



Scientific Committee on Consumer Safety

SCCS

OPINION ON

Butylphenyl methylpropional (BMHCA)

The SCCS adopted this Opinion by written procedure

on 12 August 2015

Revision of 16 March 2016

About the Scientific Committees

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

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

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

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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This opinion has been subject to a commenting period of eight weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, fragrance ingredients, Butylphenyl methylpropional, Regulation (EC) No. 1223/2009, CAS 80-54-6

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	3
1. BACKGROUND	5
2. TERMS OF REFERENCE.....	6
3. OPINION.....	7
3.1. Chemical and Physical Specifications.....	7
3.2. Function and uses.....	9
3.3. Toxicological Evaluation	9
4. CONCLUSION	44
5. MINORITY OPINION.....	45
6. REFERENCES.....	45

1. BACKGROUND

2-(4-*tert*-Butylbenzyl)propionaldehyde (BMHCA) CAS No. 80-54-6 is a fragrance ingredient used in many compounds 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 currently regulated for labelling purposes in Annex III, 83 of the Cosmetic Regulation No. 1223/2009 when present in a concentration above 10 ppm for leave-on products and above 100 ppm for rinse-off products.

The substance was introduced in Annex III of the Cosmetics Directive 76/768/EEC by the 7th amendment (2003/15/EC) on the basis of the scientific Opinion (SCCNFP/0017/98) adopted during the plenary session of 8 December 1999. Subsequently, it was taken over in the Annexes to Regulation (EC) No 1223/2009.

In October 2012, BMHCA was introduced in the Registry of submitted Harmonised Classification and Labelling intentions (<http://clp-inventory.echa.europa.eu/>), in view of a harmonised classification as CMR 2 (fertility), under the Regulation (EC) No 1272/2008. However, the substance has not been yet formally classified as a CMR2 under that Regulation.

In April 2013, the Commission has received a dossier by the International Fragrance Association (IFRA) on the safety assessment of BMHCA. This submission is intended to demonstrate the safety of the ingredient when used as a fragrance ingredient in cosmetic leave-on and rinse-off type products. IFRA recommends a safe concentration limit for BMHCA when it is used in the specific categories of cosmetic products as developed by the International Fragrance Association (IFRA).

Limits in the finished product:			
Category 1 (Lip products)	0.1 %	Category 6 (Mouthwash, toothpaste)	3.0 %
Category 2 (Deodorants/Antiperspirant)	0.2 %	Category 8 (Make up remover, nail care)	2.0 %
Category 3 (Hydroalcoholic products for shaved skin)	0.6 %	Category 9 (Shampoo, rinse-off conditioner, bar soap)	2.5 %
Category 4 (Hydroalcoholic products for unshaved skin)	1.9 %		
Category 5 (Women's facial cream, facial make-up, hand cream)	1.0 %		

2. TERMS OF REFERENCE

- 1. Does the SCCS consider 2-(4-tert-butylbenzyl)propionaldehyde (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 2-(4-tert-butylbenzyl)propionaldehyde (BMHCA) as a fragrance ingredient in cosmetic leave-on and 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

EC name: 2-(4-*tert*-Butylbenzyl)propionaldehyde
para-tert-Butyl- α -methyl-hydrocinnamaldehyde
 IUPAC name: 3-(4-*tert*-Butylphenyl)-2-methylpropanal

3.1.1.3. Trade names and abbreviations

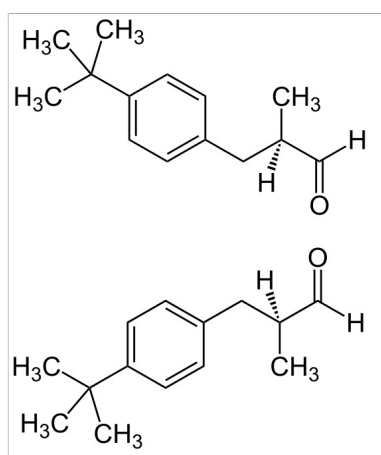
Lilial®
 Lilestralis
 Monastral Red B
 Quinacridone
 Lysmeral®Extra
 BMHCA

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

3.1.1.4. CAS / EC number

CAS: 80-54-6
 EC: 201-289-8

3.1.1.5. Structural formula



According to CLH Report, BASF SE, 30.9.2013: Lysmeral®Extra is a racemic mixture of (2*S*)-3-(4-*tert*-butylphenyl)-2-methyl-propanal and (2*R*)-3-(4-*tert*-butylphenyl)-2-methyl-propanal

3.1.1.6. Empirical formulaFormula: C₁₄H₂₀O**3.1.2. Physical form**

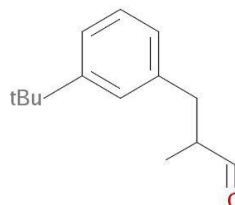
Physical state at 20°C (1013 hPa): liquid, colourless to pale yellow; odour: mildly floral, reminiscent of cyclamen and lily of the valley

3.1.3. Molecular weight

Molecular weight: 204.31

3.1.4. Purity, composition and substance codesDegree of purity (according to IFRA/IRSC dossier): ≥99.5% (w/w)
"Lysmeral technisch" (according to ECHA website): ≥97.5% (w/w)**3.1.5. Impurities / accompanying contaminants**

According to the applicant, the degree of purity is ≥99.5% (w/w). All impurities (not specified) are present at levels <0.3%. Concentration range of all impurities together: >0.5% – <1%.

According to ECHA, possible impurities: 3-(3-*tert*-butylphenyl)-2-methylpropanal, EC

number: 263-580-6, CAS number: 62518-65-4.

