Scientific Committee on Consumer Safety

SCCS

OPINION ON

Ethylzingerone - 'Hydroxyethoxyphenyl Butanone' (HEPB) - Cosmetics Europe No P98 – CAS No 569646-79-3

Submission II (eye irritation)

The SCCS adopted this Opinion
on 5 March 2019 (*)

(*) the document was technically adopted during Plenary meeting held on 26 February 2019 upon request of mandating Directorate provided that no comment were received until 5 March 2019 (deadline for the commenting period).

ACKNOWLEDGMENTS

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

This Opinion has been subject to a commenting period of a minimum eight weeks after its initial publication (from 21 December 2018 until 5 March 2019). No comment was received The conclusion did not change.

1. ABSTRACT

The SCCS concludes the following:

In light of the new studies provided, does the SCCS consider the use of Hydroxyethoxyphenyl Butanone (HEPB) safe with regard to eye irritation, when used as preservative in rinse-off, oral care and leave-on cosmetic products with a maximum concentration of 0.7%?

Based on the new information provided by the Applicant, the SCCS considers the use of Hydroxyethoxyphenyl Butanone (HEPB) as a cosmetic preservative in rinse-off, oral care and leave-on cosmetic products with a maximum concentration of 0.7 % safe with regard to eye irritation.

Keywords: SCCS, scientific opinion, Ethylzingerone - 'Hydroxyethoxyphenyl Butanone' (HEPB) - Cosmetics Europe No P98, CAS 569646-79-3, Regulation 1223/2009, SCCS/1604/18

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Ethylzingerone with INCI name 'Hydroxyethoxyphenyl Butanone' (HEPB) Cosmetics Europe No P98 - CAS No 569646-79-3 is a cosmetic ingredient not regulated under Cosmetic Regulation No 1223/2009 with the function as skin conditioning agent.

In September 2015, the Commission' services received a dossier from industry to support the safe use of Hydroxyethoxyphenyl Butanone (HEPB) when used as preservative in rinse-off, oral care and leave-on cosmetic products. In its corresponding opinion, SCCS/1582/16, the SCCS concluded that "*Based on the information provided by the applicant, a maximum concentration of 0.7 % of Hydroxyethoxyphenyl Butanone (HEPB) as a cosmetic preservative in rinse-off, oral care and leave-on cosmetic products can be considered safe*." Additionally, the SCCS expressed concerns that "*More evidence would be needed to exclude eye irritation*."

With the current submission II, received in September 2018, the applicant provides additional data on eye irritation to address the above concern of the SCCS and to support the safe use of Hydroxyethoxyphenyl Butanone (HEPB) as a preservative to concentration of up to 0.7%.

Terms of reference

In light of the new studies provided, does the SCCS consider the use of Hydroxyethoxyphenyl Butanone (HEPB) safe with regard to eye irritation, when used as preservative in rinse-off, oral care and leave-on cosmetic products with a maximum concentration of 0.7%?

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Hydroxyethoxyphenyl butanone (INCI)

3.1.1.2 Chemical names

4-(3-ethoxy-4-hydroxyphenyl)butan-2-one

3.1.1.3 Trade names and abbreviations

Ethylzingerone

R0069279A

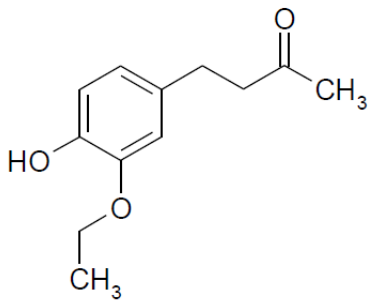
HEPB

SCCS comment
For convenience, the abbreviation HEPB will be used throughout the Opinion.

3.1.1.4 CAS / EC number

CAS: 569646-79-3
EC: not assigned

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{12}H_{16}O_3$

3.1.2 Physical form

Depending on the temperature, hydroxyethoxyphenyl butanone may appear as a solid (white powder or crystals) or as a pale yellow liquid form.

3.1.3 Molecular weight

Molecular weight: 208.25 g/mol

3.1.4 Purity, composition and substance codes

Chemical characterisation was performed using IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry and UV-Vis spectroscopy for batches 019 D-004, 019 P-001 and 020-P00!, with the following results:

- IR Spectra of the three batches were comparable.
- The ^{1}H-NMR and ^{13}C-NMR spectra of the three batches were comparable. Presence of an impurity corresponding to the alcoholic form (R0070073A) of the expected structure, (estimated value: 0.04 M/Mole in Batch 019D-004). The impurity (R0070073A) is detected in the three batches.
- UV / Vis spectra of the three batches were comparable.
- Mass spectrometry: hydroxyethoxyphenyl butanone batch 019D-004 was analysed by infusing a diluted hydro-alcoholic solution of the sample into a Thermo Fischer Scientific LTQ-Orbitrap mass spectrometer operating in the negative ion mode. The $[M-H]^-$ ion of the expected molecule $C_{12}H_{16}O_3$ was mainly observed in the mass spectrum. The MS/MS fragmentation pattern of this ion confirmed the expected structure of the molecule. The mass spectra of the two other batches 019P-001 and 020P-001 recorded using the same analytical conditions were comparable to the spectrum of batch 019D-004.

Purity of hydroxyethoxyphenyl butanone was determined by HPLC-PDA (λ=281 nm) against hydroxyethoxyphenyl butanone R0069279A batch 008L-001 primary reference standard considered as pure (100%) for batches 019D-004 and 019P-001 and against hydroxyethoxyphenyl butanone R0069279A batch 018L-002 secondary reference standard considered as 98.9% pure for batch 020P-001.
Hydroxyethoxyphenyl butanone purity:
Batch 019D-004: 95.9 ± 0.5% [w/w]
Batch 019P-001: 96.3 ± 0.8% [w/w]
Batch 020P-001: 96.2 ± 0.8% [w/w]

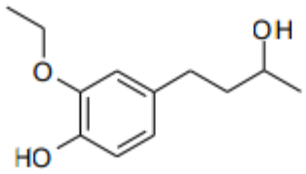
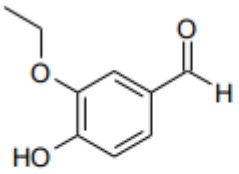
3.1.5 Impurities / accompanying contaminants

Impurities by HPLC-PDA
Separation was achieved by a linear gradient reversed phase liquid chromatography (LC) method with a photodiode array detector, Maxplot λ = 210-700nm.

The retention time of hydroxyethoxyphenyl butanone was around 8.5 minutes. One impurity (R0070073A, corresponding alcohol) was detected (Retention time R_t = 9.7 min) in the three batches, its content was determined using R0070073A 001L002 as reference standard (100%). One impurity (R0070359A, ethylvanillin) was detected (R_t = 6.3 min) in the three batches with a relative purity <0.1 %: its content was determined using R0070359A 002L001 as reference standard (100%). One impurity was detected (R_t = 7.4 min) in hydroxyethoxyphenyl butanone batches 019D004 and 019P001 with a relative UV purity < 0.1%. Three impurities were detected (R_t = 12.8 min, 14.9 min and 15.9 min) in hydroxyethoxyphenyl butanone batch 020P001 with a relative UV purity < 0.1 %. One impurity was detected (R_t = 3.6 min) in hydroxyethoxyphenyl butanone batches 019D004 and 019P001 with a relative UV purity < 0.1%. For this compound, the chemical structure 3-ethoxybenzaldehyde was proposed.

The HPLC profiles of the three batches were comparable.

According to the HPLC profile, the following impurities were identified:

R0070073A (corresponding alcohol) 2-ethoxy-4-(3-hydroxybutyl) phenol	 Exact Mass =210 Molecular Formula =C12H18O3
R0070359A (starting material) Ethylvanillin	 Exact Mass =166 Molecular Formula =C9H10O3

In the different batches, these impurities were detected in the following amounts:

R0070359A (Ethylvanillin) [µg/g]:

< 1000 in batches 019D004, 019P001 and 020P001.

R0070073A (corresponding alcohol) 2-ethoxy-4-(3-hydroxybutyl) phenol [% w/w]:

4.8 ± 0.05 in batch 019D004; 4.0 ± 0.05 in batch 019P001; 3.7 ± 0.02 in batch 020D004

Residual solvents by GC:
Acetone and ethyl acetate used in the manufacturing process of the three batches: < 0.1 % (w/w)

Heavy Metals [mg/kg]:
Batch 019D004:

As, Cd, Hg, Pb, Pd: each < 1
Al, Ba, Co, Cr, Cu, Mn, Mo, Ni, Se, Sn, Ti, V, Zn: each <5
Fe: 9

Batch 019P001:
As, Cd, Hg, Pb, Pd: each < 1
Al, Ba, Co, Cr, Cu, Mn, Mo, Ni, Se, Sn, Ti, V, Zn: each <5
Fe: 5

Batch 020 P 001:
Al, Ba, Co, Cr, Cu, Mn, Mo, Ni, Se, Sn, Ti, V, Zn: each < 1
Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, Ti,V, Zn: each < 5
Al: 6

Ash [% w/w]:

Batch 019D004: < 0.1
Batch 019P001: 0.1
Batch 020P001: 0.1

Elemental analysis:

	Theoretical values [% w/w]	Hydroxyethoxyphenyl butanone Experimental values [% w/w]		
		Batch 019D004	Batch 019P001	Batch 020P001
Carbon	69.2	69.1	69.3	68.8
Hydrogen	7.7	7.7	7.6	7.8
Oxygen	23.0	23.2	23.1	23.3

3.1.6 Solubility

Water solubility (batch 019 P001): 7.59 g/L at 20°C ± 0.5 °C
(EEC method A6 - OECD method 105)

Solubility (at 21°C) – (batch 006 L001 and 007 L001 from batch 019D004)
- Water: < 0.1 mg/mL (method not stated)
- Ethanol: ≥1000 mg/mL
- DMSO: ≥1000 mg/mL
- Corn oil: <0.1 mg/mL

SCCS Comment
The SCCS notes that the analytical file gives considerably differing values for water solubility. The issue has been clarified by the applicant and the correct value for water solubility is 7.59 g/L at 20°C ± 0.5 °C.

