SCCS/1587/17
Final version

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87)

- Submission I

The SCCS adopted this final opinion by written procedure on 14 July 2017

2017

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Scientific Committee members

Contact
European Commission
Health and Food Safety
Directorate C: Public Health, Country Knowledge and Crisis Management
Unit C2 – Country Knowledge and Scientific Committees
Office: HTC 03/073
L-2920 Luxembourg
SANTE-C2-SCCS@ec.europa.eu

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SCCS members listed below are acknowledged for their valuable contribution to this Opinion.

SCCS Members

Dr U. Bernauer
Dr L. Bodin
Dr L. Celleno
Prof. Q. Chaudhry
Prof. P.J. Coenraads (Chairperson)
Prof. M. Dusinska
Dr J. Ezendam
Prof. C. L. Galli
Dr B. Granum
Prof. E. Panteri (Rapporteur)
Prof. V. Rogiers
Dr Ch. Rousselle
Dr M. Stepnik
Prof. T. Vanhaecke
Dr S. Wijnhoven

Former Member

Prof. J. Duus-Johansen

All Declarations of Working Group members are available on the following webpage:
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This opinion has been subject to a commenting period of 9 weeks (from 13 March 2017 to 14 May 2017) after its initial publication.
There were comments received and the final version of the opinion include information on impurities under section 3.1.5, information on the conditions of pH measurement under sections 3.1.8 (page 11) with an SCCS comment on photo-stability deleted (section 3.1.9, page 12), as well as changes in the SCCS comments under sections 3.3.2.1 (page 14) and 3.3.2.2 (page 17) compared to the preliminary one. The discussion part has been revised accordingly (section 3.6). No change in the conclusions.

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1. BACKGROUND

Submission I on the UV-filter Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87) (CAS 1419401-88-9), with the chemical name 2-Ethoxyethyl (2Z)-2-cyano-2-[3-(3-methoxypropylamino)cyclohex-2-en-1-ylidene]acetate, was submitted by Cosmetics Europe in June 2016.

The ingredient Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87) was developed to be used as UV-filter in cosmetic products up to a maximum concentration of 5%.

2. Terms of reference

1. *In light of the data provided, does the SCCS consider Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87), safe when used as UV-filter in cosmetic products up to a maximum concentration of 5%?*

2. *Does the SCCS have any further scientific concerns with regard to the use of Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87) in cosmetic products?*

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87)

3.1.1.2 Chemical names

IUPAC name: 2-ethoxyethyl (2Z)-2-cyano-2-[3-(3-methoxypropylamino) cyclohex-2-en-1-ylidene]acetate

3.1.1.3 Trade names and abbreviations

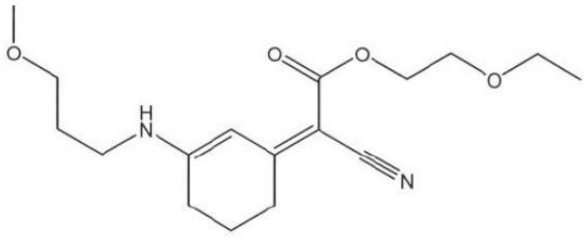
Colipa No. S 87
C-1701 B_C_3
C-1701 Merocyanine

3.1.1.4 CAS / EC number

CAS: 1419401-88-9
EC: 700-860-3

Ref.:http://www.chemical-registry.org/Chemicals/EC_700-860-3_2-ethoxyethyl-2Z-2-cyano-2-3-3-methoxypropylamino-cyclohex-2-en-1-ylidene-acetate.html

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{17}H_{26}N_2O_4$

3.1.2 Physical form

The UV filter C-1701 B_C_3 is a yellow solid consisting in form of a powder or small chunks.

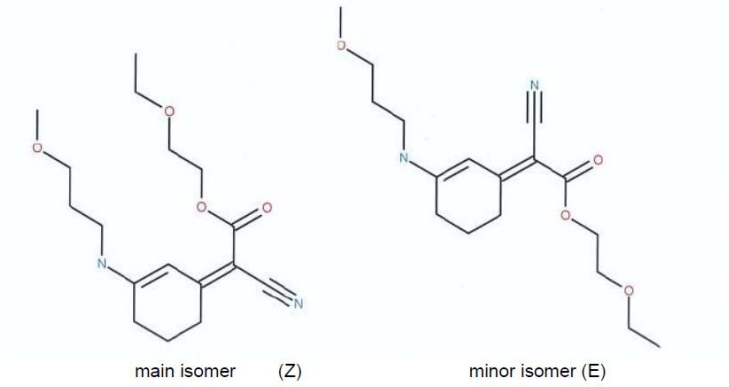
3.1.3 Molecular weight

Molecular weight: 322.41 g/mol

3.1.4 Purity, composition and substance codes

Batch/Lot:
1442/3+4
C-1701/8
0009511412

Chemical characterisation was performed by UV, FTIR and ^{1}H- and ^{13}C-NMR spectroscopy on the batches 1442/3+4 and C-1701/8. The ^{13}C-spectra showed the expected signals for the given structure. The ^{1}H-NMR results, however, showed the presence of an isomeric mixture. The non-GLP results obtained from different NMR experiments revealed a time-dependent isomerization of the test item (Z-isomer) to the corresponding E-isomer upon dissolution. The time-dependent investigation yielded equilibrium after ca. 5 hours of an isomeric mixture with a ratio of 1.98: 1.00 for Z-isomer to E-isomer.
The UV filter C-1701 B_C_3 is synthesised as Z-isomer and upon dissolution it isomerizes within 5 hours to approximately 60% Z-isomer and ca. 40% E-isomer.



Isomeric structures of the UV filter C-1701 B_C_3 based on 1H- and 13C-NMR signals

Purity of UV filter C-1701 B_C_3 was determined by quantitative ^{1}H-NMR spectroscopy with internal standard on the batches 1442/3+4 and C-1701/8.

The following table summarises the analytical profile of the three batches used in toxicological studies.

Table 1. Comparative table of the main analytical results for the three batches 1442/3+4, C-1701/8 and 0009511412

	Batches tested in toxicological studies		
	batch 1442/3+4	**batch C 1701/8**	**batch 0009511412**
Aspect	Yellow powder		
Purity / Content of Main component B_C_3 by HPLC UV (%)	96.2	97.8	98.7
1H-NMR spectroscopy (% w/w)	98.8	96.3	Not provided
Impurities content by HPLC UV			
Content of B_C (area %)	2.22	1.52	1.02
Sum of other impurities greater than 0,1% (area %)	1.41	0.53	0
Sum of other impurities lower than 0,1% (area %)	0.2	0.19	0.25
Other impurities			
Water content (% w/w)	0.13	0.09	0.07
2-Ethoxyethanol (ppm)	120	12	<10
3-methoxypropylamine (ppm)	<500	<500	<500
Diethylsulfate (ppm)	<1	<1	<1

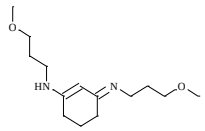
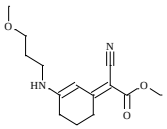
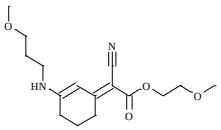
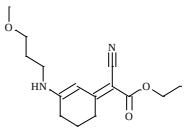
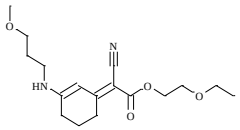
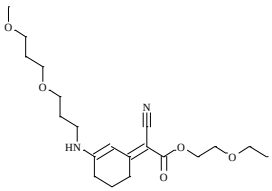
Ref.:
Meyer L. (2012) BASF SE study number 12L00108,
Meyer L. (2011) BASF SE study number 11L00388
Fux P. (2016) BASF Schweiz AG study number 16S01253

SCCS comment
NMR peak purity for the batch 0009511412 was not provided.
The applicant should provide the accurate content of 2-ethoxyethanol, diethylsulfate and 3-methoxypropylamine for all the three batches.

3.1.5 Impurities / accompanying contaminants

The impurity determinations were performed by the use of an HPLC-PDA analytical method at λ_{max} with LOD 0.05%. The structure elucidation had been done by HPLC-MS. Table 2 contains quantitative information on the main component and impurities above 0.1% and their structure proposals for the three C-1701 B_C_3 samples , which had been derived from HPLC–MS. The contents of 2-ethoxyethanol and 3-methoxypropylamine were determined by means of GC/FID using standard addition method. Diethylsulfate was quantified by means of headspace GC/MS using the standard addition method.

Table 2. Quantitative information on the main component and impurities above 0.1% and their structure proposals for the three C-1701 B_C_3 samples

Retention Time (min)		Approx. Content* (%area@380nm)			MW (Da)	Proposed Structure (and/or isomer)
LC/MS	HPLC-DAD	1442/3+4	C-1701/8	0009511412		
8.7	8.0	**	**	**	254	
21.8	21.4	<0.05	0.11	<0.05	264	
22.6	22.3	<0.05	0.18	<0.05	308	
26.1	25.9	2.20	1.57	0.99	278	 B_C
26.5	26.3	97.66	98.14	98.92	322	 B_C_3
27.5	27.4	0.12	-	-	380	

* By-products contents are calculated as described in chapter 3. Methodology

** no UV-detection @380nm, detectable at UV range 280-480 nm and by MS

\- not detected by UV and by MS (<0.001)

Ref.: BASF Schweiz AG study number 13S01712,
BASF Schweiz AG study number 16S01253,
BASF Schweiz AG study number 12Y57811,
BASF Schweiz AG study number 11B00011,
BASF Schweiz AG study number 16S01253
Meyer L 2012
Specker W. (2017) Analytical Report, 27 April 2017

SCCS comment
The applicant provided HPLC-PDA chromatograms for all the three batches, peak purity and impurities have been quantified at λmax of the test substance. According to the applicant these impurities have been chemically characterised by LC-MS. All area-% results for the impurities in the data tables were calculated from the HPLC-DAD data using a 7mg/mL test solution. However the quantification based on HPLC-DAD data has been carried out by calculating the results obtained for the concentrated solutions (7mg/mL) relative to the peak area of the main compound B_C of the diluted solution which is not accepted, unless the applicant clearly explain the dilution factor used for the calculation and the linearity range (concentrations) of the test substance. .

