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## SCCS OPINION ON Ethylzingerone – 'Hydroxyethoxyphenyl Butanone' (HEPB) (Cosmetics Europe No P98)

Ulrike Bernauer, Laurent Bodin, Leonardo Celleno, Qasim Mohammad Chaudhry, Pieter-Jan Coenraads, Maria Dusinska, Jeanne Duus-Johansen, Janine Ezendam, Eric Gaffet, Lodovico Corrado Galli, et al.

► **To cite this version:**

Ulrike Bernauer, Laurent Bodin, Leonardo Celleno, Qasim Mohammad Chaudhry, Pieter-Jan Coenraads, et al. SCCS OPINION ON Ethylzingerone – 'Hydroxyethoxyphenyl Butanone' (HEPB) (Cosmetics Europe No P98). 2016. hal-01493485

**HAL Id: hal-01493485**

**<https://hal.science/hal-01493485>**

Submitted on 27 Mar 2017

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Scientific Committee on Consumer Safety

SCCS

## **OPINION ON**

# **Ethylzingerone – 'Hydroxyethoxyphenyl Butanone' (HEPB)**

**(Cosmetics Europe No P98)**

The SCCS adopted this Opinion by written procedure

On 23 December 2016

1

## 2 **About the Scientific Committees**

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

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

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

## 13 **SCCS**

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

19

20

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36

37 © European Union, 2016

38 ISSN

ISBN

39 Doi:

ND-

40

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## **ACKNOWLEDGMENTS**

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

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Ethylzingerone - 'Hydroxyethoxyphenyl Butanone' (HEPB) - Cosmetics Europe No P98, SCCS/1582/16, Preliminary version of 23 December 2016

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## 1. 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's 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.

According to the above-mentioned dossier the safety profile of HEPB has been studied in preclinical studies conducted according to Good Laboratory Practices and following OECD guidelines whenever available. They were conducted using HEPB from batches which were well characterized analytically with a minimum purity of 95.9%.

## 2. Terms of reference

- (1) *In light of the data provided, does the SCCS consider safe 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 2.0 % under an aggregate exposure scenario for cosmetics?*
- (2) *Alternatively, what is according to the SCCS, the maximum target concentration considered safe for use of Hydroxyethoxyphenyl Butanone (HEPB) as a cosmetic preservative in rinse-off, oral care and leave-on cosmetic products under an aggregate exposure scenario for cosmetics?*
- (3) *Does the SCCS have any further scientific concerns regarding the use of Hydroxyethoxyphenyl Butanone (HEPB) in cosmetic products?*

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### 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

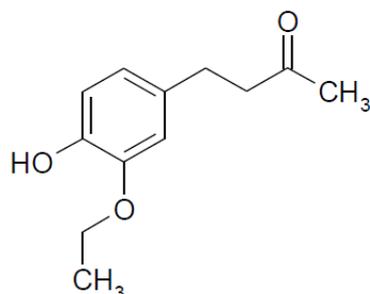
For convenience, the abbreviation HEPB will be used throughout the Opinion.

###### 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<sub>12</sub>H<sub>16</sub>O<sub>3</sub>

##### 3.1.2 Physical form

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

### 3.1.3 Molecular weight

6 Molecular weight: 208.25 g/mol

### 3.1.4 Purity, composition and substance codes

10 Chemical characterisation was performed using IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, mass spectrometry  
11 and UV-Vis spectroscopy for the batches 019 D-004, 019 P-001 and 020-P001 with the  
12 following results:

- 13 • IR Spectra of the 3 batches were comparable.
- 14 • The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of the three batches were comparable. Presence of  
15 an impurity corresponding to the alcoholic form (R0070073A) of the expected  
16 structure, (estimated value: 0.04 M/Mole in Batch 019D-004). The impurity  
17 (R0070073A) is detected in the three batches.
- 18 • UV / Vis spectra of the 3 batches were comparable.
- 19 • Mass spectrometry: hydroxyethoxyphenyl butanone batch 019D-004 was analysed  
20 by infusing a diluted hydro-alcoholic solution of the sample into a Thermo Fischer  
21 Scientific LTQ-Orbitrap mass spectrometer operating in the negative ion mode. The  
22 [M-H]<sup>-</sup> ion of the expected molecule C<sub>12</sub>H<sub>16</sub>O<sub>3</sub> was mainly observed in the mass  
23 spectrum. The MS/MS fragmentation pattern of this ion confirmed the expected  
24 structure of the molecule. The mass spectra of the two other batches 019P-001 and  
25 020P-001 recorded using the same analytical conditions were comparable to the  
26 spectrum of batch 019D-004.

28 Purity of hydroxyethoxyphenyl butanone was determined by HPLC-PDA (λ=281 nm) against  
29 hydroxyethoxyphenyl butanone R0069279A batch 008L-001 primary reference standard  
30 considered as pure (100%) for batches 019D-004 and 019P-001 and against  
31 hydroxyethoxyphenyl butanone R0069279A batch 018L-002 secondary reference standard  
32 considered as 98.9% pure for batch 020P-001.

33 Hydroxyethoxyphenyl butanone purity:

34 Batch 019D-004: 95.9 ± 0.5% [w/w]

35 Batch 019P-001: 96.3 ± 0.8% [w/w]

36 Batch 020P-001: 96.2 ± 0.8% [w/w]

### 3.1.5 Impurities / accompanying contaminants

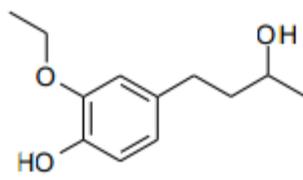
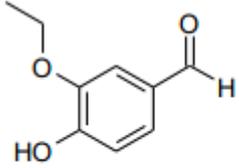
#### 41 Impurities by HPLC-PDA

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

44 The retention time of hydroxyethoxyphenyl butanone was around 8.5 minutes. One impurity  
45 (R0070073A, corresponding alcohol) was detected (Retention time R<sub>t</sub> = 9.7 min) in the  
46 three batches, its content was determined using R0070073A 001L002 as reference standard  
47 (100%). One impurity (R0070359A, ethylvanillin) was detected (R<sub>t</sub> = 6.3 min) in the three  
48 batches with a relative purity <0.1 %: its content was determined using R0070359A  
49 002L001 as reference standard (100%). One impurity was detected (R<sub>t</sub> = 7.4 min) in  
50 hydroxyethoxyphenyl butanone Batches 019D004 and 019P001 with a relative UV purity <  
51 0.1%. Three impurities were detected (R<sub>t</sub> = 12.8 min, 14.9 min and 15.9 min) in  
52 hydroxyethoxyphenyl butanone Batch 020P001 with a relative UV purity < 0.1 %. One  
53 impurity was detected (R<sub>t</sub> = 3.6 min) in hydroxyethoxyphenyl butanone batches 019D004  
54 and 019P001 with a relative UV purity < 0.1%. For this compound, the chemical structure  
55 3-ethoxybenzaldehyde was proposed.  
56

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- 1 The HPLC profiles of the three batches were comparable.  
2  
3 According to the HPLC profile, the following impurities were identified:  
4

