Scientific Committee on Consumer Safety

SCCS

OPINION ON
the safety of Butylphenyl methylpropional (p- BMHCA) in cosmetic products
- Submission II -

The SCCS adopted this Opinion by written process

on 14 December 2017

About the Scientific Committees

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

These Committees are the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and are made up of scientists appointed in their personal capacity.

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

SCCS

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

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All Declarations of Working Group members are available on the following webpage:
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1. BACKGROUND

The substance 2-(4-tert-Butylbenzyl)propionaldehyde (BMHCA, Lysmeral) CAS No. 80-54-6 with INCI name Butylphenyl methylpropional is a fragrance ingredient used in many compounds for cosmetic products as well as in non-cosmetic products.

Butylphenyl methylpropional (BMHCA) is currently regulated for labelling purposes in Annex III entry 83 of the Cosmetics Regulation No 1223/2009 when present in a concentration above 10 ppm for leave-on products and above 100 ppm for rinse-off products.

Following a proposal for a harmonised classification as Toxic for Reproduction 2 substance under Regulation (EC) No 1272/2008, a dossier on the safety assessment of BMHCA was submitted to the Commission by the International Fragrance Association (IFRA) in April 2013 (Submission I).

The SCCS issued the opinion in 2015 (SCCS/1540/14 Revision of 16 March 2016) on the safety of Butylphenyl methylpropional (BMHCA) in cosmetic products concluding that:

"The SCCS is of the opinion that BMHCA is not safe for use as fragrance ingredient in cosmetic leave-on and rinse-off type products, neither at concentration limits according to the ones set up by IFRA in 2013 (MoS = 3.6) nor at concentration limits as set up by IFRA in the revised proposal that has been submitted in 2015 belatedly (MoS = 53). In addition, no firm conclusion could be drawn on mutagenicity.

BMHCA poses a risk of inducing skin sensitisation in humans."

In March 2017, IFRA submitted to the Commission services a new safety dossier on p-BMHCA (p-Lysmeral) Submission II to address the concerns expressed by the SCCS. The dossier clearly aims to defend the use of para-isomer distinguishing between para- and meta-Lysmeral, since the SCCS addressed critics on the impurities present in BMHCA, amongst which meta-Lysmeral is a critical one.

This dossier also includes a revised proposal for maximum use levels of p-BMHCA in the finished cosmetic product types as follows:

Product types	Finished product concentration (%)
Hydroalcoholic-based fragrances (e.g. Eau de Toilette, perfume, Aftershave, Cologne)*	1.42
Deodorants	0.09
Make up products (e.g. eye make-up, make-up remover, liquid foundation, mascara, eyeliner)	0.04
Face cream	0.05
Hand cream	0.05
Body lotion	0.06
Hair styling	0.04
Bath cleansing products (e.g. soaps, shower gel, rinse-off conditioner, shampoo)	0.1
*Maximum finished product concentration for hydroalcoholics on shaved skin is 0.6%	

2. TERMS OF REFERENCE

1. *Does the SCCS consider Butylphenyl methylpropional (p-BMHCA) safe for use as a fragrance ingredient in cosmetic leave-on and rinse-off type products in a concentration limit(s) according the ones set up by IFRA as reported above?*

2. *Does the SCCS have any further scientific concerns with regard to the use of Butylphenyl methylpropional (p-BMHCA) as a fragrance ingredient in cosmetic leave-on and/or rinse-off type products?*

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Butylphenyl methylpropional

3.1.1.2 Chemical names

IUPAC name: 3-(4-*tert*-Butylphenyl)-2-methylpropanal
EC name: 2-(4-*tert*-Butylbenzyl)propionaldehyde
Benzenepropanal, 4-(1,1-dimethylethyl)-alpha-methyl-Butylphenyl methylpropional
para-tert-Bucinal; 2-(4-tert-Butylbenzyl) propionaldehyde;
para-t-Butyl-α-methyl-hydrocinnamaldehyde
α-Methyl-β-(p-t-butylphenyl)propionaldehyde

Ref.: BASF SE, 2014, 2015b, SMII: 3, 5

3.1.1.3 Trade names and abbreviations

Lilestralis
Lilial®
Lysmeral®Extra
BMHCA

Other names such as:
Lilyal
NSC 22275
pt-bucinal

Source: European Chemicals Agency, http://echa.europa.eu

Ref.: BASF SE, 2014, 2015, 2015b, SMII: 3, 4, 5

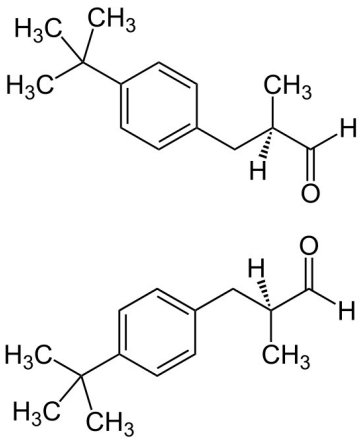
3.1.1.4 CAS / EC number

CAS: 80-54-6, containing two enantiomers, namely (2S)-3-(4-tert-butylphenyl)-2-methyl-propanal (75166-30-2) and (2R)-3-(4-tert-butylphenyl)-2-methylpropanal (CAS 75166-31-3)

EC: 201-289-8

Ref.: BASF SE, 2015b, SMII: 5

3.1.1.5 Structural formula



Lysmeral®Extra is always a racemic mixture covering two enantiomers, namely (2S)-3-(4-tert-butylphenyl)-2-methyl-propanal and (2R)-3-(4-tert-butylphenyl)-2-methylpropanal. The synthesis and isolation of the pure enantiomers is difficult due to the fact, that Lysmeral is an α-chiral aldehyde (asymmetric secondary carbon atom that is a close neighbour to the carbonyl group). The pure enantiomers would easily racemize after isolation via keto-enol tautomerism.

Ref.: Dossier BASF-IFRA

3.1.1.6 Empirical formula

Formula: $C_{14}H_{20}O$

3.1.2 Physical form

Physical state at 20°C (1013 hPa): liquid, colourless to pale yellow; odour: mildly floral, reminiscent of cyclamen and lily of the valley.

3.1.3 Molecular weight

Molecular weight: 204.31

3.1.4 Purity, composition and substance codes

The degree of para-Lysmeral (CAS 80-54-6) in BASF's quality Lysmeral®Extra is specified to be ≥ 99.0% (Reference: BASF 2010 SMII: 2). Analyses of the purity are constantly performed during production (Reference: BASF 2016 SMII: 33) and additionally before the conduct of toxicological studies (References: BASF 2014, 2015, 2016 SMII: 3, 4, 33).

Main constituent in Lysmeral®Extra, as outlined in the Certificates of Analysis:

Main constituent	Typical concentration	Remarks
para-Lysmeral *2-(4-tert-butylbenzyl)-propionaldehyde* *CAS 80-54-6*	ca. 99.4% (BASF 2013, 2016 SMII: 32, 33)	Specification: ≥ 99.0% (BASF 2010 SMII: 2)

SCCS Comment
The applicant used GC-FID method with two different GC columns (DB-1 and DB-1701) for the peak purity evaluation of the BATCH AP13-105. Peak purity was calculated based on % of area measurements to be 99.4%. Certificates of Analysis have been provided for the rest of the batches.

3.1.5 Impurities / accompanying contaminants

According to the applicant several known and unknown impurities are constantly analysed during the manufacturing process and documented in the Certificates of Analysis (Reference: BASF 2016 SMII: 33). Among the known impurities, special attention is given to the meta isomer 3-(m-tert-Butylphenyl)-2-methylpropionaldehyde (CAS 62518-65-4), which was self-classified by BASF as CMR 1B in 2011 and which has since then been subject to rigorous concentration restriction (< 0.1%) (References: BASF 2010, 2013, 2016 SMII: 2, 33, 34). TBA (4-tert-Butylbenzoic acid) is the direct autoxidation product of para-Lysmeral, which may be formed in the presence of oxygen. However, since alpha-Tocopherol (CAS 59-02-9) is added as a stabilizer directly after the production process (References: BASF 2016, 2017 SMII: 5, 36), only low concentrations of the corresponding acid are found in Lysmeral®Extra.

Ref.: BASF 2016 SMII: 33

Impurities in Lysmeral®Extra, as outlined in the Certificates of Analysis (Reference: BASF 2016 SMII: 33)

Impurity	Typical concentration	Remarks
non-specified impurities	ca. 0.1%	No further information on chemical identity available
meta-Lysmeral *3-(m-tert-Butylphenyl)-2-methylpropionaldehyde* *CAS 62518-65-4*	< 0.1% (SMII: 33, 34)	Specification: < 0.1% (SMII: 2)
Lysmerol *3-(p-tert-Butylphenyl)-2-methylpropanol* *CAS 56107-04-1*	< 0.2 % (SMII: 33)	
Ethanol *CAS 64-16-5*	ca. 0.2% (SMII: 33)	
TBA acid *4-tert-Butylbenzoic acid* *CAS 98-73-7*	< 0.01% (SMII: 33)	
TBA ester *Methyl 4-tert-butylbenzoate* *CAS 26537-19-9*	ca. 0.01% (SMII: 33)	
Alpha-Tocopherol *CAS 59-02-9*	200 ppm (SMII: 5, 36)	Added as antioxidant

SCCS comment
The applicant proceeded with chemical characterisation of the impurities using a GC-EI/MS method (BASF-Study No13L00139, SMII 32). The applicant has self-classified Meta-Lysmeral as a CMR 1B (Repr 1B) substance and therefore subjects it to rigorous

concentration restriction (< 0.1%) and surveillance. According to the analytical data provided, the Meta-Lysmeral content is < 0.1%.

3.1.6 Solubility

Water solubility: 33 mg/L at 20°C ("Flask method", OECD Guideline#105)

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow} = 4.2 (24°C, HPLC, 7, OECD Guideline#117)

3.1.8 Additional physical and chemical specifications

Melting point: <–20°C (1013 hPa)
Boiling point: 279.5°C (1013 hPa)
Flash point: 118°C
Vapour pressure: 0.0025 hPa at 20°C
Density: 0.94 at 20°C
Viscosity: 3 mm^2/sec at 23°C
pKa: Substance without any ionic structure
Refractive index: 1.503 – 1.507 at 20°C
UV_Vis spectrum: λ_{max} ≈ 263 nm

SCCS comment
The reported data do not conform with the following data reported in the literature:
- Flash point according to SMI 14 BASF 2011 a MSDS it is 79°C (Directive 92/69/EEC, A.9, closed cup).
- Viscosity according to SMI 14 BASF 2011 a MSDS it is: dynamic 12.3 mPa.s and kinematic 13 mm^2/s at 20°C and 6.01 mm^2/s at 40°C.

3.1.9 Homogeneity and Stability

General Comments to physicochemical characterisation

Further information (ECHA data dossiers): In aqueous solution and in the presence of air at pH 7 and 25°C, Lilial® (BMHCA) undergoes significant oxidation (about 30% during a period of 168 h). Thus, it can be assumed that BMHCA has a rather short life in the environment (around two weeks) and that its oxidation product, lilic acid (lysmerylic acid), is the major component to be considered in an environmental risk assessment. Given its rapid oxidation at ambient air conditions, it is furthermore reasonable to assume that BMHCA is unlikely to preserve its high purity of ≥99.5% (w/w) when being applied in toxicological studies.

Lysmeral®Extra is prevented from auto-oxidating to the corresponding acid by alpha-tocopherol, which is present in the final product at 200 ppm (References: BASF 2016, 2017 SMII: 5, 6, 35). The shelf life of Lysmeral®Extra is 730 days at 25°C (References: BASF 1998 SMII: 34). Moreover, the stability of Lysmeral®Extra as a test item is analytically monitored during the conduct of toxicological studies to make sure that only high purity substances are used throughout the experiment.

3.2 Function and uses

According to CLH Report, BASF SE, 30.9.2013:
"Lysmeral (2-(4-*tert*-butylbenzyl)propionaldehyde) is used as a fragrance in a wide number of industries. It has an intensive, radiant, floral odour with a typical lily-of-the-valley note. As a component of fragrance mixtures, the main uses include cosmetic/personal care

products and washing/cleaning products. Lysmeral may also be included as a fragrance substance in hair care products, biocidal products, coatings and paints, fillers/plasters, ink/toners, polishes/wax blends and scented articles (clothes, eraser, toys, paper articles)."

According to IRSC/IFRA Dossier, 28.3.2013:
"BMHCA (2-(4-*tert*-butylbenzyl)propionaldehyde) is a fragrance ingredient used in many compounds for dermal application in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries, as well as in non-cosmetic products such as household cleaners and detergents. BMHCA is not used in flavour applications."

According to BASF/IFRA Dossier, 24.2.2017:
"2-(4-*tert*-Butylbenzyl)propionaldehyde (BMHCA, Lysmeral) CAS No. 80-54-6 is a fragrance ingredient used in many compounds for cosmetic products as well as in non-cosmetic products such as household cleaners and detergents.
The proposed maximum use levels of BMHCA in the finished cosmetic product types are as follows:

Product types	Finished product concentration (%)
Hydroalcoholic-based fragrances (e.g. Eau de Toilette, perfume, aftershave, cologne)*	1.42
Deodorants	0.09
Make up products (e.g. eye make-up, make-up remover, liquid foundation, mascara, eyeliner)	0.04
Face cream	0.05
Hand cream	0.05
Body lotion	0.06
Hair styling	0.04
Bath products (e.g. soaps, shower gel, rinse-off conditioner, shampoo)	0.1
* Maximum finished product concentration for hydroalcoholics on shaved skin is 0.6%	

BMHCA is not used in flavour applications (Reference: BASF SE, 2016, SMII: 6) nor in lipstick, toothpaste or mouthwash products (Reference: IFRA 2015b, SMII: 19)."