3.1.6 Solubility

The water solubility of the test item at a temperature of 20 °C was found to be: 0.45 g/L (flask method OECD 105).
Insoluble in mineral oil (0.01 g/L) and very soluble in phenoxyethanol (318 g/L)
For the determination of the solubility of C-1701 B_C_3 in different cosmetic solvents the UV filter was weighed in glass vessels and dissolved in the respective cosmetic oil. The mixtures were stirred for 7 days at 25 °C. The solubility data for the UV filter C-1701 B_C_3 in cosmetic ingredients are summarised in the following Table:

Solubility of UV filter C-1701 B_C_3 (batch: C-1701 B_C_3/10) in cosmetic ingredients at 25 °C		
Solvent	**INCI**	**Solubility (% w/w)**
Protectol PE	Phenoxyethanol	31.8
Spectrasolv DMDA	Dimethyl Capramide	18.6
Transcutol CG	Ethoxydiglycol	18.3
Dottisol	Dimethyl Isosorbide	13.9
Ethanol	Alcohol	13.0
Pelemol BIP-PC	Butylphthalimide and Isopropylphthalimide	9.7
X-Tend 226	Phenethyl Benzoate	7.8
Eldew SL-205	Isopropyl Lauroyl Sarcosinate	7.2
Ronacare AP	Bis-ethylhexyl Hydroxydimethoxy Benzylmalonate	5.2
Uvinul N 539 T	Octocrylene	3.7
1,2-Propandiol	Propylene Glycol	3.3
Oxynex ST	Diethylhexyl Syringylidenemalonate	2.7

Uvinul MC 80	Ethylhexyl Methoxycinnamate	2.1
Tegosoft XC	Phenoxyethyl Caprylate	2.0
Cetiol B	Dibutyl Adipate	1.9
Finsolv EB	Ethylhexyl Benzoate	1.7
Dermofeel TC-7	Triheptanoin	0.58
Dermofeel BGC	Butylene Glycol Dicaprylate/Dicaprate	0.38
Cetiol AB	C12-15 Alkyl Benzoate	0.35
Tegosoft CT	Caprylic/Capric Triglyceride	0.31
Cetiol CC	Dicaprylyl Carbonate	0.15
Lanol 99	Isononyl Isononanoate	0.12
Isopropylpalmitate	Isopropyl Palmitate	0.12
Jojoba Oil	Jojoba Oil	0.03
Cetiol OE	Dicaprylyl Ether	0.02
Cyclomethicone DC345	Cyclomethicone	0.002
Paraffin oil	Mineral Oil	0.002
Nexbase 2006 FG	Hydrogenated Polydecene	0.001

Ref.: BASF Grenzach GmbH Data sheet Winkler S. (2013) Siemens AG study number 20120207.06 draft

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 1.7 under neutral and alkaline conditions (OECD 117, EEC A.8, GLP)
Ref.: Winkler S. (2013), Siemens AG study number 20120207.02

3.1.8 Additional physical and chemical specifications

Melting point: 85 -120 °C. The large melting range shows the presence of various crystal forms
Boiling point: 306- 315 °C
Flash point: 394 °C
Flammability: not flammable
Explosive properties: not explosive
Particle size: $D_{0.1}$= 0.858 µm, $D_{0.5}$= 1.236 µm, $D_{0.9}$= 2.942 µm. The test substance does not contain nanomaterial.
Thermal stability: Decomposition at 390 °C
Vapour pressure: /
Density: /
Viscosity: /
pKa:13.3
Refractive index: /
pH: 5.8/5.9 in a 1 % of Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate solution in water
UV-vis: λ_{max} = 385 nm

Ref.: Giesinger J. (2013), BASF Grenzach GmbH study number GIJ-Malv-Rec610
Winkler S. (2013), Siemens AG study number 20120207.01
Winkler S. (2013), Siemens AG study number 20120207.02
Winkler S. (2013), Siemens AG study number 20120207.04
Kuchta (2012), BASF SE study number SIK 12/1391
Fux P. (2013), BASF Schweiz AG study number 12B020282b

3.1.9 Stability

The characterisation of the batches used for toxicological studies showed the homogeneity of test items.
The batch C-1701 B_C_3 Lot 0009511412 was stable after being stored for 1 year at 40 °C. Neither active ingredient content nor the content and identity of impurities changed over the considered time interval.

Content of C-1701 B_C_3 Lot 0009511412 initially and after one-year storage at 40°C

Test point	Measurements performed after synthesis ("time zero")	Measurements performed after 1 year storage at 40°C	Principle of Analytical Method
Content of main component B_C_3	98.83 area%	98.73 area%	HPLC/UV
Content of B_C (Mw = 278)	0.93 area%	1.02 area%	HPLC/UV

Homogeneity and stability of C-1701 B_C_3 in toxicological test systems (PEG 300) were confirmed in dose formulation analyses conducted as part of e.g. the repeated dose toxicity studies.

Ref.: Meyer, L., BASF SE (2011), 11L00388;
Meyer, L., BASF SE (2012), 12L00108;
Fux, P. BASF Schweiz GmbH, 16S01253, 2016;
Carlson M.B. (2013), Charles River Laboratories, 20027338

SCCS comments on physicochemical characterisation
Impurities should be quantified for all the batches at λ max, retention times and HPLC-PDA chromatograms should be provided.
HPLC-MS chromatograms showing the retention time of the main compound and all the impurities, along with information on the % content and retention times of these impurities should be provided.
The applicant should provide the accurate content of 2-ethoxyethanol and 3-methoxypropylamine of all the three batches.

3.2 Function and uses

S87 is proposed to be used as a UV filter in personal care products, including suncare cosmetic formulations at a maximum concentration of 5% w/w

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

No acute toxicity study was performed on C-1701 B_C_3. However, in the existing 14-day and 90- day oral toxicity studies where C-1701 B_C_3 was administered at dose levels of

100, 300 and 1000 mg/kg/d in rats, C-1701 B_C_3 did not induce any deaths. Thus C-1701 B_C_3 is considered to be non-toxic following a single administration of up to 1000 mg/kg by the oral route.

3.3.1.1 Acute oral toxicity

/

3.3.1.2 Acute dermal toxicity

/

3.3.1.3 Acute inhalation toxicity

/

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

EpiDerm™ Skin Irritation Test

Guideline:	OECD 439 (2010), Commission Regulation (EC) No 761/2009, B.46
Test system:	EpiDerm™ model (0.6 cm²)
Replicates:	3 tissues per condition
Test substance:	C-1701 B_C_3 No. 11/0473-3
Test batch:	C-1701/8
Purity:	96.3% (HPLC)
Dose:	25 µl bulk volume (approximately 7 mg) of neat test substance upon tissue wetted with 25 µl PBS
Treatment period:	60 minutes
Post-treatment incubation time:	42 hours
Positive control:	5% (w/v) SDS in deionised water
Negative control:	PBS
Direct interaction with MTT:	Negative
Colouring of tissue:	Yes
GLP:	In compliance
Study period:	July - August 2012

Methods

A bulk volume of 25µl of the solid test material (about 7 mg) was applied onto each of three tissues, wetted with 25µL of PBS, and homogenously distributed. Control tissues were treated with 30 µl of either the negative control (PBS) or positive control (5% w/v SDS). After 60-minutes treatment (25 minutes at room temperature and 35 minutes in the incubator), the tissues were rinsed with PBS. Following a 42-hour post-treatment incubation period, cell viability was assessed by the MTT assay in which 300 µl of MTT solution was added to the tissues. After a 3-hour incubation period, the MTT solution was removed and the tissues were washed with PBS. The formed formazan was extracted by incubation of the tissues in isopropanol. The optical density was determined spectrophotometrically at 570 nm (OD570).

Results
The mean viability of the test item-treated tissues was 101%. Yellow discoloration of the tissues was observed after washing. The positive control item demonstrated appropriate sensitivity (relative viability ≤ 20%) of the tissues used under test conditions.

Relative viability of EpiDerm™ tissue samples	
Group	**Relative viability (mean ± SD, n = 3),[% NC]**
NC (PBS)	100 ± 1.31
C-1701 B_C_3 (batch: C-1701/8)	101 ± 20.85[a]
PC (5% w/v SDS)	3 ± 0.25

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

[a]: This SD was out of the acceptance limit of ≤ 20. Since all other quality criteria of the test were met and the viability values were well above the cut off for skin irritation, *i.e.* ≤ 50%, this deviation was not considered to adversely affect the results of this study.

Conclusion
The study authors conclude that, under the conditions of this *in vitro* study, C-1701 B_C_3 did not show a skin irritation potential in the EpiDerm™ skin irritation test. On the basis of this validated stand-alone *in vitro* test, C 1701 B_C_3 is not expected to be irritating to skin at the use concentration and undiluted.

Ref.: Wareing B. (2012), BASF SE study number 61V0473/11A562

SCCS comment
SCCS considers an amount of 7 mg/0.6 cm^2 or 11.67 mg/cm^2 of test substance used too low. In addition, a high variability between sample tissues was observed with a standard deviation between tissue replicates of 20.85, exceeding the recommended maximum acceptable variability of SD<18. 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

Bovine corneal opacity and permeability test (BCOP test)

Guideline:	OECD 437 (2009), Commission Regulation (EU) No 1152/2010, B.47
Test system:	Fresh bovine corneas
Replicates:	3 Corneae per test condition
Test substance:	C-1701 B_C_3 No. 11/0473-3
Test batch:	C 1701/8
Purity:	96.3% (HPLC)
Test item:	20% (w/v) suspension in deionized water
Test volume:	750µl
Treatment period:	4 hours at about 32 °C
Positive control:	20% (w/v) imidazole in deionised water
Negative control:	Deionised water
GLP:	In compliance
Study period:	July - August 2012

Methods
Freshly isolated bovine eyes from 12-16 month old donor cattle were collected from the slaughterhouse and examined for defects. Those presenting defects such as opacity, scratches, pigmentation etc. were discarded. The corneae were carefully removed from the eyes and mounted in a holder. After a first basal opacity measurement of the fresh bovine corneae, 750 µl of the test item, the positive and the negative controls were applied onto the corneae and incubated for 4 hours at about 32 °C. After the incubation phase, the test item, the positive and the negative controls were each rinsed from the corneae and the opacity was measured again. Thereafter, permeability of the corneae was determined by measuring spectrophotometrically at 490 nm the transfer of 0.5% (w/v) sodium fluorescein upon incubation in a horizontal position for 90 minutes at about 32 °C.

Results
The IVIS value of C 1701 B_C_3 did not indicate a test item-related risk of serious damage to eyes. The PC item demonstrated appropriate sensitivity (IVIS value within 2 SD of the Laboratory's historical mean value, i.e. 87.7-144.2) of the test system.

***In vitro* irritancy score (IVIS) for C-1701 B_C_3**			
Group	**Mean opacity (± SD; n = 3)[a]**	**Mean permeability (± SD; n = 3)[a]**	**IVIS (± SD; n = 3)**
C-1701 B_C_3 (20% aqueous solution)	5.5 ± 1.6	- 0.004 ± 0.002	5.4 ± 1.6
NC (deionised water)	1.5 ± 3.2	0.201 ± 0.358	4.5 ± 3.9
PC (20% w/v imidazole)	72.2 ± 6.4	3.847 ± 0.959	129.9 ± 16.4

n: number of samples, NC: negative control, PC: positive control, SD: standard deviation
[a]: A NC correction was not performed for PC and test item. The mean permeability score of the NC was out of the historical range, because the value of a single cornea was exceptionally high. Due to the unambiguous results of the test item group even without NC subtraction and because all other acceptance criteria were met, the evaluation of the study was not considered influenced by this deviation.

Conclusion
The study authors conclude that, under the conditions of this study, C-1701 B_C_3 does not cause serious eye damage.

Ref.: Remmele M. (2012), BASF SE study number 63V0473/11A563

SCCS comment
SCCS notes that due to an outlier, negative control values were not within the historical range. Consequently, negative control corrections for permeability and opacity measurements were not performed for results obtained for the positive control and the test substance. Based on the unambiguous results of the study, even without background corrections, the SCCS has accepted that C-1701 B_C_3 does not cause serious eye damage. However, an eye irritation potential cannot be excluded.