<p>R0070073A (corresponding alcohol) 2-ethoxy-4-(3-hydroxybutyl) phenol</p>	 <p>Exact Mass =210 Molecular Formula =C<sub>12</sub>H<sub>18</sub>O<sub>3</sub></p>
<p>R0070359A (starting material) Ethylvanillin</p>	 <p>Exact Mass =166 Molecular Formula =C<sub>9</sub>H<sub>10</sub>O<sub>3</sub></p>

- 5  
6 In the different batches, these impurities were detected in the following amounts:  
7

8 R0070359A (Ethylvanillin) [ $\mu\text{g/g}$ ]:  
9  
10 < 1000 in batches 019D004, 019P001 and 020P001.  
11

12  
13 R0070073A (corresponding alcohol) 2-ethoxy-4-(3-hydroxybutyl) phenol [% w/w]:  
14  
15  $4.8 \pm 0.05$  in batch 019D004;  $4.0 \pm 0.05$  in batch 019P001;  $3.7 \pm 0.02$  in batch 020D004  
16

17 **Residual solvents by GC:**

18  
19 Acetone and ethyl acetate used in the manufacturing process of the three batches: < 0.1 %  
20 (w/w)  
21

22 **Heavy Metals [mg/kg]:**

23 Batch 019D004:

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

27 Batch 019P001:

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

31 Batch 020 P 001:

32 Al, Ba, Co, Cr, Cu, Mn, Mo, Ni, Se, Sn, Ti, V, Zn : each < 1  
33 Ba, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, Ti,V, Zn : each < 5  
34 Al: 6  
35

36 Ash [% w/w]:

Opinion on Ethylzingerone - 'Hydroxyethoxyphenyl Butanone' (HEPB) - Cosmetics Europe No P98

1  
2 Batch 019D004: < 0.1  
3 Batch 019P001: 0.1  
4 Batch 020P001: 0.1

5  
6 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

8  
9

### 3.1.6 Solubility

10  
11  
12 Water solubility (batch 019 P001): 7.59 g/L at 20°C ± 0.5 °C  
13 (EEC method A6 - OECD method 105)

14  
15 Solubility (at 21°C) – (batch 006 L001 and 007 L001 from batch 019D004)  
16 - Water: < 0.1 mg/mL (method not stated)  
17 - Ethanol: ≥1000 mg/mL  
18 - DMSO: ≥1000 mg/mL  
19 - Corn oil: <0.1 mg/mL

### SCCS Comment

20  
21  
22 The SCCS notes that the analytical file gives considerably differing values for water  
23 solubility. The issue has been clarified by the applicant and the correct value for water  
24 solubility is 7.59 g/L at 20°C ± 0.5 °C.

### 3.1.7 Partition coefficient (Log P<sub>ow</sub>)

25  
26  
27  
28  
29 Log P<sub>ow</sub>: 1.46 at 22.8°C ± 1 °C  
30 (EEC method A8 - OECD method 107)

### 3.1.8 Additional physical and chemical specifications

31  
32  
33  
34 Melting point: 41 ± 2°C (DSC method)  
35 Boiling point: 328 ± 2°C at 100 to 102 kPa  
36 Flash point: /  
37 Vapour pressure: 8.7 x 10<sup>-3</sup> Pa at 25 °C  
38 Density: /  
39 Viscosity: /  
40 pKa: 10.03 (25°C and ionic strength 0.15M) for an equilibrium  
41 HO/O<sup>-</sup> (GLpKa Sirius)  
42 Refractive index: /  
43 pH: /

1 UV-Vis spectrum (200-500 nm): The UV-Vis absorption spectrum of a 0.004 g/100 ml  
2 solution of hydroxyethoxyphenyl butanone exhibited a  
3 maximum at 282nm ± 1nm; the absorbance at 282 nm  
4 was ~ 0.6.  
5  
6

### 7 **3.1.9 Stability**

8  
9 The R0069279A is stable over 2 months at 45°C in hydroalcoholic solution at 0.5 g/100 ml.  
10 It is sensitive to photostress but resistant to oxidative, heat, acid or basic stresses.  
11 Determination was performed for the three batches using batch R0069279A 001L-001 as  
12 reference.  
13

### 14 **SCCS General Comments to physico-chemical characterisation**

15  
16 Chemical characterisation of P98 was performed using IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, mass  
17 spectrometry and UV-Vis spectroscopy. Purity of P98 was determined by HPLC-PDA at λ<sub>max</sub>  
18 281 nm using reference standard or secondary reference standard and it is accepted.  
19 Impurity studies have been performed using HPLC-PDA detection at λ<sub>max</sub> of P98. Two  
20 impurities (2-ethoxy-4-(3-hydroxybutyl) phenol and ethylvanillin) have been quantified  
21 using reference standards. Four other impurities have been detected with a relative UV  
22 purity < 0.1%. In batches 019D004 and 019P001 an impurity with a relative UV purity <  
23 0.1% with a proposed chemical structure of 3-ethoxybenzaldehyde was reported.  
24 Chemical identification and characterisation of impurities is considered acceptable.

### 25 **3.2 Function and uses**

26 The ingredient HEPB is intended to be used specifically as a preservative in rinse-off, oral  
27 care and leave-on cosmetic products up to 2%.  
28

### 29 **3.3 Toxicological Evaluation**

#### 30 **3.3.1 Acute toxicity**

##### 31 **3.3.1.1 Acute oral toxicity**

32  
33  
34 No data provided.  
35  
36

##### 37 **3.3.1.2 Acute dermal toxicity**

38  
39 No data provided.  
40

##### 41 **3.3.1.3 Acute inhalation toxicity**

42  
43 No data provided.  
44

##### 45 **3.3.1.4 Acute intraperitoneal toxicity**

46  
47 No data provided.  
48

### 49 **Summary on acute toxicity**

50  
51 No acute toxicity study on any route is available for HEPB. In 14- and 90-day oral repeat  
52 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<sub>50</sub> 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:	<i>In vitro</i> Episkin <sup>SM</sup> Skin Irritation Test
Test system:	EPISKIN <sup>SM</sup> Reconstructed Human Epidermis (RHE) small (0.38 cm <sup>2</sup> ) 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.: 2

##### **SCCS Comment**

An ECVAM validated protocol was used and certificates of analysis with IC<sub>50</sub> 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

**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 edema 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.: 3

**Conclusion**

The study authors concluded that based on the conditions of this test HEPB is not classified as corrosive or 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

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1	Batch:	R0068279A 019 D 004
2	Purity:	95.9 ± 0.5% (HPLC)
3	Test item:	2 % (w/w) suspension in vehicle (yielding a pH of 5.5)
4	Vehicle:	polyethylene glycol (8 OE)
5	Dose level:	750 ± 8 µl
6	Treatment period:	30 min and 4 hours
7	Positive control:	0.5 % (w/w) Cetyl Trimethylammonium Bromide (CTAB) in distilled
8		water
9	Negative control:	nutritive medium
10	GLP:	In compliance
11	Study period:	Jan 2012 – Feb 2012

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

#### 28 29 Results:

30 The mean corneal (IVIS) scores obtained for 30-min contact time were: 4.7 ± 0.6 for the 2  
31 % HEPB suspension, 3.9 ± 0.6 for the vehicle control, and 96.0 ± 2.8 for the positive  
32 control, respectively. The mean corneal (IVIS) scores obtained for 4-hour contact time  
33 were: 5.9 ± 0.1 for the 2 % HEPB suspension and 4.5 ± 0.0 for the vehicle control.