3.3 Toxicological evaluation

3.3.1 Acute toxicity

From submission I

SCCS conclusion on acute toxicity

Acute toxicity after all relevant routes of application of BHMCA was investigated in rats and rabbits (oral, dermal, inhalation). The acute oral LD_{50} value in rats was determined to be 1390 mg/kg bw and the acute dermal LD_{50} value in rabbits >2000 mg/kg bw. Thus the acute toxicity of BMHCA can be considered moderate (oral route). An inhalation toxicity test in rats led to no mortalities but signs of systemic toxicity after exposure to a BMHCA saturated atmosphere continued to be observed for 7 hours. However, the assessment of inhalation toxicity on the basis of this study is limited due to the low volatility of BMHCA (vapour pressure: 0.0025 hPa at 20°C).

3.3.1.1 Acute oral toxicity

From submission I

SCCS overall comment on acute oral toxicity

The acute oral toxicity (LD_{50}) in rats was determined at 1390 mg/kg bw (95% confidence limits: 1019 – 1867 mg/kg bw).

Additional data from Applicant's submission II dossier

In a non-GLP, non-guideline study, the test substance was administered orally to each of 10 rats at dose levels of 1220; 2470; 5000; 10140 mg/kg bw. The animals were observed for treatment-related effects for a 14-day observation period. There were no deaths at 1220 mg/kg bw. One rat died at 2470 mg/kg bw and seven died at 5000 mg/kg bw. The highest dose was lethal for all animals. The acute oral toxicity (LD_{50}) was 3700 mg/kg bw (95% confidence limits: 2600 – 5400 mg/kg bw).

Ref.: MB Research Laboratories, 1977, SMI: 75, #1695

A further non-GLP, non-guideline screening study was conducted on groups of 2 rats (1/sex). The animals were administered, by gavage, BMHCA in vegetable oil at dose levels of 100, 500, 1000, 2000, and 5000 mg/kg bw. Observations were conducted for 13 days. One death occurred in the 2 highest dose groups. The study findings suggested that the oral LD_{50} of BMHCA in rats ranged between 1000 - 2000 mg/kg bw.

Ref.: Bush Boake Allen, 1980a, SMI: 18, #52291

3.3.1.2 Acute dermal toxicity

Additional data from Applicant's submission II dossier

There was no additional data that would have impacted the SCCS's previous conclusion (SCCS/1540/14).

3.3.1.3 Acute inhalation toxicity

Additional data from Applicant's submission II dossier

No additional data

3.3.1.4 Acute intraperitoneal toxicity

Additional data from Applicant's submission II dossier

The acute toxicity after intraperitoneal injection was investigated in the range-finding part of a guideline (OECD 474, ICH) mouse micronucleus study under GLP conditions. ICR Mice in groups of 5/sex/dose received single intraperitoneal injections of BMHCA (lot 9000349505) in corn oil at 300, 500, 700 or 1000 mg/kg bw each. Animals were observed for clinical signs after injection and daily thereafter for 3 days. Lethargy and piloerection were observed at all doses. The mice exhibited prostration, irregular breathing and crusty eyes in the two highest dose groups. Convulsions occurred at 1000 mg/kg bw, and all mice at this dose died by the third day of the study. No deaths occurred in other dose groups.

Ref.: RIFM 2000b, SMI: 91, #35691

In a GLP-compliant non-guideline study, NMRI mice (5 per sex and dose) were treated by intraperitoneal injections of BMHCA in carboxymethyl cellulose at 200 or 700 mg/kg bw. The animals were observed daily for clinical signs for 14 days following administration. Body weight determination was performed at regular intervals and gross pathology was performed. Half of the animals died in the 700 mg/kg bw dose group (4/5 males and 1/5 females) and no mortality was observed in the 200 mg/kg dose group. Unspecific signs in the form of dyspnea, apathy, staggering, spastic gait, rough fur coat and poor general condition were observed in both dose groups and body weight loss, abnormal position, twitching, tremor, tonic convulsions, skin erythema, and dehydration were found in the high-dose animals only.

Ref.: BASF SE 1981, SMI: 3, #63831

3.3.2 Irritation and corrosivity

From submission I

SCCS conclusion on irritation

Under the conditions tested, BMHCA as neat compound was revealed to be irritating to the skin and eyes of rabbits. In addition, 2% BMHCA in propylene glycol led to mild skin erythema; however, the scoring of the solvent was comparable. In general, the observed effects occurred transiently and were reversible. In a special investigation, BMHCA also displayed the potential of inducing respiratory irritation.

3.3.2.1 Skin irritation

Additional data from Applicant's submission II dossier

In a non-GLP and non-guideline study, occlusive dermal application of neat BMHCA on 2-3 rabbits for 5 minutes, 2 or 24 hours resulted in desquamation in all animals at the end of the observation period (8 days) for all exposure periods. Questionable to slight edema (reversible for all exposure periods) and erythema (reversible for 5 min exposure period) were observed. Longer exposure periods led to persisting erythema at the end of the observation period.

Ref.: BASF SE 1981, SMI: 3, #63831

3.3.2.2 Mucous membrane irritation / Eye irritation

Additional data from Applicant's submission II dossier

In a non-GLP and non-guideline study, undiluted BMHCA was applied into one eye of 3 rabbits, each without washing out after application, and animals were observed daily for 72 hours. Slight conjunctival redness was found in all animals 24 hours after application and in 1 of 3 animals 48 hours after application, resulting in a mean score of 0.7 over all animals and observation time points. No adverse findings, i.e. chemosis, iritis or corneal opacity, were observed at any time point.

Ref.: BASF SE, 1981, SMI: 3, #63831

SCCS conclusion on irritation

The data on irritation potential of BMHCA provided in Submission II do not change the SCCS's previous conclusion (SCCS/1540/14).

3.3.3 Skin sensitisation

From submission I

SCCS conclusion on skin sensitisation

BMHCA was comprehensively tested in experimental animals, mostly according to guideline procedures and under GLP conditions. Several positive LLNA resulted in EC3 values indicative for sensitisation. Depending on the solvent, the EC3 values ranged from 2.97% (in EtOH) to 13.91% (in 25% EtOH/75% DEP), and up to 18.7% by application of BMHCA in acetone/olive oil (4:1). Another LLNA with EtOH as vehicle showed SI>3 for all tested doses of BMHCA (10, 25, 50, 100%). An EC3 value of about 2.9% BMHCA in the LLNA has been substantiated by data from the International Fragrance Association directly submitted to SCCS in 2009 (SCCS, 2012). By contrast, GPMTs performed were contradictory and thus ambiguous. Finally, dermal reactions have been observed in a KAO test in guinea pigs.
Based on the animal data obtained, the overall potency classification of BMHCA is a "moderate sensitiser" (Basketter et al., 2005; SCCP, 2005 and 2012).

Local Lymph Node Assay (LLNA)

Additional data from Applicant's submission II dossier

No additional data.

Guinea pig maximization test (GPMT)

Additional data from Applicant's submission II dossier

A guinea pig maximization test was performed on 10 Hartley Dunkin guinea pigs. In the intradermal induction phase, neat BMHCA was injected and for topical induction a filter paper patch saturated with neat BMHCA was applied for 48 hours under occlusion. For topical challenge 2 weeks after the topical induction, a filter paper saturated with BMHCA was applied for 24 hours under occlusion. The challenge concentrations included the neat material and 50% in mineral oil. One concentration was applied to each flank. After patch removal, only limited signs of irritation in individual tests and control group animals were observed, but there was no indication of skin sensitisation.

Ref.: Bush Boake Allen, 1980b, SMI: 19, #52292

In a poorly reported study on an unspecified number of guinea pigs, strong sensitising effects were reported when BMHCA at 10% in an unspecified vehicle was used for induction and challenge.

Ref.: Ishihara et al., 1986, SMI: 67, #5601

Buehler test

Additional data from Applicant's submission II dossier

No additional data.

SCCS comment
The data on the sensitisation potential of BMHCA provided in Submission II do not change the SCCS's previous conclusion (SCCS/1540/14) that BMHCA is a moderate skin sensitiser.

3.3.4 Dermal / percutaneous absorption

From submission I

SCCS conclusion on dermal/percutaneous absorption
Dermal absorption studies *in vitro* demonstrated species-specific effects. The bioavailable portion was found to be much higher in rats (66.1 and 50.8%) when compared to mini pigs (0.8% and 4.9%), depending on the solvent used (methylcarbitol or ethanol). In a second study, applying two real cream formulations (that contained 0.6% BMHCA), rat skin again allowed a much higher penetration (45.2% and 78.4%) than mini pig skin (23.6% and 25.7%). Nevertheless, the fraction of bioavailable BMHCA was found strongly increased in the mini pig experiment when moving from dissolved BMHCA to real cream formulations (4.9% vs. 25.7%).
Concurrently, administration of BMHCA onto the skin of experimental animals and humans demonstrated the permeation and systemic availability of this compound. Percutaneous absorption of BMHCA in humans was lower than it was in rats (1.4 vs. 19%).
Upon dermal application of [^{14}C]-BMHCA (11.37 mg test substance in 70% ethanol on 10 cm² back skin) on 3 human volunteers for 6 hours, a mean of 1.4% (range 0.8 – 2.4%) of the applied dose was excreted in urine within 24 hours, whereas radioactivity was below the detection limit in urine samples of later time points and in all faeces and blood plasma samples. The overall mean total recovery of topical application of [^{14}C]-BMHCA was 71 ± 10%. In comparison to the *in vitro* observations, the absorption rate found in humans for ethanolic solutions of BMHCA was comparable to what has been found in excised mini pig skin. Given that the absorption of BMHCA in mini pig skin was much higher when this compound was applied via real cream formulations, it is reasonable to conclude that BMHCA might also better penetrate human skin when it is applied in cream formulations. Since there is no further experimental data on this subject, the SCCS concludes that the maximum fraction of BMHCA being absorbed by human skin might be in the range of 25% rather than at 2.4%.
In consideration of the comparability of pig skin with human skin, the dermal bioavailability of ethanolic (dissolved) BMHCA to be used in the calculation of the systemic exposure dose (SED) and margin of safety (MoS) will be set at 5% (worst case scenario based on 1% BMHCA in EtOH applied at 120 µg substance/cm^2 onto 5 cm^2 excised mini pig skin; result: total of 5.87 µg substance/cm^2 found in stripped skin and chamber fluid after 16 hrs of exposure). On the other hand, the penetration rate of BMHCA applied onto the skin as an ingredient in creamy formulations will be set at 25% (worst case scenario based on 36 µg substance/cm^2 applied onto 5 cm^2 excised mini pig skin; mean out of two experiments: total of 8.88 µg substance/cm^2 found in stripped skin and chamber fluid after 16 hrs of

exposure). The SCCS is aware of the issue that the exact identity of the cream formulations applied in the latter study remains obscure.
The results obtained from the part of the study with 1% ethanolic BMHCA can further be used to assess the SED for hydroalcoholic products to be applied on a defined surface area of shaved or unshaved skin once daily (1 x 305 cm^2/day). Here, an absorption of about 6 µg substance/cm^2 can be assumed for unshaved skin (*stratum corneum* intact). For shaved skin (*stratum corneum* compromised), however, the total absorption would be 11 µg substance/cm^2 (with the addition of the portion of 4.66 µg/cm^2 that was found sticking in the *stratum corneum* in the respective experiment; cf. above).

3.3.4.1. Dermal /percutaneous absorption *in vitro*

Additional data from Applicant's submission II dossier

Guideline:	OECD TG 428, OECD GD No. 28, SCCP/0970/06
Test system:	Frozen dermatomed human skin (200 – 400 µm)
Number of donors:	Per dose group min. 8 samples from 12 donors (< 65 years)
Membrane integrity:	Visual inspection and electrical resistance barrier integrity test, membranes with a resistance < 1 kΩ were excluded
Test substance:	BMHCA (Lysmeral Extra)
Test item:	[^{14}C]-BMHCA in 4 test formulations: 1) 70 % ethanol in water 2) "silicone in water" 3) "water in oil" 4) "oil in water"
Batch:	00046877L0 (non-radiolabeled); 969-2005 (radiolabelled)
Purity:	99.5% (non-radiolabelled, GC); 97.7 % (radiolabelled)
Dose applied:	Group 1: 1.9 % [[^{14}C]-BMHCA in formulation 1, 95.0 µg BMHCA/cm² Group 2: 0.1 % [^{14}C]-BMHCA in formulation 2, 5.0 µg BMHCA/cm² Group 3: 0.1 % [^{14}C]-BMHCA in formulation 3, 5.0 µg BMHCA/cm² Group 4: 0.1 % [^{14}C]-BMHCA in formulation 4, 5.0 µg BMHCA/cm²
Exposed area:	1 cm^2
Exposure period:	24h
Sampling period:	up to 72h post dose
Receptor fluid:	Tap water; for prolonged observation time experiments: tap water with 0.01 % sodium azide (NaN_3)
Solubility in receptor fluid:	0.033 g/L in water
Mass balance analysis:	Provided
Tape stripping:	Yes (20)
Method of Analysis:	Liquid scintillation counting
GLP:	In compliance
Study period:	July - December 2016

Human abdominal and breast skin samples were obtained from 12 different donors. The skin was dermatomed (200 - 400 µm) and then the split-thickness membranes stored frozen, at approximately -20° C until use. The dermatomed skin membranes were checked for integrity visually and by the Transepithelial/Endothelial Electrical Resistance (TEER) method prior to use. Only visually intact skin samples with a TEER (impedance value) above 1 kΩ were used. Each skin preparation was hydrated in physiological saline for about 10 minutes before mounting to the diffusion cells which were filled up with physiological saline with a protease inhibitor. The prepared diffusion cells were covered with Fixomull® Stretch and stored overnight in a refrigerator. The integrity of the skin preparations was also visually checked immediately before starting the experiment. The receptor fluid was

pumped through the receptor chambers at 2.3 mL/h. The samples were maintained at a constant temperature of 32 ± 1 °C.
Penetration of [^{14}C]-BMHCA (Lysmeral Extra) through and into human skin was assessed by a single topical application of target doses of 95.0 µg/cm² and 5.0 µg/cm² of test substance formulated in different test-substance preparations, representative of in-market cosmetic formulations: Group 1 consisted of a hydro-alcoholic preparation with 1.9 % of BMHCA in 70 % ethanol in water; Group 2 of 0.1 % BMHCA in a "silicone in water" formulation, Group 3 of 0.1 % BMHCA in a "water in oil" and Group 4 of 0.1 % BMHCA in a "oil in water" formulation. Dermal absorption of BMHCA was assessed by a two-step experimentation procedure: 24h post dosing and with prolonged observation time: 72h sampling period for each formulation, for which 6-8 cells were used.