EpiOcular™ eye irritation test

Guideline:	MatTek, Epiocular™ human cell construct : Procedure details version 3.1a; Harbell J.W. et al. (2009): COLIPA Program on Optimization of Existing *In Vitro* Eye Irritation Assays for Entry into Formal Validation: Technology Transfer and Intra/Inter Laboratory Evaluation of EpiOcular Assay for Chemicals, Poster # 378, Society of Toxicology March 2009
Test system:	EpiOcular™ human cornea model (0.6 cm^2)
Replicates:	2 tissues per condition
Test substance:	C-1701 B_C_3 No. 11/0473-3
Test batch:	C-1701/8
Purity:	96.3% (HPLC)
Dose:	50 µl bulk volume (approximately 8 mg) neat test substance upon tissue wetted with 20 µl PBS
Treatment period:	90 minutes
Post-treatment incubation time:	18 hours
Positive control:	Methyl acetate
Negative control:	Deionised water
Direct interaction with MTT:	Negative
Colouring of tissue:	Yes
GLP:	In compliance
Study period:	July - August 2012

Methods

Approximately 8 mg test item was applied onto the tissues, which were wetted with 20 µl PBS and incubated for 30 minutes. In parallel, 50 µl of the negative and positive control were handled in the same manner. The treated tissues were placed in the incubator for 90 minutes. After incubation, the tissues were rinsed with PBS to remove any residual test material and incubated for another 18 hours at standard culture conditions. Cell viability was next measured with the MTT assay. Here the medium was replaced by 300 µl of MTT solution. After a 3-hour incubation period, the MTT solution was removed and the tissues were washed with PBS. The formed formazan was extracted by incubation of the tissues in isopropanol at room temperature overnight or for at least 2 hours on a plate shaker. The optical density was determined spectrophotometrically at 570 nm (OD570).

Results

The mean viability of the test item-treated tissues was 104%, determined after an exposure period of 90 minutes with about 18 hours post-incubation. Yellow discoloration of the tissues was observed after washing. The positive control item demonstrated appropriate sensitivity (relative viability < 50%, expected tissue viability of approximately 25%) of the tissues used under test conditions.

Relative viability of EpiOcular™ tissue samples		
Group	**Relative viability**	
	Mean (n = 2) [% of NC]	**Inter-tissue variability [%]**
NC (water)	100	8.7
C-1701 B_C_3 (batch: C-1701/8) 100%	104	0.7
PC (Methyl acetate)	16	0.9

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

Conclusion

The study authors conclude that, under the experimental conditions employed, C-1701 B_C_3 did not show an eye irritation potential.

Ref.: Wareing B. (2012), BASF SE study number 62V0473/11A564

SCCS comment

This study was performed prior to the acceptance of the official guideline for the EpiOcular™ test. SCCS considers an amount of 8 mg/0.6 cm^2 or 13.33 mg/cm^2 of test substance used too low. On the basis of these results, an eye irritation potential of the test item cannot be excluded.

3.3.3 Skin sensitisation

Non-Radioactive Murine Local Lymph Node Assay (LLNA)

Guideline:	OECD 442B (2010),
Species/strain:	Female CBA/J mice
Group size:	2 animals/group (pre-test); 5 animals/group (main test)
Test substance:	C-1701 B_C_3
Batch:	1442/3+4
Purity:	100 area-% (HPLC)
Vehicle:	N,N-dimethylformamide (DMF)
Concentration:	10, 25 and 50 w/v%
Positive control:	25 vol % α-hexyl cinnamic aldehyde (HCA)
GLP:	In compliance
Study period:	October 2011 - January 2012

Methods

The concentrations used for the main test were based on a preliminary study using concentrations of 10, 25 and 50% (w/v), in which no clinical signs and no appreciable changes in body weights or auricular thickness were noted.
The test item was applied once daily at concentrations of 10, 25 and 50% to the outside of both ears (25 µL/ear for three consecutive days (days 1-3). Concurrent vehicle (DMF) and positive control items (25% (v/v) HCA in DMF were applied in the same manner. On day 5, Bromodeoxyuridine (BrdU) was administered intraperitoneally (*i.p.*) to all animals at a dose level of 5 mg/animal. All animals were sacrificed on day 6. The ears were observed and scored for erythema and/or edema. Then the auricular lymph nodes were excised for lymph node weight determination and for subsequent assessment of BrdU incorporation by means of flow cytometry. The number of BrdU-positive cells was calculated for each animal by multiplying the lymphocyte count by the ratio of BrdU-positive lymphocytes.
The stimulation index (SI) was calculated for each treated group according to the following formula:

SI = (mean number of BrdU-positive cells in the test item treated group) /
(mean number of BrdU-positive cells in the vehicle control group)

When SI > 3, the test item was considered to be a skin sensitiser.

Results

No clinical signs, including skin irritation at the application area, were observed in any animal in the test item-treated or vehicle control group. No appreciable body weight

changes were observed. In the positive control group, very slight erythema was observed in both ear auricles of all animals at approximately 1 hour after application on days 2 and 3 only.
The SI values were 1.1, 1.0 and 1.0 in the low-, mid- and high-dose groups (10, 25 and 50% (w/v)), respectively. Relevant increases in the ratio and count of BrdU-positive lymphocyte cells were noted in the Positive control group as compared to the Vehicle control group. The SI value in the Positive control group was 7.4, indicating a positive response and an adequate sensitivity of the test system.

Conclusion
Based on the study results, C-1701 B_C_3 in N,N-Dimethylformamide was considered not to possess any skin sensitising potential under the experimental conditions employed.
Therefore, C-1701 B_C_3 is not considered to be a skin sensitiser.

Ref.: Matsuda A. (2012), BASF project number 58V0473/11X188

SCCS comment
The LLNA:BrdU-ELISA uses a different cut-off than the traditional LLNA. In this non-radioactive LLNA, a substance is considered a skin sensitiser when the SI ≥ 1.6 (OECD TG442B). However, using this criterion, C-1701 B_C_3 can still be regarded to have no skin sensitisation potential.

3.3.4 Toxicokinetics

3.3.4.1 Dermal / percutaneous absorption

***In vitro* percutaneous absorption**

Guideline:	OECD 428 (2004), OECD No. 28 (2004), SCCS/1358/10, SCCS NoG 6th rev. (2006), COLIPA (1997)
Test system:	Split thickness human skin samples (200-400 µm)
Number of donors:	12 samples from 4 donors (24 to 48 years)
Membrane integrity:	Electrical resistance barrier integrity test, membranes with a resistance < 4 kΩ were excluded
Test substance:	C-1701 B_C_3
Batch:	C-1701/8
Purity:	96.3% (NMR)
Test item:	Commercial suncare formulation 758455 5, batch no. R2, containing 3% (w/w) C-1701 B_C_3
Dose applied:	2 mg/cm^2 of the test preparation (approx. 0.06 mg C-1701 B_C_3/cm^2)
Exposed area:	3.14 cm^2
Exposure period:	24 hours
Sampling period:	24 hours (0, 0.5, 1, 2, 4, 8 and 24 hours post dose)
Receptor fluid:	5% w/v bovine serum albumin in PBS
Solubility in receptor fluid:	No information
Mass balance analysis:	Provided
Tape stripping:	Yes (4 pools of 5 strips each)
Method of Analysis:	LC-MS/MS
GLP:	In compliance
Study period:	December 2012 - January 2013

Methods

Split-thickness human skin (12 samples from 4 individual donors) was mounted into static diffusion cells containing receptor fluid (Phosphate buffered saline (PBS) supplemented with bovine serum albumin (BSA); 5% w/v) in the receptor chamber. The skin surface temperature was maintained at 32 ± 1 °C throughout the experiment. An electrical resistance barrier integrity test was performed and any human skin sample exhibiting a resistance < 4 kΩ was excluded from absorption measurements. No samples were rejected. The suncare formulation was applied to the mounted human skin samples at an application rate of approximately 2 mg/cm². This quantity, as low as technically applicable, can be considered as well-representing of the use conditions.

The skin surface temperature was maintained at 32 ± 1 °C throughout the experiment. Exposure was terminated at 24 hours post dose by washing the skin surface rinsed twice with an aqueous solution of Sodium dodecyl sulfate (SDS, 2% w/v) and then twice with water, to reflect in-use conditions. For each washing step, the skin wash was aspirated with a pipette and the skin was dried with a tissue paper swab. An additional tissue paper swab was used after the last water rinse. The soap and water were retained for analysis in a single pooled sample (skin wash). The pipette tips and tissue paper swabs were retained. The cells were dismantled and the donor chamber retained for analysis (donor chamber wash). The underside of the skin was dried with additional tissue swabs. The receptor chambers were emptied, and bulk receptor fluid retained. The chambers were rinsed with methanol (40 mL) and the wash retained (receptor wash). The skin was divided into exposed skin and unexposed skin (i.e. the area of skin under the cell flange). The stratum corneum was removed from the skin by tape stripping. Afterwards, the epidermis was separated from the dermis by the heat separation technique. Exposed skin, unexposed skin, skin washes, tissue swabs, pipette tips and tape strips were extracted in a suitable solvent and all samples were analysed by LC-MS/MS. All cumulative receptor fluid values were calculated from data which included values less than the lower limit of quantification (LLOQ, 1 ng/mL). Any receptor fluid value below the LLOQ was assigned the nominal value of the LLOQ (1 ng/mL), representing the "worst-case" result for absorbed test item. Values below the LLOQ were observed up to 2-4 hours post dose. The solubility of the test item in the receptor fluid was not rate limiting for absorption.

Results

The distribution and the absorption of the test item are summarised in the following table.

Distribution/absorption of C-1701 B_C_3 (batch: C-1701/8) 24 hours after application in a typical suncare formulation (3% w/w[a]) to split-thickness human skin		
Distribution	**Fraction of applied dose mean ± SD (n = 12) [%]**	**Amount mean ± SD (n = 12) [µg/cm²]**
Total dislodgeable dose	93.73 ± 5.48	61.79 ± 3.61
Stratum corneum	0.79 ± 0.46	0.52 ± 0.30
Epidermis (without *stratum corneum*)	0.57 ± 0.48	0.37 ± 0.32
Dermis	0.35 ± 0.41	0.23 ± 0.27
Total unabsorbed dose	94.71 ± 5.06	62.44 ± 3.34
Total absorbed dose	0.72 ± 0.63	0.48 ± 0.42
Dermal delivery	**1.63 ± 1.02**	**1.08 ± 0.67**
Mass balance	96.34 ± 4.57	63.51 ± 3.01

[a]: nominal concentration; a test item concentration of 3.23% (w/w) was determined by LC-MS/MS

n: number of samples, SD: standard deviation

Total dislodgeable dose: donor chamber wash + skin wash + tissue swabs + pipette tips
Total unabsorbed dose: total dislodgeable dose + *stratum corneum* + unexposed skin
Total absorbed dose: cumulative receptor fluid + receptor rinse + receptor chamber wash
Dermal delivery: total absorbed dose + dermis + epidermis (without *stratum corneum*);
Mass balance: total unabsorbed dose + epidermis (without *stratum corneum*) + dermis + total absorbed dose

Conclusion

Under the conditions of this *in vitro* study, C-1701 B_C_3 in a representative suncare cosmetic formulation at the concentration of 3% (w/w) penetrated through split-thickness human skin to a low extent. At 24h post dose, the amount considered as absorbed was estimated to be at maximum 1.08 ± 0.67 µg/cm^2 corresponding to 1.63 ± 1.02% of the applied dose.