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

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Ref.: 4

#### 40 **SCCS Comment**

41 The SCCS notes that the permeability (OD) values of the vehicle control are much higher  
42 (8-fold for the 4-hour treatment period) than the negative control, suggesting that the  
43 vehicle polyethylene glycol itself might have an adverse effect on the test system.  
44 Therefore, the results of the BCOP assay are inconclusive. An appropriate vehicle should be  
45 used for which it can be demonstrated that it has no adverse effect on the test system.  
46 The SCCS further notes that the BCOP assay (OECD 437, 2009) is only able to identify  
47 severe eye irritants and cannot discriminate between slightly irritant, moderately irritant  
48 and severely irritant.

### 51 **3.3.3 Skin sensitisation**

#### 53 **Local Lymph Node Assay (LLNA)**

54  
55 Guideline: OECD 429; EC B.42  
56 Species/strain/sex: mouse, CBA/J, female  
57 Group size: 4

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1	Test substance:	R0069279A
2	Batch:	R0069279A 019 D 004
3	Purity:	95.9 ± 0.5%
4	Vehicle:	acetone:olive oil (4:1; v/v)
5	Concentration:	1, 10, 25, 50 and 75 %
6	Vehicle control:	acetone:olive oil (4:1; v/v) (AOO)
7	Positive control:	$\alpha$ -hexylcinnamaldehyde (HCA) at 25 % (v/v) in AOO
8	GLP:	In compliance
9	Study period:	March 2011 – July 2011

10  
11 Concentrations for the main study were based on findings from a preceding irritation test, in  
12 which no increase in skin thickness was noted at test item concentrations of 5.0, 25.0, 50.0  
13 and 75.0 % (w/v).

14 Seven treatment groups each comprising 4 mice received either the test substance at 1, 10,  
15 25, 50 or 75% (w/v) in AOO, AOO alone as negative control or HCA at 25 % in vehicle as  
16 positive control. The test item or controls were applied on the dorsum of both ears for three  
17 consecutive days. After a 2-day rest lymphocyte proliferation was assessed in the pooled  
18 auricular lymph nodes by measuring the incorporation of 3H-TdR. The values were used to  
19 calculate stimulation indices (SI).

20 Results:

21 The positive control HCA generated a SI value of 3.38. The test substance induced a small  
22 increase in lymphoproliferation. SI values of 1.16, 1.24, 1.43, 1.53 and 1.78 were obtained  
23 at test substance concentrations of 1%, 10%, 25%, 50% and 75%, respectively.

24 Therefore the study authors concluded that R0069279A did not induce skin sensitisation in  
25 the LLNA.

26  
27 Ref.: 23

### 28 **SCCS comment**

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

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## 34 **3.3.4 Toxicokinetics**

### 36 **3.3.4.1 Dermal / percutaneous absorption**

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39	Guideline:	OECD 428 (November 2004); SCCS 1358/10
40	Species/strain:	Split-thickness human abdomen skin from 4 donors aged 30 – 41 63 years and one split-thickness breast skin sample from a 42 female donor aged 58 years
43	Membrane integrity:	sample exhibiting a resistance < 4 k $\Omega$ excluded
44	Replicates:	12 skin samples from 12 different donors
45	Method:	Dermatomed thawed skin mounted on static diffusion cells and 46 exposed to radiodiluted test item
47	Test substance:	R0069279A (non-labelled); [ <sup>14</sup> C]-R0069279A (labelled)
48	Batch:	R0069279A 019 P 001 (non-labelled material)
49		CFQ41364 (labelled material)
50	Purity:	96.0% ± 1.3 % (non-labelled material)
51		98.0 % (labelled material; radiochemical purity)
52	Test item:	radiodiluted [ <sup>14</sup> C]-R0069279A in a cosmetic formulation; 53 104.2 % of target (2.0 % (w/w)
54	Exposed membrane area:	3.14 cm <sup>2</sup>
55	Dose applied:	2.11 mg/cm <sup>2</sup>
56	Sampling period:	24 hours

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1 Receptor fluid: calcium- and magnesium-free phosphate buffered saline (PBS)  
 2 containing bovine serum albumin (5%, w/v)  
 3 Mass balance analysis: Provided  
 4 Tape stripping: Yes (20)  
 5 Method of Analysis: Liquid Scintillation Counting (LSC)  
 6 GLP: In compliance  
 7 Study period: June – July 2012; draft report Nov 2012  
 8

9 The in vitro percutaneous absorption of [<sup>14</sup>C]-HEPB by using a typical lipophilic skin care  
 10 formulation containing HEPB at the concentration of 2% was determined in human  
 11 dermatomed skin. Any split-thickness human skin sample exhibiting a resistance less than 4  
 12 kΩ was excluded from subsequent absorption measurements.

13 Human dermatomed skin samples were mounted onto diffusion cells. Calcium- and  
 14 magnesium-free PBS was used as the receptor fluid. A quantity of 2 mg/cm<sup>2</sup> of skin care  
 15 formulation was applied to the skin surface. After 24 hours of exposure, the skin surface  
 16 was rinsed-off, simulating in-use conditions. 24 hours after application, the stratum  
 17 corneum was removed by 20 tape strippings and the penetration, mass balance and  
 18 distribution of [<sup>14</sup>C]-HEPB were determined by measuring its concentration in relevant  
 19 compartments (e.g. skin wash, epidermis, dermis, and receptor fluid) by using LSC.

20  
21 Results

22 Mean recovery of the applied test material (mass balance) was 82.4% of the applied dose.  
 23 The mean systemically available dose of HEPB sum of amounts measured in living  
 24 epidermis/dermis and receptor fluid after application of a typical skin care formulation  
 25 containing this preservative ingredient at 2% was estimated to be 36.8±17% of the applied  
 26 dose (16.0±7.4 µg equiv/cm<sup>2</sup>).  
 27  
 28

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

	Cell Number and Donor Number												Mean	SD
	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		
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

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

	Cell Number and Donor Number												Mean	SD
	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		
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 [<sup>14</sup>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.: 6; 7

**SCCS Comment**

The dedicated study evaluating the recovery rate did not give 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.