Absorption of BHMCA was evaluated by collecting receptor fluid every hour from 0 to 8h post dose, then every 2 hours from 8 to 24h post dose. After the exposure time of 24h and after the sampling period, skin membranes were washed with sodium-laurylethersulfate, diluted 1:140 w/w in tap water, followed by tap water. The tape-stripping procedure was performed on dried skin samples. Twenty tape strips were taken and pooled into three samples (the first 2 tapes as sample 1, the subsequent 9 tapes as sample 2 and the last 9 samples as sample 3) for analysis. The remaining skin from the 24h experiments was separated into dermis and epidermis by heat separation and subsequently analysed. The remaining skin of the 72h prolonged observation experiments and the skin of the control experiments were not separated into epidermis and dermis, but were extracted immediately after the stripping procedure or application.
No rate limiting effects on the diffusion process by saturation of the aqueous receptor fluid were present. The stability of the test item over the exposure period was assessed. The concentration of test-substance preparations of > 80.2 % and mean radiochemical purities of > 87.8 % radiolabelled [^{14}C] were determined over the application period.

Results

In the 24h experiments, the mean total recoveries ranged between 80.44 and 97.32 % (with individual values between 74.43 and 119.37 %) of the applied dose. Lysmeral is volatile and major parts of the test substance evaporated during the exposure period and were recovered in the charcoal filter.
Given the evaporation observed, the recovery range expressed as percentage was 80.44 ± 1.83% and 84.67 ± 13.80% for formulation 1, 83.08 ± 3.28% and 88.72 ± 2.97% for formulation 2, 97.32 ± 3.91% and 91.01 ± 13.82% for formulation 3, 96.21 ± 2.98% and 87.88 ± 3.44% for formulation 4, after 24h and 72h post-exposure, respectively.
Under these test conditions, 5.31 ± 2.22% (4.85 ± 2.03 µg), 3.50 ± 1.31% (0.16 ± 0.06µg), 4.83 ± 3.54% (0.23 ± 0.17µg), and 4.77 ± 2.16% (0.23 ± 0.1µg) of the applied dose of ^{14}C Lysmeral were recovered as absorbed dose in the 24h absorption experiments for the hydro-alcoholic solution, the "silicone in water", the "water in oil", and the "oil in water" based formulations, respectively. When an additional 48 hours post-observation period was included after the 24h exposure, 5.29 ± 2.52% (5.07 ± 2.42µg), 5.04 ± 2.60% (0.21 ± 0.11µg), 7.82 ± 5.42% (0.39 ± 0.27 µg), and 4.97 ± 2.26% (0.23 ± 0.10µg) of the applied dose of ^{14}C-Lysmeral were recovered as absorbed dose after 72h for the hydro-alcoholic solution, the "silicone in water", the "water in oil", and the "oil in water" based formulations, respectively.

In the 72h experiments, the residues of BMHCA in the skin preparations were differentiated into an extractable portion and a non-extractable portion. The non-extractable portion of BMHCA in living skin is assumed to be bound to the skin matrix and therefore represents a non-absorbable fraction excluded from the final calculations:
Percentage of BMHCA in living skin not extractable = Mean percent of the applied dose in skin residue (+ 1SD) * 100 / Mean percent of the applied dose in skin residue (+ 1SD) + skin extract (+ 1SD)

- "Ethanol in water" = (0.32 + 0.11) * 100 / (0.32 + 0.11 + 1.31 + 0.33) = 21%

- "Silicone in water" = (0.25 + 0.10) * 100 / (0.25 + 0.10 + 0.71 + 0.24) = 27%
- "Water in oil" = (0.24 + 0.15) * 100 / (0.24 + 0.15 + 0.50 + 0.48) = 28%
- "Oil in water" = (0.18 + 0.06) * 100 / (0.18 + 0.06 + 0.28 + 0.12) = 38%

Conclusion

The dermal penetration data using the hydro-alcoholic vehicle showed that an additional 72h observation time did not result in any evident movement of BMHCA from different skin compartments (i.e. the skin reservoir) to the receptor fluid. Therefore, the fraction found in the epidermis was not included as bioavailable. For the other vehicles, the dose associated to the remaining skin (dermis+ epidermis) was reduced by the non-extractable portion determined in the living skin. The percentage of dermally absorbed BMHCA was calculated as follows:

- "Ethanol in water" (24h): (Absorbed dose+1SD) + (Dermis+1SD) = 5.31+2.22+0.71+0.28 = 8.52%
- "Water in oil": (Absorbed dose+1SD) + ((Epidermis+1SD) + (Dermis+1SD) * 72%) = 4.83+3.54+((0.74+0.31+0.73+0.35)*72%) = 9.90%
- "Oil in water" (24h): (Absorbed dose+1SD) + (Epidermis+1D) + (Dermis+1SD) * 62%) = 4.77+2.16+((0.69+0.31+0.78+0.17)*62%) = 8.14%

Ref.: BASF SE, 2016a, SMII, 7

SCCS comment

The electrical resistance of the human skin samples was far below the 10 kΩ threshold for intact skin. In addition, according to SCCS/1358/10, recovery should be between 85 - 115%. The overall recovery of BMHCA tested in formulations 1 ("ethanol in water") and 2 ("silicone in water") was not within this acceptance range, even under the semi-occlusive conditions used.

According to SCCS/1564/15, in the case of substances with very low dermal absorption and limited permeation (e.g. colourants or UV-filters with high molecular weight and low solubility), the epidermis may be excluded when it is demonstrated that no movement of the chemicals from the skin reservoir to the receptor fluid occurs. BMHCA does not fulfil these criteria. Therefore, all BMHCA present in the living epidermis has to be taken into account for the dermal absorption.

Based on significant deviations from the SCCS requirements, the mean + 2 SD should have been taken for potential MoS calculation as follows:

- "Ethanol in water" (24h) = (Absorbed dose+2SD) + (Epidermis+2SD) + (Dermis+2SD) = (5.31+2*2.22) + (1.50+2*0.49) + (0.71+2*0.28) = 13.5%
- "Silicone in water" (24h) = (Absorbed dose+2SD) + (Epidermis+2SD) + (Dermis+2SD) = (3.50+2*1.31) + (0.96+2*0.18) + (0.64+2*0.23) = 8.5%
- "Water in oil" (24h): (Absorbed dose+2SD) + (Epidermis+2SD) + (Dermis+2SD) = (4.83+ 2*3.54) + (0.74+2*0.31) + (0.73+2*0.35) = 14.7%
- "Oil in water" (24h): (Absorbed dose+2SD) + (Epidermis+2D) + (Dermis+2SD) = (4.77+2*2.16) + (0.69+2*0.31) + (0.78+2*0.17) = 11.5%.

3.3.4.2. Dermal /percutaneous absorption *in vivo*

Additional data from Applicant's submission II dossier

No additional data.

3.3.5 Repeated dose toxicity

From submission I

SCCS conclusion on subacute and subchronic dose toxicity

The toxicity of BMHCA after repeated application was investigated in several species. Decreases in body weights and food consumption and/or clinical signs of toxicity were observed after subacute oral administration of BMHCA at doses of ≥50 mg/kg bw/day (rats) and ≥200 mg/kg bw/day (dogs). In oral studies, rats were found to be more sensitive than dogs to this compound irrespective of the length of treatment. Clinical chemistry and histopathological examinations repeatedly revealed adverse effects on the liver and male reproductive system. Decreases in plasma cholinesterase activity levels in both sexes of rats were observed after oral exposure to ≥25 mg/kg bw/day for 90 days. In addition, effects on adrenal glands in females were also observed at the same dose levels. From this most meaningful oral study, with respect to the doses administered, a NOAEL of 5 mg/kg bw/day can be derived for systemic effects.

On the other hand, dermal administration in rats for 5 days led to adverse effects (including testicular toxicity) only at excessive dose levels (2000 mg/kg bw/day). No 90-day studies on dermal or inhalative administration were available.

3.3.5.1 Repeated dose short-term oral / dermal / inhalation toxicity

Additional data from Applicant's submission II dossier

The results of screening studies provided (BASF SE, 2011b, SMI: 15, #59014 and Givaudan, 2009, SMI: 60, #57411) confirmed the known potential of BMHCA (orally for 5-14 days, at 50-250 mg/kg bw/d) to affect the reproductive organs in rats.

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

Additional data from Applicant's submission II dossier

No additional data.

3.3.5.3 Chronic (> 12 months) toxicity

Additional data from Applicant's submission II dossier

No additional data.

3.3.6 Mutagenicity / Genotoxicity

From submission I

The applicant's overall conclusion on mutagenicity/genotoxicity

Based on the data provided, the applicant came to the following conclusion on the overall mutagenicity/ genotoxicity: No genotoxic/mutagenic potential was found in bacterial gene mutation assays with *S. typhimurium* or *E. coli* strains in the presence or absence of metabolic activation. BMHCA also did not induce gene mutations at the *Hprt* locus in Chinese hamster V79 cells. Structural and numerical chromosomal aberrations were found

in the absence of S9, while no aberration occurred in its presence in CHO cells. Intraperitoneal treatment of mice with BMHCA did not induce increases in the incidence of chromosomal aberrations in bone marrow cells. Hence occasionally emerging clastogenicity *in vitro* remained unconfirmed *in vivo*. Based on the data available, BMHCA can be considered not mutagenic/genotoxic.

SCCS comment and conclusion

SCCS disagrees with the applicant's conclusion. Neither *in vitro* gene mutation nor *in vitro* chromosomal damage can be excluded based on the data provided. Similarly, due to the lack of sufficient and detailed information, it is also impossible to draw a firm conclusion from the *in vivo* micronucleus report provided.

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Additional data from Applicant's submission II dossier

Guideline:	OECD 471
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, Escherichia coli strain WP2uvrA
Replicates:	Three experiments, triplicate plates
Test substance:	BMHCA (Lilestralis Pure: 32229)
Batch:	A100423A (purity: > 99%)
Concentrations:	Experiment I – Plate incorporation test: ±S9 mix: S. typhimurium strains: 0, 1.5, 5, 15, 50, 150, 500, 1500 µg/plate; E. coli strain: 0, 50, 150, 500, 1500, 5000 µg/plate Experiment II – Pre-incubation test: -S9 mix: S. strains TA100 and TA1537: 0, 0.15, 0.5, 1.5, 5, 15, 50, 150 µg/plate -S9 mix: S. strain TA1535 and E. coli strain: 0, 0.05, 0.15, 0.5, 1.5, 5, 15, 50 µg/plate -S9 mix: S. strain TA98: 0, 0.5, 1.5, 5, 15, 50, 150, 500 µg/plate +S9 mix: all S. strains: 0, 1.5, 5, 15, 50, 150, 500, 1500 µg/plate; E. coli strain: 0, 5, 15, 50, 150, 500, 1500, 5000 µg/plate Experiment III (confirmatory test) – Plate incorporation test: +S9 mix: S. strain TA1535: 0, 50, 100, 150, 200, 300 µg/plate
Vehicles:	DMSO
Positive Controls:	-S9 mix: N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG): 2 µg/plate for WP2uvrA, 3 µg/plate for TA100, 5 µg/plate for TA1535; 9-Aminoacridine (9AA): 80 µg/plate for TA1537; 4-Nitroquinoline-1-oxide (4NQO): 0.2 µg/plate for TA98 +S9 mix: 2-Aminoanthracene (2AA): 1 µg/plate for TA100; 2 µg/plate for TA1535 and TA1537, 10 µg/plate for WP2 uvrA; Benzo(a)pyrene (BP): 5 µg/plate for TA98
Negative controls:	Vehicle control
GLP:	In compliance
Study period:	06 Jan 2011 – 30 Jun 2011

Material and methods

BMHCA was tested for mutagenicity in the reverse mutation assay with and without metabolic activation in S. typhimurium strains TA1535, TA1537, TA98, TA100 and E. coli

strain WP2 uvrA using both the Ames plate incorporation and pre-incubation methods at up to seven dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (induced with Phenobarbitone/β Naphthoflavone, 10% liver S9 in standard co-factors). The dose range for the first experiment was determined in a preliminary toxicity assay and ranged between 1.5 and 5000 mg/plate, depending on bacterial strain type. The experiment was repeated (pre-incubation method) using fresh cultures of the bacterial strains and fresh test item dilutions. The test item dose range was slightly expanded, based on the results of Experiment 1, and ranged between 0.05 and 5000 µg/plate, depending on bacterial strain type and presence or absence of S9-mix. Additional dose levels and an expanded dose range were selected in both experiments. This was done in order to achieve both four non-toxic dose levels and the toxic limit of the test item. In addition, a third experiment was performed to confirm whether a two-fold increase in TA1535 revertant colony frequency, noted in Experiment 1, was real or spurious. The experiment was carried out using bacterial strain TA1535 (presence of S9-mix only) and employed a narrowed test item dose range of 50, 100, 150, 200 and 300 µg/plate.