Ref.: Blackstock C. (2013), Charles River Laboratories study number 792670

SCCS comment

The electrical resistance of the human skin samples used was below the 10 kΩ threshold for intact skin. In addition, a typical suncare formulation containing 3% (w/w) C-1701 B_C_3 was tested, whereas C-1701 B_C_3 is intended to be used in cosmetic products up to a maximum concentration of 5% (w/w). According to the SCCS Notes of Guidance, several concentrations, including the highest concentration of the test substance in a typical formulation, should be tested.

3.3.4.2 Other studies on toxicokinetics

3.3.5 Repeated dose toxicity

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

Repeated Dose (14 days) oral toxicity

Guideline: /
Species/strain: Rat, Grl:Wi (Han)
Group size: 5/sex/dose
Test substance: C-1701 B_C_3
Batch: 1442/3+4
Purity: 98.8%; dose calculations were not corrected for purity
Vehicle: Polyethylene glycol 300
Dose levels: 0, 100, 300 or 1000 mg/kg bw/day
Dose volume: 5 mL/kg bw
Route: oral
Administration: gavage
Duration: 14 days
GLP: in compliance
Study period: October 2011- May 2013 (in life phase ended August 2012)

Animals received test substance for 14 days. During the treatment period all animals were assessed repeatedly for mortality and clinical signs of toxicity. Body weights and food consumption were recorded at regular intervals. On the day of scheduled necropsy, urine samples were collected after overnight fasting and blood samples were taken for the

assessment of haematology and clinical chemistry parameters. At necropsy, all animals were examined macroscopically and selected organ weights were determined. Organs/tissues of all high dose group and control group animals were processed and examined microscopically for histopathological findings. The dose formulations used in this study were analysed for test item concentration and homogeneity.

Results

Stability analyses demonstrated that the test item is stable in PEG 300 at room temperature and protected from light for 24 hours and under refrigerated conditions (2-8 °C) and protected from light for 10 days at concentrations bracketing those used in the present study. All dose formulations used in this study were formulated appropriately and remained within the concentration acceptance criterion (*i.e.*, difference between analytically determined mean concentration and nominal concentration ≤ 15%). Homogeneity testing showed that the formulation technique used produced homogenous dose formulations.

No mortalities and no toxicologically relevant test item-related changes in haematology, clinical chemistry and urinalysis parameters were observed. Except for the liver, no relevant test item-related changes in organ weights were noted on the day of scheduled necropsy. Macroscopical and histopathological examinations revealed no adverse test item-related gross lesions or microscopic findings in both male and female rats. Treatment of male rats with the test item resulted in clinical signs (discoloured fur, mild to moderate dehydration, mild to moderate excess salivation, hunched posture, rales, decreased motor activity, swelling in the axillary region and ptosis), reductions in body weight gain and food consumption, and increased liver weights at the high dose level of 1000 mg/kg bw/day . Females at the same dose level showed clinical signs (discoloured fur, mild dehydration, urine-stained abdominal fur and chromorhinorrhea) and increased liver weights. Increased liver weights were also seen in females treated at 300 mg/kg bw/day.

In the absence of concomitant macroscopical and histopathological findings, the increased liver weights noted in both sexes at 1000 mg/kg bw/day and in females also at 300 mg/kg bw/day were not considered adverse.

Conclusion

Under the conditions of this dose range-finding toxicity study, the NOAEL for C-1701 B_C_3 was established at 300 mg/kg bw/day for male and female rats. Dose levels of 100, 300 and 1000 mg/kg bw/day were selected for the subsequent 90-day repeated dose oral toxicity study in rats.

Ref.: Carlson M.B. (2013), BASF project number 99C0284/11X221

SCCS comment

A dose level of 1000 mg/kg body weight/day in C-1701 produced general toxicity, indicating that this dose is an appropriate maximum tolerated dose (MTD) for subsequent studies.

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

Oral

Guideline:	OECD 408; US EPA OPPTS 870.3100
Species/strain:	Wistar (Crl:WI(Han)) rats
Group size:	10 /sex/group
Test substance:	C-1701 B_C_3
Batch:	C-1701/8
Purity:	96.3% (^{1}H-NMR)

Vehicle:	Polyethylene glycol 300
Dose levels:	0, 100, 300 or 1000 mg/kg bw/day
Dose volume:	5 mL/kg bw
Route:	oral
Administration:	gavage
Duration:	90 days
GLP:	in compliance
Study period:	May 2012- May 2013 (in life phase ended August 2012)

During the treatment period animals were observed for mortality, general clinical observations detailed observations, body weights and food consumption at defined intervals. Functional observation battery (FOB) and locomotor activity assessments were performed in week 12. Vaginal lavage samples were collected daily for the last 28 days of the treatment period and on the day of scheduled necropsy for estrous cycle evaluations. Ophthalmological examinations were conducted on all animals, once during the acclimatisation period and once prior to scheduled necropsy. Blood samples for clinical pathology examinations, haematology and clinical chemistry parameters were collected on the day of scheduled necropsy from all rats after an overnight fasting period. For the assessment of urinalysis parameters, only urine from female animals (obtained after overnight fasting on the day of necropsy) was taken. On the day of scheduled necropsy, all animals were examined macroscopically and the weights of selected organs were determined. Full histopathology was performed on the preserved organs/tissues of all premature decedents and of the animals of the control and high dose groups. Due to lesions observed in high-dose group animals, the liver was also examined microscopically in low- and mid-dose group animals. All gross lesions of all animals were examined. Male reproductive assessments were conducted including sperm motility, sperm concentration, sperm morphology and spermatid counts. The dose formulations used in this study were analysed for test item concentration and homogeneity using a validated HPLC method. Stability analyses demonstrated that the test item is stable in PEG 300 at room temperature and protected from light for 24 hours and under refrigerated conditions (2-8 °C) and protected from light for 10 days at concentrations bracketing those used in the present study.

Results

Analysis of the dose formulations used in this study revealed all actual concentrations were within the acceptance criteria of ± 15% of the nominal concentrations. All dose formulation samples met acceptance criteria for homogeneity (the relative standard deviation [RSD] of concentrations was < 5% for each group).

Daily test item administration at 1000 mg/kg bw/day resulted in clinical signs, consisting of urine-stained abdominal fur, increased incidence of dehydration and excess salivation. Body weight gains were slightly lower in males as compared with concurrent controls. After the start of the study, food consumption was slightly and transiently decreased in males and females. The bilirubin test in urine was positive for the female rats. Haematological and clinical chemistry examinations mainly revealed slight decreases in red blood cell parameters (haemoglobin concentration and haematocrit in males, mean corpuscular haemoglobin concentration (MCHC) in females, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) in both sexes) and increased reticulocyte counts and bilirubin concentrations in both sexes. Leukocyte and lymphocyte counts were slightly increased in the female rats. Liver weights were moderately increased in males and females, with minimal centrilobular hepatocellular hypertrophy as histopathological correlate noted in 5/10 males and 8/10 females. There were statistically significant changes in other organ weights, but no patterns, trends, or associated microscopic findings to identify them as being toxicologically relevant. Slightly lower testicular spermatid count and spermatid density occurred in the male rats; however, these differences were not considered to be adverse because there were no corresponding reductions in absolute testicular weights and no microscopic correlations in testicular histology.

At 300 mg/kg bw/day, urine-stained abdominal fur and increased incidence of dehydration were noted in males and females. Minor differences occurred in single haematology parameters, but without consistency across genders. The bilirubin test in urine was positive for the female rats. These findings were not considered adverse. Liver weights were slightly increased in both sexes, but without any histopathological correlates or any evidence of an impaired organ function by clinical chemistry parameters. Therefore, these liver weight changes were not considered adverse, but to be a test item-related adaptive response.

Following test item administration at 100 mg/kg bw/day, dehydration was observed in 3/10 females and the bilirubin test in the urine was positive in female rats. In the absence of any other effects, these differences from controls were not considered to be adverse. No test item-related effects were observed in the male rats.

Conclusion

Under the conditions of this study, the No Observed Adverse Effect Level (NOAEL) for C-1701 B_C_3 was established at 300 mg/kg bw/day for male and female rats. C-1701 B_C_3 was found to be of low toxicity and no adverse effects on male/female reproductive organs have been observed after repeated administration for 90 days *via* gavage.

Ref.: Carlson M.B. (2013), BASF project number 50C0473/11X497

SCCS comment

Administration of C-1701 B_C_3 by oral gavage to rats once a day for 90 days at a dose of 1000 mg/kg/day resulted in no test article-related gross findings, although liver weight changes with associated microscopic liver findings (centrilobular hypertrophy) were noted. There were statistically significant changes in other organ weights, but there were no patterns, trends or associated microscopic findings to identify them as being toxicologically relevant. Administration of C-1701 B_C_3 by oral gavage to rats once a day for 90 days at a dose of 100 or 300 mg/kg/day resulted in no test article-related gross findings. Organ weight changes in liver (increased) only in females at the 300 mg/kg/day dose level but no microscopic findings in the liver. Therefore SCCS agrees with the NOAEL of 300 mg/kg/day.

3.3.5.3 Chronic (> 12 months) toxicity

/

3.3.6 Reproductive toxicity

3.3.6.1 Fertility and reproduction toxicity

Reproduction/developmental screening study in rats

Guideline:	OECD 421; US EPA OPPTS 870.3550
Species/strain:	Rat/Crl:WI(Han)
Group size:	10/sex/dose (a total of 80 rats)
Test substance:	C-1701 B_C_3 suspended in polyethylene glycol 300
Batch:	C-1701/8
Purity:	96.3% (^{1}H-NMR); dose calculations were not corrected for purity
Dose levels:	0, 100, 250 or 700 mg/kg bw/day
Dose volume:	5 mL/kg bw

Route: Oral
Exposure period: 14 days prior to cohabitation, through cohabitation, and continuing through the day before necropsy for male rats or through day 4 of lactation (DL4) for females rats that delivered a litter.
GLP: in compliance
Study period: June 2012-May 2013 (in life phase ended August 2012)

This screening study was designed to provide initial information on possible effects on reproduction and/or development, either at an early stage of assessment of toxicological properties of a compound. This test was not designed to provide complete information on all aspects of reproduction and development.

The choice of tested doses was based on a range-finding maternal toxicity study (Carlson M.B. (2013), CRL study number 20027339) in pregnant Crl:WI(Han) female rats at dose levels of 0 (vehicle control), 100, 300 and 1000 mg/kg bw/day on DGs 6-20. In this range-finding study, clinical signs such as urine-stained abdominal fur, slight to moderate excess salivation and ungroomed coat occurred in a generally dose-dependent manner in each of the dose groups. Additionally, dehydration, piloerection, discolored urine, soft or liquid feces, hunched posture, scant feces, decreased motor activity, and discolored fur occurred in the 300 and/or 1000 mg/kg bw/day whereas ptosis, thin body condition, and hyperpnoea occurred in a single rat at 1000 mg/kg bw/day. Maternal body weights/changes, food consumption, gravid uterine weights and terminal body weights were reduced and absolute and relative liver weights were increased in the 1000 mg/kg bw/day group. On the basis of the observed effects, the dose level of 700 mg/kg bw/day was expected to produce maternal toxicity.