1 Results from a structurally similar compound (zingerone) are indicative of almost complete  
2 and rapid absorption and excretion (see section 3.3.11). Based on this information and on  
3 physico-chemical properties of HEPB (molecular weight: 208.25 g/mol; water solubility of  
4 7.59 g/mol and log  $P_{ow}$  of 1.46), 100 % oral absorption is taken for MoS calculation.  
5

#### 6 3.3.4.3 Toxicokinetics in humans

7  
8 No data provided.  
9

#### 10 3.3.4.4 Toxicokinetics in vitro

11  
12 Guideline: /  
13 Test system: Human intestinal epithelial Caco-2 cells  
14 Test substance: R0069279A  
15 Batch: R0069279A 019 P 001  
16 Purity: not mentioned  
17 GLP: in compliance  
18 Study period: June – August 2014  
19

20 In each experiment and if applicable, the respective reference compounds (propranolol:  
21 highly permeable; labetalol: moderately permeable; ranitidine: poorly permeable;  
22 colchicine: P-glycoprotein substrate) were tested concurrently with HEPB, and the data were  
23 compared with historical values determined at the testing facility. Only limited information  
24 was provided on the experimental setting. In short, the apical-basolateral (A-B)  
25 permeability of HEPB in Caco-2 cells (pH 6.5/7.4) was determined and compared to that of  
26 the reference compounds. A test concentration of 10  $\mu$ M HEPB was used.  
27

#### 28 Results:

29 The test item has a mean apparent permeability coefficient of  $62.2 \times 10^{-6}$  cm/s. Based on  
30 this result of the test item and the reference compounds, the test item should be highly  
31 permeable.  
32

Ref.: 22

#### 34 **SCCS comment**

35 The study report is extremely poor. However, it can be inferred from the results of the  
36 study, that penetration of HEPB through the Caco-2 monolayer is high.  
37  
38  
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### 40 3.3.5 Repeated dose toxicity

#### 42 3.3.5.1 Repeated Dose (14 days) oral / dermal / inhalation toxicity

##### 44 *Oral*

45  
46 A 14-day oral repeat dose study was performed as a preliminary (range-finder) study for a  
47 90-day oral toxicity study. The study was performed in August 2011, the final report dates  
48 from March 2013. Four groups of male and female Wistar rats, respectively, each  
49 comprising 5 animals, received dose levels of 100, 300 and 1000 mg/kg bw/d of HEPB (test  
50 item R0069279A, batch R0069279A 019 D 004) in 0.5 % (w/v) carboxymethylcellulose  
51 (CMC) in water and 2 % Tween® 80 (v/v) by oral gavage. Control groups received vehicle  
52 only. Animals were observed twice daily for clinical signs and body weight and food  
53 consumption were monitored. At the end of the treatment period, blood and urine samples  
54 were taken, animals were subjected to gross examination and specified organs were

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1 collected. Histopathological examination was carried out on heart, kidneys, liver and spleen  
2 from all rats of the vehicle control and high dose group.  
3 There were no clinical signs or mortalities in any of the groups during the treatment period.  
4 There were no test item-related changes in any of the parameters evaluated in  
5 haematology, coagulation, clinical chemistry and urine. An increase in absolute and relative  
6 liver weight was observed in high dose males. There were no test item-related gross  
7 pathological findings in test-item treated animals. In high-dose males, a 6 % increase in  
8 mean platelet volume not accompanied by any significant changes in platelet count was also  
9 observed. A dilatation of the uterus was observed in one female of the mid-dose and one  
10 female of the high-dose.  
11 Based on the results of this 14-d oral repeat dose study, 100, 300 and 1000 mg/kg bw/d  
12 were chosen for the 90-day oral toxicity study.

Ref.: 20

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

20 Guideline: OECD TG 408 (Sep 1998)  
21 Species/strain: Rat, Wistar rats-HsdHan<sup>TM</sup>:WIST  
22 Group size: 10 / sex / dose  
23 Test substance: R0069279A  
24 Batch: R0069279A 019 D 004  
25 Purity: 95.9 ± 0.5 %  
26 Vehicle: 0.5 % (w/v) carboxymethylcellulose (CMC) in water and 2 % Tween®  
27 80 (v/v)  
28 Dose levels: 0, 100, 300 and 1000 mg/kg bw/d  
29 Dose volume: 10 ml/kg  
30 Route: oral  
31 Administration: gavage  
32 GLP: In compliance  
33 Study period: Sep 2011 – Oct 2012

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

**Results**

51 No mortalities were observed throughout the study period. No ocular abnormalities were  
52 observed in the ophthalmological examinations. Transient clinical signs (slight to moderate  
53 salivation, dragging gait, hypoactivity, splayed hind-limbs) appeared shortly (5-15 min)  
54 after gavage in both sexes and persisted up to 30 min in animals dosed at 300 mg/kg bw/d.  
55 At the highest dose, these signs appeared approximately 5-15 min post dose and persisted  
56 up to 1 hour (60 min) post dose in most rats. For 2 rats, hypoactivity and splayed hindlimbs  
57 with dragging gait persisted for more than an hour. In FOB, grip strength of hindlimbs was

1 significantly reduced in mid- and high-dose females. Landing hindlimbs foot splay values  
2 were significantly lower in males of all dose levels and in low dose females. At 300 and 1000  
3 mg/kg bw/d, hindlimb grip strength in females was statistically significantly ( $p < 0.0001$ )  
4 different from controls. Motor activity was statistically significantly different from controls in  
5 males and females at 1000 mg/kg bw/d lower values of stereotypic time (interval 6) and  
6 total stereotypic time in males and lower values of stereotypic time, ambulatory time and  
7 horizontal and ambulatory counts (interval 6) in females, respectively. The study authors  
8 interpreted statistical variations in motor activity as incidental as there were no  
9 accompanying changes in muscle tone. Food consumption was statistically significantly  
10 reduced in high-dose males and females (about -12 % and -16%, respectively, compared to  
11 controls). In high-dose males, statistically significant (about -10 % compared to controls)  
12 lower body weights were observed. There were no test-item related alterations in  
13 haematology, coagulation and clinical chemistry or urine parameters in any of the treated  
14 dose groups in both sexes. Gross pathology revealed no changes in rats up to 300 mg/kg  
15 bw/d. At the highest dose level, thickening and white multiple raised foci of nonglandular  
16 mucosa in the stomach was observed in 5/10 males and 3/10 females. The macroscopic  
17 lesions were associated with mild to moderate epithelial hyperplasia in the non-glandular  
18 stomach (8/10 males and 6/10 females) and with mild to moderate leucocytic infiltration of  
19 the mucosa (2/10 males and 1/10 females). In addition, mild mucosal erosion was observed  
20 in the glandular stomach (1/10 males).

## 21 22 Conclusion

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

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Ref.: 24

## 32 **SCCS comment**

33 Macroscopic lesions and microscopic changes in the stomach were observed at the highest  
34 dose tested (1000 mg/kg bw/d) in a 90-day oral repeat-dose toxicity study in rats.  
35 Therefore a local NOAEL of 300 mg/kg bw/d is taken as NOAEL for the oral uptake route.

36  
37 Concerning systemic effects which are of higher relevance for dermal uptake of cosmetics,  
38 the SCCS considers 100 mg/kg bw/d as NOAEL based on clinical signs, motor activity scores  
39 and significantly reduced hindlimb grip strength in female animals at 300 and 1000 mg/kg  
40 bw/d.

41 Clinical signs, mainly hypoactivity and splayed hindlimbs with dragging gait, as observed at  
42 the mid-dose were more pronounced in the high-dose group, and indicate acute treatment-  
43 related effects. To which extent these effects are fully reversible (between daily dosing) was  
44 very poorly documented.

45 Concerning motor activity, some statistically significant variations compared to controls  
46 were observed at the highest dose, further motor activity scores with a trend for lower  
47 values were observed at the highest dose (not statistically significant); thus, observations  
48 from motor activity support the findings from clinical observations.