Source: European Chemicals Agency

http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d8c081b-f7a8-0b62-e044-00144f67d249/AGGR-258209a0-74ce-45e9-9cbe-cefc17754be9_DISS-9d8c081b-f7a8-0b62-e044-00144f67d249.html#section_1.1
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 mm²/sec at 23°C
 pKa: Substance without any ionic structure
 Refractive index: 1.503 – 1.507 at 20°C
 UV_Vis spectrum: $\lambda_{\max} \approx 263$ nm

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 (of the order of two weeks) and that its oxidation product, lilic acid (lysmerylic acid), is the major species 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 $\geq 99.5\%$ (w/w) when being applied in toxicological studies.

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.”

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: / (BASF SE 1981; RIFM#63831)
 Species/strain: Rat (Sprague-Dawley)
 Group size: 5 rats/sex
 Test substance: BMHCA
 Batch: no data
 Purity: /
 Vehicle: 0.5% aqueous carboxymethyl cellulose
 Dose levels: 681; 1000; 1470; 2150, 3160 mg/kg bw
 Administration: oral (gavage), 10 ml/kg bw
 GLP: /

Study period: 6 July 1979 – 29 August 1979

The test substance was administered by oral administration to each of 5 fasted rats per sex at dose levels of 681, 1000, 1470, 2150 and 3160 mg/kg bw. The test substance was applied in a 0.5% aqueous carboxymethyl cellulose preparation. Animals were observed for treatment-related effects for a 14-day observation period. After the observation period, animals were sacrificed and a gross pathological examination was conducted.

Results:

Mortality was observed, starting 1 day after application of the test substance and unspecific clinical signs of toxicity, i.e. poor general state, rough fur coat, drunken and spastic gait, dyspnoea, apathy and abnormal position were observed in all dose groups. Necropsy of the animals that had died revealed dilated hearts, congestions and discoloured livers, while sacrificed animals showed no gross-pathological findings.

Conclusion:

The acute oral toxicity (LD₅₀) in rats was determined at 1390 mg/kg bw (95% confidence limits: 1019 – 1867 mg/kg bw).

3.3.1.2. Acute dermal toxicity

Guideline: / (BASF SE 1981; RIFM#63831)

Species/strain: Rat, strain unspecified

Group size: 3 rats

Test substance: BMHCA

Batch: no data

Purity: unclear

Vehicle: aqueous carboxymethyl cellulose

Dose levels: 2000 mg/kg bw

Administration: dermal, single application

GLP: no

Study period: 6 July 1979 – 29 August 1979

Three rats were treated by a single dermal (occlusive) application of BMHCA at 2000 mg/kg bw dissolved in carboxymethyl cellulose. The animals were observed for a period of 14 days for mortality, body weight gain and clinical signs of toxicity. At termination the animals were sacrificed and a gross pathological examination was conducted.

Results:

No mortality was observed, and clinical signs of toxicity as well as of irritation, i.e. dyspnoea, agitation, apathy, staggering, rough fur coat, lacrimation, poor general condition and slight erythema/oedema followed by desquamation were reversible within the observation period.

Conclusion:

Dermal LD₅₀ in rats was >2000 mg/kg bw.

Second study:

Under non-GLP conditions (ref: MB Research Laboratories, 1977, RIFM# 1695), test substance was administered dermally to each of 10 rabbits at a dose level 5000 mg/kg bw. The animals were observed for treatment-related effects for a 14-day observation period. After the observation period, animals were sacrificed and a gross pathological examination was conducted. No deaths or systemic clinical signs were observed. Signs of irritation consisted of mild to moderate redness and oedema. Necropsy showed mottled or yellow

kidneys in two animals and sloughing skin in all animals. Conclusion: LD₅₀ in rabbits >5000 mg/kg bw.

Third study:

Under GLP conditions (ref: Cosmopolitan Safety Evaluation, 1979, RIFM# 15027), test substance was applied to the shaved back of each of 3 animals per sex (rabbits) under occlusive condition for 24 hours at a dose level of 5 mL/kg bw (corresponding to about 4700 mg/kg bw). The animals were observed over a 14-day period, then sacrificed and a gross necropsy was conducted. No deaths occurred. Clinical signs included erythema and thickened wrinkled skin, which persisted through day nine but declined thereafter. Gross pathology revealed no indication for systemic effects. Conclusion: LD₅₀ in rabbits >4700 mg/kg bw.

3.3.1.3. Acute inhalation toxicity

Guideline: / (BASF SE 1981; RIFM#63831), according to Smyth et al., 1962

Species/strain: Rat (Sprague-Dawley)

Group size: 6 rats/sex

Test substance: BMHCA

Batch: no data

Purity: unclear

Vehicle: atmosphere

Dose levels: saturated atmosphere

Administration: Inhalation

GLP: no

Study period: 6 July 1979 – 29 August 1979

12 rats were exposed to an atmosphere saturated with BMHCA at 20°C for 7 hours. The animals were observed for treatment-related effects for a 14-day observation period. After the observation period, animals were sacrificed and a gross pathological examination was conducted.

Result: Although no mortality, clinical signs of toxicity or organ findings were observed.

SCCS conclusion on acute toxicity:

The acute toxicity after all relevant routes of application of BHMCA was investigated in rats and rabbits (oral, dermal, inhalation). The acute oral LD₅₀ value in rats was determined to be 1390 mg/kg bw and the acute dermal LD₅₀ 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).