Results

Equivocal findings were observed in this study for the Salmonella strain TA 1535 in the plate incorporation test with and without metabolic activation. Increased numbers of revertant colonies were observed for TA 1535 in the first experiment (plate incorporation method) but not in the follow-up pre-incubation test. The increase observed consisted of an isolated statistically significant increase in colony frequency at non-bacteriotoxic concentrations, noted in one single concentration (150 µg/plate) in the presence of S9. This finding was not reproducible in a confirmatory plate incorporation test. At higher test item concentrations, a concentration dependent increase of colony numbers associated with a sparse bacterial background lawn was noted for TA 1535 in experiment 1 and 3. The authors suggest that this increase in colony number might have resulted from residual histidine levels that were available to a small number of surviving His- bacteria in the presence of bacteriotoxic BMHCA concentrations (although likely, this has not been confirmed experimentally). These histidine levels would allow the surviving His- bacteria to undergo several additional cell divisions: resulting colonies do therefore not represent revertant (mutant) colonies.

SCCS comment

The SCCS disagrees with the applicant's conclusion and considers the results obtained as positive. In Exp I BMHCA was shown to be positive in S. typhimuruim TA1535, both ±S9-mix (almost 10 fold increase in revertants, starting from 150 µg/plate –S9-mix and 500 µg/plate +S9-mix). In Exp II –S9-mix BMHCA was not tested at the same concentrations, but only up to 50 µg/plate. In Exp III, BMHCA was tested only with S9-mix at up to 300 µg/plate.

Ref.: Innospec Ltd., 2011a, SMII: 16

Guideline:	OECD 471
Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, Escherichia coli strain WP2uvrA
Replicates:	Two experiments, triplicate plates
Test substance:	BMHCA
Batch:	not stated (source: Sigma–Aldrich, St. Louis, MO, USA, purity: > 90%)
Concentrations:	±S9 mix:
	Preliminary test:

	All strains: 0.05, 0.25, 0.5, 2.5, 5.0, 25 µM/plate (corresponding to 10, 51, 102, 510, 1022, 5108 µg/plate based on the molecular weight of 204.31 g/mol) Main test: S. typhimurium and E. coli strains: 0.01; 0.02; 0.05; 0.07; 0.1; 0.2; 1.0; 2.0; 10.0 µM/plate (corresponding to 2; 4; 10; 14; 20; 40; 200; 400; 2000 µg/plate based on the molecular weight of 204.31 g/mol)
Vehicles:	DMSO
Positive Controls:	-S9 mix: - sodium azide (SA): 1 µg/plate for TA1535 and TA100 - 9-aminoacridine (9AA): 50 µg/plate for TA1537 - 2-nitrofluorene (2NF): 2 µg/plate for TA98 - methyl methanesulfonate (MMS): 500 µg/plate for WP2 uvrA +S9 mix: - 2-aminoanthracene (2AA): 1 µg/plate for TA98 and TA100; 10 µg/plate for TA1535, TA1537, WP2 uvrA - benzo[a]pyrene (BaP): 50 µg/plate for TA98, TA100, WP2 uvrA, 100 µg/plate for TA1535; 50, 100, 500 µg/plate for TA1537
Negative controls:	Vehicle control
GLP:	No
Published:	Yes, date of publication: 2014

Material and methods

BMHCA was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (liver postmitochondrial supernatant of rats treated with phenobarbital/β-naphthoflavone) according to the pre-incubation test method. In a pre-test, the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and Escherichia coli strain WP2 uvrA were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 0.05 – 25 µM/plate (10-5108 µg/plate) to check solubility and cytotoxicity. For the main test, concentrations ranging from 0.01-10.0 µM/plate were tested.

Results

In the preliminary test, BMHCA was cytotoxic in strains TA1535 and TA1537 in the absence of S9 at a concentration of 0.25 µM/plate (51 µg/plate). In the presence of S9, cytotoxicity occurred at 0.25 µM/plate (51 µg/plate) in TA1537 and 0.5 µM/plate (102 µg/plate) in TA1535 and at 5 µM/plate (510 µg/plate) in TA98 and WP2*uvr*A.
In the main mutagenicity assay, BMHCA did not increase the number of revertant colonies in any of the bacterial strains tested at non-cytotoxic concentrations, either with or without the metabolic activator S9.

Conclusion

BMHCA was considered to be non-mutagenic in this bacterial gene mutation test, with or without S9-mix metabolic activation, when tested up to cytotoxic concentrations.

Ref.: Di Sotto et al., 2014 a, b, SMII: 11, 12

SCCS comment

The study has several limitations: it was not conducted under GLP conditions; positive controls used did not clearly demonstrate positive response. The positive control substance, i.e. BaP for TA1535, did not induce mutant frequency up to the concentration of 500 µM/plate. BMHCA was tested in low concentrations. No data on historical controls are

provided. Overall, the results have limited value and no firm conclusion can be drawn from this study.

Guideline:	OECD 476
Test system:	L5178Y mouse lymphoma cell line (Tk+/-)
Replicates:	Two independent experiments, each two parallel cultures
Test substance:	BMHCA (Lilestralis pure: 32229)
Batch:	A100423A (purity: > 99%)
Concentrations:	Preliminary test: ±S9 mix (4 h exposure) and -S9 mix (24 h exposure): 7.97, 15.94, 31.88, 63.75, 127.5, 255, 510, 1020, 2040 µg/mL Main test: Experiment I: -S9 mix (4 h exposure): 4, 8, 16, 20, 24, 28, 32, 36 µg/mL +S9 mix (4 h exposure): 8, 16, 32, 40, 48, 56, 64, 72 µg/mL Experiment II: -S9 mix (24 h exposure): 1.25, 2.5, 5, 10, 15, 20, 25, 30 µg/mL +S9 mix (4 h exposure): 20, 30, 40, 50, 55, 60, 65, 70 µg/mL
Vehicle controls:	DMSO
Positive Controls:	-S9 mix: ethyl methanesulfonate (EMS), 150 µg/mL +S9 mix: Cyclophosphamide (CP), 2 µg/mL
GLP:	Yes
Study period:	25 Jun 2010 – 22 Jun 2011

Material and methods

The *in vitro* mammalian cell gene mutation assay was conducted to investigate the potential of BMHCA dissolved in DMSO to induce gene mutations at the TK +/- locus of the L5178Y mouse lymphoma cell line. Prior to the main study, a preliminary toxicity test was performed on cell cultures using a 4-hour exposure time both with and without metabolic activation (S9, liver post mitochondrial supernatant of rats treated with phenobarbital/β-naphthoflavone) and using a 24-hour exposure without S9-mix. The dose range used was 7.97 to 2040 µg/mL for all three exposure groups.
The following main study was performed in two independent experiments, using two parallel cultures each. In the first experiment of the main study, BMHCA treatments were performed in duplicate (A + B) both with and without metabolic activation (S9-mix) at eight dose levels of the test item (4 - 36 µg/mL in the absence of S9-mix, and 8 - 72 µg/mL in the presence of metabolic activation), vehicle and positive controls. The treatment vessels were incubated at 37°C for 4 hours with continuous shaking. In the second experiment of the main study, the dose range of the test item was 1.25 - 30 µg/mL in the absence and 20 – 70 µg/mL in the presence of S9-mix. The treatment vessels were incubated at 37°C with continuous shaking for 24 hours in the absence of metabolic activation and 4 hours in the presence of S9-mix.

Results

In the preliminary test, toxicity in the form of marked reductions in %Relative Survival Growth (%RSG) was observed in all three of the exposure groups starting at 31.88 µg/mL (15% RSG, -S9). At the end of the exposure periods, precipitation of test item was observed at and above 127.5 µg/mL in the 4h exposure groups, and at and above 255 µg/mL in the 24h exposure group and increased in intensity as the concentration increased.
In both experiments of the main test performed, a marked test item-induced toxicity in both the absence and presence of S9-mix, as indicated by the %RSG and Relative Total Growth (RTG) values was observed (Exp. I: 4 h, -S9: at/above 32 µg/mL (31% RSG); 4 h, +S9:

at/above 64 µg/mL (40% RSG), Exp. II: 24 h, -S9: at/above 15 µg/mL (44% RSG), 4 h, +S9: at/above 50 µg/mL (18 % RSG)). The test item did not induce any statistically significant or dose-related increases in the mutant frequency at any of the concentrations, neither in the absence nor presence of metabolic activation, including the concentration in the absence of metabolic activation exceeding the upper limit of acceptable toxicity (10% - 20% RSG).
The vehicle control mutant frequency values were within the acceptable range and the positive controls produced marked increases in the mutant frequency demonstrating the sensitivity of the assay and the efficacy of the S9-mix. Precipitation of the test item was not observed at any of the concentrations tested in both main study experiments.

Conclusion

Under the conditions of the study, BMHCA did not induce any toxicologically significant increases in the mutant frequency at the Tk +/- locus in L5178Y cells and is therefore considered to be non-mutagenic in mammalian cells.

Ref.: Innospec Ltd., 2011b, SMII: 17

Guideline:	Comparable to OECD 487
Test system:	Human peripheral blood lymphocytes (two healthy non-smoker males, less than 40 years old, supplied by AVIS (Italian Association of Voluntary Blood donors))
Replicates:	Each treatment on cells of 2 donors, each in 2 separate cultures (i.e. 4 cultures/treatment)
Test substance:	BMHCA
Batch:	Not stated (source: Sigma–Aldrich, St. Louis, MO, USA, purity: > 90%)
Concentrations:	5, 10, 25, 35, 50, 100, 250, 500 µM
Vehicles:	BMHCA: dissolved in ethanol (50 % v/v), diluted in RPMI 1640 medium to avoid precipitation
Positive controls:	ethyl methanesulfonate (EMS): 120 µM Colcemid (COL): 0.02 µM
Negative controls:	DMSO
GLP:	No
Published:	Yes, date of publication: 2014

Material and methods

BMHCA was tested for its clastogenic and aneugenic potential *in vitro* on peripheral blood lymphocytes of two healthy non-smoker males (less than 40 years old). Prior to the main test, the cytotoxicity of BMHCA on the peripheral blood lymphocytes was evaluated by scoring at least 1000 cells per treatment for the presence of one, two, three or more nuclei and determining the nuclear division index (NDI). The cells that did not undergo mitosis were not included in the count. Genotoxicity was assayed in the main tests starting from the highest concentration to concentrations at which neither necrosis nor cytotoxic or cytostatic effects were observed. The cultured lymphocytes, supplemented with Cytochalasin-B (6.25 µM final concentration), were treated for 24h at 37°C with test material at concentrations of 5, 10, 25, 35, 50, 100, 250 and 500 µM in the absence of an exogenous source of metabolic activation. Each treatment was carried out on the cells obtained from two donors and in two separate cultures (i.e. four cultures were set up for each treatment group). For each treatment, at least 1000 lymphocytes were scored to determine the NDI value, and at least 2000 binucleated cells (BNCs) were examined for the presence of micronuclei. A positive response was defined as a statistically significant increase of MN frequencies in the treated cultures respect to the vehicle.

Results

The preliminary cytotoxicity test showed that at the concentration of 100 µM, BMHCA reduced the cell proliferation, inducing a less than 70% value of NDI and early signs of cytotoxicity. At 250 and 500 µM, the NDI was not applicable due to the advanced necrosis. BMHCA, when tested on the human lymphocyte cultures at non-cytotoxic concentrations of 5 - 50 µM for 24 hours, did not increase the mean micronuclei frequency in binucleated cells in comparison with the vehicle. The positive controls, EMS and COL increased the micronuclei frequency significantly, showing that the lymphocytes were suitable for detecting both clastogenic and aneuploidic damage.

Conclusion

It was shown that BMHCA revealed no potential to induce clastogenic or aneuploidic damage under the chosen testing conditions.

Ref.: Di Sotto et al., 2014 a, b, SMII: 11, 12

SCCS comment
The study was not performed under GLP. It was performed without metabolic activation. Only information from public literature is available. Limited information is provided on treatment of cells, cytotoxicity and how the study was done. Also, no data on historical controls are provided. Results have limited value.

Guideline/method:	Alkaline Comet assay according to published literature (Aviello et al., 2010, J. Cell. Mol. Med. 14, 2006–2014)
Test system:	Human colonic epithelial cells (HCEC, obtained from Fondazione Callerio Onlus, Trieste, Italy)
Replicates:	Three experiments
Test substance:	BMHCA
Batch:	not stated (source: Sigma–Aldrich, St. Louis, MO, USA, purity: > 90%)
Concentrations:	100 µM
Vehicles:	DMSO
Positive control:	H2O2, 75 µM
Negative controls:	Vehicle control
GLP:	No
Published:	Yes, date of publication: 2014

Material and methods

BMHCA was tested for its potential to induce DNA damage in an indicator test in the form of the alkaline Comet assay in Human colonic epithelial cells (HCEC, obtained from Fondazione Callerio Onlus, Trieste, Italy). Prior to the main test, the cytotoxicity on HCEC cells was evaluated by the neutral red uptake assay. The cells were seeded in 96-well plates and allowed to adhere for 48 h. Thereafter, they were incubated with serial dilutions of the test substance in the range between 1 – 300 µM for 24 h and subsequently with the neutral red dye solution for 3 h, and the absorbance was read at 532 nm.
In the main test, DNA damage was evaluated by the alkaline comet assay. HCEC were seeded in 6 well-plates. After 48 h, the cells were incubated with 100 µM for 24 h and subsequently, cells were trypsinised. Aliquots of cell suspension were centrifuged and pellets were collected, mixed with 0.85% low melting point agarose and laid on pre-coated glass slides. The slides were then suspended at 4°C for 1 h for lysis and electrophoresed in

alkaline buffer at 26 V, and 300 mA for 20 min. After neutralization in Tris-HCl, the gels were stained with ethidium bromide. Images were analyzed using a Leica microscope equipped with image analysis Comet Assay™ software.