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 1.46 at 22.8°C ± 1 °C

(EEC method A8 - OECD method 107)

3.1.8 Additional physical and chemical specifications

Melting point:	41 ± 2°C (DSC method)
Boiling point:	328 ± 2°C at 100 to 102 kPa
Flash point:	/
Vapour pressure:	8.7 x 10^{-3} Pa at 25 °C
Density:	/
Viscosity:	/
pKa:	10.03 (25°C and ionic strength 0.15M) for an equilibrium HO/O^- (GLpKa Sirius)
Refractive index:	/
pH:	/
UV-Vis spectrum (200-500 nm):	The UV-Vis absorption spectrum of a 0.004 g/100 ml solution of hydroxyethoxyphenyl butanone exhibited a maximum at 282nm ± 1nm; the absorbance at 282 nm was ~ 0.6.

3.1.9 Stability

The R0069279A is stable over 2 months at 45°C in hydroalcoholic solution at 0.5 g/100 ml. It is sensitive to photostress but resistant to oxidative, heat, acid or basic stresses. Determination was performed for the three batches using batch R0069279A 001L-001 as reference.

SCCS General Comments to physico-chemical characterisation
Chemical characterisation of P98 was performed using IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry and UV-Vis spectroscopy. Purity of P98 was determined by HPLC-PDA at λmax 281 nm using reference standard or secondary reference standard and it is accepted.
Impurity studies were performed using HPLC-PDA detection at λmax of P98. Two impurities (2-ethoxy-4-(3-hydroxybutyl) phenol and ethylvanillin) were quantified using reference standards. Four other impurities were detected with a relative UV purity < 0.1%. In batches 019D004 and 019P001, an impurity with a relative UV purity < 0.1% with a proposed chemical structure of 3-ethoxybenzaldehyde was reported.
Chemical identification and characterisation of impurities are considered acceptable.

3.2 Function and uses

The ingredient HEPB is intended to be used specifically as a preservative in rinse-off, oral care and leave-on cosmetic products up to 0.7%.

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

No data provided.

3.3.1.2 Acute dermal toxicity

No data provided.

3.3.1.3 Acute inhalation toxicity

No data provided.

3.3.1.4 Acute intraperitoneal toxicity

No data provided.

Summary on acute toxicity
No acute toxicity study on any route is available for HEPB. In 14- and 90-day oral repeat dose studies using dose levels of 100, 300 and 1000 mg/kg bw/d, no deaths occurred. Hence, it can be assumed that the oral LD_{50} would be higher than 1,000 mg/kg/d (i.e. the substance is of low acute oral toxicity).

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

***In vitro* Acute Skin Irritation: Reconstructed Human Epidermis Test Method**

Guideline:	*In vitro* Episkin^{SM} Skin Irritation Test
Test system:	EPISKIN^{SM} Reconstructed Human Epidermis (RHE) small (0.38 cm^2) model (Skin Ethic Laboratories)
Replicates:	3 tissue batches, 3 replicates per batch
Test substance:	R0069279A
Test batch:	R0069279A 019 D 004
Purity:	95.9 % (HPLC)
Test items:	neat (100% powder)
Dose level:	10 mg
Treatment period:	15 minutes
Post-treatment incubation time:	42 hour ± 1 hour
Positive control:	5% (w/v) aqueous solution of sodium dodecyl sulphate (SDS)
Negative control:	PBS+
Direct interaction with MTT:	Negative
Colouring of epidermis:	No information provided in the study report
GLP:	In compliance
Study period:	13.12.2011 – 17.1.2012

10 ± 2 mg of undiluted HEPB and 10 µl of each of the controls were applied onto the slightly wetted reconstructed human epidermis. After 15 min incubation at room temperature, the tissues were rinsed with PBS+ and cultivated in fresh medium for 42 hours at 37° C. At the end of the 42-hour post-treatment incubation period, the epidermis was prepared for cell viability measurement (spectrophotometric MTT conversion test). Culture medium was kept frozen for IL-1α measurement. The amounts of IL-1α released in the culture medium were

determined by a classic quantitative sandwich enzyme immunoassay technique (ELISA). The amounts of IL-1α released were not determined for the positive control.

Results
When compared to the negative control, there were no changes in viability rates and IL-1α concentrations following application of HEPB. Mean cell viability values obtained for HEPB, negative and positive controls were 100%, 100% and 8.3%, respectively. The mean value of final IL-1α release was 10 pg/mL for HEPB.

Based on the results of this study, the authors concluded that undiluted HEPB can be considered as potentially non-irritant to the skin.

Ref.: 1

SCCS comment
An ECVAM validated protocol was used and certificates of analysis with IC_{50} values (for SDS) for each tissue batch were presented in annex of the study report. Barrier function has been checked by determination of the concentration at which SDS reduces the viability of the tissues by 50% and the obtained values fell within the defined acceptance range.

3.3.2.2 Mucous membrane irritation / Eye irritation

Submission I:

Bovine Corneal Opacity and Permeability (BCOP) Test Method

Guideline:	OECD 437 (September 2009)
Test system:	Isolated bovine eyes (corneas)
Replicates:	3 corneas per condition
Test substance:	R0069279A
Batch:	R0068279A 019 D 004
Purity:	95.9 ± 0.5% (HPLC)
Test item:	20 % (w/w) suspension of test substance in vehicle (yielding a pH of 5.5)
Vehicle:	polyethylene glycol (8 OE)
Dose level:	750 ± 8 µl
Treatment period:	4 hours ± 10 min
Positive control:	20% (w/w) imidazole in NaCl 0.9%
Negative control:	NaCl 0.9% in sterile water
GLP:	In compliance
Study period:	Nov 2011 – Feb 2012

Bovine eyes (from cattle less than 12 months old) were collected at slaughterhouses and prepared within 4 hours of collection. Eyes that were too big or were presenting defects were rejected. After the pre-incubation and equilibration period of the corneas of at least 1 hour at 32 ± 1°C, 750 µl of the test item, positive, negative and vehicle control were applied onto the corneas for 4 hours. At the end of the contact period, corneas were rinsed and prepared to measure opacity (changes in light transmission) and permeability (evaluation of transfer of 5 mg/ml fluorescein through the cornea by measuring the optical density at 490 nm of the media in the ocular posterior compartment). The *in vitro* irritancy score (IVIS), which is the combination of opacity and permeability, was then calculated. Negative, vehicle and positive controls were tested according to the same experimental conditions.

Results
After 4 hours of contact, the mean IVIS was 13.9 ± 0.6 for a HEPB suspension at 20% in polyethylene glycol. Detachment of the epithelium and oedema were observed. The mean IVIS for the vehicle polyethylene glycol and the positive controls were 6.7 ± 0.7 and 182.1 ± 2.7, respectively.

Ref.: 2

Conclusion
The study authors concluded that based on the conditions of this test, HEPB is not classified as corrosive or as a severe eye irritant.

SCCS comment
The SCCS notes that the permeability (OD) values of the vehicle control are much higher (20-fold) than for the negative control, suggesting that the vehicle polyethylene glycol itself might have an adverse effect on the test system. Therefore, the results of the BCOP assay are inconclusive. An appropriate vehicle should be used for which it can be demonstrated that it has no adverse effect on the test system.

Bovine Corneal Opacity and Permeability (BCOP) Test Method

Guideline:	OECD 437 (September 2009) with some deviations
Test system:	Isolated bovine eyes (corneas)
Replicates:	3 corneas per condition
Test substance:	R0069279A
Batch:	R0068279A 019 D 004
Purity:	95.9 ± 0.5% (HPLC)
Test item:	2 % (w/w) suspension in vehicle (yielding a pH of 5.5)
Vehicle:	polyethylene glycol (8 OE)
Dose level:	750 ± 8 µl
Treatment period:	30 min and 4 hours
Positive control:	0.5 % (w/w) Cetyl Trimethylammonium Bromide (CTAB) in distilled water
Negative control:	nutritive medium
GLP:	In compliance
Study period:	Jan 2012 – Feb 2012

Bovine eyes (from cattle less than 12 months old) were collected at slaughterhouses and prepared within 4 hours of collection. Eyes that were too big or that had defects were rejected. After the pre-incubation and equilibration period of the corneas of at least 1 hour at 32 ± 1°C, 750 µl of the test item, positive, negative and vehicle control were applied onto the corneas for 30 minutes or 4 hours. At the end of each contact period, corneas were rinsed and prepared for measurement of opacity (changes in light transmission) and permeability (evaluation of transfer of 5 mg/ml fluorescein through the cornea by measuring the optical density at 490 nm of the media in the ocular posterior compartment). The corneal (IVIS) score, which is the combination of opacification and permeability, was then calculated. Negative, vehicle and positive controls were tested according to the same experimental conditions except that the 4-hour treatment period was not performed on the positive control. According to the study authors, this method uses a prediction model that allows a classification of the products into four categories of irritation potential, i.e. from the lowest to the highest ocular irritation potential: slightly irritant, moderately irritant, moderately irritant to irritant and irritant to severely irritant.

Results:
The mean corneal (IVIS) scores obtained for 30-min contact time were: 4.7 ± 0.6 for the 2% HEPB suspension, 3.9 ± 0.6 for the vehicle control, and 96.0 ± 2.8 for the positive control, respectively. The mean corneal (IVIS) scores obtained for 4-hour contact time were: 5.9 ± 0.1 for the 2 % HEPB suspension and 4.5 ± 0.0 for the vehicle control.

The study authors concluded that under the conditions of this study, HEPB has at most a slight ocular irritation potential when tested at 2%.