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: / (RIFM 1984a, RIFM #1795, ECETOC 1995, RIFM #50448)

Species/strain: Rabbit / New Zealand White

Group size: 3 females
Test substance: BMHCA
Batch: 856'564
Purity: 97.8%
Vehicle: /
Dose level: 0.5 mL neat (undiluted) substance for 4 hours (semi-occlusive)
Dose volume: 0.5 mL
Exposure: 4 hours
Observation: Skin readings at 1, 24, 48, 72, 168 hours after patch removal
GLP: yes
Study period: May and/or June 1984

The procedures used were based on the test for skin irritation described in Annex V of EEC Directive 79/831. An amount of 0.5 mL of the neat test substance was placed evenly over a 2.5 cm square of surgical lint B.P. The lint square was then placed onto the animal's skin, on the left flank and immediately caudal to the last rib, with the test material in contact with the skin. A second material was applied in the same manner to the skin of the right flank, bilateral to the first material. Two other materials were applied caudal to the sites dosed with the first two materials. The lint patches were held in place by encircling the trunk of the animal with an elastic adhesive bandage which also served as a semi-occlusive barrier to the treated skin. All rabbits were treated in this manner, except that the relative position of the test materials was varied. The treated sites were assessed for signs of reaction to treatment at 1, 24, 48, 72 and 168 hours after patch removal and scored according to the grades in OECD test guideline 404.

Results:

Erythema were observed in all animals with a mean score of 1.7 (24 hrs), 2.0 (48 hrs) and 2.3 (72 hrs). Oedema was also observed in all animals with a mean score of 2.3 (24 hrs), 2.7 (48 hrs) and 2.7 (72 hrs). Erythema and oedema were still present at the end of the observation period of 7 days (mean score of 1.7 and 1.3, respectively) and a marked desquamation from skin surface was observed in all animals. The mean scores at 24, 48 and 72 hours after patch removal for erythema and oedema were 2.0 and 2.6, respectively.

Conclusion:

Under the conditions of the study, neat BMHCA was irritating to the skin of rabbits.

SCCS comment:

The SCCS noted that 32 test substances were tested in this study. As information regarding the non-relevant test substances has been retracted from the study report, the exact study period for the test substance BMHCA cannot be identified.

The SCCS also noted that four different test substances were tested on each animal simultaneously and that a control site (untreated skin) was not evaluated in the study.

The observation period in the study was only 7 days (14 days observation period in the *in vivo* test guideline study, both EC and OECD).

Under the conditions of the study, the test substance BMHCA (purity 97.8%) is a moderate skin irritant.

Additional information:

1. Confirmation of irritating potential (ref. RIFM 1985a, RIFM #3099): GLP-like study under the same test conditions including semi-occlusive application for 4 hours of undiluted BMHCA (purity of 95%), 4 rabbits: mean oedema score of 1.7 and a mean erythema score of 1.9 (readings: 24, 48, 72 hrs). Desquamation at the end of the 7-day observation in all animals.

2. GLP-like study (ref: Bush Boake Allen, 1980c, RIFM#52290) with enhanced and prolonged application on 6 female rabbits. A 0.5-ml aliquot of BMHCA at 2% in propylene glycol was placed on pads attached to adhesive wrapping (24 hours, occlusive) on both flanks. The sites were scored according to Draize at 1 and 48 hours after patch removal. No oedema was observed on either flank. At the 1-hr reading, very slight (3/6) to well-defined erythema (1/6) was observed on the abraded and intact flank. No erythema was observed at the 48-hrs reading. The primary irritation score was 0.5 and was similar to that observed with propylene glycol.

3. Further indication of the skin irritating properties of BMHCA was given in studies with dermal application, e.g. acute dermal toxicity study or range finding studies for skin sensitization in rabbits, mice and Guinea pigs as well as in the induction phase of Human repeated insult patch tests (see below). In addition, BMHCA has been included as a chemical known to cause skin irritation in the validation of *in vitro* tests for skin irritation (reference: Fentem et al. A pre-validation study on *in vitro* tests for acute skin irritation: results and evaluation by the Management Team. Toxicology in Vitro 15, 57-93, 2001, RIFM#38267).

3.3.2.2. Mucous membrane irritation

Guideline: OECD 405 (Roche 1987, RIFM#34335)

Species/strain: Rabbit /New Zealand White

Group size: 3

Test substance: BMHCA

Batch: 140'852

Purity: no data

Vehicle: diethyl phthalate (10 and 30% BMHCA)

Dose level: 0.1 mL neat substance

Dose volume: 0.1 mL (instillation in the conjunctival sac of the eye)

Observation: 1, 24, 48, 72 hrs, 7 days, 14 days

GLP: yes

Study period: May 1987

The irritant effect of the test substance was investigated by instillation of 0.1 ml of the neat test substance and concentrations of 10% and 30% in diethyl phthalate into the conjunctival sac of one eye of each animal; the untreated eye served as control. The eyes were not rinsed. The effects on the cornea, iris and conjunctivae (reddening, swelling, ulceration) were scored after 1, 24, 48 and 72 hours according to the method of Draize.

Results:

No effects on the cornea or iris were observed. Conjunctival redness (score 1) was observed at 1 and 24 hours after instillation of both concentrations of BMHCA dissolved in diethyl phthalate. Conjunctival redness (score 1) and chemosis and discharge (score 1) were observed with the neat material up to 72 hours and 24 hours, respectively, and were absent by day 7.

Conclusion:

Under the conditions of the study, neat BHMCA revealed only mild and transient effects, while concentrations of 10% and 30% led only initially to minimal mild effects. All findings were reversible.

Additional information:

Confirming GLP study (ref. Cosmopolitan Safety Evaluation, 1979a, RIFM #15026): 0.1 mL aliquot of an 8% BMHCA in propylene glycol; instilled to the right eye of each 6 albino New Zealand rabbits. Untreated left eyes served as control. Scoring was done according to the scheme of Draize at 24, 48 and 72 hours and at 4 and 7 days, a re-evaluation was made on

day 10 in three animals. Only slight effects occurred in individual animals. Corneal effects were noted up to 72 hours after instillation, while iris effects lasted until day 7 in a single animal. Conjunctival effects such as redness, chemosis, and discharge were observed. Conjunctival redness (slight to moderate) was observed. These effects were absent on day 4. Chemosis (very slight to severe) was observed in 4/6 rabbits. This effect was absent on day 4. Conjunctival discharge (slight to moderate) was observed in 3/6 animals. These effects were absent at 72 hours. The primary irritation score was 15.7 (24 hrs), 4.8 (48 hrs), 2.7 (72 hrs), 0 (4 days), and 0.83 (7 days).