Results

In the preliminary test, BMHCA at concentrations ranging from 1 to 300 µM did not affect HCEC cell viability after 24 h exposure. The vehicle DMSO (0.1% v/v) did not modify the response, while DMSO at higher concentration (20% v/v) and used as positive control, significantly reduced HCEC viability.
In the main test, BMHCA at the tested non-toxic concentration of 100 µM induced no DNA damage in the form of an increase in DNA tail after electrophoresis compared to the vehicle and following a 24 hour exposure. The positive control (H2O2) increased the DNA tail significantly, indicating induction of single strand breaks. In summary, no evidence was found for BMHCA to induce single-strand breaks.

Conclusion

BMHCA was considered to induce no DNA damage in the form of single-strand breaks under the conditions of this indicator test in human colonic epithelial cells.

Ref.: Di Sotto et al., 2014 a, b, SMII: 11, 12

SCCS comment

The comet assay experiment was not performed under GLP. So far there is no OECD TG for the comet assay *in vitro*. Only 24h exposure was used though it would also be required to use short (3-4 h) treatment as during 24h exposure DNA repair is taking place and thus effects may not be detected. In a preliminary cytotoxicity test neither of the used concentrations induced cytotoxicity. The concentrations should range from non-toxic up to mildly toxic (around 80% viability). Testing only one concentration of BMHCA of 100 µM in the comet assay is not justified and results of the test would have limited value.

3.3.6.2 Mutagenicity / Genotoxicity in vivo

Additional data from Applicant's submission II dossier

No additional data.

Overall discussion and conclusion on mutagenicity/genotoxicity

From submission II

The applicant's overall conclusion on mutagenicity/genotoxicity

The mutagenic/genotoxic potential of BMHCA was investigated in a wide range of validated and scientifically robust studies *in vitro* and *in vivo*. The overall picture of several bacterial reverse mutation assays performed over more than 3 decades is mostly consistent. The majority of mutagenicity data in bacteria provide no evidence for a mutagenic potential of BMHCA. However, equivocal findings were reported in one of the submitted Ames tests for Salmonella strain TA1535 but this study is considered insufficient in terms of procedure and reporting (Innospec Ltd., 2011a, SMII: 16). Moreover, this observation in TA1535 was not confirmed in the respective pre-incubation test and no corresponding increases of other strains (i.e. TA100) were observed. Further, this finding is in contrast to the results of a GLP and guideline Ames plate incorporation test (Reference: RIFM, 1999b, SMI: 89, #35168)

and the Ames pre-incubation test in line with OECD TG 471 reported in literature (References: Di Sotto et al., 2014 a, b, SMII: 11, 12). Further, sporadic but no relevant increases in the mean number of revertant colonies were reported for the Salmonella strain TA1538 (without metabolic activation only) (Roche, 1984, SMI: 107,). These findings were not reproducible in further trials and followed no concentration response and the study is considered to have limited validity, since spontaneous revertant frequencies were unusually low. The lack of biological relevance of this variation is confirmed by the results in TA98. In this tester strain, investigating the same type of mutagenic lesions, no effects/variations were observed.

Two different mutagenicity studies in mammalian cells investigating the same mutagenic endpoint (gene mutation at both the HPRT- and the tk+/- locus) supported the absence of a mutagenic potential of BMHCA (BASF SE, 2010a, SMI: 12; Innospec Ltd., 2011b, SMII: 17). Although methodological shortcomings exist, the highly sensitive indicator test for DNA damage that was reported in the literature, namely the Comet assay in human colonic epithelial cells, provided further evidence for the absence of BMHCA's DNA-damaging potential (Di Sotto et al., 2014 a, b, SMII: 11, 12).

Thus, the negative result generated in mammalian cells as well as the absence of an effect in the Comet assay support the weight of evidence that BMHCA is non-genotoxic *in vitro*. BMHCA was found to induce structural and numerical chromosomal aberrations in the absence of a metabolic system, while no induction occurred in the presence of metabolic activation in Chinese hamster ovary cells and it is therefore considered clastogenic in CHO cells (RIFM 2000a, SMI: 94,). However, these cells have previously been shown to generate a high percentage of false-positive results compared with other cell types, e.g. primary human cells, cell lines with functional p53 etc., and are consequently considered of questionable value in the investigation of this endpoint (Fowler et al, 2012, SMII: 13). A chromosomal damage potential of BMHCA was not observed in a non-GLP but a scientifically reliable micronucleus test in human peripheral lymphocyte cultures that is comparable to OECD 487 (Di Sotto et al., 2014 a, b, SMII: 11, 12). Thus, BMHCA does not appear to have the potential to induce clastogenic or aneugenic damage in primary human peripheral lymphocytes under the chosen testing conditions. Therefore, no conclusive result with regards to chromosomal damage was observed *in vitro*.

The absence of a relevant potential of induction of chromosomal aberrations was confirmed by an *in vivo* micronucleus assay where no relevant increase in the incidence of micronuclei in bone marrow cells was observed following i.p. application of BMHCA to mice (RIFM 2000b, SMI: 95,). Systemic bioavailability was clearly demonstrated by the PCE/total erythrocyte ratio in the top dose group at 24 hours sacrifice interval (-15% or -30% of control [male; female]). This ratio evidenced cytotoxicity in the bone marrow as target tissue of the test substance/metabolites after intraperitoneal administration.

Overall, BMHCA is unlikely to pose a genotoxic hazard to humans in a weight of evidence. Some isolated equivocal findings in a few *in vitro* assays were not considered relevant due to lack of reproducibility and insufficiencies in terms of procedure and reporting. *In vivo*, there was no evidence of a genotoxic potential of BMHCA in a micronucleus assay following i.p. application in mice.

SCCS overall comment on mutagenicity/genotoxicity based on studies from submission I and II

In its previous Opinion (SCCS/1540/14) the SCCS concluded that neither *in vitro* gene mutation nor *in vitro* chromosomal damage could be excluded based on the data provided in submission I. Similarly, due to the lack of sufficient and detailed information, it was also impossible to draw a firm conclusion from the *in vivo* micronucleus report provided.

Based on the analysis of additional reports provided in submission II, the SCCS considers that the data does not allow to exclude potential genotoxic effects of BMHCA because:

1. In the tests on gene mutations in bacteria:
    - BMHCA was confirmed to induce gene mutations in TA1535 strain (Ref. Innospec Ltd., 2011a, SMII: 16)
    - The study by Di Sotto et al. (2014a, b) using the Ames test was considered to be of limited value as: the positive controls used did not clearly demonstrate

positive response, no information on historical controls was available and BMHCA was tested in low concentrations,

2. In the tests on chromosomal aberrations *in vitro*:
   - The study by Di Sotto et al. (2014a) using a micronucleus test on human peripheral blood lymphocytes was considered to be of limited value as: BMHCA was tested without metabolic activation, limited information was provided on the treatment of cells, cytotoxicity and how study was done and no information on historical controls was available,
3. In the comet assay *in vitro*:
   - The study by Di Sotto et al. (2014a) using human colonic epithelial cells was considered to be of limited value as: only 24h exposure was used though shorter incubation times (3-4h treatment) should also have been used, at least 3-5 concentrations ranging from non-toxic up to mildly toxic (around 80% viability) should be used, testing only one concentration of 100 µg/mL was not justified.

Based on analysis of data provided in submission I and additionally in submission II, the SCCS maintains its previous opinion that no firm conclusion can be drawn on the mutagenicity of BMHCA.

3.3.7 Carcinogenicity

From submission I

SCCS conclusion on carcinogenicity

No carcinogenicity data are available for BMHCA. Currently there is no evidence from repeated dose studies that BMHCA is able to induce hyperplasia or neoplasia.

Additional data from Applicant's submission II dossier

No additional data.

3.3.8 Reproductive toxicity

From submission I

SCCS conclusion on reproductive toxicity

Adverse effects of BMHCA on the male reproductive system have been consistently observed in several repeated dose and reproduction toxicity studies. A NOAEL of 25 mg/kg bw/day in male rats with regard to this endpoint is substantiated by studies applying the compound for 5 days, 90 days or in the frame of a 1-generation study over 6 weeks prior to mating. It is to be emphasised that reproductive toxicity already became occasionally visible after a single application of 50 mg/kg bw/day. In all investigations available, testicular toxicity in rats was accompanied by signs of systemic toxicity. By contrast, other species such as mice and dogs were less sensitive. In dogs, a NOAEL of 40 mg/kg bw/day has been established based on the onset of testicular toxicity after treatment periods of 2 weeks and 3 months. So, from the animal data available, male rats revealed as most sensitive species with regard to BMHCA-mediated testicular toxicity. On the other hand, in female rats developmental toxicity was accompanied by systemic toxicity and was already found at lower concentrations. Here, a NOAEL based on developmental toxicity is to be set at 5 mg/kg bw/day. This value is identical to the one defined for general systemic toxicity in rats based on repeated dose (90-days) toxicity studies. Since the onset of developmental toxicity was tightly accompanied by maternal toxicity, the malformations and tissue

variations observed likely resulted from general fetotoxicity rather than from specific teratogenicity.

3.3.8.1 Two generation reproduction toxicity

Additional data from Applicant's submission II dossier

No additional data.

3.3.8.2 Other data on fertility and reproduction toxicity

Additional data from Applicant's submission II dossier

Guideline/method:	OECD 443, Modified Extended one-generation reproduction toxicity study
Species/strain:	Rat/Wistar (strain Crl:WI(Han))
Group size:	35 male and 35 female rats per group for diet control, placebo alginate control, low- and mid-dose groups 40 male and 40 female rats per group for high-dose group (F0 parental generation) 10 male and 10 female rats as positive control (Cohort 3 – developmental immunotoxicity)
Test substance:	BMHCA (Lysmeral encapsulated)
Batch:	1420-0552/201400167 (purity/content: 17.7 g/100 g, (3-(4-tert-butylphenyl)-2-methylpropanoic acid): 0.2 g/100g, Reference: BASF SE, 2015, SMII: 4))
Dose levels:	Target: 0, 1, 3, 10 mg/kg bw/d
Encapsulated in the diet:	0, 75, 230, 750 ppm (corresponding to 0, 13, 41 and 133 ppm active ingredient (a.i.))
Placebo alginate:	750 ppm consisting of 67.6 % Glycerin (Lot: GR335), 20.6 % Alginat BR- L (Lot: G2600301) and 11.8 % Alginat BR-GM (Lot: G7708901). The nucleus consists of 100 % sunflower oil, refined (Lot: 5603206)
Positive control:	Cyclophospamide monohydrate (Batch: MKBS0021V, purity: 99.9% and 6.9% water) used for Cohort 3 – (Immunotoxicity)
Route:	Oral (diet (microcapsules of Lysmeral homogenously added to food))
Exposure period:	F0 animals: approximately 2 weeks prior to breeding and continuing through breeding (up to two weeks), and for a maximum of 6 post-mating weeks (males) or gestation (three weeks) and lactation (three weeks) for females. Selected F1 offspring (cohorts 1A, 1B, 2A, 2B, 3, 4A and 4B) were maintained on the test diet until sacrifice or one day before.
Exposure frequency:	daily
GLP:	Yes
Study period:	21 April 2015 - 30 Jan 2017

The study was performed to fulfil the requirements of a decision on a substance evaluation pursuant to Article 46(1) of the REACh regulation, not for the purposes of the cosmetic safety evaluation.

Material and methods

BMHCA (Lysmeral encapsulated) was investigated in an extended one-generation reproduction toxicity study to obtain general information on the possible effects on the integrity and performance of the male and female reproductive systems, including gonadal function, estrous cyclicity, mating behaviour, conception, gestation, parturition, lactation

and weaning, as well as on growth and development of the offspring. This study also provided information on neonatal morbidity, mortality, target organs of the pups and preliminary data on prenatal and postnatal developmental toxicity including possible effects on the embryonic, fetal and pre-adult development of the nervous and immune systems as well as alterations in endocrine function including thyroid perturbations.

The test substance was administered to groups of 35 male and 35 female healthy young Wistar rats in the control, low- and mid-dose groups and to 40 male and 40 female healthy young Wistar rats in the high dose groups (F0 parental generation) as a homogeneous addition to the food in concentrations of 75, 230 and 750 ppm (corresponding to 13, 41 and 133 ppm of the active ingredient or to target dose levels of 1, 3 and 10 mg/kg bw/d due to its content of 17.7%). The negative control group was fed a plain diet and an additional placebo control group was dosed with Placebo Alginat (encapsulated) without BMHCA via the diet in parallel.

F0 animals were treated at least for 13 days prior to mating to produce a litter (F1generation). Mating pairs were from the same dose group. Pups of the F1 litter were selected (F1 rearing animals) and assigned to 7 different cohorts, which continued in the same fashion as their parents and which were subjected to specific post-weaning examinations. Cohort 1B was selected to produce F2 pups. F1 Cohort 1B animals selected for breeding were continued in the same dose group as their parents, and the breeding programme was repeated to produce a F2 litter. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 Cohort 1B parental animals. Test diets containing BMHCA (encapsulated) were offered continuously throughout the study.

