test as well (ref. 70), 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.

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.

Carcinogenicity
No data provided.

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 **SO2374** 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.

Toxicokinetics
In a satellite study of the above OECD 422 Screening test, three groups of eight males and eight females received the non-ground active substance **SO2374** by gavage for 2 weeks at dose-levels of 100, 300 or 1000 mg/kg/day for blood plasma concentration measurements.

Blood plasma levels were around or < 0.500 ng/mL, the limit of quantification. 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, high lipophilicity and high melting point 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.

Phototoxicity
Slight opacity was already reported for the maximum test concentration of 0.928 µg/ml of the ground active substance (poor solubility).

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 i.e. < 10µg/ml. Therefore, the test chemical is also likely to act as a phototoxin under various exposure conditions *in vivo* (Commission Directive 2000/33/EC Annex V method B41). The SCCS, however, recognises that the 3T3 NRU PT test may also provide false positive results. In that case, it is then common practice, as a second tier, to further evaluate the biological effects on a reconstructed human skin model with some barrier properties while carefully checking for the solvents (Ceridono et al. 2012, AR1). However, the follow-up study using a SkinEthic 3D skin model cannot be used because the test items already showed cytotoxicity without UVA irradiation. Likewise, the information provided for the second follow-up study using fresh human skin discs does not allow concluding on the phototoxic potential of the test substance. Thus, based on the submitted *in vitro* data, the SCCS cannot judge the phototoxic potential of S86.

From both a phototoxicity (photoirritation) and photosensitisation study in guinea pigs, no firm conclusion on the phototoxicity potential of the ground active substance (in SO2771) can be drawn due to too short exposure time to the test items before irradiation. A longer application time (even under occlusion) should have been considered.
A photomutagenicity study using the Ames test was clearly negative under the conditions of the study.

Whereas the *in vitro* and *in vivo* tests do not give firm evidence of phototoxicity, the potential to elicit such reactions after application on the human skin can as yet not be excluded. Several substances that are used as sunscreens do occasionally show this paradoxical effect.

Properly conducted phototoxicity tests should be provided.

Human data
No data provided.

4. CONCLUSION

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.

5. MINORITY OPINION

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Annex

Toxicological Evaluation of by-products and impurities

Safety evaluation for the solubiliser, Eumulgin L

The manufacturer has provided the following safety assessment to the applicant:
- Eumulgin® L was not toxic or harmful in an acute toxicity test in the rat.
- The dermal penetration is low. With respect to the precautionary principle, Eumulgin® L is classified as an eye irritant and as a skin irritant.
- Eumulgin® L is not considered to be a skin sensitizer.
- **A NOAEL of 80 mg/kg bw** was established in a 90 day study.
- No potential for gene mutations was detected in the Ames test with Eumulgin® L.

Ref. 47

Under these conditions, Eumulgin L could be considered as safe at the maximum concentration of 0.5% on healthy and on altered and irradiated skin, in the general scenario (18g/day).

Safety evaluation for the preservative, benzoic acid

The SCCP opinion on benzoic acid and sodium benzoate (SCCP/0891/05, dated June 2005) stated that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in leave-on products up to 0.5%.

Ref. 48

At the maximum concentration of 10% of S02771 in the finished product, the maximum concentration of benzoic acid is 0.1%. In these conditions, benzoic acid could be considered as safe for the intended use.

Safety evaluation for impurities

Ethanol and Hydrazone are not classified CMR under Regulation (EC) No 1272/2008. N-Methylpyrrolidone and Hydrazine are classified CMR under Regulation (EC) No 1272/2008.

Summary regarding CMR properties of two residual impurities

Residual N-Methylpyrrolidone

N-Methylpyrrolidone is covered by index number 606-021-00-7 of Regulation (EC) No 1272/2008 in Annex VI, as toxic for reproduction category 2. Therefore, this classification of the substance in Regulation (EC) No 1272/2008 shows that it meets the criteria for classification as **toxic for reproduction,** in accordance with Article 57 (c) of REACH.
ECHA states the 20th May 2011, this substance is not relevant for the identification of the substance as SVHC in accordance with Article 57(c).
See also SCCS Opinion (2011) on this substance.

Ref. 5, 6 & 7

Residual Hydrazine

Hydrazine is covered by index number 007-008-00-3 of Regulation (EC) No 1272/2008 in Annex VI, as carcinogen. Carc. IB. Therefore, this classification of the substance in Regulation (EC) No 1272/2008 shows that it meets the criteria for classification as ECHA stated the 26th May 2011, this substance is not relevant for identification of SVHC in accordance with Article 57(c) of REACH.

Ref. 8 & 9

Maximum concentration of residual impurities in the cosmetic products

Impurities	**S02374** specifications (maximum percentage)	(**S02771** specifications (maximum percentage)	Percentage in sunscreen product with 10% of S02771 (maximum percentage)
NMP	< 0.5%	< 0.225%	< 0.0225%
Ethanol	< 0.5%	< 0.225%	< 0.0225%
Dihydrazone	< 0.5%	< 0.225%	< 0.0225%
Hydrazine	< 2 ppm	< 1 ppm	Under limits of identification
Benzyl rg = 0.39	< 0.1%	< 0.045%	< 0.0045%
rg = 0.68	< 0.22%	< 0.099%	< 0.0099%
rg = 0.70	< 0.42%	< 0.189%	< 0.0189%

Ref. 21 & 28