3.3.2.3. Respiratory irritation

Special investigation (RIFM 1986a, RIFM#3433): The potential of BMHCA to induce acute respiratory irritation was investigated in four female albino mice per group. The animals were placed in individual polycarbonate restraining holders with the snout projecting into a cylindrical exposure chamber of approximately 2.6 L. After a 10-min pre-treatment period with exposure to room air only, the animals were exposed for 1 – 5 min to nebulized BMHCA at concentrations of 69.8, 256.9 and 815.4 µg/L. At the end of exposure, the animals were removed from the chamber and again exposed to room air. Animal respiration was recorded for 30 s periods at 3, 5, and 15 min after the exposure period. The percent decreases in respiration rates were 2.6%, 15.2%, and 41.2% at concentrations of 69.8, 256.9, and 815.4 µg/L, respectively, thus being indicative for respiratory irritation.

SCCS conclusion on irritation:

Under the conditions tested, BMHCA as neat compound revealed 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.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline: OECD 406, OPPTS 870.2600 (RIFM 2001a,b,c,d; RIFM #37065, #37066, #37067, #37068; Isola & Lalko 2001, RIFM #38352; Lalko et al. 2004, RIFM #45218)
 Species/strain: Mice (CBA/CA/Ola/Hsd)
 Group size: 4 males per group
 Test substance: BMHCA
 Batch: 658003
 Purity: 98.6%
 Vehicle: Ethanol (EtOH), diethyl phthalate (DEP), EtOH:DEP = 1:3 or 3:1
 Concentrations: 0.3, 1, 3, 10, 30, 50% (w/v)
 Positive control: α -hexylcinnamal in acetone
 GLP: yes

Method: Each group of four male mice was treated once daily for three consecutive days by topical application to the dorsal surface of each ear lobe (application volume of 25 µL). On day six, all mice were injected with 250 µL of [³H]-thymidine in sterile saline. Five hours later, mice were sacrificed.

Results: The estimated concentrations for the EC3 values were 2.97% (743 µg/cm²) for ethanol; 4.17% (1043 µg/cm²) for DEP; 13.91% (3478 µg/cm²) for EtOH:DEP=1:3, 8.85%

(2213 µg/cm²) for EtOH:DEP=3:1. Based on these results, it was concluded that BMHCA has the potential to induce dermal sensitisation in the murine LLNA.

Additional information:

1. First additional murine LLNA (according to OECD 406 and 429, GLP; ref.: RIFM 2001b, RIFM #41235): Groups of 4 female CBA/Ca/Ola/Hsd mice received a 25-ml aliquot of BMHCA (batch: 9000422'182, purity: 97.8%) in acetone:oil (4:1) to the dorsal surface of each ear for 3 consecutive days. The SI value was 0.8, 1.3, 1.3, 1.9, and 3.6 with 1%, 2.5%, 5%, 10% and 25%, respectively. Thus, BMHCA showed no potential for skin sensitisation up to a concentration of 10%, but was positive at 25%.

2. Second additional murine LLNA (ref.: RIFM 2001d, RIFM#41328) with BMHCA (batch: 9000418'269, purity: 98.2%) neat or dissolved in EtOH. SI values of 3.3, 9.8, 24.3 and 38.5 were observed for 10%, 25%, 50% and the neat test substance indicative for a skin sensitising property at all concentrations.

3. Third additional murine LLNA (Basketter 2001, RIFM#38311; Basketter 2003, RIFM#42276; Gerberick 2004, RIFM#45016): BMHCA was tested on groups of 4 female CBA/Ca mice at 1%, 2.5%, 10%, 25% and 50% in acetone:olive oil (4:1) resulting in SI values of 1.30, 2.47, 2.02, 3.71 and 9.26, respectively. Calculated EC3 = 18.7%. This result is in accordance with a comparative QSAR study (ref.: Patlewicz, 2003, RIFM #52041) in which an EC3 value of 19.7% is reported.

Guinea pig maximization test (GPMT)

Guideline: OECD 406, EEC 84/449 (Roche 1990; RIFM #16177)

Species/strain: Guinea pig (Himalayan)

Group size: 20 females (test group), 10 females (control) 2 females (pre-test intracutaneous), 4 females (pre-test epicutaneous)

Test substance: BMHCA

Batch: 180949

Purity: 99.1%

Vehicle: Ethanol (EtOH)

Concentration: 5% (0.1 mL, intradermal induction); 7 days later: topical booster with neat substance (dermal induction, occlusive, 48 hrs); 14 days later: 10%, 30%, or neat substance (epicutaneous challenge, occlusive, 48 hrs)

GLP: yes

Readings: 24 or 48 hrs after patch removal.

Results: At 24 or 48 hours after challenge, none of the treated animals (0/20) showed any skin reaction in form of erythema or oedema. Based on this, it was concluded that BMHCA exhibited no potential to induce dermal sensitisation in the GPMT test when tested as neat substance at challenge according to Magnusson and Kligman test conditions.