Dose formulation and control substance, PEG 300, were administered for 14 days prior to cohabitation, throughout cohabitation and continuing through the day before necropsy for male rats or through day 4 of lactation (DL4) for female rats that delivered a litter. Female rats that did not deliver a litter were euthanised on an estimated day 25 of gestation (DG 25).

A complete necropsy was performed in the main study on all parental (P) generation rats, and selected tissues were weighed, retained and processed for histopathological examination. All surviving filial (F1) generation pups were euthanised on postnatal day 5 (PND 5), and examined for gross lesions. In this study, mortality (P and F1 generations), clinical signs (P and F1 generations), body weights (P and F1 generations), feed consumption, estrous cyclicity, mating and fertility parameters, natural delivery, litter observations, macroscopic findings (P and F1 generations), selected organ weights and microscopic findings (incl. sperm staging in males) were assessed.

Dose formulation samples were collected for concentration and homogeneity analysis by means of a HPLC method. Stability analyses were performed and demonstrated that the test item is stable in the vehicle at room temperature and protected from light for 24 hours and under refrigerated conditions (2-8 °C) and protected from light for 10 days at concentrations bracketing those used in the present study.

Results

Analysis of dose formulation samples revealed accurate preparation. The test item was homogeneously distributed in the vehicle.

Administration of the test item at dose levels of 100, 250 and 700 mg/kg bw once daily by oral gavage resulted in urine-stained abdominal fur in male and female rats. Mean body weight gains were slightly decreased (53% of the control group mean value) during the first week of study (days 1-8) in P generation male rats at 700 mg/kg bw/day. In P generation female rats at the same dose level, mean body weight gains were slightly decreased (83%

of the control group mean value) throughout the overall gestation period (DGs 0-20). Mean food consumption values were slightly decreased (90 to 88% of the control group mean value) during the first week in P generation male and female rats and the first week of pregnancy (DGs 0-7; 93 to 92% of the control group mean value) in female rats at 700 mg/kg bw/day.

There were no test item-related effects on estrous cycle, mating and fertility parameters, gestation and lactation. Reproductive organ weights were not altered by the administration of the test item.

Mean pup weights per litter on DLs 1 and 5 were slightly reduced (9 and 14% reduction, relative to control group mean values, respectively) in the 700 mg/kg bw/day group (reflecting decreased body weight change in P generation females during gestation (17% reduction, relative to the control group mean value) and also the slightly higher mean litter size (11.2 versus 10.4 in the control group)). It is known from literature (Fleeman *et al*., 2005) that reductions in fetal body weights frequently occur concurrent with reduced maternal food consumption and maternal body weights, as seen in the current study results.

Histopathological examinations did not reveal any test item-related effects. There were no adverse clinical signs or gross lesions in the F1 generation pups attributed to administration of the test item to the P generation dams.

Body weight gain [g] in P generation females treated by oral gavage with C-1701 B_C_3				
Study period	**Dose level [mg/kg bw/day]**			
	0	**100**	**250**	**700**
P generation female rats				
Precohabitation [Days 1-14]	10.1 ± 6.7	14.0 ± 6.1	15.6 ± 7.6	17.7 ± 5.5
Gestation [DG 0-20]	102.0 ± 11.5	101.2 ± 14.8	105.5 ± 12.8	84.9 ± 8.2**
Lactation [DL 1-5]	13.2 ± 11.4	10.4 ± 11.6	18.1 ± 8.4	18.2 ± 7.3

DG: day of gestation, DL: day of lactation; P: parental
All values are given as mean ± standard deviation (n = 8-10 pregnant females)
** significantly different from the control group value ($p \leq 0.01$), Dunnett's test

Body weights per litter [g] in F1 generation pups of dams treated with C-1701 B_C_3				
Study period	**Dose level [mg/kg bw/day]**			
	0	**100**	**250**	**700**
F1 generation pups				
Lactation [DPP 1]	5.8 ± 0.4	5.9 ± 0.5	5.5 ± 0.4	5.3 ± 0.4*
Lactation [DPP 5]	9.3 ± 1.4	9.7 ± 1.2	8.8 ± 0.8	8.0 ± 0.6*

DPP: day *post partum*, F: filial
All values are given as mean ± standard deviation (n = 8-10 delivered litters with liveborn pups)
* Significantly different from the control group value ($p \leq 0.05$), Dunnett's test

Conclusion
Under the conditions of this study, the NOAEL for parental toxicity of C-1701 B_C_3 was considered to be 250 mg/kg bw/day given the signs observed at the highest tested dose (urine-stained abdominal fur, mean body weight gains and mean food consumption values slightly decreased).

The NOAEL for reproductive toxicity was considered to be 250 mg/kg bw/day, based on the reductions in mean pup weights per litter at 700 mg/kg bw/day, which were probably related to maternal toxicity, as the reductions in pup weights were concurrent with decreased maternal body weights and a slightly higher litter size. Further, these reductions in mean pup weights per litter were not observed in the lower dose groups, where evidence of maternal toxicity was not apparent.

Based on the study results, C-1701 B_C_3 did not display adverse effects on reproduction parameters.

Ref.:
Carlson M.B. (2013), CRL study number 20027339 (range-finding study)
Carlson M.B. (2013), Charles River Laboratories study number 20027631, BASF project number 80R0473/11X498

SCCS comment
SCCS agrees with a NOAEL of 250 mg/kg bw/day for the parental toxicity as well as for the reproductive toxicity.

3.3.6.2 Developmental toxicity

Guideline:	OECD 414
Species/strain:	Rat/ Crl:WI(Han)
Group size:	25 pregnant female rats/group (a total of 100 rats)
Test substance:	C 1701 B_C_3
Batch:	C-1701/8
Dose levels:	0, 100, 250 and 700 mg/kg bw/day on GDs 6-20
Dose volume:	5 mL/kg bw
Route:	Oral gavage
Exposure period:	from gestation day 6 to gestation day 20
Positive control:	
GLP:	In compliance
Study period:	April 2012- May 2013 (in life phase ended August 2012)

Methods

The choice of tested doses was based on a range-finding maternal toxicity study (Carlson M.B. (2013), CRL study number 20027339) in pregnant Crl:WI(Han) female rats at dose levels of 0 (vehicle control), 100, 300 and 1000 mg/kg bw/day on DGs 6-20. In this range-finding study clinical signs such as urine-stained abdominal fur, slight to moderate excess salivation and ungroomed coat occurred in a generally dose-dependent manner in each of the dose groups. Additionally, dehydration, piloerection, discolored urine, soft or liquid feces, hunched posture, scant feces, decreased motor activity, and discolored fur occurred in the 300 and/or 1000 mg/kg bw/day whereas ptosis, thin body condition, and hyperpnoea occurred in a single rat at 1000 mg/kg bw/day. Maternal body weights/ changes, food consumption, gravid uterine weights and terminal body weights were reduced and absolute and relative liver weights were increased in the 1000 mg/kg bw/day group. On the basis of the observed effects, the dose level of 700 mg/kg bw/day was expected to produce

maternal toxicity and the dose levels of 100, 250 and 700 mg/kg bw/day were selected for the main prenatal developmental toxicity study.

All female rats were euthanised on DG 21 and examined for ovarian and uterine contents, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed blind to dose group. The following parameters and end points were evaluated: viability, clinical signs, body weights, body weight changes, food consumption, mating performance, gross observations, ovarian and uterine contents, gravid uterine weights, and fetal sex, fetal body weights, and fetal gross external, soft tissue, and skeletal alterations, as well as ossification site averages. Dose formulation samples were collected for concentration and homogeneity analysis by means of a HPLC method.
Stability analyses demonstrated that the test item is stable in the vehicle at room temperature and protected from light for 24 hours and under refrigerated conditions (2-8 °C) and protected from light for 10 days at concentrations bracketing those used in the present study.

Results

Analysis of the dose formulation samples revealed all actual concentrations were within the acceptance criteria of ± 15% of the respective theoretical concentrations. All dose formulation samples met acceptance criteria for homogeneity (the relative standard deviation [RSD] of concentrations was < 5% for each group). Control substance samples contained no detectable concentrations of the test substance.

Urine-stained abdominal fur, dehydration (based on skin turgor), and red perinasal substance occurred in the 700 mg/kg bw/day group. These clinical signs were attributed to administration of the test item. Additional clinical signs included but were not limited to excess salivation, thin body condition, urine-stained perivaginal area, all of which occurred in a single animal in the 700 mg/kg bw/day group; these clinical signs were also attributed to test item administration. Urine-stained abdominal fur also occurred in an increased number of animals at 250 mg/kg bw/day, and dehydration was noted in a single animal on a single occasion. No test item-related clinical signs were observed at 100 mg/kg bw/day.

Mean maternal body weights and body weight changes (absolute and corrected for gravid uterine weights) were reduced at 700 mg/kg bw/day, and mean absolute body weight gain between DGs 6 and 21 was reduced by 24% when compared to the control group value. Likewise, mean absolute and relative food consumption values in this dose group were reduced by 14% and 12%, respectively when compared to the respective control group values during this same interval. Mean body weight and body weight changes and food consumption values were not affected by the administration of the test substance in the other dose groups.

Slight reductions in fetal body weight averages (approximately 7%) were noted at 700 mg/kg bw/day. Fetal morphology examinations revealed reduced numbers of ossified caudal vertebrae and hind limb tarsals, metatarsals, and phalanges at 700 mg/kg bw/day. No test item-related effects were observed at 100 and 250 mg/kg bw/day.

Overall, daily test item administration at 700 mg/kg bw/day from DGs 6-20 caused maternal toxicity, as evidenced by clinical signs, significantly reduced food consumption and significantly reduced body weight and body weight changes. There were no compound-related effects regarding pregnancy or Caesarean-sectioning examination parameters. Mean fetal body weights were slightly reduced at 700 mg/kg bw/day. Fetal examinations revealed reductions in the mean number of ossification sites in the caudal vertebrae and hind limbs, but no test item-related effects regarding the incidence of malformations and other variations. The reductions in the mean number of ossification sites at the caudal vertebrae and hind limbs were morphological correlates of the reductions in fetal body weight averages, which occurred at a maternally toxic dose level. It is known from the literature (Fleeman *et al*., 2005) that reductions in fetal body weights and delays in ossification

frequently occur concurrent with reduced maternal food consumption and maternal body weights, as seen in the current study results.

At 250 mg/kg bw/day, a higher incidence of urine-stained abdominal fur was present in the dams. Mild dehydration (based on skin turgor) occurred in a single rat on a single occasion. In the absence of any other changes, these findings were not considered as adverse. No embryo-fetal effects were observed.

Neither maternal nor embryo-fetal effects were observed at 100 mg/kg bw/day.