49 In female animals, hindlimb grip strength exhibited a dose-dependent decrease with  
50 increasing concentration reaching statistical significance ( $p < 0.0001$ ) at 300 and 1000  
51 mg/kg bw/d. The effect can be considered strong (Cohen's  $d$ : -3.066 at 300 mg/kg).  
52 Although histopathology of femoral muscles, brain or sciatic nerves did not indicate  
53 treatment-related changes, clinical signs as well as the results from FOB testing indicate  
54 neurotoxicity. Reductions in hindlimb grip strength are considered adverse due to the  
55 following reasons:

56 (1) the effect is accompanied by other neurological findings (e.g. decreased motor activity  
57 (with dose-dependent tendency), hypoactivity, effects on hindlimb foot splay);

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1 (2) Although the observed clinical signs such as hypoactivity and splayed hindlimbs might  
2 be considered reversible, reversibility of findings from FOB has not been demonstrated  
3 (3) the histopathological procedure as performed in the study (H&E staining) might not be  
4 adequate to reliably detect effects on the nerves and furthermore, nerves other than sciatic  
5 nerves might be of relevance  
6 (4) possible manifestations in the form of neurotransmitter changes have not been  
7 assessed; (5) similar observations pointing to neurotoxicity (e.g. hypoactivity, spread-out  
8 hindlimbs and dragging gait) were also described in an independently performed prenatal  
9 developmental toxicity study in rats.

10  
11  
12 By using Benchmark calculation for reduced hindlimb grip strength in female animals, a  
13 BMD<sub>10%</sub> of 220 mg/kg bw/d and a BMDL<sub>10%</sub> of 110 mg/kg bw/d was calculated.  
14  
15

### 16 3.3.5.3 Chronic (> 12 months) toxicity

17  
18 No data provided.  
19  
20  
21

## 22 3.3.6 Reproductive toxicity

### 23 24 3.3.6.1 Fertility and reproduction toxicity

25  
26 No data provided.  
27

### 28 3.3.6.2 Developmental toxicity

29  
30 Guideline: OECD TG 414 (2001)  
31 Species/strain: Rat, Wistar  
32 Group size: 24  
33 Test substance: R0069279A  
34 Batch: R0069279A 019 D 004  
35 Purity: 95.9 ± 0.5 %  
36 Vehicle: 0.5 % (w/v) carboxymethylcellulose (CMC) in water and 2 % Tween®  
37 80 (v/v)  
38 Dose levels: 0, 100, 300 or 1000 mg/kg bw/day  
39 Dose volume: 10 ml/kg  
40 Controls: Vehicle  
41 Route: oral  
42 Administration: gavage on gestation days (GD) 5 - 19.  
43 GLP: in compliance  
44 Study period: October 2011 - Sep 2013  
45

46 Dose selection was based on the outcome of a GLP-compliant preliminary developmental  
47 toxicity study according to OECD TG 414, repeatedly administering three oral gavage  
48 dosages of HEPB to pregnant Wistar rats on gestation days (GD) 5-19.

49 In the definitive study, HEPB was administered by daily oral (gavage) doses of 0, 100, 300  
50 or 1000 mg/kg/day to mated Wistar female rats (24/group) during the sensitive period of  
51 organogenesis (day 5 through day 19 of gestation, the day of detection of sperm positive  
52 vaginal smear/vaginal plug being designated as gestation day 0 (GD 0)). Maternal  
53 evaluations and measurements included daily clinical signs and body weight/food intake  
54 measured at designated intervals. The dams were killed on GD 20 and subjected to  
55 macroscopic examination. All required litter parameters were recorded and fetuses were  
56 sexed, weighed and submitted to external examination. About one half of the fetuses were

1 also examined for soft tissue anomalies, and remaining fetuses were examined for skeletal  
2 anomalies.

### 3 Results:

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

19 The study authors concluded that HEPB had no teratogenic potential based on the outcome  
20 of this study. The study authors set the maternal and developmental NOAEL at 1000 mg/kg  
21 bw/d.

22  
23 Ref.: 15; 16

### 24 **SCCS Comment**

25 Hypoactivity and effects on hindlimbs were also observed in a 90-day oral repeated-dose  
26 toxicity study (see section 3.3.5.2).

27 Using Fisher's exact test instead of the Chi2, the number of dams with any resorptions is  
28 only significant compared to control animals at 1000 mg/kg. In Chi2, the significant  
29 difference was observed at 300 and 1000 mg/kg. The number of resorptions and  
30 implantation losses calculated with the Kruskal-Wallis test did not show any significant  
31 differences compared to the controls. All means analysed were within historical control  
32 ranges. Altogether, this led the SCCS to conclude that no toxicologically meaningful changes  
33 were observed.

## 36 **3.3.7 Mutagenicity / Genotoxicity**

### 38 **3.3.7.1 Mutagenicity / Genotoxicity *in vitro***

#### 40 **Bacterial Reverse Mutation Test**

41  
42 Guideline: OECD TG 471 (1997)  
43 Test system: *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and  
44 TA1537  
45 Replicates: 2 experiments  
46 Test substance: R0069279A  
47 Batch: R0069279A 019 D004  
48 Purity: 95.9 ± 0.5 %  
49 Concentrations: Experiment I: with and without S9-mix, 0.32, 1.6, 8, 40, 200, 1000  
50 and 5000 µg/plate  
51 Experiment II: with and without S9-mix, 39.06<sup>\*)</sup>, 78.13<sup>\*)</sup>, 156.3,  
52 312.5, 625, 1250, 2500, 5000 µg/plate  
53 Treatment: Experiment I: direct plate incorporation, with and without S9-mix  
54 Experiment II: direct plate incorporation without S9-mix and pre-  
55 incubation with S9-mix  
56 Vehicle: DMSO  
57 Negative Control: DMSO

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1 Positive Control: without S9: 2-nitrofluorene (TA98); sodium azide (TA 100; TA1535);  
2 9-aminoacridine (TA1537), mitomycin C (TA102)  
3 with S9: benzo[a]pyrene (TA98); 2-aminoanthracene (TA100, TA102;  
4 TA1535; TA 1537)  
5 GLP: In compliance  
6 Study period: March – June 2011  
7

8 (\*) Concentration used for strains TA1537 and TA102 in the presence of S9-mix only using  
9 0.05 ml additions  
10

11 The test item was evaluated in two independent experiments in the absence and presence  
12 of metabolic activation (S9-mix from livers of Aroclor 1254 treated rats). Experiment I was  
13 performed according to the direct plating incorporation method, experiment II with S9-mix  
14 was performed according to the pre-incubation method. HEPB was soluble in DMSO. Known  
15 recommended mutagens were used as positive controls; three plates per treatment  
16 condition were used.  
17

#### 18 Results:

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

21 In experiment I, evidence of toxicity ranging from a slight thinning of the background  
22 bacterial lawn and/or a marked reduction in revertant numbers to a complete killing of the  
23 test bacteria was observed at 1000 and/or 5000 µg/plate in strains TA1537 and TA102 in  
24 the absence and presence of S9-mix. In experiment II evidence of toxicity ranging from a  
25 slight thinning in the background bacterial lawn and/or a marked reduction in revertant  
26 numbers to a complete killing of the test bacteria was observed in strains TA98 and TA1537  
27 in the presence of S9-mix and TA102 in the absence of S9-mix at 2500 µg/plate and/or  
28 5000 µg/plate and in strain TA102 at 1250 µg/plate and above in the presence of S9-mix.