Additional information:

Contradictory GPMT study: Unilever 1988b (RIFM #7091): OECD 406, GLP conditions, Dunkin/Hartley guinea pigs (5 males/5 females). Intradermal induction: 3 pairs of intradermal injections consisting of 1) 0.1 mL of 50% Freund's Complete Adjuvant (FCA) in 0.01% dodecylbenzene sulphonate (Dobs)/0.9% physiological saline (Dobs/saline), 2) 0.1 mL of BMHCA at 1% in Dobs/saline, and 3) 0.1 mL of BMHCA in Dobs/saline. Topical induction: 1 week later, patch saturated with neat BMHCA applied for 48 hrs under occlusion. Challenge: 12-14 days after induction, patch saturated with BMHCA at 25% in acetone/PEG400 24 hours under occlusion. Readings: 24 and 48 hrs after patch removal. Reactions were observed in all 10 test animals. Rechallenge 1 week later with 0.25% and 2.5% in 6% acetone/PEG400. Reactions were observed in 1/10 and 9/10 animals at 0.25% and 2.5%, respectively, indicative for a clear sensitising potential of BMHCA.

Buehler test

Guideline: OECD 406, EEC 84/449 (Cosmopolitan Safety Evaluation 1979b, RIFM #15028)

Species/strain: Guinea pig (Hartley albino)

Group size: 10 males

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: Ethanol (EtOH)

Concentration: 2% (topical induction: 5 x 0.1 mL for 24 hrs on 9 cm², occlusive); 18 days later: epicutaneous challenge (2%, 5 x 0.05 mL on 4 cm², occlusive, 24 hrs)

GLP: yes

Readings: 30 min after induction, 24, 48 and 72 hrs after challenge.

Results: Signs of skin irritation were transiently observed during the induction phase in 1 out of 10 animals. No positive response in animals after challenge with 2%. Based on this, it was concluded that BMHCA exhibited a low potential to induce dermal sensitisation in Guinea pigs in the Buehler test, when tested as 2.0% ethanolic solution.

Other sensitization testing

The sensitising potential of BMHCA was tested in groups of 10 Himalayan guinea pigs according to the KAO test procedure (Roche 1982, RIFM#56769). Induction: topical patch application of neat (100%) or 30% ethanolic solution under occlusion for 24 h on days 0, 3, 7, 10 within 2 weeks. At day 7: simultaneous treatment by two intradermal injections of 0.1 mL Freund's complete adjuvant (FCA) on application sites. Challenge: occlusive epicutaneous application of 1% in ethanol (day 22) or of 1% in vaseline (day 35). Scoring: 24 and 48 hrs after removal of the patch.

Results: 30% BMHCA in EtOH: 5/10 animals were positive 24 and 48 hrs after challenge. Neat BMHCA: 6/10 animals were positive. Thus BMHCA induced dermal sensitisation in guinea pigs in the KAO test, when tested as neat or 30% ethanolic solution.

SCCS conclusion on skin sensitization:

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).

3.3.4. Dermal / percutaneous absorption**3.3.4.1. Dermal / percutaneous absorption *in vitro***

Explorative *in vitro* screening study on the percutaneous absorption and penetration of BMHCA in excised skin of mini pigs and naked rats (no data on animal strains, compound batches and purities; Roche 1982d, RIFM#56763):

12 µl/cm² of 1% [¹⁴C]-BMHCA (dissolved in methylcarbitol or EtOH, equal to 120 µg/cm² substance) was applied to a 5 cm² area of excised skin and rubbed into the skin for 30 seconds. 1, 6, 16 and 24 hrs later the amount of labelled material in the *stratum corneum*, stripped skin, and the chamber liquid was determined.

Recovery mini pigs: 6 hrs after exposure to BMHCA in methylcarbitol: 1.9% (2.22 µg/cm²) in the *stratum corneum*, 0.8% (0.91 µg/cm²) in the stripped skin, 0% in the chamber fluid; 16 hrs after exposure to BMHCA in EtOH: 3.9% (4.66 µg/cm²) in the *stratum corneum*, 4.1% (4.94 µg/cm²) in the stripped skin, 0.8% (0.93 µg/cm²) in the chamber fluid.

Recovery rats: 24 hrs after exposure to BMHCA in methylcarbitol: 7.0% (8.35 µg/cm²) in the *stratum corneum*, 61% (73.23 µg/cm²) in the stripped skin, 5.1% (6.14 µg/cm²) in the chamber fluid; 16 hrs after exposure to BMHCA in EtOH: 4.8% (5.81 µg/cm²) in the *stratum corneum*, 30% (36.05 µg/cm²) in the stripped skin, 20.8% (24.93 µg/cm²) in the chamber fluid.

Thus the bioavailable portion (chamber fluid, stripped skin) in hairless rat skin is significantly higher when compared to the skin of mini pigs.

Additional *in vitro* study (Roche 1986c, RIFM#56764): Same test procedure, [¹⁴C]-BMHCA incorporated in two different cream formulations (termed "cream 02_TD2=A", and "cream 04_TD1=B") at 0.6%. Unfortunately, the study does not provide the exact identity of the cream formulations used ("bases" that may have been used include: vaseline, hydrated wool-wax-alcohol ointment, hydrated hydrophilic ointment, polyethylene glycol ointment, and other "suitable" vehicles). Applied dose 6 mg/cm² (36 µg/cm² active substance, area of 5 cm²).

Recovery naked rats: 16 hrs after exposure to BMHCA in cream A/cream B: 2.6/2.1% (0.93/0.74 µg/cm²) in the *stratum corneum*, 21.6/39.5% (7.79/14.22 µg/cm²) in the remaining skin, 23.6/38.9% (8.49/14.01 µg/cm²) in the chamber fluid. Again, the penetration rates using the mini pig skin samples were lower. Data for mini pigs after 16 hours: 17.5/19.1% (6.30/6.89 µg/cm²) in the remaining skin and 6.1/6.6% (2.19/2.38 µg/cm²) in the chamber fluid. Therefore the fraction that might become bioavailable was 23.6 and 25.7%, respectively (mean for both kinds of cream formulations: 25%).