Conclusion

Under the conditions of this study with the C-1701 B_C_3, the No Observed Adverse Effect Levels (NOAELs) for maternal and embryo-fetal toxicity were established at 250 mg/kg bw/day.

Reductions in fetal body weight averages and reductions in the mean number of ossification sites in the caudal vertebrae and hind limbs occurred at 700 mg/kg bw/day, and were considered related to maternal toxicity, as these effects were concurrent with decreased maternal food consumption and body weights. These reductions in fetal body weights and ossification sites were not observed at lower dose levels, including 250 mg/kg bw/day, where evidence of maternal toxicity was not apparent.

Considering that test item-related slight reduction in fetal body weight and retardation of ossification were seen only in association with maternal toxicity, C-1701 B_C_3 was considered to have no selective embryotoxicity or teratogenicity.

Ref.:
Carlson M.B. (2013), CRL study number 20027339 (range-finding study)
Carlson M.B. (2013), BASF project number 30R0473/11X499

SCCS comment

SCCS agrees with a NOAEL of 250 mg/kg bw/day for maternal toxicity as well as for the embryo-fetal toxicity.

3.3.7 Mutagenicity / Genotoxicity

3.3.7.1 Mutagenicity / genotoxicity *in vitro*

Bacterial Reverse Mutation Test (AMES)

Guideline:	OECD 471; Commission Regulation (EC) No 440/2008, B.13/B.14; US EPA OPPTS 870.5100
Test system:	Salmonella typhimurium strains TA1535, TA100, TA1537, TA98 and E. coli WP2 uvrA
Replicates:	Triplicate plates
Test substance:	C-1701 B_C_3
Batch:	1442/3+4
Purity:	98.8%
Solvent:	DMSO
Concentrations:	0, 33, 100, 333, 1 000, 2 625 and 5 250 µg/plate.

Treatment:	Exp. 1: Standard plate test (SPT) and Exp. 2: preincubation test (PIT), both with and without a mammalian metabolic activation system, incubation 48-72 h
Negative control:	DMSO
Positive control:	with S9-mix: 2 Aminoanthracene (2-AA), without S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 4-Nitro-o-phenylenediamine (NOPD), 9 Aminoacridine (AAC), 4 Nitroquinoline-N-oxide (4 NQO)
GLP:	in compliance
Study period:	10 April 2011 - 31 January 2012

The test substance C-1701 B_C_3 was tested for mutagenicity in the Salmonella typhimurium / Escherichia coli reverse mutation assay both in the standard plate test (SPT) and in the preincubation test (PIT) with and without metabolising system (S9 mix), obtained from phenobarbital/β-naphthoflavone-induced rats using the Salmonella strains TA 1535, TA 100, TA 1537, TA 98 and Escherichia coli WP2 uvrA.
The stability of the test item at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.
Bacteriotoxicity was detected by a decrease in the number of revertants, clearing or diminution of the background lawn and/or reduction in the titer. Precipitation of the test item was recorded. Individual plate counts and the mean number of revertant colonies per plate were determined for mutagenicity assessment.
The test item was considered positive in this assay if a dose-related and reproducible increase in the number of revertant colonies, *i.e.* nearly doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or with S9-mix, was noted.
A test substance was considered non-mutagenic if the number of revertant colonies for all tester strains was within the historical NC range under all experimental conditions in two independent experiments. Negative and positive controls were in accordance with the OECD guideline.

Results

Bacteriotoxicity (decrease in the number of his^+ revertants, slight reduction in the titer) was observed in the SPT and PIT depending on the strain and test conditions at or from about 2625 µg/plate onward. No test item precipitation was found with and without S9-mix.
C-1701 B_C_3 did not induce a biologically relevant increase in the number of revertant colonies over background, either with S9-mix or without S9-mix in two independent experiments (SPT and PIT).
The results of the NC and PC items performed in parallel corroborated the validity of this study, since the values fulfilled the acceptance criteria. The number of revertant colonies in the NC plates was within the range of the historical NC data for each tester strain, with and without S9-mix. In addition, the PC items both with and without S9-mix induced a significant increase in the number of revertant colonies within the range of the historical PC data or above.

Conclusion

C-1701 B_C_3 up to 5250 µg/plate was not mutagenic in the bacterial reverse mutation test (Ames test) neither in the absence nor in the presence of a mammalian metabolic activation system S9-mix under the experimental conditions of the study.

Ref.: Woltkowiak C. (2012)

***In vitro* Micronucleus Test in human lymphocytes**

Guideline:	OECD 487 (2010)
Species/strain:	Cultured human peripheral blood lymphocytes from two female volunteers (pooled blood)
Replicates:	Duplicate cultures, two independent experiments
Test substance:	C-1701 B_C_3
Batch:	1442/3+4
Purity:	98.8%
Concentrations:	Exp1: -S9 mix: 750, 900, 1050 µg/mL (3 h),+S9 mix: 750, 900, 1000 µg/mL (3 h), -S9 mix: 80, 110, 155 µg/mL (24 h) Exp1:-S9 mix: 400, 800, 1000 µg/mL (3 h), +S9 mix: 800, 950, 1000 µg/mL (3 h)
Solvent/negative control:	0.85% saline
Positive Controls:	-S9 mix: Mitomycin C (MMC), Vinblastine (VIN) +S9 mix: Cyclophosphamide (CPA)
Vehicle:	DMSO
GLP:	In compliance
Study period:	27 October 2011 - September 12, 2012

In an *in vitro* micronucleus assay, C-1701 B_C_3 (purity/content: 98.8%; batch: 1442/3+4) was tested using duplicate human lymphocyte cultures prepared from the pooled blood of two female donors in two independent experiments for clastogenicity and aneugenicity assessment. The maximum concentrations analysed were determined following a preliminary cytotoxicity experiment. Cytotoxicity was assessed as reduction in the replication index (RI). Suitable maximum concentrations for analysis were selected with special regard to the steep concentration-related toxicity observed.
Treatments were conducted 48 hours following mitogen stimulation with Phytohaemagglutinin (PHA). Cells were exposed to the test item in the vehicle DMSO for 3 hours (followed by 21 hours recovery) in the absence and the presence of a mammalian metabolic activation system (S9-mix from the liver of Aroclor 1254 induced male Sprague Dawley rats). In addition, cells were exposed for 24 hours (equivalent to approximately 1.5 to 2 times the average generation time of cultured lymphocytes from the panel of donors used in this laboratory; no recovery) in the absence of S9-mix.
Negative and positive controls were in accordance with the OECD guideline.
All cultures were sampled 24 hours after the beginning of treatment (*i.e.* 72 hours after culture initiation). A total of 1000 binucleate cells from each culture (2000 cells/concentration) was analysed for micronuclei. The test item was considered to induce clastogenic and/or aneugenic events if a statistically significant increase in the frequency of binucleate cells with micronuclei (MNBN) at one or more concentrations was observed, an incidence of MNBN cells at such a concentration that exceeded the normal range in both replicates was seen and a concentration-related increase in the proportion of MNBN cells was noted.

Results

Experiment 1

Treatment of cells with the C-1701 B_C_3 for 3 hours in the absence of S9-mix in Experiment 1 resulted in mean frequencies of MNBN cells that were significantly higher than those observed in concurrent NCs at the highest two concentrations analysed (900 and 1050 µg/mL, giving 23% and 69% reductions in RI, respectively). The MNBN cell frequencies exceeded the 95th percentile of the observed historical NC range (0.1-1.0%) in one culture at 900 µg/mL and both cultures at 1050 µg/mL and there was evidence of a concentration-related response. However, the MNBN frequencies in both cultures at 900 µg/mL were below the upper limit of the historical NC range (2.40%) and the only

concentration at which the MNBN frequencies exceeded this range (1050 µg/mL) gave 69% reduction in RI (greater than the target RI range of 50-60%). The data therefore showed evidence of micronucleus induction under this treatment condition, but primarily at a cytotoxic concentration at which increased MNBN frequency might be a secondary effect of cytotoxicity.

Treatment of cells for 3 hours in the presence of S9-mix resulted in frequencies of MNBN cells that were significantly higher than those observed in concurrent NCs at the highest concentration analysed (1000 µg/mL, giving 39% reduction in RI). The MNBN cell frequencies exceeded the 95th percentile of the historical NC range (0.1-1.1%) in both cultures at 1000 µg/mL.
Treatment of cells for 24 hours in the absence of S9-mix resulted in frequencies of MNBN cells that were similar to (and not significantly different from) those observed in concurrent NCs at all concentrations analysed. The MNBN cell frequencies in all treated cultures fell within the 95% percentile of the historical NC range (0.1-1.4%).

Experiment 2

Treatment of cells for 3 hours in the absence of S9-mix resulted in frequencies of MNBN cells that were significantly higher than those observed in concurrent NCs at the highest two concentrations analysed (800 and 1000 µg/mL, giving 31% and 39% reductions in RI, respectively). The MNBN cell frequencies exceeded the 95% percentile of the historical NC range (0.1-1.0%) in both cultures at 800 and 1000 µg/mL and exceeded the upper limit of the historical NC range at 1000 µg/mL with evidence of a concentration-related increase in MNBN cell frequency, thus fulfilling the criteria for a positive response. The data from Experiment 2 in the absence of S9-mix therefore confirmed the evidence of micronucleus induction seen in Experiment 1 at concentrations giving moderate levels of cytotoxicity.
Treatment of cells for 3 hours in the presence of S9-mix resulted in frequencies of MNBN cells that were significantly higher than those observed in concurrent NCs at all three concentrations analysed (800, 950 and 1000 µg/mL, giving 14%, 30% and 46% reductions in RI, respectively). The MNBN cell frequencies exceeded the 95% percentile of the historical NC range (0.1-1.1%) in one culture at 800 µg/mL and in both cultures at 950 and 1000 µg/mL, with evidence of a concentration-related increase in MNBN cell frequency.

Because of the positivity observed after 3 hours treatment, treatment of cells for 24 hours in the absence of S9-mix was not considered necessary in Experiment 2.
The data therefore showed evidence of micronucleus induction in the presence of S9-mix in Experiments 1 and 2.

Conclusion

C-1701 B_C_3 induced micronuclei in cultured human peripheral blood lymphocytes when tested for 3 hours in the absence and presence of a mammalian metabolic activation system. In the same test system, the test item did not induce micronuclei when tested up to cytotoxic concentrations for 24 hours in the absence of metabolic activation.

Ref: Lloyd M. (2012)

SCCS comment

C-1701 B_C_3 was positive in an *in vitro* micronucleus assay. After 3h treatment both with and without S9-mix, a statistically significant and concentration-dependent increase in the number of cells with micronuclei was observed in both experiments.
The SCCS notes a discrepancy in the highest concentrations used in the MN tests (>750 µg/mL) and in the solubility of the test substance in water (450 µg/mL) as reported in the paragraph 3.1.6 Solubility.