Ref.: 3

SCCS comment
The SCCS notes that the permeability (OD) values of the vehicle control are much higher (8-fold for the 4-hour treatment period) than the negative control, suggesting that the vehicle polyethylene glycol itself might have an adverse effect on the test system. Therefore, the results of the BCOP assay are inconclusive. An appropriate vehicle should be used for which it can be demonstrated that it has no adverse effect on the test system.
The SCCS further notes that the BCOP assay (OECD 437, 2009) is only able to identify severe eye irritants and cannot discriminate between slightly irritant, moderately irritant and severely irritant.

Submission II

Bovine Corneal Opacity and Permeability (BCOP) Test Method

Guideline:	OECD 437 (October 2017)
Test system:	Isolated bovine eyes (corneas)
Replicates:	3 corneas per condition
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9± 0.5% (HPLC)
Test item:	0.7% (w/w) suspension in vehicle (yielding a pH of 5.8)
Vehicle:	30% (w/w) propylene glycol (batch no DGA17d0318) in distilled water
Dose level:	750 ± 8 µl
Treatment period:	10 ± 1 min
Positive control:	10% (w/w) NaOH in sterile water
Negative control:	0.9% (w/v) NaCl in sterile water
GLP:	In compliance
Study period:	May - June 2018

Bovine eyes (from cattle 6-8 months old) were collected at slaughterhouses and prepared within 4 hours of collection. Eyes that were too big or that had defects were rejected. After the pre-incubation and equilibration period of the corneas of at least 1 hour at 32 ± 1°C, 750 µl of the test item, positive, negative and vehicle control were applied onto the corneas for 10 ± 1 min. At the end of the contact period, corneas were rinsed and prepared for measurement of opacity (changes in light transmission) and permeability (evaluation of transfer of 4 mg/ml fluorescein through the cornea by measuring the optical density at 490 nm of the media in the ocular posterior compartment). The corneal (IVIS) score, which is the combination of opacification and permeability, was then calculated. Negative, vehicle and positive controls were tested according to the same experimental conditions.

Results
The mean corneal (IVIS) scores obtained for 10-min contact time were: 0.7 ± 0.9 for the 0.7% HEPB suspension, 1.0 ± 1.2 for the vehicle control, and 249.6 ± 4.1 for the positive control, respectively.

Conclusion
The study authors concluded that under the conditions of this study HEPB does not require classification for eye irritation or serious eye damage.

Ref.: 4

SCCS comment
According to SCCS, the test substance should have been tested using the protocol for a non-surfactant solid, not a liquid. Based on the study conducted, no conclusion can be made on the classification of HEPB for eye irritation or serious eye damage.

Reconstructed Human Cornea-Like Epithelium (RhCE) Test Method

Guideline:	OECD 492 (July 2017)
Test system:	SkinEthic reconstructed HCE® model (surface area 0.5 cm^2)
Replicates:	Three per condition
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9± 0.5% (HPLC)
Test item:	0.67% (w/w) suspension in vehicle (yielding a pH of 5.8)
Vehicle:	Propylene glycol
Dose level:	30 µl (60 µl/cm^2)
Treatment period:	30 ± 2 min
Positive control:	Methyl acetate
Negative control:	Ca^{2+}/Mg^{2+}-free phosphate buffered saline (PBS^-)
Direct interaction with MTT:	Negative
Colour interference:	Negative
GLP:	In compliance
Study period:	June 2018

Preliminary tests were performed to detect the ability of the test item and the vehicle to directly reduce MTT as well as its colouring potential. Following the preliminary tests, the eye irritation potential of the test item formulation was assessed in the main test. Hereto, 30 µl of test item, vehicle positive and negative controls were applied topically on triplicate tissues pre-moistened with 10ul PBS^- and incubated at 37°C for 30 ± 2 min. At the end of the treatment period, each tissue was rinsed with PBS^-, incubated for 30 ± 2 min with fresh medium, blotted on absorbent material and then incubated with MTT solution for another 3 hours ± 15 min. After incubation time, tissues were rinsed with PBS and extracted in isopropanol overnight. Cell viability was assessed by measuring the optical density of the extracts at 570nm.

Results
Neither the test item nor the vehicle were found to have direct MTT reducing properties or colouring potential.
Mean cell viability values obtained were: 89.9 ± 3.7% for the 0.67% HEPB suspension, 91.9 ± 3.5% for the vehicle control, 100 ± 6.7% for the negative control and 22.7 ± 2.8% for the positive control, respectively.

Conclusion
The study authors concluded that under the conditions of this study, HEPB, as well as the vehicle substance propylene glycol, does not require classification for eye irritation or serious eye damage.

Ref.: 5

SCCS conclusion on eye irritation
Based on the newly submitted data, SCCS considers that HEPB is not irritating to the eye at 0.7% (w/w) in propylene glycol.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429; EC B.42
Species/strain/sex:	mouse, CBA/J, female
Group size:	4
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9 ± 0.5%
Vehicle:	acetone:olive oil (4:1; v/v)
Concentration:	1, 10, 25, 50 and 75 %
Vehicle control:	acetone:olive oil (4:1; v/v) (AOO)
Positive control:	α-hexylcinnamaldehyde (HCA) at 25 % (v/v) in AOO
GLP:	In compliance
Study period:	March 2011 – July 2011

Concentrations for the main study were based on findings from a preceding irritation test, in which no increase in skin thickness was noted at test item concentrations of 5.0, 25.0, 50.0 and 75.0 % (w/v).
Seven treatment groups each comprising 4 mice received either the test substance at 1, 10, 25, 50 or 75% (w/v) in AOO, AOO alone as negative control or HCA at 25 % in vehicle as positive control. The test item or controls were applied on the dorsum of both ears for three consecutive days. After a 2-day rest, lymphocyte proliferation was assessed in the pooled auricular lymph nodes by measuring the incorporation of 3H-TdR. The values were used to calculate stimulation indices (SI).

Results:
The positive control HCA generated a SI value of 3.38. The test substance induced a small increase in lymphoproliferation. SI values of 1.16, 1.24, 1.43, 1.53 and 1.78 were obtained at test substance concentrations of 1%, 10%, 25%, 50% and 75%, respectively.
Therefore the study authors concluded that R0069279A did not induce skin sensitisation in the LLNA.

Ref.: 6

SCCS comment
HEPB induced a dose-dependent increase of lymphocyte proliferation in the LLNA that resulted in a SI value of 1.78 at the highest dose (75%) tested. HEPB is not considered to be a skin sensitiser under the conditions of the test.

3.3.4 Toxicokinetics

3.3.4.1 Dermal / percutaneous absorption

Guideline:	OECD 428 (November 2004); SCCS 1358/10
Species/strain:	Split-thickness human abdomen skin from 4 donors aged 30 – 63 years and one split-thickness breast skin sample from a female donor aged 58 years
Membrane integrity:	sample exhibiting a resistance < 4 kΩ excluded
Replicates:	12 skin samples from 5 different donors
Method:	Dermatomed thawed skin mounted on static diffusion cells and exposed to radiodiluted test item
Test substance:	R0069279A (non-labelled); [^{14}C]-R0069279A (labelled)
Batch:	R0069279A 019 P 001 (non-labelled material) CFQ41364 (labelled material)
Purity:	96.0% ± 1.3 % (non-labelled material) 98.0 % (labelled material; radiochemical purity)
Test item:	radiodiluted [^{14}C]-R0069279A in a cosmetic formulation; 104.2 % of target (2.0 % (w/w)
Exposed membrane area:	3.14 cm^2
Dose applied:	2.11 mg/cm²
Sampling period:	24 hours
Receptor fluid:	calcium- and magnesium-free phosphate buffered saline (PBS) containing bovine serum albumin (5%, w/v)
Mass balance analysis:	Provided
Tape stripping:	Yes (20)
Method of Analysis:	Liquid Scintillation Counting (LSC)
GLP:	In compliance
Study period:	June – July 2012; draft report Nov 2012

The *in vitro* percutaneous absorption of [^{14}C]-HEPB by using a typical lipophilic skin care formulation containing HEPB at the concentration of 2% was determined in human dermatomed skin. Any split-thickness human skin sample exhibiting a resistance less than 4 kΩ was excluded from subsequent absorption measurements.
Human dermatomed skin samples were mounted onto diffusion cells. Calcium- and magnesium-free PBS was used as the receptor fluid. A quantity of 2 mg/cm^2 of skin care formulation was applied to the skin surface. After 24 hours of exposure, the skin surface was rinsed-off, simulating in-use conditions. 24 hours after application, the stratum corneum was removed by 20 tape strippings and the penetration, mass balance and distribution of [^{14}C]-HEPB were determined by measuring its concentration in relevant compartments (e.g. skin wash, epidermis, dermis, and receptor fluid) by using LSC.

Results
Mean recovery of the applied test material (mass balance) was 82.4% of the applied dose. The mean systemically available dose of HEPB sum of amounts measured in living epidermis/dermis and receptor fluid after application of a typical skin care formulation containing this preservative ingredient at 2% was estimated to be 36.8±17% of the applied dose (16.0±7.4 µg equiv/cm²).