Summary of the study Roche 1986c:

Exposure	Skin surface		Horny layer		Remaining skin		Chamber fluid	
	µg/cm ²	%	µg/cm ²	%	µg/cm ²	%	µg/cm ²	%
0.6% in cream A								
Intact skin of naked rats								
1 h	29.48	81.9	0.69	1.9	5.82	16.2	0.01	-
6 h	25.09	69.7	0.72	2.0	8.16	22.7	2.03	5.6
16 h	18.79	52.2	0.93	2.6	7.79	21.6	8.49	23.6
Intact skin mini pigs								
1 h	34.31	95.3	0.57	1.6	1.12	3.1	-	-
6 h	31.74	88.2	0.87	2.4	3.22	8.9	0.17	0.5
16 h	26.55	73.8	0.96	2.7	6.30	17.5	2.19	6.1
0.6% in cream B								
Intact skin of naked rats								
1 h	29.11	80.9	0.81	2.3	6.08	16.9	-	-
6 h	7.75	21.5	1.09	3.0	20.8	57.8	6.36	17.8
16 h	7.03	19.5	0.74	2.1	14.22	39.5	14.01	38.9
Intact skin of mini pigs								
1 h	34.55	96.0	0.47	1.3	0.98	2.7	-	-
6 h	28.51	79.2	0.97	2.7	5.47	15.2	1.05	2.9
16 h	25.89	71.9	0.84	2.3	6.89	19.1	2.38	6.6

Based on the dermal absorption studies *in vitro*, it becomes obvious that skin penetration depends on time and species, but less on the kind of formulation.

3.3.4.2. Dermal /percutaneous absorption *in vivo*

3.3.4.2.1. Humans

Guideline: Human dermal penetration study according to an approved study protocol following SOPs (Huntingdon Research Center 1994, RIFM #23500)

Species/strain: Human

Group size: 3 male volunteers

Method: cf. above

Test substance: [¹⁴C]-BMHCA

Batch: Labelled: 19154-162-40 (215.7 µCi/mg, radiochemical purity: >98%); Unlabelled: 6252-91

Purity: no data

Dose volume: 4 x 0.25 mL = 1 mL [¹⁴C]-BMHCA (mean dose 14.7 µCi), vehicle: 70% EtOH in water

Exposure time: 6 hrs

Samples: 5 adhesive tape strips; urine samples until 120 hrs after application; faeces until 5 days after application; blood samples until 72 hrs after application

GLP: yes

Procedure: Penetration of [¹⁴C]-BMHCA through human skin was evaluated in three healthy volunteers. Compound dissolved in 70% EtOH was applied to a square area of skin 10 cm x 10 cm in four 0.25 ml portions on the back. The application sites were occluded with gauze dressing for 6 hrs. Washed areas of treated skin (2.5 cm x 2.5 cm) were stripped with five successive applications of adhesive tape and then occluded with fresh gauze dressing for 114 hrs. Radioactivity was measured in all patches and cleaning swabs. Urine was collected 9 times during the 120 hrs after sample application, and faeces were collected daily for 5 days. Blood samples were taken at intervals until 72 hrs.

Results: Although the mean amount of radioactivity dispensed was 20.89 ± 0.23 µCi, the mean dose remaining on the backs of the volunteers and thus available for absorption was 14.70 ± 1.95 µCi (11.37 ± 1.51 mg BMHCA). Following the topical application a mean of 1.38% was excreted in urine during the first 24 hrs. Afterwards no radioactivity could be detected in the urine anymore, and none was found in faeces or blood samples.

Table: Radioactivity excreted in the urine [% of dose administered]

Sample collection period (h)	Subject number			Mean ± SD
	1	2	3	
0 - 2	0.01	0.02	ND	0.01 ± 0.01
2 - 4	0.16	0.24	0.10	0.17 ± 0.07
4 - 6	0.24	0.18	0.16	0.19 ± 0.04
6 - 12	0.13	1.20	0.37	0.57 ± 0.56
12 -24	0.30	0.76	0.26	0.44 ± 0.28
24 -48	ND	ND	ND	
48 - 72	ND	ND	ND	
72 - 96	ND	ND	ND	
96 - 120	ND	ND	ND	
Total	0.84	2.40	0.89	1.38 ± 0.89

SD Standard deviation, ND Not detected

The amounts of radioactivity on adhesive tapes at 6 hrs and at 120 hrs declined with each successive tape stripping; the mean total amount of dose removed by successive tape stripping was 0.1127% at 6 hrs and 0.0012% at 120 hrs. A mean 63.12% of the applied radioactivity was recovered from the gauze dressing used to occlude the site of application between 0 - 6 hrs; a further 3.76% was removed by washing the skin using an EtOH swab at 6 hrs and 3.06% was recovered from gauze dressings used to occlude the treated areas of skin during 6 - 120 hrs. Based on this, it was concluded that limited percutaneous

absorption and systemic bioavailability occurs when [¹⁴C]-BMHCA is applied onto the skin of human volunteers.

3.3.4.2.2. Rats

Guideline: Comparable to OECD TG 417 and 427, Comparative oral and dermal absorption study according to an approved study protocol following SOPs (Huntingdon Research Center 1995, RIFM #23697)

Species/strain: Rat (Lister-Hooded)

Group size: 18 males

Test substance: [¹⁴C]-BMHCA

Batch: Labelled: 19154-162-40 (215.7 µCi/mg, radiochemical purity: >98%); Unlabelled: 6252-91

Purity: no data

Dose levels: 6.75 mg/kg bw; vehicle: 70% EtOH in water

Application volume: 0.2 mL/rat

Exposure time: 6 hrs

Route: Topical dermal application with occlusive dressing (9 cm²) on the back

Method: Sacrifice at 0.5, 1, 3, 6, 12, 24, 48, 72 and 120 hours after application; tape strippings, numerous organs and tissues; urine samples, blood samples until 120 hrs after application

GLP: yes

Results: Up to 120 hours after application of BMHCA, a mean cumulative total of 14.6% of the dose was excreted in urine, 0.8% was recovered in cage washings and 2.0% was excreted via faeces, whereas levels in expired air traps were not detectable. The remaining radioactivity in all tissues investigated was 1.2% of the initial dose (site of application site). The maximum urinary excretion rate was observed 6 - 12 hours after dermal application. The highest concentration of ¹⁴C was recovered in the liver ($c_{\max} = 15.6 \mu\text{g/g}$ tissue representing 0.826% of given dose/g tissue). Overall, a relation of c_{\max} and the blood perfusion rate of a respective tissue is indicated based on the findings for highly perfused tissues (i.e. lungs, heart) and poorly perfused tissues (i.e. skin, fat). The mean total proportion of dose in excreta and tissues was about 19%.