- Cohort 1A (Reproductive PND90); Puberty: Yes; Approx. age at necropsy: 13 weeks
- Cohort 1B (Reproductive = F1 parental animals); Puberty: Yes; Approx. age at necropsy: 19-25 weeks
- Cohort 2A (Neurotoxicity PND75-90); Puberty: Yes; Approx. age at necropsy: 11 weeks
- Cohort 2B (Neurotoxicity PND22); Puberty: No; Approx. age at necropsy: 3 weeks
- Cohort 3 (developmental immunotoxicity); Puberty: Yes; Approx. age at necropsy: 8-9 weeks
- Cohort 4A (Cholinesterase PND22); Puberty: No; Approx. age at necropsy: 3 weeks
- Cohort 4B (Cholinesterase adult); Puberty: Yes; Approx. age at necropsy: 11-12 weeks

The parents' and the pups' state of health was checked each day, and parental animals were examined for their mating and reproductive performances. Food consumption of the F0 and F1 parents and F1 rearing animals was determined regularly once weekly and weekly during gestation (days 0 - 7, 7 - 14, 14 - 20) and lactation periods (days 1 - 4, 4 - 7, 7 - 14 and 14 - 21). In general, body weights of F0 and F1 parents and F1 rearing animals were determined once weekly. However, during gestation and lactation F0/F1 females were weighed on gestation days (GD) 0, 7, 14 and 20 and on postnatal days (PND) 1, 4, 7, 14 and 21. A detailed clinical observation (DCO) was performed in all F0 parents and F1 animals in cohorts 1A, 1B, 2A, 3 and 4B before initial test substance administration (only F0 parents) and, as a rule, thereafter at weekly intervals. Estrous cycle data were evaluated for F0 and cohort 1B (=F1 generation) females over a two weeks (F0 females) or three weeks (F1 females) time period prior to mating until evidence of mating occurred. In all cohort 1A females, vaginal smears were collected after the vaginal opening until the first cornified smear (estrous) was recorded. The estrous cycle was also evaluated in cohort 1A females for 2 weeks around PND 75. Moreover, the estrous stage of each female was determined on the day of scheduled sacrifice. An auditory startle response test was carried out in all animals of cohort 2A on PND 24. A functional observational battery examination (FOB) was performed in all animals of cohort 2A on PND 69. Motor activity was measured in all animals of cohort 2A on PND 68. The F1 and F2 pups were sexed on the day of birth (PND 0) and were weighed on the first day after birth (PND 1) as well as on PND 4, 7, 14 and 21. Their viability was recorded. At necropsy, all pups were examined macroscopically (including weight determinations of brain, spleen and thymus in one pup/sex/litter).

Anogenital distance (defined as the distance from the anus [centre of the anal opening] to the base of the genital tubercle) measurements were conducted in a blind randomized fashion, using a measuring ocular on all live male and female pups on PND 1. All surviving male pups were examined for the presence or absence of nipple/areola anlagen on PND 13. If nipple/areola anlagen were recorded, all surviving male pups were carefully re-examined one day prior to necropsy. Time of sexual maturation, i.e. day of vaginal opening (females) or balanopreputial separation (males), of all F1 pups brought up beyond weaning was recorded. Blood samples for clinical pathological investigations were withdrawn from 10 selected F0 and cohort 1A animals per sex and group. Further blood samples were taken from 10 surplus (culled) PND 4 pups per sex and group as well as from 10 surplus PND 22 pups per sex and group. Blood samples for acetyl cholinesterase investigations (AChE) were withdrawn from 10 selected F0 animals per sex and group as well as from 10 surplus (culled) PND 4 and 10 PND 22 (=cohort 4A) pups per sex and group and in all cohort 4B animals.

Various sperm parameters (motility, sperm head count, morphology) were assessed in the F0 and F1 generation males at scheduled sacrifice or after appropriate staining. All F0 and F1 parental animals were assessed by gross pathology (including weight determinations of several organs) and subjected to an extensive histopathological examination; special attention being paid to the organs of the reproductive system. A quantitative assessment of primordial and growing follicles in the ovaries was performed for all control and high-dose F1 parental females.
All F1 rearing animals were assessed by different pathological, neuro- and histopathological examinations.

Results

The stability of the test substance preparations over a period of 35 days at ambient temperature and the homogeneous distribution of the test substance in the diet was analytically verified. The mean recovery of BMHCA from the diet preparation in the first analysis ranged between 60 and 80% of the expected values, the recovery rates in the remaining 4 analyses were 63 - 95%, 92 - 102%, 81 - 107% and 86 - 97% of the expected values. With regard to the very low concentration of BMHCA in the applied formulation as well as in the diet preparations, and the high complexity of the extraction and analytical method, these recovery rates were considered acceptable and demonstrated the correctness of the diet preparations. The overall mean dose of BMHCA throughout all study phases and across all cohorts was approx. 1.4 mg/kg mg/kg bw/d in the 75 ppm group, approx. 4.5 mg/kg bw/d in the 230 ppm group and approx. 15.1 mg/kg bw/d in the 750 ppm group indicating that the targeted dose levels were achieved or exceeded.

There were no test substance-related mortalities or adverse clinical observations noted in any of the groups. In particular, regularly conducted detailed clinical observations revealed no effects at all.

The high-dose of the test substance led to some adverse systemic effects in the F0 parental rats and F1 offspring. In the 10 mg/kg bw/d F0 females and F1 females of Cohort 1B, food consumption was consistently reduced during lactation (F0 females: 5% and F1 cohort B1 females: 13% below placebo-control). The food consumption of all animals in other cohorts in all dose groups remained unchanged.

Organ weights: absolute and relative ovary weights were reduced significantly in a dose-dependent manner in the F0 females. The weight decrease (absolute 97.067 mg; relative 0.045%) was below the historical control range values (absolute 109.542–130.320 mg; relative: 0.046 – 0.056%). This change was judged by the authors to be "attributed to physiological differences in the phases of the sexual cycle and not treatment-related". This reasoning is not clear, since the values were mean values compared to the mean values from the control animals.

Table 1. F0 ovary weight change (%) relative to the placebo controls. **p <=0.01

Dose (mg/kg bw/d)	0	1	3	10
Absolute ovary weight	100	99	94	**88****
Relative ovary weight	100	96	93	**89****

In the high-dose F0 parental females, body weights were consistently reduced during gestation and the first two weeks into lactation, which was caused by a reduced body weight gain during different sections of premating and gestation. No such effects were observed in the high-dose F0 parental males. Body weights of the high-dose Cohort 1A, Cohort 1B, Cohort 2A and Cohort 4B males were below the concurrent control throughout the in-life period after weaning (up to 11%). The difference gained statistical significance in Cohorts 1B and 2A, but was consistently present in all these cohorts. High-dose F1 females of Cohort 1B were similarly affected, and the decrease of body weight persisted throughout gestation and lactation period for the F2 litters. The high-dose F1 females of Cohort 1B were also affected by a reduction of body weight gain during pregnancy. Although all these changes were not consistent and mild, a substance relationship is considered as likely.

In addition, there were some changes in blood and enzyme parameters in F0 and F1 females at 10 mg/kg bw/d such as prolonged prothrombin time (i.e. reduced synthesis of coagulation factors), increased γ-glutamyl transferase (GGT) activity and reduced albumin levels indicative of an altered metabolic activity of the liver cells. A prolonged prothrombin time was also noted for the corresponding F0 and F1 males at this dose. In F0 females at 10 mg/kg bw/d higher red blood cell (RBC) counts, hemoglobin and hematocrit values were detected. This effect was also present at 10 mg/kg bw/d in F1 males and females, both with higher RBC and haemoglobin values.

Regarding pathology, the target organ was the liver. In the high-dose F0 females and Cohort 1A and 1B, a significant increase in absolute and relative liver weights was observed. When assessed histopathologically, these increases were associated with minimal to slight centrilobular hypertrophy accompanied by minimal to slight apoptosis/single cell necrosis of hepatocytes. Furthermore, periportal vacuolation and multinucleated hepatocytes were noted in a few animals. All of these findings together were considered as treatment-related and adverse. At 3 mg/kg bw/d a significant liver weight increase in F0, Cohort 1A and 1B females was within the historical control range values and occurred without a histopathological correlate, thus, it was clearly considered not adverse. There were no indications from clinical examinations or from gross and histopathology that BMHCA (encapsulated) adversely affected the fertility or reproductive performance of the F0 and F1 parental animals up to and including the high dose of 10 mg/kg bw/d.

Estrous cycle data, on the whole sperm quality of males, mating behaviour, conception, gestation, parturition, lactation and weaning as well as sexual organ weights and gross and histopathological findings of these organs (specifically the differential ovarian follicle count) were comparable between the rats of all groups including control and ranged within the historical control data of the test facility.

The only notable findings were slightly higher incidences of abnormal sperm in the cauda epididymidis in the high-dose F0 males (9.8+/-13.2% compared with 6.3+/-0.6% in the control males, mean +/-SD respectively). However, this effect was not present in the corresponding high-dose F1 males, had no influence on fertility and reproductive performance of the affected males and had no testicular histopathological correlate. Thus, the adversity and toxicological relevance of this finding is rather questionable.

For all liveborn male and female pups of the F0 and F1 parents, no test substance-induced signs of developmental toxicity were noted at dose levels as high as 3 mg/kg bw/d. Postnatal survival, pup body weight gain as well as post-weaning development of the offspring of this test group until puberty remained unaffected by the test substance. Furthermore, clinical and/or gross necropsy examinations of the F1 and F2 pups revealed no adverse findings. Pup body weight development of the high-dose F1 and F2 offspring was affected as these offspring weighed about 14-15% less than control after birth and did not recover until weaning. Organ weight changes observed at this dose were considered to be secondary to the changes in body weight.

There was no influence on postnatal pup survival.

Measurement of thyroid hormones revealed no effect caused by the test item, either in the F0 parental animals or in the F1 offspring.

Anogenital distance of all test substance treated F1 pups were comparable to the concurrent placebo-control values. Anogenital distance of the high-dose F2 male and female pups was statistically significantly below the concurrent placebo-control values (about 4%, respectively) and at the lower limit of historical control. In contrast, anogenital index of the high-dose male and female F1 and F2 pups were statistically significantly above the concurrent placebo-control values. Thus, the observed findings were solely a consequence of the lower body weight and not considered as a specific treatment-related effect.

The incidence of present nipples/areolas revealed no test substance-related effect. No treatment-related adverse effects were noted for the vaginal opening in all female F1 offspring or preputial separation in male F1 offspring, indicating no influence on sexual maturation of the F1 progeny. An observed 1 day delay in preputial separation of the male F1 offspring (10 mg/kg bw/d) was well within the historical control range of the test facility and can be attributed to the general developmental delay. It is thus not considered to be a direct test substance-related effect on male sexual maturation. No effect at all on the timing of male puberty was noted in the lower-dose groups.

Lower peripheral acetylcholinesterase (AChE) activities in serum erythrocytes and diaphragm tissue were found in male pups at PND 4 and in females at PND 76 of the high dose group, while no changes were found in the animals of the opposite sex at these time points. Although these results were not fully conclusive an inhibitory effect of the compound on the peripheral AChE activity in pups and adolescent rats cannot be excluded. However, no corresponding clinical signs of developmental neurotoxicity were evident in male and female F1 offspring at any dose level. There were no compound related effects on motor activity, auditory startle habituation, and in-the-field observation battery following exposure to the test compound in these animals.

The only notable finding in neurobehavioral testing was lower maximum amplitudes in the auditory startle response test of the high-dose F1 males of Cohort 2A. However, in comparison to corresponding vehicle control data and high-dose F1 Cohort 2A female data the placebo control values were rather unusually high. Moreover, no such findings were noted in the high dose F1 females and no corresponding effects were recorded for startle response latency. Thus, this isolated observation was not considered as a treatment-related effect.

Neuropathology examinations in the form of brain weight determination, brain length and width measurements as well as brain morphometry and neuropathological examination by light microscopy did not reveal any neurotoxicological treatment-related findings.

There was no evidence that the test substance produced any developmental immunotoxicity. Neither T-cell dependent anti-SRBC IgM antibody response, nor absolute and relative lymphocyte subpopulation cell counts in the spleen tissue (B-, T-lymphocytes,

CD4-, CD8- T lymphocytes and natural killer (NK) cells) displayed any treatment-related changes.

Conclusion

The extended one-generation reproduction toxicity study is predominantly designed to focus on reproductive and developmental effects that may occur as a result of pre- and postnatal exposure to a substance as well as an evaluation of systemic toxicity in pregnant and lactating females and young and adult offspring. Under the conditions of this study, the NOAEL for fertility and reproductive performance in the F0 and F1 parental rats was 10 mg/kg bw/d, the highest dose tested.

The NOAEL for developmental toxicity in the F1 and F2 progeny was 3 mg/kg bw/d (equivalent to a mean overall oral dose of 4.5 mg/kg bw/d), based on reduced pup body weights in the F1 and F2 offspring, which were observed at 10 mg/kg bw/d. As these weight reductions were only observed in the presence of maternal toxicity, including lower weight gain during pregnancy, they are not considered as an indication for specific developmental toxicity.

The NOAEL for developmental neurotoxicity (DNT) for the F1 progeny is 10 mg/kg bw/d, the highest dose tested. Although an inhibitory effect at this dose on the peripheral AChE activity in pups and adolescent rats cannot be excluded, there were no corresponding effects evident in the neurobehavioral or neuropathological examinations.

The NOAEL for developmental immunotoxicity (DIT) for the F1 progeny is 10 mg/kg bw/d, the highest dose tested.

The NOAEL for general, systemic toxicity is 3 mg/kg bw/d (equivalent to a mean overall oral dose of 4.5 mg/kg bw/d) for the F0 and F1 parental as well as adolescent animals, based on evidence for distinct liver toxicity, as well as corresponding effects on food consumption, body weights and clinical pathological parameters, which were observed at 10 mg/kg bw/d predominantly in females.

Ref.: BASF SE, 2015, 2017b, SMII, 4, 8

SCCS comment

The SCCS agrees that NOAEL for fertility and reproductive as well as systemic toxicity of BMHCA in this study is 10 mg/kg bw/d and the NOAEL for developmental toxicity is 3 mg/kg bw/d. However, the SCCS does not agree with the developmental neurotoxicity NOAEL since inhibition of AChE in different tissues at 10 mg/kg bw/d should be considered adverse.
Based on the overall assessment, the NOAEL value of 3 mg/kg bw/d could be applied for MoS calculation.

3.3.8.3 Developmental Toxicity

Additional data from Applicant's submission II dossier

No additional data.