29 Concerning mutation, no increases in revertant numbers were observed that were  
30 statistically significant following treatment of all the test strains in the presence or absence  
31 of S9-mix. The positive control chemicals all induced large increases in revertant numbers in  
32 the appropriate strains.  
33

#### 34 Conclusion

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

36 Ref.: 1  
37

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

39  
40 Guideline: OECD TG 476 (1997)  
41 Test system: Mouse lymphoma cell line L5178Y [*hprt* locus]  
42 Replicates: duplicate  
43 Test substance: R0069279A  
44 Batch: R0069279A 019 D004  
45 Purity: 95.9 ± 0.5 %  
46 Concentrations: Experiment I:  
47 without S9-mix: 300, 600, 900, 1100, 1300, 1500, 1900 and 2083  
48 µg/ml  
49 with S9-mix: 300, 600, 1300, 1500 and 1900 µg/ml  
50 Experiment II:  
51 400, 800, 1200, 1400, 1600, 1700, 1800, 1900, 2000 and 2083  
52 µg/ml (with and without S9-mix)  
53 Treatment: Experiment I and II: 3 hour treatment without and with S9-mix; an  
54 expression period of 7 days and a selection period of 12-13 days  
55 Vehicle: DMSO  
56 Negative Control: DMSO, diluted 100-fold in treatment medium  
57 Positive Control: 4-nitroquinoline 1-oxide (without S9-mix)

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1 benzo[a]pyrene (with S9-mix)  
2 GLP: In compliance  
3 Study period: July – August 2011  
4

5 Concentrations selected for the mutation experiments were based on the results of a  
6 cytotoxicity range-finder experiment using 2083 µg/ml as highest concentration; at this  
7 concentration, precipitate was observed, which did not persist during incubation. No marked  
8 changes in osmolality or pH were observed at 2083 µg/ml, but excessive toxicity was  
9 observed at this dose level.

10 Both experiments used a 3-hour treatment. Known mutagens in the presence  
11 (Benzo(a)pyrene, BP) or absence of S9-mix (4-nitroquinoline 1-oxide, NQO) were tested at  
12 two different concentrations and served as positive controls. Negative controls consisted of  
13 cultures treated with the vehicle DMSO diluted 100-fold in the treatment medium.  
14

#### 15 Results

16 In experiment I, the highest concentrations analysed were 2083 µg/ml in the absence of  
17 S9-mix and 1900 µg/ml in the presence of S9-mix, which gave relative survival rates of 14  
18 % and 12 %, respectively. In experiment II, the highest concentration analysed was 2083  
19 µg/ml both in the absence and in the presence of S9-mix, which resulted in relative survival  
20 rates of 49 % and 27 %, respectively.

21 In both experiments no statistically significant increases in mutant frequency were observed  
22 following treatment with any concentration of HEPB tested in the absence or presence of  
23 S9-mix. Two concentrations of the respective positive controls revealed a clear response.  
24

#### 25 Conclusion

26 Under the test conditions used HEPB was not mutagenic in this gene mutation test in  
27 mammalian cells.  
28

29  
30 Ref.: 18

#### 31 **SCCS comment**

32 The required level of toxicity (10-20% relative survival after the highest concentration) was  
33 not reached in experiment II.  
34  
35

#### 36 **In Vitro Micronucleus Test in Cultured Human Lymphocytes**

37  
38 Guideline: OECD TG 487 (2010)  
39 Test system: lymphocyte cultures from the pooled blood of two male donors  
40 Replicates: duplicate (four replicates for vehicle)  
41 Test substance: R0069279A  
42 Batch: R0069279A 019 D 004  
43 Purity: 95.9 ± 0.5 %  
44 Concentrations: Experiment I: without S9-mix 1500, 1900, 1980 and 2020 µg/ml  
45 Experiment II: with S9-mix 1500, 1780, 1860 and 1900 µg/ml  
46 Experiment III: without S9-mix 100, 250, 300 and 350 µg/ml  
47 Treatment: Experiment I: 3 h treatment  
48 Experiment II: 3 h treatment  
49 Experiment III: 24 h treatment  
50 Vehicle: DMSO  
51 Negative control: Vehicle  
52 Positive controls: Mitomycin C (MMC) (without S9-mix)  
53 Vinblastin (VIN) (without S9-mix)  
54 Cyclophosphamide (CPA) (with S9-mix)  
55 GLP: In compliance  
56 Study period: March - April 2011  
57

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1  
2 HEPB was assayed for the induction of micronuclei in cultured human peripheral blood  
3 lymphocytes from the pooled blood from two male volunteers in the absence and presence  
4 of metabolic activation (S9-mix prepared from the livers of Aroclor 1254-treated rats). The  
5 highest concentration in each test condition was selected on the basis of solubility and  
6 cytotoxicity criteria.  
7 Duplicate cultures were treated with each concentration of HEPB or with known clastogens  
8 in the presence (cyclophosphamide) or absence of S9-mix (mitomycin C and vinblastine).  
9 Vehicle-treated cultures (DMSO, four replicates) were used as negative controls.  
10 Blood cultures were incubated in the presence of the mitogen phytohaemagglutinin (PHA)  
11 for 48 hours and then treated for 24 or 3 hours in the absence or presence of S9-mix,  
12 respectively. Cells were harvested 72 hours after the beginning of incubation. Cytochalasin  
13 B was added after the 3-hour treatments or before the 24-hour treatments. Lymphocyte  
14 preparations were stained and examined microscopically for determining the replication  
15 index (RI) and the proportion of micronucleated binucleated (MNBN) cells when selected.  
16 Where possible, 2000 binucleate cells per concentration (one thousand from each replicate)  
17 were analysed blindly.

18  
19 Results

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

32  
33 Conclusion

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

37  
38  
39 Ref.: 17

#### 39 **SCCS comment**

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

#### 45 46 **SCCS conclusion on mutagenicity**

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

53  
54

55 3.3.6.2 Mutagenicity / Genotoxicity <i>in vivo</i>
---

56  
57 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 <sup>+</sup>
Source of light:	Atlas CPS+ equipped with 2 anti-UVB filters
UVA irradiance:	about 1.7 mW/ cm <sup>2</sup>
Irradiation dose:	5 J/cm <sup>2</sup>
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 <sup>+</sup>
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<sup>2</sup>. 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<sup>2</sup> displayed 90 % and 75 % viability, respectively, compared to non-irradiated cells. In the preliminary cytotoxicity test, no concentration leading to 50 % mortality (IC<sub>50</sub>) 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.

1 Conclusion

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

5 Ref.: 5

6  
7 **SCCS comment**

8  
9 In view of the absence of indications of phototoxicity at 1000 µg/ml, the SCCS agrees that  
10 under the conditions of the test, phototoxicity of HEPB is unlikely.