***In Vitro* Micronucleus Test using Reconstructed skin Micronucleus (RSMN) assay in EpiDerm™**

Guideline:	OECD Guideline not available
Species/strain:	EpiDermTM tissues come from MatTek Corporation (Ashland, MA, USA)
Replicates:	Two independent experiments, triplicate tissue
Test substance:	C-1701 B_C_3
Batch:	0009511412
Purity:	98.8%
Concentrations:	10, 20, 25, 30, 35, 40, 45, 50, and 60 mg/mL
Treatment:	First experiment 2-day regime (2x24 h), 2. Experiment 3-day regime (3x 24h)
Solvent/negative control:	acetone
Positive Controls:	Mitomycin C (MMC),
Vehicle:	acetone
GLP:	In compliance
Study period:	November 2, 2015 – March 16, 2016

The genotoxic potential of C-1701 B_C_3 (purity/content: 98/73% by HPLC, batch 0009511412), was assessed for induction of micronuclei in the reconstructed skin micronucleus assay (RSMN) in EpiDerm™ on the basis on an expert recommended protocol (Dahl et al, 2011) derived from the general *in vitro* micronucleus OECD Guideline 487.
Tissues were treated by application of 10 µL of the test article/vehicle mixture at the appropriate concentration on the top surface of the tissue. EpiDermTM tissues come from MatTek Corporation (Ashland, MA, USA) and are multi-layered, differentiated tissues consisting of basal, spinous, granular and cornified layers resembling the normal human epidermis (Curren et al., 2006).
Cytotoxicity was assessed by calculating the cytokineseis-block proliferation index (CBPI) and determining the relative viable cell count (RVCC), whichever parameter came first.
In the preliminary cytotoxicity and the 1st definitive micronucleus assay, EpiDermTM tissues were treated twice, 24 hours apart, and tissues were processed at 48 hours (2-day dosing regimen). In the confirmatory micronucleus assay, the tissues were treated three times, 24 hours apart, and tissues were processed at 72 hours (3-day dosing regimen).
The preliminary cytotoxicity test was conducted by exposing a single tissue per concentration to vehicle alone and 15 concentrations of the test article ranging from 0.006 to 100 mg/mL (corresponding to the maximum recommended concentration). Both Micronucleus assays were conducted with 9 concentrations using triplicate tissues.
The highest dose level evaluated for micronuclei was selected to give 50 to 60% cytotoxicity (CBPI relative to the vehicle control or reduction in RVCC, whichever comes first) and at least two additional dose levels, demonstrating moderate to minimal toxicity, were also evaluated.

Results
In the preliminary assay, ≥ 50% cytotoxicity by calculating CBPI relative to vehicle control was observed at concentrations ≥ 50 mg/mL, while cytotoxicity RVCC determination was not observed at any concentrations. Precipitate was observed on the tissue at concentrations ≥ 50 mg/mL at the end of treatment.
Based on these results, the definitive micronucleus assay was conducted at concentrations ranging from 10 to 60 mg/mL. A 50 to 60% cytotoxicity by calculating CBPI relative to vehicle control was observed in the 3 replicates at the concentrations of 25 and 30 mg/mL, while cytotoxicity by RVCC determination was not observed at any concentration. The concentrations selected for scoring micronuclei were 10, 20, 25, and 30 mg/mL. One thousand binucleated cells per tissue were scored for the presence of micronuclei. The percentage of micronucleated binucleated cells in the test article-treated tissues was not significantly increased relative to the vehicle control at any concentration tested.
Since the result of the micronucleus assay using a 2-day dosing regimen was negative, a

confirmatory assay was conducted with a 3-day dosing regimen at concentrations ranging from 8 to 35 mg/mL.
In the confirmatory micronucleus assay, cytotoxicity of 50 to 60% (determined by calculating CBPI relative to vehicle control) was observed at the concentrations of 24 and 26 mg/mL, while cytotoxicity (RVCC) was not observed at any other concentrations. The concentrations selected for scoring micronuclei were 8, 20, and 26 mg/mL. The percentage of micronucleated binucleated cells in the test article-treated tissues was not significantly increased relative to the vehicle control at any concentration tested.
In addition, in the definitive and confirmatory micronucleus assays, the percentage of micronucleated binucleated cells in the vehicle control was within the acceptable historical control range and the percentage of micronucleated binucleated cells in the positive control was statistically increased and also within the historical positive range.

Conclusion
Based on the findings of this study, it was concluded that C-1701 B_C_3 was negative for the induction of micronuclei in the reconstructed skin micronucleus assay (RSMN) in EpiDerm™.

Ref.: Shambhu R. (2016)

Conclusion on genotoxicity
The genotoxic potential of C 1701 B_C_3 was evaluated in an extensive battery of *in vitro* studies including the bacterial reverse mutation test, a micronucleus test in cultured human lymphocytes and also in a reconstructed skin micronucleus assay (RSMN assay) in EpiDerm™ model.
When tested for gene mutation in a bacterial system (in independent experiments) with and without addition of a mammalian metabolic activation system, the UV filter C 1701 B_C_3 was shown to be non-mutagenic *in vitro*.
The potential of C-1701 B_C_3 to induce clastogenicity and/or aneugenicity was assessed in two separate *in vitro* micronucleus tests.
In the *in vitro* micronucleus test in cultured human peripheral blood lymphocytes, C-1701 B_C_3 induced micronuclei when tested for 3 hours in the absence and presence of a mammalian metabolic activation system. A reconstructed skin micronucleus assay (RSMN assay) in EpiDerm™ model was done as an alternative to an *in vivo* test. In this test, C-1701 B_C_3 did not induce any increase in the frequency of micronuclei at any tested concentrations showing a sufficient cytotoxicity (50-60% of cytotoxicity). This model currently under validation has already been demonstrated to be sensitive to the clastogenic and aneugenic activity of variety of chemicals and is considered as especially relevant for chemicals for which human exposure is expected to be dermal. In addition, the Epiderm™ model has been shown to be more permeable than human skin and the applied dose is higher in this test than expected in human. Thus, the exposure conditions in this model are assumed to be maximal.

Taken together, the results obtained in the available *in vitro* test battery, addressing all relevant endpoints of genotoxicity, indicate that the UV filter C-1701 B_C_3 is non-mutagenic and non-genotoxic.

SCCS comment
The SCCS considers RSMN assay a promising new *in vitro* approach designed to assess genotoxicity of dermally-applied compounds. However, the validation of the test is still ongoing, while a search of the open literature on RSMN so far has not indicated satisfactory data relating to indirectly acting genotoxic chemicals.
Furthermore, in the RSMN assay on S87 (GLP study) submitted by the applicant, only Mitomycin C was used (a direct-acting clastogen) as a positive control substance. To prove the validity of the assay, additional genotoxins with a different mode of action should be

applied as a positive control (e.g. cyclophosphamide, indirectly acting clastogen and vinblastine, direct aneugen).

Furthermore, SCCS is of the opinion that the reconstructed skin micronucleus EpiDerm assay alone cannot be used to overrule the positive result in the *in vitro* micronucleus test. Based on the data provided by the Applicant, a genotoxic potential of S87 cannot be excluded.

3.3.7.2 Mutagenicity / genotoxicity *in vivo*

3.3.8 Carcinogenicity

/

3.3.9 Photo-induced toxicity

3.3.9.1 Phototoxicity / photo-irritation and photosensitisation

***In vitro* 3T3 NRU phototoxicity test**

Guideline:	OECD 432; Commission Regulation (EC) No 440/2008, B.41
Species:	Balb/c 3T3 cells
Test substance:	C-1701 B_C_3
Batch:	C-1701/8
Purity:	96.3%
Vehicle:	aqueous Dimethyl sulfoxide (DMSO, 1.0% v/v)
Exposure duration:	24 h
Concentrations:	-UVA: 0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, 1000.0 µg/mL; +UVA: 0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, 1000.0 µg/mL
GLP:	In compliance
Study period:	July – September 2012

Photo-cytotoxicity was estimated using the neutral red uptake (NRU) method. Two independent experiments (Experiment 1 and 2) were carried out, both with and without irradiation by means of an ultraviolet A (UVA) source. According to an initial range-finding phototoxicity test conducted for the determination of experimental concentrations, the following concentrations were tested in aqueous Dimethyl sulfoxide (DMSO, 1.0% v/v) in both main experiments:

- without UVA irradiation (-UVA)
0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, 1000.0 µg/mL
- with UVA irradiation (+UVA)
0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, 1000.0 µg/mL

After an attachment period of about 24 hours, the cells were pre-incubated with the test item or the positive control (PC) item Chlorpromazine (CPZ) for 1 hour in the dark. Then one microtiter plate per substance (test item or PC item) was irradiated for 50 minutes with the UVA irradiation source (approximately 5 J/cm²). The respective reference plates treated in parallel were kept in the dark for the same period. About 24 hours after end of treatment, the cytotoxicity was determined by measuring the NRU using a microplate reader. In

addition, the pH value, osmolarity, test item solubility (precipitation) and cell morphology in the cultures were assessed.

The prediction model is based on the comparison of two equi-effective cytotoxic concentrations (EC50 values) obtained in concurrently performed experiments in the absence (-UVA) and presence (+UVA) of UVA irradiation, which are used to calculate a photo-irritancy factor (PIF): PIF = EC50(-UVA) / EC50(+UVA)
If a test substance is only cytotoxic after irradiation (+UVA), a C PIF has to be calculated using the highest test concentration (Cmax) applied in the experimental part in the absence of UV light (-UVA): C PIF = Cmax(-UVA) / EC50(+UVA)

Results
After treatment with the test item, cytotoxic effects indicated by neutral red absorbance values of below 50% of control were observed in Experiments 1 and 2 in the presence of UVA irradiation and only in Experiment 2 in the absence of UVA irradiation at the highest concentration. Without UVA irradiation, in Experiment 2 there was a decrease in the cell number at 1000 µg/mL (EC50: 958.1 µg/mL). The cell densities were not distinctly reduced. In addition, with UVA irradiation, there was a decrease in the cell number at 1000 µg/mL (Experiment 1: EC50 of 998.7 µg/mL; Experiment 2: EC50 of 758.4 µg/mL). The cell densities were not distinctly reduced. Based on the EC50 values a C PIF of 1.0 (no phototoxic potential) was obtained in Experiment 1 and a PIF of 1.3 (no phototoxic potential) was obtained in Experiment 2.
The vehicle controls (DMSO) clearly fulfilled the acceptance criteria. The PC item led to the expected cytotoxicity both with and without UVA irradiation (PIF: 28.7 and 44.2 in each experiment, respectively). Osmolarity and pH values were not influenced by the test item treatment. No precipitation was noted in Experiments 1 and 2 at the end of the exposure period.

Conclusion
Under the experimental conditions of this study, C-1701 B_C_3 was not a phototoxic substance in the *in vitro* 3T3 NRU phototoxicity test using Balb/c 3T3 cells.