Table 1 **Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [^{14}C]-R0069279A in Test Preparation 819193 (2%, w/w) to Human Split-Thickness Skin**

	Cell Number and Donor Number													
	Cell 1 0317	Cell 2 0317	Cell 3 0394	Cell 4 0394	Cell 5 0394	Cell 6 0378	Cell 7 0313	Cell 8 0313	Cell 9 0313	Cell 10 0348	Cell 11 0348	Cell 12 0348	Mean	SD
Skin Wash	10.37	16.54	31.34	28.97	26.68	13.31	22.44	12.21	26.67	14.93	29.88	17.12	20.87	7.61
Tissue Swabs	9.50	18.33	25.45	35.76	29.13	14.54	18.61	22.18	31.21	19.79	20.23	20.49	22.10	7.29
Pipette Tips	1.15	0.14	0.14	0.26	0.63	0.12	0.19	0.11	0.37	0.41	0.25	0.16	0.33	0.30
Donor Chamber Wash	9.31	0.14	0.73	0.23	0.52	0.38	1.02	0.29	4.90	0.64	1.13	0.44	1.64	2.74
Dislodgeable Dose 24 h	30.33	35.15	57.65	65.22	56.97	28.34	42.27	34.80	63.14	35.78	51.48	38.21	44.95	13.20
Stratum Corneum 1-5	0.20	0.10	0.18	0.57	0.51	0.10	0.12	0.57	0.24	0.12	0.14	0.10	0.24	0.19
Stratum Corneum 6-10	0.09	0.06	0.13	0.35	0.29	0.05	0.09	0.22	0.18	0.09	0.14	0.07	0.15	0.10
Stratum Corneum 11-15	0.06	0.07	0.14	0.26	0.33	0.03	0.08	0.14	0.12	0.08	0.12	0.06	0.12	0.09
Stratum Corneum 16-20	0.04	0.06	0.12	0.29	0.13	0.04	0.07	0.08	0.12	0.06	0.13	0.04	0.10	0.07
Stratum Corneum	0.39	0.28	0.57	1.48	1.27	0.21	0.35	1.01	0.66	0.35	0.53	0.27	0.61	0.42
Unexposed Skin	0.22	0.11	0.04	0.02	0.08	0.08	0.11	0.15	0.04	0.10	0.01	0.05	0.08	0.06
Total Unabsorbed	30.95	35.54	58.26	66.72	58.32	28.64	42.72	35.96	63.84	36.23	52.02	38.53	45.64	13.43
Epidermis	0.50	0.46	0.53	1.15	0.97	0.41	0.30	0.47	0.42	0.55	0.53	0.36	0.55	0.25
Dermis	3.47	2.32	2.13	1.53	1.70	2.98	1.16	1.99	1.03	2.45	1.87	2.04	2.06	0.70
Receptor Fluid	65.33	52.62	*26.12	*16.33	*20.70	43.96	29.26	44.77	*15.73	40.34	*13.97	34.53	°33.64	°16.21
Receptor Chamber Wash	1.35	0.51	0.31	N.C.	0.46	0.72	0.46	0.70	0.24	0.48	0.21	0.74	0.56	0.32
Total Absorbed	66.68	53.12	26.43	16.33	21.16	44.68	29.71	45.47	15.97	40.81	14.18	35.27	34.15	16.51
Dermal Delivery	70.65	55.90	29.09	19.01	23.84	48.07	31.17	47.92	17.42	43.81	16.58	37.66	36.76	16.98
Mass Balance	101.60	91.44	87.35	85.73	82.16	76.71	73.89	83.88	81.25	80.04	68.59	76.19	82.40	8.68

*=Results calculated from data less than 30 d.p.m. above background;
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 3 **Distribution of [^{14}C]-R0069279A (µg equiv./cm^2) at 24 h Post Dose Following Topical Application of [^{14}C]-R0069279A in Test Preparation 819193 (2%, w/w) to Human Split-Thickness Skin**

	Cell Number and Donor Number													
	Cell 1 0317	Cell 2 0317	Cell 3 0394	Cell 4 0394	Cell 5 0394	Cell 6 0378	Cell 7 0313	Cell 8 0313	Cell 9 0313	Cell 10 0348	Cell 11 0348	Cell 12 0348	Mean	SD
Skin Wash	4.53	7.22	13.68	12.65	11.65	5.81	9.80	5.33	11.64	6.52	13.04	7.48	9.11	3.32
Tissue Swabs	4.15	8.00	11.11	15.62	12.72	6.35	8.13	9.68	13.62	8.64	8.83	8.95	9.65	3.18
Pipette Tips	0.50	0.06	0.06	0.11	0.28	0.05	0.08	0.05	0.16	0.18	0.11	0.07	0.14	0.13
Donor Chamber Wash	4.07	0.06	0.32	0.10	0.23	0.16	0.44	0.13	2.14	0.28	0.49	0.19	0.72	1.19
Dislodgeable Dose 24 h	13.24	15.35	25.17	28.48	24.87	12.38	18.45	15.19	27.57	15.62	22.48	16.68	19.62	5.76
Stratum Corneum 1-5	0.09	0.04	0.08	0.25	0.22	0.04	0.05	0.25	0.10	0.05	0.06	0.04	0.11	0.08
Stratum Corneum 6-10	0.04	0.03	0.06	0.15	0.13	0.02	0.04	0.10	0.08	0.04	0.06	0.03	0.06	0.04
Stratum Corneum 11-15	0.03	0.03	0.06	0.12	0.15	0.01	0.03	0.06	0.05	0.04	0.05	0.02	0.05	0.04
Stratum Corneum 16-20	0.02	0.03	0.05	0.13	0.06	0.02	0.03	0.03	0.05	0.03	0.06	0.02	0.04	0.03
Stratum Corneum	0.17	0.12	0.25	0.65	0.56	0.09	0.15	0.44	0.29	0.15	0.23	0.12	0.27	0.18
Unexposed Skin	0.10	0.05	0.02	0.01	0.03	0.03	0.05	0.06	0.02	0.04	0.00	0.02	0.04	0.03
Total Unabsorbed	13.51	15.52	25.44	29.13	25.46	12.50	18.65	15.70	27.87	15.82	22.71	16.82	19.93	5.86
Epidermis	0.22	0.20	0.23	0.50	0.42	0.18	0.13	0.20	0.18	0.24	0.23	0.16	0.24	0.11
Dermis	1.51	1.01	0.93	0.67	0.74	1.30	0.51	0.87	0.45	1.07	0.82	0.89	0.90	0.31
Receptor Fluid	28.53	22.98	*11.41	*7.13	*9.04	19.20	12.78	19.55	*6.87	17.61	*6.10	15.08	°14.69	°7.08
Receptor Chamber Wash	0.59	0.22	0.14	N.S.	0.20	0.31	0.20	0.31	0.10	0.21	0.09	0.32	0.25	0.14
Total Absorbed	29.12	23.20	11.54	7.13	9.24	19.51	12.98	19.86	6.97	17.82	6.19	15.40	14.91	7.21
Dermal Delivery	30.85	24.41	12.70	8.30	10.41	20.99	13.61	20.93	7.61	19.13	7.24	16.45	16.05	7.42
Mass Balance	44.36	39.93	38.14	37.43	35.87	33.50	32.26	36.63	35.48	34.95	29.95	33.27	35.98	3.79

The applicant concluded that the mean recovery rate of 82.4% of the applied dose obtained in this study was a bit low. However, similar to greater losses of radioactivity were obtained in a dedicated study evaluating the recovery rate of [^{14}C]-HEBP 24 hours after simple spiking (Craig, 2014b, the study is described in the appendix). Therefore it was concluded that the low recovery rate obtained in the *in vitro* percutaneous absorption study would not put the scientific validity of this study into question.

Ref.: 7, 8

SCCS comment

The dedicated study evaluating the recovery rate did not provide any explanation for the low recovery. As HEPB possesses a low vapour pressure, evaporation appears unlikely. Nevertheless, in order to avoid loss, occlusive conditions could be used in dermal absorption studies.

According to SCCS 1358/10, recovery should be at least 85%. A recovery of at least 85% was only obtained for 4 cells evaluated. Thus, eliminating all other cells leads to only 4 evaluable cells from 2 different donors, which is not in line with SCCS 1358/10.
The chosen cut-off value of 4 kΩ for barrier function assessment is not of common practice (threshold of 10 kΩ is normally used) and may lead to an overestimation of the results.

The SCCS notes that dermal penetration was only investigated using a cosmetic formulation consisting of 93% of an oily phase and 7 % of water phase. However, appropriate studies should be performed for the intended uses in representative cosmetic formulations.

Based on significant deviations from requirements, the SCCS considers the study unacceptable. Instead, in the absence of adequate experimental data, a default value of 50% dermal absorption is taken for MoS calculation according to SCCS 1564/15.

3.3.4.2 Toxicokinetics in laboratory animals

No data provided.

Results from a structurally similar compound (zingerone) are indicative of almost complete and rapid absorption and excretion (see section 3.3.11). Based on this information and on physico-chemical properties of HEPB (molecular weight: 208.25 g/mol; water solubility of 7.59 g/mol and log P_{ow} of 1.46), 100% oral absorption is taken for MoS calculation.

3.3.4.3 Toxicokinetics in humans

No data provided.

3.3.4.4 Toxicokinetics *in vitro*

Guideline:	/
Test system:	Human intestinal epithelial Caco-2 cells
Test substance:	R0069279A
Batch:	R0069279A 019 P 001
Purity:	not mentioned
GLP:	in compliance
Study period:	June – August 2014

In each experiment and if applicable, the respective reference compounds (propranolol: highly permeable; labetalol: moderately permeable; ranitidine: poorly permeable; colchicine: P-glycoprotein substrate) were tested concurrently with HEPB, and the data were compared with historical values determined at the testing facility. Only limited information was provided on the experimental setting. In short, the apical-basolateral (A-B) permeability of HEPB in Caco-2 cells (pH 6.5/7.4) was determined and compared to that of the reference compounds. A test concentration of 10 µM HEPB was used.

Results
The test item has a mean apparent permeability coefficient of 62.2 x 10^{-6} cm/s. Based on this result of the test item and the reference compounds, the test item should be highly permeable.