SCCS overall comment on reproductive toxicity based on studies from submission I and II

In the previous Opinion (SCCS/1540/14) the SCCS concluded that based on the study in which pregnant female rats were exposed to BMHCA at 5, 15 or 45 mg/kg bw/d (BASF SE, 2004, RIFM# 52014), a NOAEL based on developmental toxicity could be set at 5 mg/kg bw/day. This value was identical to the one defined for general systemic toxicity in rats

based on repeated dose (90-days) toxicity studies (Givaudan, 1990a, RIFM #12144, Givaudan, 1990i, RIFM #12143).
However, based on the study provided with submission II, the SCCS considers that the NOAEL for developmental toxicity should be set 3 mg/kg bw/d.

3.3.9 Toxicokinetics

From submission I

SCCS conclusion on toxicokinetics

Quantitative data on the toxicokinetics of BMHCA are available from rat, mouse, rabbit, guinea pig, dog and rhesus monkey and human studies. Given its physicochemical properties, BMHCA is likely to have high bioavailability via the oral route. After oral and dermal administration to experimental animals and humans, there is clear evidence of systemic absorption of BMHCA. However, in humans compared to rats, only limited percutaneous absorption of BMHCA (in EtOH) could be observed *in vivo* (1.4% vs. 19%).
Species-specific differences in the metabolism of BMHCA have been identified both *in vitro* and *in vivo*. Lysmerylic acid was the main hepatic metabolite in all species tested. Quantitative evaluation of metabolic profiles for different species in an *in vitro* metabolism study demonstrated much higher levels of p-tert-butylbenzoic acid (TBBA) formation by rat hepatocytes when compared to other species. In particular, TBBA levels observed in human hepatocytes were about 4-fold lower compared to rat hepatocytes at corresponding concentrations. Comparative assessment of the urinary metabolites in different animal species again uncovered differences in the urinary excretion of TBBA (and p-tert-butyl-hippuric acid, TBHA), with rats being the species that predominantly forms TBBA. However, the differences observed between rats and monkeys did not mirror the 4-fold difference in TBBA formation as seen with rat and human liver microsomes *in vitro*.

3.3.9.1 Metabolism *in vitro*

Additional data from Applicant's submission II dossier

No additional data.

3.3.9.2 Toxicokinetics in laboratory animals

Additional data from Applicant's submission II dossier

No additional data.

3.3.9.3 Toxicokinetics in humans

Additional data from Applicant's submission II dossier

Guideline/Method:	Explorative metabolism and excretion study after a single oral dose according to an ethically approved protocol
Species:	Human
Group size:	5 healthy volunteers (3 females, 2 males, age range: 23 – 32 years)
Test substances:	BMHCA
Batch:	no data (purity: no data)
Dose level:	5.26 mg/volunteer
Vehicle:	Ethanol

Route:	Oral
Exposure:	Single
Application procedure:	52.6 mg BMHCA dissolved in ethanol using a 10 mL volumetric flask. Each volunteer received a chocolate-coated edible waffle cup containing 1 mL spiked ethanol (exact 5.26 mg BMHCA, equivalent to 25.7 µM) and approximately 20 mL coffee, milk or water, depending on the choice of the volunteers
Urine samples:	immediately prior to exposure up to 48h after exposure
GLP:	No data
Study period:	No data

Material and methods

The metabolism and excretion kinetics of BMHCA was investigated in an explorative study in human volunteers after application of a single oral dosage. The study was performed in accordance with the ethical standards of the Declaration of Helsinki (1964) and was approved by the Ethics Commission of the Ruhr University Bochum (Reg. No.:5105-14). The primary intention of this investigation was to develop a human biomonitoring method (HBM) including identification of suitable biomarkers of exposure in human urine and basic toxicokinetics. In addition, urinary conversion factors (CF) were deduced from the toxicokinetics results to allow the back-calculation of the exposure doses of BMHCA from urinary metabolite levels of the 40 adult volunteers.
Five healthy subjects (3 females, 2 males) were orally dosed once with BMHCA. Each volunteer received a chocolate-coated edible waffle cup containing 1 mL spiked ethanol (exact 5.26 mg lysmeral, equivalent to 25.7 µmol) and approximately 20 mL coffee, milk or water, depending on the choice of the volunteers. Urine was collected immediately before and for 48h after administration and frozen (< - 20°C) until analysis. The BMHCA metabolites lysmerol, lysmerylic acid, hydroxylated lysmerylic acid and 4-tert-butylbenzoic acid (TBBA) were determined in all urine samples by a newly developed UPLC-MS/MS (ultra-high-pressure liquid chromatography combined with tandem mass spectrometry) method. The derived conversion factors (CFs) were applied to spot urines samples of 40 health subjects (33 males, 7 females). The toxicokinetic variables for the urinary excretion of the BMHCA metabolites were evaluated individually for each subject. Where appropriate, means, standard deviations (SD) and medians were calculated. The amount of metabolites excreted after 3, 6, 12 and 24h were obtained by linear interpolation.

Results

The peak amounts of the 4 metabolites were excreted between 3 and 6h after oral BMHCA application. The primary metabolites lysmerol (2) and lysmerylic acid (3) appeared slightly earlier in the urine than the secondary metabolites hydroxyl-lysmerylic acid (9) and TBBA (4). After 12 and 24h more than 90 and 97%, respectively, of the BMHCA metabolites were excreted in the urine. The authors regarded excretion of these metabolites as complete by 48h after the oral intake.
After 48h, the urinary excreted metabolites of BMHCA are dominated by TBBA (4) representing about 14.3% of the administered dose, followed by lysmerol (2), yielding 1.82% of the dose after 48 h. Hydroxy-lysmerylic acid (9) and lysmerylic acid (3) represented only 0.20% and 0.16% of the dose, respectively. In total, the 4 metabolites represented about 16.5% of the dose. Average times for peak excretion (tmax) were 2.2 h and 4.64 h for lysmerol (2) and TBBA (4) and 3.1 h for both lysmerylic acid (3) and hydroxyl-lysmerylic acid (9). After 24 h, between 95% (TBBA) and 99% (lysmerol) were excreted. The elimination half-lives (t½) were found to be lower for the primary metabolites lysmerol (2) and lysmerylic acid (3) (1.19 h and 1.25 h, respectively) than for the secondary metabolites hydroxyl-lysmerylic acid (9) and TBBA (4) (1.39 h and 1.40 h, respectively).
Volunteers CF values were applied to 40 urine samples collected by subjects of the general population. Creatinine standardised urinary BMHCA metabolite levels were used for back

calculation of the uptake dose. The CF derived with the molar sum of all four metabolites (2 + 3 + 9 + 4) yielded a median uptake dose of 224 µg/d.

Conclusion

This explorative metabolism study confirmed that TBBA, lysmerol, lysmerylic acid and hydroxyl-lysmerylic acid are major urinary BMHCA metabolites in humans. Therefore, they can be considered as possible biomarkers for assessing exposure in human biomonitoring studies. While TBBA is quantitatively the most dominant BMHCA metabolite in urine, its specificity might be hampered by other sources of TBBA apart from BMHCA. The three other metabolites, carrying the full BMHCA backbone, are considered as specific to BMHCA representing about 2% of the oral dose.

Peak excretion for all metabolites occurred between 2 and 5h after oral application, with the primary metabolites (lysmerol and lysmerylic acid) being excreted about 1h earlier than the secondary metabolites (hydroxylated lysmerylic acid and TBBA). More than 90% of all measured lysmeral metabolites were excreted after 12h, with the renal excretion being virtually complete after 48h. After this time period, TBBA, lysmerol, lysmerylic acid and hydroxyl-lysmerylic acid represent on average 14.3, 1.82, 0.20 and 0.16%, respectively, of the dose administered. In total, the 4 metabolites determined represent about 16.5% of the dose. Back-calculation of the exposure dose in 40 adult subjects from the general population resulted in median daily doses of 140–220 µg/d BMHCA, depending on the inclusion or exclusion of TBBA in the combined urinary conversion factors.

Ref.: Scherer et al., 2016, SMII: 22

3.3.10 Photo-induced toxicity

From submission I

SCCS conclusion on photo-induced toxicity

Based on the data and studies available, BMHCA is unlikely to exhibit photo-induced toxicity (irritation or sensitisation) in guinea pigs.

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

Additional data from Applicant's submission II dossier

No additional data.

3.3.10.2 Photomutagenicity / photoclastogenicity

Additional data from Applicant's submission II dossier

No additional data.

3.3.11 Human data

From submission I

SCCS conclusion on human data

There is no evidence that BMHCA exhibits photo-induced toxicity. However, undiluted BMHCA is a proven skin irritant. In most HRIPT studies, BMHCA – when being dissolved in a mixture of ethanol and diethyl phthalate – did not provoke skin sensitising reactions after dermal application at concentrations of up to 25%. Conversely, BMHCA dissolved in

petrolatum already caused positive skin reactions in this assay at concentrations of 5%, thus demonstrating the influence of the vehicle being used to administer the compound onto skin. Additional data from clinical populations also point to sensitising properties of BMHCA, albeit at only low frequencies. Reactions were only occasionally observed at concentrations of <5%. Overall, mainly based on clinical studies, the SCCS considers BMHCA as an "established contact allergen in humans", an opinion it has held since 2012 (SCCS, 2012).

3.3.11.1. Irritation

Additional data from Applicant's submission II dossier

No additional data.

3.3.11.2. Sensitisation

Additional data from Applicant's submission II dossier

No additional data.

3.3.11.3. Other clinical data

Recently, the working group of Schnuch et al. 2015 analysed the frequency of sensitisation to 26 fragrances in 5451 products including BMHCA to be labelled according to current EU legislation.
Use volumes were provided by the International Fragrance Association (IFRA). Data on sensitization frequency generated by the Information Network of Departments of Dermatology (IVDK) network between 2007 and 2009 and specifically 2008 were used. Results of patch testing on the 26 labelled fragrances (1870 patients (in 2008: n=823) were analysed. The proportion of reactions to single constituents in breakdown testing in fragrance mix positives from testing the standard series was extrapolated to the study population (n = 1870) yielding the frequency of sensitisation to single constituents. The relative frequency of sensitisation was calculated as the share of sensitisation to a single allergen (%) relative to the total of sensitisation (=100%) to fragrances. Sensitisation exposure quotient (SEQ) as an estimate of sensitisation risk associated with exposure to the respective fragrance was calculated as the quotient of the relative frequency of sensitisation divided by the relative frequency of use. The SEQ varied greatly, offering a ranking regarding risk of sensitisation.
Although BMHCA was highly used in terms of the relative volume sold (standardised market share) of 19.42%, only 0.7% of the 1870 patients tested showed a positive allergic reaction. The share of positive reactions to BMHCA was calculated to be 2.9% (confidence interval (CI): 1.5 - 4.9) and the resulting sensitisation exposure quotient (SEQ) of 0.15 indicated a low risk of sensitisation.

Ref.: Schnuch et al., 2015 (SMII: 23)

SCCS comment
The study confirms that, while BMHCA is a sensitiser, risk of sensitisation at current use levels is low.

3.3.12 Special investigations

Additional data from Applicant's submission II dossier

The possible estrogenic activity of BMHCA (source: Sigma, Poole, UK, purity: ≥ 95%) was examined in an explorative screening assay in MCF7 human breast cancer cells *in vitro*. At 3.000.000-fold molar excess, BMHCA partially displaced [^{3}H]-estradiol from recombinant human estrogen receptors ERα and ERβ and from cytosolic estrogen receptor of MCF7 cells. At concentrations in the range of 5×10^{-5} to 5×10^{-4} M it increased the expression of a stably integrated estrogen-responsive reporter gene (ERE-CAT) and of the endogenous estrogen-responsive *pS2* gene in MCF7 cells. However, the increase was clearly below the positive control 17β-estradiol (10^{-8} M). BMHCA led to an increase of the proliferation of the estrogen-dependent MCF7 cells over 7 days. This effect was inhibited by the anti-estrogen fulvestrant, suggesting an ER-mediated mechanism.
Although the extent of stimulation of proliferation over 7 days was lower with BMHCA than with 10^{-8} M 17β-estradiol, given a longer time period of 35 days the extent of proliferation with 10^{-4} M increased to the same magnitude as observed with 10^{-8} M 17β-estradiol over 14 days. Based on these observations the authors concluded that BMHCA is able to induce estrogenic responses in the MCF7 human breast cancer cell line *in vitro*.

Ref.: Charles and Darbre, 2009, SMII: 10

However, *in vitro* receptor-binding alone does not inform whether specific exposures to that substance may lead to adverse effects *in vivo*. For BMHCA, there is ample evidence from a variety of *in vivo* studies of a lack of adverse effects on female reproductive organs or fertility. Especially the most recent extended one-generation reproduction toxicity study led to no effects on fertility or on reproductive or endocrine organs (see section 6.8.2). Furthermore, the adverse testicular effects of BMHCA in sensitive species appear not to be endocrine-related, but due to overt toxicity to seminiferous tissues including a clear threshold.
BMHCA (no data on source, batch or purity) and its main metabolite p-tert-butylbenzoic acid (TBBA) (no data on source, batch or purity) were tested for agonist and antagonist activities against human RARα, RARβ and RARγ receptors under GLP conditions. The tested concentrations ranged between 0.0013 – 100 µM. All treatment concentrations were performed in triplicate. DMSO was used as solvent and examined as negative control. Agonists (9-cis-retinoic acid, all-trans-retinoic acid) and antagonists (BMS195614, CD2665) were used as reference compounds. For all treatment groups, the DMSO concentration was normalised to a final concentration of 0.1%. 100 µl of each treatment medium was dispensed into triplicate assay wells pre-dispensed with the Reporter Cells. Assay plates were incubated at 37°C for 24 h. In the agonist assays, BMHCA and TBBA exhibited no agonist activity towards human RARα and RARβ receptors. BMHCA showed very low-level, non-dose-dependent agonist activity towards human RARγ receptor (about 2.5 fold activation at 4.0 µM only), which is finally considered as biologically not relevant. In the antagonist assays, none of the test compounds showed antagonist activity towards human RARα and RARβ and RARγ receptors.