SCCS conclusion on dermal/percutaneous absorption:

Dermal absorption studies *in vitro* demonstrated species-specific effects. The bioavailable portion was found much higher in rats (66.1 and 50.8%) when compared to mini pigs (0.8% and 4.9%), thereby 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 permeation and systemic availability of this compound. Percutaneous absorption of BMHCA in humans was lower when compared with rats (1.4 vs. 19%).

Upon dermal application of [¹⁴C]-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 [¹⁴C]-BMHCA was $71 \pm 10\%$. 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/cm² onto 5 cm² excised mini pig skin; result: total of 5.87 µg substance/cm² 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 ingredient of creamy formulations will be set at 25% (worst case scenario based on 36 µg substance/cm² applied onto 5 cm² excised mini pig skin; mean out of two experiments: total of 8.88 µg substance/cm² 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 cm²/day). Here, an absorption of about 6 µg substance/cm² can be assumed for unshaved skin (*stratum corneum* intact). For shaved skin (*stratum corneum* compromised), however, the total absorption would be 11 µg substance/cm² (with the addition of the portion of 4.66 µg/cm² that was found sticking in the *stratum corneum* in the respective experiment; cf. above).

3.3.5. Repeated dose toxicity

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

3.3.5.1.1. Oral Studies

1st Study: BASF SE, 2006b, RIFM#53648

Guideline/Method: Explorative oral and toxicokinetics study

Species/strain: Rats (Wistar), mice (C57BL)

Group size: 5 males/group (rat and mice)

Test substances: BMHCA, lysmerylic acid

Batch: no data

Purity: no data

Vehicle: Olive oil

Doses: 0, 50 mg/kg bw/day (5 mL/kg bw/day), once daily

Route: Oral (gavage)

Exposure: up to 14 days

GLP: yes

Study period: 4 October 2004 – 19 October 2004

Method: BMHCA in comparison to lysmerylic acid (metabolically formed oxidation product) was investigated for induction of genital organ effects. Food consumption and body weights were determined weekly. Examinations for clinical signs or mortality were conducted once daily. Sub-groups of 5 animals were anaesthetised after days 1, 2, 3, 4 and at the end of the treatment period (day 15).

Results: Rats: No mortality occurred and no clinical signs were observed. Body weight gains were found to be decreased by 25% and 20% below controls after application of BMHCA for 14 days. Slight to severe testicular atrophy with an incidence of 2/5 animals for BMHCA after a single application, and in all animals after longer application periods was observed (cf. 3.3.8.). Mice: No mortality occurred and no clinical signs were observed. BMHCA led to a reduction in the ratio of normal to abnormal sperm in animals after exposure of 3 and 4

days. Macroscopic and microscopic evaluation of the testes revealed no pathological changes.

2nd Study: Givaudan, 1990c, Givaudan, 1990a, RIFM#12138, 12144

Guideline/Method: Explorative oral study

Species/strain: Rats

Group size: 5 males/group; 4 males/control group

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: Rape oil

Doses: 0, 25, 50, 100, 200, 400 mg/kg bw/day (10 mL/kg bw/day), once daily

Route: Oral (gavage)

Exposure: 5 days

GLP: no

Method: The animals were observed for mortality, clinical signs, and body weight changes. At termination the animals were sacrificed and following a gross necropsy, several organs such as the liver, kidneys, and testes were weighed. The testes of all animals were microscopically examined.

Results: No mortality occurred. Loss of hair, hunched posture and lethargy were noted in the 2 highest dose groups, haematuria and paresis of the forelegs were noted in the highest dose group. An initial loss of body weight was observed at 50 – 400 mg/kg bw/day. Gross findings included mild liver effects in some rats at 50 – 200 mg/kg bw/day and in all rats at 400 mg/kg bw/day group, small prostate (100 – 400 mg/kg bw/day), and small seminal vesicles (200 – 400 mg/kg bw/day), and reddened testes in 1/8 rats at 200 mg/kg bw/day and in 6/8 rats at the highest dose group. Kidney weights were decreased in the high dose group, and a dose-related decrease in the testes weights was observed at doses of ≥ 100 mg/kg bw/day. Histology of the testes revealed degeneration and loss of germ cells in the seminiferous epithelium at ≥ 50 mg/kg bw/day.

Subacute administration of BMHCA to male rats led to treatment-related effects at ≥ 50 mg/kg bw/day including dose-related testicular findings. From this study the NOAEL derived was suggested at 25 mg/kg bw/day. The NOAEL of 25 mg/kg bw/day has been confirmed in a subsequent study (refs.: Givaudan, 1991, RIFM #16176).

3rd Study: Givaudan, 1990b, RIFM#12139

Guideline/Method: Explorative oral and toxicokinetics study

Species/strain: Mice

Group size: 5 males/group

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: Rape oil

Doses: 0, 100 mg/kg bw/day (5 mL/kg bw/day), once daily

Route: Oral (gavage)

Exposure: 5 days

GLP: no

Method: The animals were observed for mortality, clinical signs, and body weight changes. At termination the animals were sacrificed and following a gross necropsy, testes and epididymides were examined by histopathology.