Ref.: 9

SCCS comment
The study report is extremely poor. However, it can be inferred from the results of the study that penetration of HEPB through the Caco-2 monolayer is high.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral / dermal / inhalation toxicity

Oral

A 14-day oral repeat dose study was performed as a preliminary (range-finder) study for a 90-day oral toxicity study. The study was performed in August 2011, the final report dates from March 2013. Four groups of male and female Wistar rats, respectively, each comprising 5 animals, received dose levels of 100, 300 and 1000 mg/kg bw/d of HEPB (test item R0069279A, batch R0069279A 019 D 004) in 0.5 % (w/v) carboxymethylcellulose (CMC) in water and 2% Tween® 80 (v/v) by oral gavage. Control groups received vehicle only. Animals were observed twice daily for clinical signs and body weight and food consumption were monitored. At the end of the treatment period, blood and urine samples were taken, animals were subjected to gross examination and specified organs were collected. Histopathological examination was carried out on heart, kidneys, liver and spleen from all rats of the vehicle control and high-dose group.
There were no clinical signs or mortalities in any of the groups during the treatment period. There were no test item-related changes in any of the parameters evaluated in haematology, coagulation, clinical chemistry and urine. An increase in absolute and relative liver weight was observed in high-dose males. There were no test item-related gross pathological findings in test-item treated animals. In high-dose males, a 6% increase in mean platelet volume not accompanied by any significant changes in platelet count was also observed. A dilatation of the uterus was observed in one female in the mid-dose and one female in the high-dose groups.
Based on the results of this 14-d oral repeat dose study, 100, 300 and 1000 mg/kg bw/d were chosen for the 90-day oral toxicity study.

Ref.: 10

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

Oral

Guideline:	OECD TG 408 (Sep 1998)
Species/strain:	Rat, Wistar rats-HsdHan™:WIST
Group size:	10 / sex / dose
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9 ± 0.5 %
Vehicle:	0.5 % (w/v) carboxymethylcellulose (CMC) in water and 2% Tween® 80 (v/v)
Dose levels:	0, 100, 300 and 1000 mg/kg bw/d
Dose volume:	10 ml/kg
Route:	oral
Administration:	gavage
GLP:	In compliance
Study period:	Sep 2011 – Oct 2012

The test item was stable for 8 days in vehicle suspension; thus dosing formulations were prepared accordingly. Animals received daily oral gavage doses for 90 consecutive days. Detailed clinical examination was performed prior to test item administration and weekly thereafter. Animals were observed regularly for mortality, morbidity and clinical signs. Ophthalmological examination was performed in control and high-dose animals prior to and at the end of the treatment. Functional observational battery (FOB) tests were performed between treatment day 82 and 83. Food consumption and body weight were determined on a more or less regular basis throughout the administration period.
At the end of the treatment period (day 91), blood was taken for haematology and clinical chemistry and urine was taken for microscopic examination and investigation of selected parameters. All animals were subjected to gross necropsy and selected organs were weighed and preserved. Histopathological examination was performed on all preserved organs from the control and high-dose group and in addition, all gross lesions from all rats were examined microscopically. The stomach was examined in all dose groups.

Results
No mortalities were observed during the study period. No ocular abnormalities were observed in the ophthalmological examinations. Transient clinical signs (slight to moderate salivation, dragging gait, hypoactivity, splayed hind-limbs) appeared shortly (5-15 min) after gavage in both sexes and persisted up to 30 min in animals dosed at 300 mg/kg bw/d. At the highest dose, these signs appeared approximately 5-15 min post dose and persisted up to 1 hour (60 min) post dose in most rats. For 2 rats, hypoactivity and splayed hindlimbs with dragging gait persisted for more than an hour. In FOB, grip strength of hindlimbs was significantly reduced in mid- and high-dose females. Landing hindlimbs foot splay values were significantly lower in males of all dose levels and in low-dose females. At 300 and 1000 mg/kg bw/d, hindlimb grip strength in females was statistically significantly ($p<0.0001$) different from controls. Motor activity was statistically significantly different from controls in males and females at 1000 mg/kg bw/d lower values of stereotypic time (interval 6) and total stereotypic time in males and lower values of stereotypic time, ambulatory time and horizontal and ambulatory counts (interval 6) in females, respectively. The study authors interpreted statistical variations in motor activity as incidental as there were no accompanying changes in muscle tone. Food consumption was statistically significantly reduced in high-dose males and females (about -12 % and -16%, respectively, compared to controls). In high-dose males, statistically significant (about -10 % compared to controls) lower body weights were observed. There were no test-item related alterations in haematology, coagulation and clinical chemistry or urine parameters in any of the treated dose groups in both sexes. Gross pathology revealed no changes in rats up to 300 mg/kg bw/d. At the highest dose level, thickening and white multiple raised foci of nonglandular mucosa in the stomach was observed in 5/10 males and 3/10 females. The macroscopic lesions were associated with mild to moderate epithelial hyperplasia in the non-glandular stomach (8/10 males and 6/10 females) and with mild to moderate leucocytic infiltration of the mucosa (2/10 males and 1/10 females). In addition, mild mucosal erosion was observed in the glandular stomach (1/10 males). Histopathological examination revealed moderate dilatation of uterus with cervix in 2/10 high-dose females and mild dilatation of uterus with cervix in 1/10 high-dose females.

Conclusion
The study authors considered clinical (neurological) signs observed at the mid- and high-dose as non-adverse treatment-related effects due to their transient nature. However, the observed macroscopic lesions and microscopic changes in the stomach of high-dose animals were considered test-item related and associated with lower body weight (males) and food consumption (males and females). Thus, an NOAEL of 300 mg/kg bw/d was derived from this study.

Ref.: 11

SCCS comment
Macroscopic lesions and microscopic changes in the stomach were observed at the highest dose tested (1000 mg/kg bw/d) in a 90-day oral repeat-dose toxicity study in rats, indicating a local NOAEL of 300 mg/kg bw/d for the oral uptake route.

Concerning systemic effects, which are of higher relevance for dermal uptake of cosmetics, the SCCS considers 100 mg/kg bw/d as systemic NOAEL based on clinical signs, motor activity scores and significantly reduced hindlimb grip strength in female animals at 300 and 1000 mg/kg bw/d.
Clinical signs, mainly hypoactivity and splayed hindlimbs with dragging gait as observed at the mid-dose, were more pronounced in the high-dose group, and indicate acute treatment-related effects. To what extent these effects are fully reversible (between daily dosing) was very poorly documented.
Concerning motor activity, some statistically significant variations compared to controls were observed at the highest dose, further motor activity scores with a trend for lower values were observed at the highest dose (not statistically significant); thus, observations from motor activity support the findings from clinical observations.
In female animals, hindlimb grip strength exhibited a dose-dependent decrease with increasing concentration reaching statistical significance ($p<0.0001$) at 300 and 1000 mg/kg bw/d. The effect can be considered strong (Cohen's d: -3.066 at 300 mg/kg). Although histopathology of femoral muscles, brain or sciatic nerves did not indicate treatment-related changes, clinical signs as well as the results from FOB testing indicate neurotoxicity. Reductions in hindlimb grip strength are considered adverse due to the following reasons:
(1) the effect is accompanied by other neurological findings (e.g. decreased motor activity (with dose-dependent tendency), hypoactivity, effects on hindlimb foot splay);
(2) Although the observed clinical signs such as hypoactivity and splayed hindlimbs might be considered reversible, reversibility of findings from FOB has not been demonstrated
(3) the histopathological procedure as performed in the study (H&E staining) might not be adequate to reliably detect effects on the nerves and furthermore, nerves other than sciatic nerves might be of relevance
(4) possible manifestations in the form of neurotransmitter changes have not been assessed; (5) similar observations pointing to neurotoxicity (e.g. hypoactivity, spread-out hindlimbs and dragging gait) were also described in an independently performed prenatal developmental toxicity study in rats.

By using Benchmark calculation for reduced hindlimb grip strength in female animals, a $BMD_{10\%}$ of 220 mg/kg bw/d and a $BMDL_{10\%}$ of 110 mg/kg bw/d was calculated.

3.3.5.3 Chronic (> 12 months) toxicity

No data provided.

3.3.6 Reproductive toxicity

3.3.6.1 Fertility and reproduction toxicity

No data provided.

3.3.6.2 Developmental toxicity

Guideline:	OECD TG 414 (2001)
Species/strain:	Rat, Wistar
Group size:	24
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9 ± 0.5 %
Vehicle:	0.5 % (w/v) carboxymethylcellulose (CMC) in water and 2 % Tween® 80 (v/v)
Dose levels:	0, 100, 300 or 1000 mg/kg bw/day
Dose volume:	10 ml/kg
Controls:	Vehicle
Route:	oral
Administration:	gavage on gestation days (GD) 5 – 19.
GLP:	in compliance
Study period:	October 2011 – Sep 2013

Dose selection was based on the outcome of a GLP-compliant preliminary developmental toxicity study according to OECD TG 414, repeatedly administering three oral gavage dosages of HEPB to pregnant Wistar rats on gestation days (GD) 5-19.
In the definitive study, HEPB was administered by daily oral (gavage) doses of 0, 100, 300 or 1000 mg/kg/day to mated Wistar female rats (24/group) during the sensitive period of organogenesis (day 5 through day 19 of gestation, the day of detection of sperm positive vaginal smear/vaginal plug being designated as gestation day 0 (GD 0)). Maternal evaluations and measurements included daily clinical signs and body weight/food intake measured at designated intervals. The dams were killed on GD 20 and subjected to macroscopic examination. All required litter parameters were recorded and fetuses were sexed, weighed and submitted to external examination. About one half of the fetuses were also examined for soft tissue anomalies, and remaining fetuses were examined for skeletal anomalies.

Results:
No deaths were observed. Animals given HEPB at 100 mg/kg bw/d did not show any clinical signs while transient clinical signs of hypoactivity were observed on a single occasion in 6 out of 24 rats given 300 mg/kg bw/d. At 1000 mg/kg bw/d, most animals showed clinical signs of hypoactivity within 10 to 45 min of dosing, particularly splayed hindlimbs and dragging gait. The study authors therefore considered these effects as non-adverse due to their transient nature. Dosing with HEPB did not produce any changes in maternal body weight, maternal body weight gain or food consumption. There was a statistically significant increase in dams with resorptions in mid- and high-dose groups when compared to the vehicle control group (16.67, 38.1, 43.48 and 47.84 % animals with any resorption at 0, 100, 300 and 1000 mg/kg bw/d). Nevertheless, there were no changes in mean early and late resorptions in these groups when compared to the vehicle control group. Therefore, this increase was considered as incidental. No other adverse changes in maternal and litter parameters occurred- and there were no fetuses with external malformations. The visceral and skeletal variations observed were of the type and incidence commonly observed in rats of this strain and age.
The study authors concluded that HEPB had no teratogenic potential based on the outcome of this study. The study authors set the maternal and developmental NOAEL at 1000 mg/kg bw/d.