Ref.: Cetto V. (2012)

Skin photosensitization study in guinea pigs

Guideline/method:	No guideline available
Species/strain:	Guinea pig / Hartley Crl:HA
Group size:	Main study: 10 animals/group (with UV irradiation) and 5 animals/group (without UV irradiation), Positive control: 2 groups with 5 animals/group
Test substances:	C-1701 B_C_3 (solution 50% (w/v) in N,N-Dimethylformamide)
Batch:	C-1701/8
Purity:	96.3% (^{1}H-NMR)
Concentration:	10, 25 or 50 w/v% (preliminary study) and 50% (w/v) (main study)
Volume:	Duplicate 0.5 mL samples
Route:	
Negative control:	
Positive control:	3,3',4',5 Tetrachlorosalicylanilide (TCSA) in acetone.
Source of light:	Dermaray®-200 type UV irradiator
Irradiation:	In the main study the actual values of irradiance, intensity and duration of irradiation were 8.10-8.85 J/cm², 5.4-5.9 mW/cm² and 1500 s for UVA light and 0.093-0.098 J/cm², 0.93-0.98 mW/cm² and 100 s for UVB light, respectively
Observations:	1, 4, 24 and 48 hrs after application
GLP:	In compliance

Study period: 28 August 2012- 9 January 2013

The concentrations of dose formulations used in this study were verified by means of a HPLC method. The stability of another batch of C-1701 B_C_3 (batch: 1442/3+4) at 1 and 50% (w/v) was confirmed for a storage duration of 4 hours in tight containers at room temperature. Dose formulations in this study were used within 2 hours after preparation.
In the main assay, a test item concentration of 50% (w/v) was used for induction and challenge.

Results

No clinical signs were observed in any animal in the test item or vehicle control group. The animals gained weight in a normal range during the course of the study.
During the induction period, slight erythema (score = 0.5) was observed in 2 animals each at the induction sites with DMF in the vehicle control and UV irradiated test item groups starting prior to the fourth induction until prior to the last (sixth) induction. No erythema was observed at any induction site with the test item in the UV-irradiated or UV non-irradiated test item group.
No erythema was observed at any challenge site with the test item in the vehicle control group, the test item groups (with or without UV irradiation) or at any challenge site with DMF in the UV-irradiated test item group.
In the PC groups, slight erythema (score = 0.5) was observed prior to the fourth induction at the induction sites with TCSA in the UV-irradiated and UV non-irradiated groups. The degree of erythema increased thereafter and erythema was still observed at 24 hours after the last induction. At the challenge sites with TCSA, slight erythema was observed in the UV non-irradiated group and mild or marked erythema was observed in the UV irradiated group. Therefore, the skin photosensitising potential of TCSA was confirmed and it was demonstrated that this study was conducted under the appropriate conditions.
Analysis of dose formulations revealed appropriate dosing with the test item. The mean measured concentrations at the first and second preparations were 113.5% and 98.9% of the nominal concentration, respectively and were considered acceptable.

Conclusion

Based on the results obtained, under the conditions of this study, C-1701 B_C_3 displayed no skin photoirritating or photosensitising potential when tested up to 50% (w/v) in DMF.

Ref: Matsuda A. (2013) Ina Research Inc. study number ZB12180
BASF project number 47H0473/11X539

3.3.9.2 Phototoxicity / photomutagenicity / photoclastogenicity

Photomutagenicity in a Salmonella typhimurium and Escherichia coli reverse mutation assay

Guideline:	Based on OECD 471; EC 440/2008, B.13/14, SCCNFP/0690/03
Species/strain:	Salmonella typhimurium strains TA1537, TA98, TA100, TA102 and Escherichia coli strain WP2
Replicates:	Triplicates in 3 individual experiments
Test substance:	C-1701 B_C_3
Solvent:	DMSO
Batch:	C-1701/8
Purity:	96.8%
UV source:	Dr. Hönle Sol 500 solar simulator
UVA doses:	TA 1537, TA 98, T100 and WP2: 486 mJ/cm^2, TA102 324 mJ/cm^2
UVB doses:	The filter H1 was used to keep the UVB irradiation as low as possible.

Concentrations: Pre- Experiment: 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate
Experiment I and II: 33, 100, 333, 1000, 2500, 5000 µg/plate
Positive controls: -UVA: Sodium azide (TA100, 10 µg/plate), 4-Nitro-o-phenylene-diamine (TA1537, 50 µg/plate; TA98, 10 µg/plate), methyl methane sulfonate (WP2 and TA102, 3.0 µL/plate). +UVA: 8-methoxypsoralen (WP2, TA102; 125 µg/plate).
Treatment:
GLP: In compliance
Date: 24 October 2012 – 13 May 2013

Methods
This study was performed to investigate the potential of C-1701 B_C_3 to induce gene mutations under irradiation with artificial sunlight according to the plate-incorporation test (Experiment I) and the pre-incubation test (Experiment II) using the Salmonella typhimurium strains TA1537, TA98, TA100, TA102 and Escherichia coli strain WP2. These strains were chosen since they tolerate relatively high doses of ultraviolet (UV) irradiation used to assess the possible photomutagenic potential of sunblockers.
The assay was performed in three independent experiments including a pre-experiment for dose selection for the main experiments. Each concentration, including the controls, was tested in triplicate.

Results
Precipitation of the test item was observed in the overlay agar in the test tubes at 5000 µg/plate in all experiments. No precipitation of the test item was observed on the incubated agar plates.
The plates incubated with the test item showed normal background growth up to 5000 µg/plate without metabolic activation with irradiation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with irradiation and without metabolic activation. No substantial increase in revertant colony numbers of any of the 5 tester strains was observed following test item treatment under irradiation with artificial sunlight at any concentration tested.
The appropriate reference mutagens used as PCs showed a distinct increase of induced revertant colonies over background, thus confirming sensitivity of the test system.

Conclusion
Under the experimental conditions reported, C-1701 B_C_3 did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used. Therefore, C 1701 B_C_3 was non-mutagenic in this Salmonella typhimurium and Escherichia coli photomutagenicity assay.

Ref.: Sokolowski A. (2013)

3.3.10 Human data

/

3.3.11 Special investigations

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3.4 Exposure assessment

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3.5 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

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SCCS comment
On the basis of data provided, the SCCS could not exclude the genotoxic potential of S87. Hence, the MoS calculation is not applicable.

3.6 Discussion

Physicochemical properties
SCCS cannot accept the quantification for the impurities based on HPLC-DAD data of the concentrated and the diluted solution of the test substance, unless the applicant clearly explain the dilution factor used for the calculation and the linearity range (concentrations) of the test substance.

Function and uses
S87 is proposed to be used as a UV filter in personal care products, including suncare cosmetic formulations at a maximum concentration of 5% w/w.

Toxicological Evaluation
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Acute toxicity
On the basis of data provided, C-1701 B_C_3 is not considered to be acutely toxic.

Irritation and corrosivity
The skin irritation potential of neat substance C 1701 B_C_3 has been tested *in vitro* according to OECD TG 439 (2010) using the Epiderm™ model. The amount of test item brought onto the tissues was too low . On the basis of this *in vitro* study, a skin irritation potential cannot be excluded.
On the basis of the results obtained for C 1701 B_C_3 in the BCOP assay (OECD 437, 2009), it can be concluded that C 1701 B_C_3 does not cause serious eye damage. The eye irritation potential of C 1701 B_C_3 has been tested *in vitro* using the EpiOcular™ test system. The study was performed prior to the acceptance of the official guideline of this test. The amount of test item brought onto the tissues was too low. On the basis of these results, an eye irritation potential of the test item cannot be excluded.

Skin sensitisation
The skin sensitising potential of C 1701 B_C_3 was assessed in the LLNA:BrdU-ELISA assay. Based on the results of this study, C 1701 B_C_3 is regarded to be a non-skin sensitiser.

Toxicokinetics
An *in vitro* dermal absorption experiment using split thickness human skin samples was conducted according to OECD TG 428 and indicated low dermal absorption of C-1701 B_C_3. The substance was tested in a typical suncare formulation at a concentration of 3% (w/w). A systemically-available dose of C-1701 B_C_3 of 1.08 ± 0.67 µg/cm² was measured.
According to the SCCS Notes of Guidance, several concentrations, including the highest concentration of the test substance in a typical formulation, should be tested.

Repeated dose toxicity
Administration of C-1701 B_C_3 by oral gavage to rats once a day for 90 days at a dose of 1000 mg/kg/day resulted in no test article-related gross findings, although liver weight changes with associated microscopic liver findings (centrilobular hypertrophy) were noted. There were statistically significant changes in other organ weights, but there were no patterns, trends or associated microscopic findings to identify them as being toxicologically relevant. Administration of C-1701 B_C_3 by oral gavage to rats once a day for 90 days at a dose of 100 or 300 mg/kg/day resulted in no test article-related gross findings, organ weight changes in liver (increased) only in females at the 300 mg/kg/day dose level and no

microscopic findings in the liver. Therefore, based on these results, a NOAEL of 300 mg/kg/day may be derived.

Reproductive toxicity
Based on the results of a reproduction/developmental screening study in rats, the NOAEL for parental toxicity of C 1701 B_C_3 was considered to be 250 mg/kg bw/day given the signs observed at the highest tested dose (urine-stained abdominal fur, mean body weight gains and mean food consumption values slightly decreased). The NOAEL for reproductive toxicity was considered to be 250 mg/kg bw/day, based on the reductions in mean pup weights per litter at 700 mg/kg bw/day, which were probably related to maternal toxicity.

Based on the results of a developmental toxicity study in rats, a NOAEL for maternal and for embryo-fetal toxicity was established at 250 mg/kg bw/day. Indeed, reductions in fetal body weight averages and reductions in the mean number of ossification sites in the caudal vertebrae and hind limbs occurred at 700 mg/kg bw/day, and were considered related to maternal toxicity, as these effects were concurrent with decreased maternal food consumption and body weights. These reductions in fetal body weights and ossification sites were not observed at lower dose levels, including 250 mg/kg bw/day, where evidence of maternal toxicity was not apparent.

C-1701 B_C_3 was considered to be of no concern regarding embryotoxicity or teratogenicity.

Mutagenicity / genotoxicity
The genotoxicity of C-1701 B_C_3 was investigated in the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy. C-1701 B_C_3 did not induce gene mutations in 4 strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537) nor in the E. coli WP2 uvrA strain up to the concentration of 5250 µg/plate in the absence and in the presence of a rat liver metabolic activation system (S-9 MIX). However, C-1701 B_C_3 clearly induced micronuclei in cultured human peripheral blood lymphocytes in the absence and presence of S-9 mix. In this test, which was subsequently performed, the 3D human reconstructed skin micronucleus did not indicate any mutagenic effect of C-1701 B_C_3, but in the SCCS's opinion, based on available published data, the test was not satisfactorily validated for indirectly acting genotoxins. Moreover, the SCCS considers that the EpiDerm assay cannot be used alone to overrule the positive result in the *in vitro* micronucleus test. Based on all the data provided by the Applicant, a genotoxic potential of C-1701 B_C_3 cannot be excluded.

Carcinogenicity
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Photo-induced toxicity
The data provided did not show any evidence for phototoxicity.

Human data
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Special investigation
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4. CONCLUSION

In light of the data provided, does the SCCS consider Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87), safe when used as UV-filter in cosmetic products up to a maximum concentration of 5%?

Based on the data provided, the SCCS is of the opinion that genotoxic potential of Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87) cannot be excluded. Therefore, the SCCS cannot conclude on the safety of S87. More evidence is needed to exclude the genotoxicity concern regarding S87.

Does the SCCS have any further scientific concerns with regard to the use of Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (S87) in cosmetic products?

On the basis of the studies provided, skin and eye irritation potential of the test item cannot be excluded. Dermal penetration data using 5% of the test material should also be provided.

5. MINORITY OPINION

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