11  
12  
13 **3.3.9.2 Phototoxicity / photomutagenicity / photoclastogenicity**

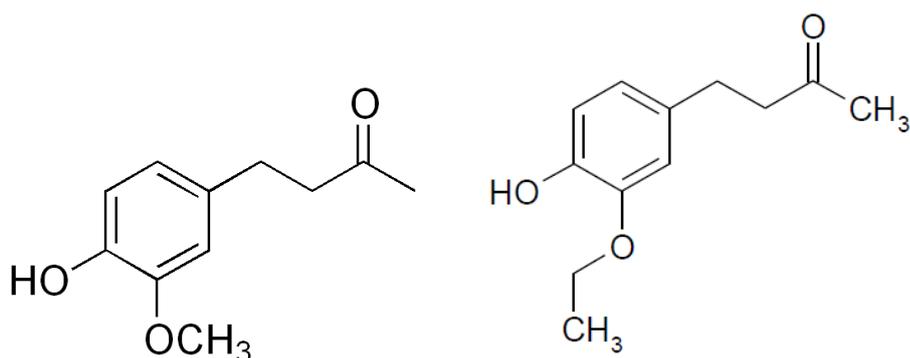
14 No data provided.

15  
16  
17  
18 **3.3.10 Human data**

19 No data provided.

20  
21  
22 **3.3.11 Special investigations**

23  
24 Excretion and metabolism of zingerone, a structurally similar compound to HEPB was  
25 described in a publication from the open literature. The structural difference between  
26 zingerone (4-(4-Hydroxy-3-methoxyphenyl)butan-2-one; CAS 122-48-5) and  
27 ethylzingerone (HEPB) consists in a methylene group in the alkoxy-chain at the aromatic  
28 ring of the molecules. Therefore, results obtained from zingerone may allow conclusions to  
29 be drawn on the excretion and metabolism of HEPB.



34 Zingerone

35 Ethylzingerone (HEPB)

36  
37 After oral administration of a single dose of 100 mg/kg bw of zingerone to male albino  
38 (Wistar-derived) rats, urine and faeces were collected in 24 hr periods. For identification of  
39 metabolites, free and total fraction (after hydrolysis) were analysed. Within 24 hours, a  
40 mean of 95 % of the applied dose had been excreted via urine. A mean of 56 % of the  
41 applied dose was excreted unmetabolised (i.e. as parent compound), the remainder was  
42 excreted as metabolites (most prominent identified metabolites: zingerol (11 % of the  
43 applied dose; 4-(3,4-dihydroxyphenyl)butan-2-one (6%) and homovanillic acid (5%). In 24  
44 hr – 48 hr urine samples, only traces of zingerone and zingerol were detected. These results

1 demonstrate that zingerone is almost completely absorbed, metabolised and readily  
2 excreted after oral intake.

3  
4 Ref.: 19

### 5 3.4 Exposure assessment

6  
7 No data provided.

### 9 3.5 Safety evaluation (including calculation of the MoS)

#### 13 CALCULATION OF THE MARGIN OF SAFETY

#### 16 3.5.1 Aggregate exposure from the use as a cosmetic preservative in rinse-off, 17 oral care and leave-on cosmetic products

18  
19 Absorption through the skin (default) DAp (%) = 50 %  
20 Amount of cosmetic product applied daily A (g/d) = 17.4 g/d  
21 Concentration of ingredient in finished product C (%) = 2 %  
22 Typical body weight of human = 60 kg  
23 Systemic exposure dose (SED) =  
24  $A \text{ (g/d)} \times 1000 \text{ mg/g} \times C \text{ (\%)} / 100 \times Dap \text{ (\%)} / 100 / 60$  = 2.9 mg/kg  
25 bw/d

26  
27 No adverse observed effect level NOAEL = 100 mg/kg bw/d  
28 (systemic NOAEL, 90d oral repeat dose toxicity study, rat)  
29 No adjustment, 100 % oral absorption

MOS	NOAEL/SED = 34.5
-----	------------------

#### 33 3.5.2 Oral care cosmetics

34  
35 For oral hygiene products, the relative calculated daily exposure to cosmetic product is 34.7  
36 mg/kg bw/d (2.16 mg/kg bw/d for toothpaste (adults) and 32.54 mg/kg bw/d for  
37 mouthwash), according to the 9<sup>th</sup> 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	0.694	100	144.1
Toothpaste (adults)	0.043	100	2326
Mouthwash	0.651	100	153.6

39 No adjustment, 100 % oral absorption

1 **3.5.3 Dermally applied cosmetic products**  
2

Product type	SED [mg/kg bw/d]	NOAEL [mg/kg bw/d]	MoS
All types of products	2.52	100	39.7
Rinse-off skin & hair cleansing products	0.09	100	1111
Leave-on skin and hair care products	2.24	100	44.6
Make-up products	0.19	100	526.3
Leave-on skin and hair care products and make-up products combined	2.42	100	41.3

3  
4 **The following parameters were used to determine SED for dermally applied**  
5 **cosmetic products:**  
6

7  
8 **Concentration of ingredient in finished product C (%) = 2 %**  
9 **Typical body weight of human = 60 kg**

10 **Systemic exposure dose (SED) =**  
11  **$A \text{ (g/d)} \times 1000 \text{ mg/g} \times C \text{ (\%)} / 100 \times D_{ap} \text{ (\%)} / 100 / 60$**   
12 **bw/d**

13  
14 **No adverse observed effect level NOAEL = 100 mg/kg bw/d**  
15 **(systemic NOAEL, 90d oral repeat dose toxicity study, rat)**  
16 **No adjustment, 100 % oral absorption**  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

**Exposure calculated according to Table of the SCCS NoG, 9<sup>th</sup> 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
	Eyeliners	0.005	0.08
Oral care cosmetics	Toothpaste	0.14	2.16
	Mouthwash	2.16	32.54
<b>TOTAL</b>		<b>± 17.4</b>	<b>269</b>

**3.6 Discussion*****Physico-chemical properties***

Depending on the temperature, hydroxyethoxyphenyl butanone 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  $8.7 \times 10^{-3}$  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 2%.

***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 1 000 mg/kg/d (i.e. the substance is of low acute oral toxicity).

***Local toxicity***

Based on an in vitro Episkin<sup>SM</sup> Skin Irritation Test performed with the neat substance, HEPB can be considered as potentially non-irritant to the skin.

1 Data on mucous membrane irritation based on the Bovine Corneal Opacity and Permeability  
2 (BCOP) Test Method are inconclusive.

### 3 4 ***Skin Sensitisation***

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6 HEPB is not considered to be a skin sensitiser.

### 7 8 ***Dermal absorption***

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10 The in vitro percutaneous absorption of HEPB by using a typical lipophilic skin care  
11 formulation containing HEPB at the concentration of 2% was determined in human  
12 dermatomed skin. A recovery of at least 85 % was only obtained for 4 cells evaluated. The  
13 number of 4 evaluable cells is not in line with the SCCS criteria. Further, the in vitro  
14 percutaneous absorption has not been determined in aqueous cosmetic formulation.

15 Based on significant deviations from requirements, the SCCS considers the study not  
16 acceptable. Instead, in the absence of adequate experimental data, a default value of 50 %  
17 dermal absorption is taken for MOS calculation according to SCCS 1564/15.

### 18 19 ***Repeated dose toxicity***

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21 Macroscopic lesions and microscopic changes in the stomach were observed at the highest  
22 dose tested (1000 mg/kg bw/d) in a 90-day oral repeat-dose toxicity study in rats.  
23 Therefore a local NOAEL of 300 mg/kg bw/d is taken as the NOAEL for the oral uptake  
24 route.