Ref.: 12, 13

SCCS comment
Hypoactivity and effects on hindlimbs were also observed in a 90-day oral repeated-dose toxicity study (see section 3.3.5.2).
Using Fisher's exact test instead of the Chi2, the number of dams with any resorptions is only significant compared to control animals at 1000 mg/kg. In Chi2, the significant difference was observed at 300 and 1000 mg/kg. The number of resorptions and implantation losses calculated with the Kruskal-Wallis test did not show any significant differences compared to the controls. All means analysed were within historical control ranges. Altogether, this led the SCCS to conclude that no toxicologically meaningful changes were observed.

3.3.7 Mutagenicity / Genotoxicity

3.3.7.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD TG 471 (1997)
Test system:	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537
Replicates:	2 experiments
Test substance:	R0069279A
Batch:	R0069279A 019 D004
Purity:	95.9 ± 0.5 %
Concentrations:	Experiment I: with and without S9-mix, 0.32, 1.6, 8, 40, 200, 1000 and 5000 µg/plate Experiment II: with and without S9-mix, 39.06*), 78.13*), 156.3, 312.5, 625, 1250, 2500, 5000 µg/plate
Treatment:	Experiment I: direct plate incorporation, with and without S9-mix Experiment II: direct plate incorporation without S9-mix and pre-incubation with S9-mix
Vehicle:	DMSO
Negative Control:	DMSO
Positive Control:	without S9: 2-nitrofluorene (TA98); sodium azide (TA 100; TA1535); 9-aminoacridine (TA1537), mitomycin C (TA102) with S9: benzo[a]pyrene (TA98); 2-aminoanthracene (TA100, TA102; TA1535; TA 1537)
GLP:	In compliance
Study period:	March – June 2011

(*) Concentration used for strains TA1537 and TA102 in the presence of S9-mix only using 0.05 ml additions

The test item was evaluated in two independent experiments in the absence and presence of metabolic activation (S9-mix from livers of Aroclor 1254 treated rats). Experiment I was performed according to the direct plating incorporation method, experiment II with S9-mix was performed according to the pre-incubation method. HEPB was soluble in DMSO. Known recommended mutagens were used as positive controls; three plates per treatment condition were used.

Results
The test item was completely soluble in the aqueous assay system at all concentrations tested and in each of the experiments performed.

In experiment I, evidence of toxicity ranging from a slight thinning of the background bacterial lawn and/or a marked reduction in revertant numbers to a complete killing of the test bacteria was observed at 1000 and/or 5000 µg/plate in strains TA1537 and TA102 in the absence and presence of S9-mix. In experiment II, evidence of toxicity ranging from a slight thinning in the background bacterial lawn and/or a marked reduction in revertant numbers to a complete killing of the test bacteria was observed in strains TA98 and TA1537 in the presence of S9-mix and TA102 in the absence of S9-mix at 2500 µg/plate and/or 5000 µg/plate and in strain TA102 at 1250 µg/plate and above in the presence of S9-mix.
Concerning mutation, no increases in revertant numbers were observed that were statistically significant following treatment of all the test strains in the presence or absence of S9-mix. The positive control chemicals all induced large increases in revertant numbers in the appropriate strains.

Conclusion
HEPB was not mutagenic in this bacterial gene mutation test.

Ref.: 14

Mammalian Cell Gene Mutation Test in Mouse Lymphoma Cells (hprt locus)

Guideline:	OECD TG 476 (1997)
Test system:	Mouse lymphoma cell line L5178Y [*hprt* locus]
Replicates:	duplicate
Test substance:	R0069279A
Batch:	R0069279A 019 D004
Purity:	95.9 ± 0.5 %
Concentrations:	Experiment I: without S9-mix: 300, 600, 900, 1100, 1300, 1500, 1900 and 2083 µg/ml with S9-mix: 300, 600, 1300, 1500 and 1900 µg/ml Experiment II: 400, 800, 1200, 1400, 1600, 1700, 1800, 1900, 2000 and 2083 µg/ml (with and without S9-mix)
Treatment:	Experiment I and II: 3 hour treatment without and with S9-mix; an expression period of 7 days and a selection period of 12-13 days
Vehicle:	DMSO
Negative Control:	DMSO, diluted 100-fold in treatment medium
Positive Control:	4-nitroquinoline 1-oxide (without S9-mix) benzo[a]pyrene (with S9-mix)
GLP:	In compliance
Study period:	July – August 2011

Concentrations selected for the mutation experiments were based on the results of a cytotoxicity range-finder experiment using 2083 µg/ml as highest concentration; at this concentration, precipitate was observed, which did not persist during incubation. No marked changes in osmolality or pH were observed at 2083 µg/ml, but excessive toxicity was observed at this dose level.
Both experiments used a 3-hour treatment. Known mutagens in the presence (Benzo(a)pyrene, BP) or absence of S9-mix (4-nitroquinoline 1-oxide, NQO) were tested at two different concentrations and served as positive controls. Negative controls consisted of cultures treated with the vehicle DMSO diluted 100-fold in the treatment medium.

Results
In experiment I, the highest concentrations analysed were 2083 µg/ml in the absence of S9-mix and 1900 µg/ml in the presence of S9-mix, which gave relative survival rates of

14% and 12%, respectively. In experiment II, the highest concentration analysed was 2083 µg/ml both in the absence and in the presence of S9-mix, which resulted in relative survival rates of 49% and 27%, respectively.
No statistically significant increases in mutant frequency were observed in either experiment following treatment with any concentration of HEPB tested in the absence or presence of S9-mix. Two concentrations of the respective positive controls revealed a clear response.

Conclusion
Under the test conditions used, HEPB was not mutagenic in this gene mutation test in mammalian cells.

Ref.: 15

SCCS comment
The required level of toxicity (10-20% relative survival after the highest concentration) was not reached in experiment II.

***In vitro* Micronucleus Test in Cultured Human Lymphocytes**

Guideline:	OECD TG 487 (2010)
Test system:	lymphocyte cultures from the pooled blood of two male donors
Replicates:	duplicate (four replicates for vehicle)
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9 ± 0.5 %
Concentrations:	Experiment I: without S9-mix 1500, 1900, 1980 and 2020 µg/ml Experiment II: with S9-mix 1500, 1780, 1860 and 1900 µg/ml Experiment III: without S9-mix 100, 250, 300 and 350 µg/ml
Treatment:	Experiment I: 3 h treatment Experiment II: 3 h treatment Experiment III: 24 h treatment
Vehicle:	DMSO
Negative control:	Vehicle
Positive controls:	Mitomycin C (MMC) (without S9-mix) Vinblastin (VIN) (without S9-mix) Cyclophosphamide (CPA) (with S9-mix)
GLP:	In compliance
Study period:	March - April 2011

HEPB was assayed for the induction of micronuclei in cultured human peripheral blood lymphocytes from the pooled blood from two male volunteers in the absence and presence of metabolic activation (S9-mix prepared from the livers of Aroclor 1254-treated rats). The highest concentration in each test condition was selected on the basis of solubility and cytotoxicity criteria.
Duplicate cultures were treated with each concentration of HEPB or with known clastogens in the presence (cyclophosphamide) or absence of S9-mix (mitomycin C and vinblastine). Vehicle-treated cultures (DMSO, four replicates) were used as negative controls.
Blood cultures were incubated in the presence of the mitogen phytohaemagglutinin (PHA) for 48 hours and then treated for 24 or 3 hours in the absence or presence of S9-mix, respectively. Cells were harvested 72 hours after the beginning of incubation. Cytochalasin B was added after the 3-hour treatments or before the 24-hour treatments. Lymphocyte preparations were stained and examined microscopically for determining the replication index (RI) and the proportion of micronucleated binucleated (MNBN) cells when selected. Where possible, 2000 binucleate cells per concentration (one thousand from each replicate) were made subject to blind analyses.

Results
No marked changes in osmolality or pH were observed at the highest concentration tested in the range-finder (2083 µg/ml), compared to the concurrent vehicle controls.
When compared to concurrent solvent controls, treatment of cultures with positive controls CPA, MMC and VIN resulted in consistent significant increases in MNBN frequencies, thus validating the sensitivity of the test system and procedure used. The MNBN cell frequencies in all treated cultures under both treatment conditions without S9-mix fell within the normal ranges with the exception of one culture at 1980 µg/ml following the 3-hour treatment, which fell slightly below the normal range and was considered of no biological relevance. In the experiment with S9-mix, the MNBN cell frequency exceeded the normal range in one of the cultures where a 64% reduction in replication index was observed; therefore the study authors considered this observation in a single culture at high toxicity of little or no biological relevance.

Conclusion
Under the test conditions used, HEPB did not induce biologically relevant increases in the number of cells with micronuclei in cultured human peripheral blood lymphocytes and consequently was not mutagenic (clastogenic/aneugenic) in this micronucleus test.

Ref.: 16

SCCS comment
Results for the 3-hour incubation experiment were from a repeat experiment. In the initial trial, steep concentration-related toxicity was observed under these two treatment conditions and a concentration giving the required level of toxicity could not be identified. The experiment was therefore repeated using more closely spaced concentrations at the upper end of the concentration range.

SCCS conclusion on mutagenicity
HEPB was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations. HEPB did not induce gene mutations in bacteria nor in mammalian cells when evaluated at the hprt locus. Exposure of human lymphocytes with HEPB did not result in an increase of micronucleated binucleated cells. Based on the present available tests, HEPB can be considered to have no genotoxic potential and additional tests are unnecessary.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

No data provided.