Ref.: Indigo Biosciences, 2016, SMII: 15

SCCS comment
In *in vitro* experiments a potential BMHCA estrogenic activity has been noted but at a lower concentration than observed for the reference. However, as only estrogenic activity was considered, the SCCS cannot exclude an endocrine mediated mode of action for BMHCA.

3.3.13 Safety evaluation (including calculation of the MoS)

Based on analysis of data provided in submission I and additionally in submission II, the SCCS is of the opinion that genotoxicity potential of BMHCA cannot be excluded. Therefore, the SCCS cannot conclude on the safety of BMHCA.

CALCULATION OF THE MARGIN OF SAFETY

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3.3.14 Discussion

Physicochemical properties

Based on the previous SCCS Opinion (SCCS/1540/14):
BMHCA is a colourless to pale yellow liquid carrying a mildly floral odour, reminiscent of cyclamen and lily of the valley. It is commercially available at a purity of ≥97.5% (w/w). According to the applicant the degree of purity can be as high as ≥99.5% (w/w). Possible impurities include 3-(3-*tert*-butylphenyl)-2-methylpropanal and lysmerylic (lilac) acid. The latter compound results from air oxidation in aqueous solutions at pH7 and 25°C.

General toxicity

Based on the previous SCCS Opinion (SCCS/1540/14) and submission II:
The acute toxicity after all relevant routes of application of BHMCA was investigated in rats and rabbits. Based on the LD_{50} values obtained, the acute (lethal) toxicity of BMHCA can be considered moderate (>1300 mg/kg bw, oral route) or low (>2000 mg/kg bw, dermal route). However, a single oral application of 50 mg BMHCA per kg body weight in male rats already led to testicular atrophy in 2 out of 5 animals. An inhalation toxicity test in rats led to no mortalities but to signs of systemic toxicity after exposure to a saturated atmosphere.
The data on acute toxicity of BMHCA provided in Submission II do not change the previous SCCS conclusion (SCCS/1540/14).

Repeated dose toxicity

Based on the previous SCCS Opinion (SCCS/1540/14):
The toxicity of BMHCA after repeated oral application was investigated in several species. Decreases in body weights and food consumption and/or clinical signs of toxicity were observed after subacute oral administration of BMHCA at doses of ≥50 mg/kg bw/day (rats) and ≥200 mg/kg bw/day (dogs). Clinical chemistry and histopathological examinations repeatedly revealed adverse effects on the liver and male reproductive system (testicular toxicity). In a 90-day GLP study in rats BMHCA dose-dependently induced systemic toxicity in both sexes at levels of ≥25 mg/kg bw/day and testicular toxicity in males at ≥50 mg/kg bw/day. Thus oral NOAEL values of 5 mg/kg bw/day and 25 mg/kg bw/day were derived for systemic effects and reproductive effects, respectively.

Reproductive toxicity

Based on the previous SCCS Opinion (SCCS/1540/14) and submission II:
Adverse effects of BMHCA on the male reproductive system have been consistently observed in several repeated dose and reproduction toxicity studies. A NOAEL of 25 mg/kg bw/day in male rats with regard to this endpoint is substantiated by studies applying the compound for 5 days, 90 days or in the frame of a 1-generation study over 6 weeks prior to

mating. In all investigations available, testicular toxicity in rats was accompanied by signs of systemic toxicity. By contrast, other species such as mice and dogs were less sensitive. In dogs, a NOAEL of 40 mg/kg bw/day has been established based on the onset of testicular toxicity after treatment periods of 2 weeks and 3 months. So, from the animal data available, male rats revealed as the most sensitive species with regard to BMHCA-mediated testicular toxicity. On the other hand, in female rats developmental toxicity was accompanied by systemic toxicity and found already at lower concentrations. Here, a NOAEL is to be set at 5 mg/kg bw/day. This value is identical to the one defined for general systemic toxicity in rats based on repeated dose toxicity studies. The data available point to rats as most sensitive animal species tested. Toxicokinetic studies revealed that hepatic metabolism of BMHCA in rats results in significantly higher levels of p-tert-butylbenzoic acid (TBBA) when compared to other species. The SCCS is aware of older short-term studies applying TBBA to rats via the oral route and suggesting that this metabolite may also exert testicular toxicity (along with systemic toxicity). However, the doses applied in these studies from the 1960s – 1980s were high and the quality of the studies generally low. The data available therefore do not support the conclusion that this metabolite would be mainly responsible for the testicular effects observed with BMHCA in rats.

In the extended one-generation reproduction toxicity study which results were provided in submission II, the NOAEL for general, systemic toxicity of BMHCA applied in encapsulated form at 1, 3, or 10 mg/kg bw/d, was established at 3 mg/kg bw/d for the F0 and F1 parental as well as adolescent animals, based on evidence for distinct liver toxicity. This value was further supported by corresponding effects on food consumption, body weights and clinical pathological parameters, which were observed at 10 mg/kg bw/d predominantly in females. The NOAEL for fertility and reproductive toxicity of BMHCA in this study could be established at 10 mg/kg bw/d. The NOAEL for developmental toxicity in the F1 and F2 progeny was 3 mg/kg bw/d (equivalent to a mean overall oral dose of 4.5 mg/kg bw/d), based on reduced pup body weights in the F1 and F2 offspring, which were observed at 10 mg/kg bw/d. As these weight reductions were only observed in the presence of maternal toxicity, including lower weight gain during pregnancy, they are not considered as an indication for specific developmental toxicity.

Irritation/sensitisation

Based on the previous SCCS Opinion (SCCS/1540/14):
BMHCA as neat compound is irritating to the skin and eyes of rabbits. A solution of 2% BMHCA in propylene glycol led to mild skin erythema. In general the observed effects occurred transiently and were reversible. In a special investigation, BMHCA also displayed the potential of inducing respiratory irritation at high concentrations (starting at about 70 µg/L in the atmosphere). In humans 10 and 20% BMHCA (dissolved in 75% ethanol/25% diethyl phthalate) led to faint, minimal erythema in 1 and 2 out of 25 volunteers, respectively.
According to its sensitising potential, BMHCA was comprehensively tested in experimental animals. Several positive LLNA are available. Depending on the solvent used, the EC3 values ranged from 2.97% (in EtOH) to 13.91% (in 25% EtOH/75% DEP), and up to 18.7% by application of BMHCA in acetone/olive oil (4:1). An EC3 value of about 2.9% BMHCA in the LLNA has been substantiated by data from the International Fragrance Association (SCCS, 2012). Based on the animal data obtained, the overall potency classification of BMHCA is ”moderate sensitiser”. In most HRIPT studies, BMHCA, dissolved in EtOH/DEP, was unable to induce skin sensitisation at concentrations of up to 25%. However, BMHCA dissolved in petrolatum caused positive reactions already at concentrations of 5%. Additional data from clinical investigations also pointed to sensitising properties of BMHCA. However, reactions were rare at concentrations of <5%. In 2012, SCCS considered BMHCA as “established contact allergen in humans”. In light of an experimentally substantiated EC3 value of 2.9% (BMHCA in EtOH), the concentrations of this compound suggested to be permitted in finished products of up to 3% must be considered too high. Based on human

(patch-test) data it can be concluded that, while BMHCA is a sensitiser, risk of sensitisation at current use levels is low.

Dermal absorption

Based on the previous SCCS Opinion (SCCS/1540/14) and submission II:
Administration of BMHCA onto the skin of both experimental animals and humans demonstrated permeation and systemic availability of this compound. Further, *in vitro* studies demonstrated solvent dependent and species specific effects. The bioavailable portion was found much higher in rats (>50%) when compared to mini pigs (<5%). Applying real cream formulations of 0.6% BMHCA, again rat skin allowed a much higher absorption (>45%) than mini pig skin (about 25%). In the latter the fraction of bioavailable BMHCA increased from 4.9% (EtOH solution) to 25% (cream formulation).
In vivo, percutaneous absorption of BMHCA in humans was lower when compared with rats (1.4 vs. 19%). The range in 3 volunteers observed was 0.8 – 2.4% (excreted in urine within 24 hours). So, the absorption found in humans for ethanolic solutions of BMHCA was comparable to that was has been found in excised mini pig skin.
In the study on percutaneous study provided in submission II the SCCS identified significant deviations from the SCCS requirements. The electrical resistance of the human skin samples was far below the 10 kΩ threshold for intact skin. In addition, according to SCCS 1358/10, recovery should be between 85 - 115%. The overall recovery of BMHCA tested in formulations 1 ("ethanol in water") and 2 ("silicone in water") was not within this acceptance range, even under the semi-occlusive conditions used. According to SCCS 1564/15, in the case of substances with very low dermal absorption and limited permeation (e.g. colourants or UV-filters with high molecular weight and low solubility), the epidermis may be excluded when it is demonstrated that no movement of the chemicals from the skin reservoir to the receptor fluid occurs. BMHCA did not fulfil these criteria. Therefore, all BMHCA present in the living epidermis had to be taken into account for the dermal absorption. Based on these deviations from the SCCS requirements, the mean + 2 SD should be taken for potential MoS calculation, i.e.: "Ethanol in water" (24h) = 13.5%, "Silicone in water" (24h) = 8.5%, "Water in oil" (24h) = 14.7%, "Oil in water" (24h) = 11.5%.

Mutagenicity

Based on the previous SCCS Opinion (SCCS/1540/14) and submission II:
In its previous Opinion (SCCS/1540/14) the SCCS concluded that neither *in vitro* gene mutation nor *in vitro* chromosomal damage could be excluded based on the data provided in submission I. Similarly, due to the lack of sufficient and detailed information, it was also impossible to draw a firm conclusion from the *in vivo* micronucleus report provided.
Based on the analysis of additional reports provided in submission II the SCCS considers that the data do not allow excluding potential genotoxic effects of BMHCA because:

- In the tests on gene mutations in bacteria:
    - BMHCA was confirmed to induce gene mutations in TA1535 strain
    - The study based on the Ames test was considered to be of limited value as: positive controls used did not clearly demonstrate positive response, no information on historical controls was available and BMHCA was tested in low concentrations,
- In the tests on chromosomal aberrations *in vitro*:
    - The study on micronucleus test on human peripheral blood lymphocytes was considered to be of limited value as: BMHCA was tested without metabolic activation, limited information was provided on treatment of cells, cytotoxicity or on study methodology and no information on historical controls was available,
- In the comet assay *in vitro*:
    - The study on human colonic epithelial cells was considered to be of limited value as: only 24h exposure was used though shorter incubation times (3-4h

treatment) should have also been used, at least 3-5 concentrations ranging from non-toxic up to mild toxic (around 80% viability) should be used, testing only one concentration of 100 µg/mL was not justified.

Based on analysis of data provided in submission I and additionally in submission II, the SCCS maintains its previous opinion that no firm conclusion can be drawn on mutagenicity of BMHCA.

Carcinogenicity

Based on the previous SCCS Opinion (SCCS/1540/14):
No specific investigations available. There is no evidence from repeated dose studies in animals that BMHCA is capable of inducing cancer.

Toxicokinetics

Based on the previous SCCS Opinion (SCCS/1540/14) and submission II:
Quantitative data on the toxicokinetics of BMHCA are available from rat, mouse, rabbit, guinea pig, dog and rhesus monkey and humans. Given its physicochemical properties, BMHCA is likely to have high bioavailability via the oral route. Similarly, data after dermal administration clearly demonstrates that BMHCA becomes systemically available in animals and humans.
Species specific differences in the metabolism of BMHCA have been identified *in vitro* as well as *in vivo*. Still, lysmerylic acid (oxidation product) was the main hepatic metabolite in all species tested. Quantitative evaluation of the metabolic profiles in different species *in vitro* demonstrated much higher levels of *p-t*-butyl-benzoic acid (TBBA) formation by rat hepatocytes when compared to other species. Older studies with rats also provided some evidence of testicular toxicity induced by TBBA, suggesting that this metabolite might be involved in the effects triggered upon application of its parent.
TBBA levels observed in human hepatocytes were about 4-fold lower compared to rat hepatocytes at corresponding concentrations. Comparative assessment of the urinary metabolites in different animal species again uncovered differences in the urinary excretion of TBBA (and TBHA), with rats being the species that predominantly forms TBBA. However, the differences observed between rats and monkeys did not mirror the 4-fold difference in TBBA formation as seen with rat and human liver microsomes *in vitro*. Therefore, the available information on species differences is not sufficient to conclude that rats are more sensitive than humans.
The data on metabolism of BMHCA provided in submission II confirmed that TBBA, lysmerol, lysmerylic acid and hydroxyl-lysmerylic acid are major urinary BMHCA metabolites in humans. Peak excretion for all metabolites occurred between 2 and 5 h after oral application, with the primary metabolites (lysmerol and lysmerylic acid) being excreted about 1 h earlier than the secondary metabolites (hydroxylated lysmerylic acid and TBBA). After 48 h, TBBA, lysmerol, lysmerylic acid and hydroxyl-lysmerylic acid represent on average 14.3, 1.82, 0.20 and 0.16%, respectively, of the dose administered.

Human data

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4. CONCLUSION

1. Does the SCCS consider Butylphenyl methylpropional (p-BMHCA) safe for use as a fragrance ingredient in cosmetic leave-on and rinse-off type products in a concentration limit(s) according the ones set up by IFRA as reported above?

Based on analysis of data provided in submission I and additionally in submission II, the SCCS is of the opinion that genotoxicity potential of BMHCA cannot be excluded. Therefore, the SCCS cannot conclude on the safety of BMHCA.

2. Does the SCCS have any further scientific concerns with regard to the use of Butylphenyl methylpropional (p-BMHCA) as a fragrance ingredient in cosmetic leave-on and/or rinse-off type products?

Evaluation of this substance by other scientific bodies (under REACH) will also need to be taken into consideration for any future assessment of the substance.

5. MINORITY OPINION

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6. REFERENCES

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