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

### 32 33 ***Mutagenicity***

34  
35 HEPB was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene  
36 mutations, structural and numerical chromosome aberrations. HEPB did not induce gene  
37 mutations in bacteria nor in mammalian cells when evaluated at the hprt locus. Exposure of  
38 human lymphocytes with HEPB did not result in an increase of micronucleated binucleated  
39 cells. Based on the present available tests, HEPB can be considered to have no genotoxic  
40 potential and additional tests are unnecessary.

### 41 42 ***Carcinogenicity***

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### 45 46 ***Reproductive toxicity***

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

### 50 51 ***Toxicokinetics***

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

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**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**

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**4. CONCLUSION**

- 1. In light of the data provided, does the SCCS consider safe 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 2.0 % under an aggregate exposure scenario for cosmetics?*

Based on the information provided by the applicant, 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 2.0 % under an aggregate exposure scenario for cosmetics is not considered safe.

It is of note that a maximum concentration of 2.0 % under an aggregate exposure scenario for cosmetics could be considered safe if more reliable and supporting data on *in vitro* dermal penetration was provided.

- 2. Alternatively, what is, according to the SCCS, the maximum target concentration considered safe for use of Hydroxyethoxyphenyl Butanone (HEPB) as a cosmetic preservative in rinse-off, oral care and leave-on cosmetic products under an aggregate exposure scenario for cosmetics?*

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.

- 3. Does the SCCS have any further scientific concerns regarding the use of Hydroxyethoxyphenyl Butanone (HEPB) in cosmetic products?*

The SCCS does not have any other concerns.

**5. MINORITY OPINION**

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## 6. REFERENCES

Toxicity References *in italics* are not submitted as full reports in the present dossier. They consist of reports of dose-range finding studies that can be provided upon request and of documents/guidelines that can be downloaded from the internet (URL provided).

1. 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
2. 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<sup>SM</sup> model; EPISKIN Study No 11-BPL-1065
3. 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
4. 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
5. 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.
6. 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.
7. Craig S (2014b). Hydroxyethoxyphenyl Butanone (R0069279A): Assessment of Recovery of Radiolabelled R0069279A in a Skin Care Formulation. Study No 793009.
8. Dekant W (2013). Expert review. Scientific opinion on the NOAEL of ethylzingerone to be used in safety assessment.
9. Dekant W (2014). Expert review. Maximum safe concentrations of 4-(3-ethoxy-4-hydroxyethoxyphenyl butanone; common name, ethylzyngerone) when used as a preservatives in cosmetic formulations.
10. ECVAM <http://ecvam.jrc.it/> Method Validation – Regulatory Acceptance – Skin Irritation
11. ECVAM <http://ecvam-dbalm.jrc.ec.europa.eu/> Protocols: Permeability Assay on Caco-2 Cells DB-ALM Protocol n° 142.
12. EFSA (2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission related to Flavouring Group Evaluation 22: Ring-substituted phenolic substances from chemical groups 21 and 25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000) Flavouring Group Evaluation 22: Ring-substituted

- 1 phenolic substances from chemical groups 21 and 25. QUESTION N° EFSA-Q-2003-  
2 165. The EFSA Journal. Vol 393. pp 1-78  
3  
4 13. Kroes (2007) Application of the Threshold of toxicological concern (TTC) to the  
5 safety evaluation of cosmetic ingredients. Food and Chemical Toxicology; 2007; Vol.  
6 45, pp. 2533-2562.
- 7 14. Latha M (2013a). Hydroxyethoxyphenyl Butanone (R0069279A, Batch  
8 R0069279A 019 D 004): Prenatal Developmental Toxicity Study of R0069279A in  
9 Wistar Rats by Oral Route. Advinus Study No. G7939.
- 10  
11 15. Latha M (2013b). Hydroxyethoxyphenyl Butanone (R0069279A, Batch  
12 R0069279A 019 D 004): Preliminary Prenatal Developmental Toxicity Study of  
13 R0069279A in Wistar Rats by Oral Route. Advinus Study No. G7938.
- 14  
15 16. Lloyd M (2011). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A  
16 019 D 004): Induction of Micronuclei in Cultured Human Peripheral Blood  
17 Lymphocytes. Covance Study No. 8244262
- 18  
19 17. Lloyd M (2012). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A  
20 019 D 004): Mutation at the *hprt* locus of L5178Y Mouse Lymphoma Cells using the  
21 Microtitre® Fluctuation Technique. Covance Study No. 8250242
- 22  
23 18. Monge P (1976). The metabolism of Zingerone, a pungent principle of ginger.  
24 Xenobiotica. Vol. 6. No 7. pp 411-423
- 25  
26 19. Ravi M (2013). Hydroxyethoxyphenyl Butanone (R0069279A, Batch R0069279A  
27 019 D 004): 14-Day Repeated Dose Toxicity Study of R0069279A in Wistar Rats by  
28 Oral Route. Advinus Study No. G7935.
- 29  
30 20. SCCS (2012). The SCCS'S notes of guidance for the testing of cosmetic  
31 substances and their safety evaluation 8th revision. SCCS/1501/12. Available at  
32 [http://ec.europa.eu/health/scientific\\_committees/consumer\\_safety/docs/sccs\\_s\\_006](http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_006.pdf)  
33 [.pdf](http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_006.pdf)
- 34  
35 21. Tang J (2014). Hydroxyethoxyphenyl Butanone (R0069279A): ADME-Tox Study  
36 of R0069279A. Study No 100016337.
- 37  
38 22. Verma R (2011). Hydroxyethoxyphenyl Butanone (R0069279A, Batch  
39 R0069279A 019 D 004): Skin Sensitization Study of R0069279A by Local Lymph  
40 Node Assay in Mice. JRF Study No. 409-1-01-2126
- 41  
42 23. Yogesha BN (2012). Hydroxyethoxyphenyl Butanone (R0069279A, Batch  
43 R0069279A 019 D 004): 90-Day Repeated Dose Toxicity Study of R0069279A in  
44 Wistar Rats by Oral Route. Advinus Study No. G7937
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## Appendix

### 6.1.1.1

#### 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); [ <sup>14</sup> 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 [ <sup>14</sup> C]-R0069279A in a cosmetic formulation; 105.85 % of target (2.0 % (w/w))
Replicates:	8
Exposed membrane area:	3.14 cm <sup>2</sup>
Dose applied:	ca. 2.0 mg/cm <sup>2</sup>
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 dated 23 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.

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1 Results:

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

2  
3 The mean mass balance for the aluminium foil samples terminated at 0 h post dose was  
4 94.20% of the applied dose and this decreased to 64.01% at 24 h post dose. The results  
5 showed a loss of [14C]-R0069279A when applied in Test Preparation 819181 (2%, w/w) to  
6 aluminium foil. Recovery at 24 h post dose was variable ranging from 54.54% to 74.57% of  
7 the applied dose. Recovery was less variable at 0 h post dose ranging from 86.92% to  
8 98.48% of the applied dose. This confirmed that there were losses during the processing of  
9 the recovery samples.