3.3.8 Carcinogenicity

No data provided.

3.3.9 Photo-induced toxicity

3.3.9.1 Phototoxicity / photo-irritation and photosensitisation

Guideline: OECD TG 432 (2004)
Test system: Balb/c 3T3 mouse fibroblasts, clone 31

Replicates:	2 irradiated and 2 non-irradiated microplates (6 wells per concentration)
Test substance:	R0069279A
Batch:	R0069279A 019 D 004
Purity:	95.9 ± 0.5 % (HPLC)
Test concentrations:	3.91, 7.81, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml
Vehicle:	PBS^+
Source of light:	Atlas CPS+ equipped with 2 anti-UVB filters
UVA irradiance:	about 1.7 mW/ cm^2
Irradiation dose:	5 J/cm^2
Irradiation duration:	about 50 minutes
Incubation time:	60 ± 10 minutes (after test substance addition) 20 ± 2 hours (after irradiation)
Positive control:	chlorpromazine (0.781 – 200 µg/ml without irradiation; 0.039 – 10 µg/ml with irradiation)
Negative control:	non-irradiated cells in PBS^+
GLP:	in compliance
Study period:	Feb – March 2014

Sensitivity of the cells to UVA irradiation was investigated using irradiation doses of 1, 5, 9 and 11 J/cm^2. In a preliminary cytotoxicity experiment, cytotoxicity was investigated using test item concentrations from 3.906 – 1000 µg/ml and an incubation time of 24 ± 3 hours. Irradiation doses and test item concentrations for the definitive irradiation experiment were based on the outcome of these investigations.
In the definitive experiments, 2 microplates each were used for irradiated and non-irradiated conditions. Cells were seeded in microplates and incubated for 24 ± 3 hours. Test substance or positive controls were then applied onto the cells, followed by a 60 ± 10 min incubation period. Irradiation plates were then irradiated for 48 (positive control) or 47 (test item) minutes, while non-irradiated plates were kept in the dark at room temperature. After an incubation period of 20 ± 2 hours, cells were checked for morphology and a neutral red uptake viability assay was subsequently conducted. The photo irritation factor (PIF) and the mean photo effect (MPE) were calculated by "Phototox 2.0" software.

Results
Sensitivity of cells to light was demonstrated as cells irradiated with 5 and 9 J/cm^2 displayed 90% and 75% viability, respectively, compared to non-irradiated cells. In the preliminary cytotoxicity test, no concentration leading to 50% mortality (IC_{50}) could be established. Thus, a PIF could not be calculated for the test item and the phototoxic potential was evaluated by MPE. For HEPB, an MPE of 0.016 ± 0.022 was calculated.

Conclusion
A test substance is considered not phototoxic when the MPE is < 0.1. The positive control chlorpromazine revealed a PIF of 40.933 (i.e. >6 indicating phototoxic potential). Based on these results, the study authors concluded that HEPB is not phototoxic.

Ref.: 17

SCCS comment
In view of the absence of indications of phototoxicity at 1000 µg/ml, the SCCS agrees that under the conditions of the test, phototoxicity of HEPB is unlikely.

3.3.9.2 Phototoxicity / photomutagenicity / photoclastogenicity

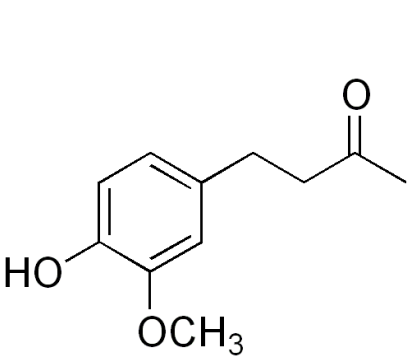
No data provided.

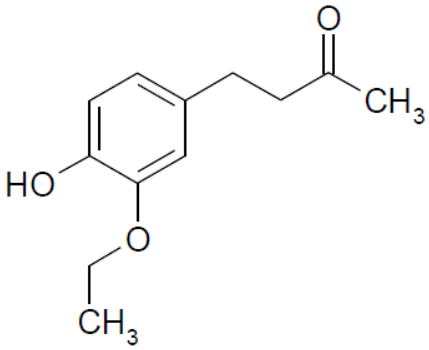
3.3.10 Human data

No data provided.

3.3.11 Special investigations

Excretion and metabolism of zingerone, a structurally similar compound to HEPB, was described in a publication from the open literature. The structural difference between zingerone (4-(4-Hydroxy-3-methoxyphenyl)butan-2-one; CAS 122-48-5) and ethylzingerone (HEPB) consists in a methylene group in the alkoxy-chain at the aromatic ring of the molecules. Therefore, results obtained from zingerone may allow conclusions to be drawn on the excretion and metabolism of HEPB.





Zingerone Ethylzingerone (HEPB)

After oral administration of a single dose of 100 mg/kg bw of zingerone to male albino (Wistar-derived) rats, urine and faeces were collected in 24 hr periods. For identification of metabolites, free and total fraction (after hydrolysis) were analysed. Within 24 hours, a mean of 95% of the applied dose had been excreted via urine. A mean of 56% of the applied dose was excreted unmetabolised (i.e. as parent compound), the remainder was excreted as metabolites (most prominent identified metabolites: zingerol (11 % of the applied dose; 4-(3,4-dihydroxyphenyl)butan-2-one (6%) and homovanillic acid (5%). In 24 hr – 48 hr urine samples, only traces of zingerone and zingerol were detected. These results demonstrate that zingerone is almost completely absorbed, metabolised and readily excreted after oral intake.

Ref.: 18

3.4 Exposure assessment

No data provided.

3.5 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

3.5.1 Aggregate exposure from the use as a cosmetic preservative in rinse-off, oral care and leave-on cosmetic products

Absorption through the skin (default) DAp (%) = 50 %
Amount of cosmetic product applied daily A (g/d) = 17.4 g/d
Concentration of ingredient in finished product C (%) = 0.7 %
Typical body weight of human = 60 kg
Systemic exposure dose (SED) =
A (g/d) x 1000 mg/g x C (%)/100 x DAp (%)/100 /60 kg = 1.02 mg/kg bw/d

No adverse observed effect level NOAEL = 100 mg/kg bw/d
(systemic NOAEL, 90d oral repeat dose toxicity study, rat)
No adjustment, 100 % oral absorption

MoS NOAEL/SED = 98.0

3.5.2 Oral care cosmetics

For oral hygiene products, the relative calculated daily exposure to cosmetic product is 34.7 mg/kg bw/d (2.16 mg/kg bw/d for toothpaste (adults) and 32.54 mg/kg bw/d for mouthwash), according to the 9th revision of the SCCS NoG (SCCS 1564/15).

Product type	Calculated relative daily exposure [mg/kg bw/d]	NOAEL [mg/kg bw/d]	MoS based on NOAEL
Toothpaste (adults) and mouthwash combined	0.243	100	412
Toothpaste (adults)	0.015	100	6667
Mouthwash	0.228	100	439

No adjustment, 100 % oral absorption

3.5.3 Dermally applied cosmetic products

Product type	SED [mg/kg bw/d]	NOAEL [mg/kg bw/d]	MoS
All types of	0.880	100	114

products			
Rinse-off skin & hair cleansing products	0.032	100	3125
Leave-on skin and hair care products	0.783	100	128
Make-up products	0.065	100	1539
Leave-on skin and hair care products and make-up products combined	0.848	100	118

The following parameters were used to determine SED for dermally applied cosmetic products:

Concentration of ingredient in finished product C (%) = 0.7 %
Typical body weight of human = 60 kg
Systemic exposure dose (SED) =
A (g/d) x 1000 mg/g x C (%)/100 x DAp (%)/100 /60 kg

No adverse observed effect level NOAEL = 100 mg/kg bw/d
(systemic NOAEL, 90d oral repeat dose toxicity study, rat)
No adjustment, 100 % oral absorption

Exposure calculated according to the following Table of the SCCS NoG, 9th revision:

Type of exposure	Product	g/d	mg/kg bw/d
Rinse-off skin & hair cleansing products	Shower gel	0.19	2.79
	Hand wash soap	0.20	3.33
	Shampoo	0.11	1.51
	Hair conditioner	0.04	0.67
Leave-on skin & hair care products	Body lotion	7.82	123.20
	Face cream	1.54	24.14
	Hand cream	2.16	32.70
	Deo non-spray	1.50	22.08
	Hair styling	0.40	5.74
Make-up products	Liquid foundation	0.51	7.90
	Make-up remover	0.50	8.33
	Eye make-up	0.02	0.33
	Mascara	0.025	0.42
	Lipstick	0.06	0.90
	Eyeliner	0.005	0.08
Oral care cosmetics	Toothpaste	0.14	2.16
	Mouthwash	2.16	32.54
TOTAL		**17.4**	**269**

3.6 Discussion

Physico-chemical properties

Depending on the temperature, HEPB may appear as a solid (white powder or crystals) or as a pale yellow liquid form. Reported purities in the different batches of HEPB were 95.9% and higher. Two impurities (2-ethoxy-4-(3-hydroxybutyl) phenol (up to 4.8% in different batches reported) and ethylvanillin (< 1000 mg/g)) have been quantified using reference standards. Four other impurities have been detected with a relative UV purity < 0.1%. Water solubility is 7.59 g/L at 20°C, Log Pow is 1.46 at 22.8°C and the vapour pressure is 8.7×10^{-3} Pa at 25 °C. The substance is stable over 2 months at 45°C in hydroalcoholic solution at 0.5g/100ml. It is sensitive to photostress but resistant to oxidative, heat, acid or basic stresses.

Function and uses

The ingredient HEPB is intended to be used specifically as a preservative in rinse-off, oral care and leave-on cosmetic products up to 0.7%.

Toxicological Evaluation

Acute toxicity

No acute toxicity study on any route is available for HEPB. In 14- and 90-day oral repeat dose studies using dose levels of 100, 300 and 1000 mg/kg bw/d, no deaths occurred. Hence, it can be assumed that the oral LD50 would be higher than 1000 mg/kg/d (i.e. the substance is of low acute oral toxicity).

Local toxicity

Based on an *in vitro* Episkin^{SM} Skin Irritation Test performed with the neat substance, HEPB can be considered as potentially non-irritant to the skin.
A Reconstructed Human Cornea-Like Epithelium test indicates that HEPB can be considered to be non-irritating to the eye at 0.7% (w/w) in propylene glycol.

Skin Sensitisation

HEPB is not considered to be a skin sensitiser.

Dermal absorption

The *in vitro* percutaneous absorption of HEPB by using a typical lipophilic skin care formulation containing HEPB at the concentration of 2% was determined in human dermatomed skin. A recovery of at least 85% was only obtained for 4 cells evaluated. The number of 4 evaluable cells is not in line with the SCCS criteria. Further, the *in vitro* percutaneous absorption has not been determined in aqueous cosmetic formulation.
Based on significant deviations from requirements, the SCCS considers the study not acceptable. Instead, in the absence of adequate experimental data, a default value of 50% dermal absorption is taken for MoS calculation according to SCCS 1564/15.

Repeated dose toxicity

Macroscopic lesions and microscopic changes in the stomach were observed at the highest dose tested (1000 mg/kg bw/d) in a 90-day oral repeat-dose toxicity study in rats, indicating a local NOAEL of 300 mg/kg bw/d for the oral uptake route.
Concerning systemic effects, which are of higher relevance for dermal uptake of cosmetics, the SCCS considers 100 mg/kg bw/d as the systemic NOAEL based on clinical findings indicating an acute neurotoxic effect and based on FOB findings (statistically significantly reduced hindlimb grip strength observed in females at 300 and 1000 mg/kg bw/d and statistically significant and non-significant findings in some motor activity scores). It is of note that indications for neurotoxicity were not only observed in a 90-day oral repeat dose study in rats but also in a developmental toxicity study performed in rats.

Mutagenicity

HEPB was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations. HEPB did not induce gene mutations in bacteria nor in mammalian cells when evaluated at the hprt locus. Exposure of human lymphocytes with HEPB did not result in an increase of micronucleated binucleated cells. Based on the present available tests, HEPB can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity

/

Reproductive toxicity

Based on the findings of a developmental toxicity study, it can be concluded that HEPB is not a developmental toxicant. A separate study addressing fertility has not been performed.

Toxicokinetics

Physico-chemical properties and *in vivo* experimental findings obtained from a structurally similar compound (zingerone) are indicative of almost complete and rapid absorption and excretion. 100% oral absorption is therefore assumed for MoS calculation.

Phototoxicity

In view of the absence of indications of phototoxicity at 1000 µg/ml, the SCCS considers that phototoxicity of HEPB is unlikely.

Human data

/

4. CONCLUSION

In light of the new studies provided, does the SCCS consider the use of Hydroxyethoxyphenyl Butanone (HEPB) safe with regard to eye irritation, when used as preservative in rinse-off, oral care and leave-on cosmetic products with a maximum concentration of 0.7%?

Based on the new information provided by the Applicant, the SCCS considers the use of Hydroxyethoxyphenyl Butanone (HEPB) as a cosmetic preservative in rinse-off, oral care and leave-on cosmetic products with a maximum concentration of 0.7% safe with regard to eye irritation.

5. MINORITY OPINION

/

6. REFERENCES

1. Brémond C (2012). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Primary cutaneous tolerance – Prediction of the acute irritant potential on human reconstructed epidermis EPISKIN^{SM} model; EPISKIN Study No 11-BPL-1065.

2. Cannamela N (2012a). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Ocular primary irritation B.C.O.P. – Study performed on the isolated bovine cornea measurement of the bovine corneal opacity and permeability. IEC Report No 120053RD.

3. Cannamela N (2012b). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Ocular primary irritation B.C.O.P. – Study performed on the isolated bovine cornea measurement of the bovine corneal opacity and permeability. IEC Report No 120055RD.

4. Cannamela N (2018). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Ocular primary irritation B.C.O.P. – Study performed on the isolated bovine cornea measurement of the bovine corneal opacity and permeability. IEC Report No 180839RD.

5. Simpson H (2018): Hydroxyethoxyphenyl Butanone: Evaluation of Ocular Irritation *In vitro* Using the HCE® Reconstructed Human Corneal Model. Test Facility Study No. 782900, Report No. 40166.

6. Verma R (2011). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Skin Sensitization Study of R0069279A by Local Lymph Node Assay in Mice. JRF Study No. 409-1-01-2126.

7. Craig S (2014a). Hydroxyethoxyphenyl Butanone (R0069279A): The *In vitro* Percutaneous Absorption of R0069279A in a Direct Skin Care Formulation Through Human Skin Through Human. Study No 791813.

8. Craig S (2014b). Hydroxyethoxyphenyl Butanone (R0069279A): Assessment of Recovery of Radiolabelled R0069279A in a Skin Care Formulation. Study No 793009.

9. Tang J (2014). Hydroxyethoxyphenyl Butanone (R0069279A): ADME-Tox Study of R0069279A. Study No 100016337.

10. Ravi M (2013). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): 14-Day Repeated Dose Toxicity Study of R0069279A in Wistar Rats by Oral Route. Advinus Study No. G7935.

11. Yogesha BN (2012). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): 90-Day Repeated Dose Toxicity Study of R0069279A in Wistar Rats by Oral Route. Advinus Study No. G7937.

12. Latha M (2013a). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Prenatal Developmental Toxicity Study of R0069279A in Wistar Rats by Oral Route. Advinus Study No. G7939.

13. Latha M (2013b). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Preliminary Prenatal Developmental Toxicity Study of R0069279A in Wistar Rats by Oral Route. Advinus Study No. G7938.

14. .Bowen R (2011). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Reverse Mutation in five histidine-requiring strains of *Salmonella typhimurium*. Covance Study No. 8244263.

15. Lloyd M (2012). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Mutation at the *hprt* locus of L5178Y Mouse Lymphoma Cells using the Microtitre® Fluctuation Technique. Covance Study No. 8250242.

16. Lloyd M (2011). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): Induction of Micronuclei in Cultured Human Peripheral Blood Lymphocytes. Covance Study No. 8244262.

17. Cannamela N (2014). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A 019 D 004): 3T3 NRU Phototoxicity Test. Study performed on a permanent mouse fibroblast cell line (3T3). IEC Report No 140156RD.

18. Monge P (1976). The metabolism of Zingerone, a pungent principle of ginger. Xenobiotica. Vol. 6. No 7. pp 411-423.

7. GLOSSARY OF TERMS

See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 141

8. LIST OF ABBREVIATIONS

See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 141

9. Appendix

Assessment of Recovery of Radiolabelled R0069279A in a Skin Care Formulation.
This study has been performed in order to explain the low recovery rate in the dermal absorption study.

Guideline:	/
Method:	Determination of recovery of test item when applied to aluminium foil
Test substance:	R0069279A (non-labelled); [^{14}C]-R0069279A (labelled)
Batch:	R0069279A 019 P 001 (non-labelled material) CFQ41708 (labelled material)
Purity:	96.0% ± 1.3 % (non-labelled material) 92.2 % (labelled material; HPLC purity)
Test item:	radiodiluted [^{14}C]-R0069279A in a cosmetic formulation; 105.85 % of target (2.0 % (w/w)
Replicates:	8
Exposed membrane area:	3.14 cm^2
Dose applied:	ca. 2.0 mg/cm²
Sampling period:	24 hours
Receptor fluid:	calcium- and magnesium free phosphate buffered saline (PBS) containing bovine serum albumin (5%, w/v)
Mass balance analysis:	Provided
Tape stripping:	not applicable
Method of Analysis:	Liquid Scintillation Counting (LSC)
GLP:	In compliance
Study period:	experimental phase: Feb 2013, Report dated23 October 2014; draft report Nov 2012

Sections of aluminium foil, ca 3 x 3 cm, were cut out, positioned on the receptor chamber of the diffusion cells already connected to the waterbath (heated to maintain a surface temperature of 32°C ± 1°C) in a fume hood. The receptor chambers were filled with phosphate buffered saline and a magnetic stirring bar was introduced in each cell. The test item (radiodiluted test item in a combination of one oily and two water-based preformulations) was applied over the exposed surface of 8 samples of aluminium foil. The donor chambers were left open to the atmosphere. To accurately quantify the radioactivity applied to the aluminium foil, seven weighed aliquots of the test preparation were taken throughout and analysed by liquid scintillation counting (LSC). At 0 h post dose, the exposure was terminated for 4 samples by placing the aluminium foil and the donor chamber into pre-weighed pots containing ethanol (40 mL). The same procedure was carried out for a further 4 samples at 24 h post dose.
The radiochemical was then extracted from the test system and each sample was sonicated for ca 10 min. The donor chambers were removed from the pots and duplicate aliquots were analysed by LSC.

Results:

Test Preparation	819181	
Target R0069279A Concentration Actual R0069279A Concentration by Radioactivity	2% (w/w) 2.12% (w/w)	
Application Rate of Test Preparation (mg/cm^2) Application Rate of R0069279A (μg equiv./cm^2)	1.89 mg/cm^2 39.94 μg equiv./cm^2	
Distribution	% Applied Dose	
Termination Timepoint (h)	0	24
Mass Balance	94.20	64.01

The mean mass balance for the aluminium foil samples terminated at 0 h post dose was 94.20% of the applied dose and this decreased to 64.01% at 24 h post dose. The results showed a loss of [14C]-R0069279A when applied in Test Preparation 819181 (2%, w/w) to aluminium foil. Recovery at 24 h post dose was variable ranging from 54.54% to 74.57% of the applied dose. Recovery was less variable at 0 h post dose ranging from 86.92% to 98.48% of the applied dose. This confirmed that there were losses during the processing of the recovery samples.