Result: No mortality occurred and there was no effect on any of the parameters investigated, including testes or epididymides.

4th Study in rabbits (BASF SE, 2008a, RIFM#55472): 5 male rabbits (Himalayan) were treated with BMHCA in water and Tween 80 orally by gavage for 15 days at doses of 0, 30, 100 and 300 mg/kg bw/day. Application volume was 10 mL/kg bw. Parameters observed:

mortality, clinical signs, behaviour, morbidity, body weights. A gross necropsy was conducted with special attention of the reproductive system. Results: No mortality occurred. No test substance related findings on clinical observations, body weights and food consumption were observed in all dosing groups. A diffuse degeneration of the seminiferous tubules combined with a moderate oligospermia and a moderate mixed inflammation in the epididymides was observed in 1/5 animals of the low dose group (30 mg/kg bw/day). In the mid-dose group, reduced testes and epididymides sizes with severe diffuse degeneration of seminiferous tubules and severe atrophy plus aspermia in the left epididymides was observed in 1/5 animals. A dose response relationship could not be observed.

5th Study: BASF SE, 2008c, RIFM#55474

Guideline/Method: Explorative oral screening study

Species/strain: Dogs (Beagle)

Group size: 4 males/group

Test substance: BMHCA

Batch: 00036077L0

Purity: 99.1%

Vehicle: Gelatine capsules

Doses: 0, 40, 200 and 1000 mg/kg bw/day (5 mL/kg bw/day), once daily

Route: Oral (gavage)

Exposure: 2 weeks

GLP: yes

Study period: 1 June 2007 – 14 June 2007

Method: This study was intended to clarify whether testicular toxicity after oral administration of BMHCA occurs in a non-rodent species. Due to vomitus and diarrhoea at 1000 mg/kg bw/day, the dose was lowered to 500 mg/kg bw/day, starting on study day 3. Parameters: food consumption, clinical examinations; clinical chemistry and haematology, urinalyses. All animals were subjected to a gross pathology and histopathology.

Results: Retardation of body weight gain or body weight loss together with decreases in food efficiency were observed in combination with vomitus and soft faeces/diarrhoea in all animals at ≥ 200 mg/kg bw/day. Liver weight increases between 30-40% above control values, and centrilobular hypertrophy of hepatocytes were observed at ≥ 200 mg/kg bw/day. A range of clinical parameters were altered in highly dosed animals. Massive diffuse degeneration of seminiferous tubules combined with hyperplasia of Leydig cells in testes and aspermia and epithelial vacuolation in the epididymides was found in one dog in the mid-dose group.

A follow-up study (BASF SE, 2008b, RIFM#55473) with a higher number of animals per dose group (10 males) and additional examinations prior and during the test substance administration period was performed. Doses: 0 and 200 mg/kg body bw/day for 2 weeks. BMHCA led to decreased body weight gain including severe body weight loss, induced anaemia, increased values of urea and creatinine, organ weight changes in the liver and the male-reproductive organs (testes, prostate). The main target organs were the male reproductive organs: organ weights of testes and prostate were decreased and histomorphological correlates were noted; altered sperm quality and reduced sperm motility were noted.

6th Study: RIFM, 1990c, RIFM#12141

Guideline/Method: Explorative oral screening study

Species/strain: Rhesus monkeys (*Macaca mulatta*)

Group size: 2 males

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: Food

Doses: 0, 100 mg/kg bw/day, once daily

Route: Oral (suspended in fluid baby food)

Exposure: 5 days

GLP: no

Method: The animals were observed for mortality, clinical signs, and body weight changes. Animals were sacrificed and subjected to a complete necropsy. All organs and tissues were examined grossly and testes and epididymides were examined by histopathology.

Results: No effects on body weights or histopathological alterations in the testis were noted. Minimal inflammatory reaction was found in one epididymis of one animal and small hollow spaces in the epithelium of one epididymis of the other. The testes of both animals were found free of lesions. Thus, general and testicular toxicity was not observed under the conditions of this study.

3.3.5.1.2. Dermal Study

Study: Givaudan, 1991, RIFM #16176; US EPA (TSCAT), 1991a, RIFM#53120

Guideline/Method: Explorative comparative oral/dermal study

Species/strain: Rats

Group size: 5 males/group

Test substance: BMHCA

Batch: no data

Purity: 99.1%

Doses: 0, 250, 500, 1000, 2000 mg/kg bw/day

Route: Dermal (occluded)

Exposure: 5 days, once daily

GLP: no

Method: In the dermal part of the study, the neat (undiluted) substance was applied to the shaved back under an occlusive dressing for 6 hours on 5 consecutive days. The animals were observed for mortality, clinical signs, and body weight changes. At termination the animals were sacrificed, a complete necropsy was performed and microscopic examination of the testes and epididymides was conducted.

Results: No mortality was observed. Slight decrease in body weights and marked testicular atrophy were noted at 2000 mg/kg bw/day. Some effects on seminiferous tubules, decreases of the number of germ cells, and increases of the number of degenerating germ cells (including giant cells) were observed in combination with degenerating germ cells in epididymides and the occurrence of spermatocele at the highest dose tested. No clinical signs or substance-related necropsy findings were observed. No further observations were performed in this study to assess adverse effects other than testicular toxicity.

3.3.5.1.3. Inhalation Route

Not available

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

3.3.5.2.1. Oral Studies

1st Study: Givaudan, 1990a, RIFM #12144, Givaudan, 1990i, RIFM #12143

Guideline/Method: Subchronic study according to OECD 408, US EPA 540/9-82-025

Species/strain: Rats (Albino)

Group size: 14 males and 14 females/group

Test substance: BMHCA

Batch: no data

Purity: 97.8%

Vehicle: Rape oil
Doses: 0, 2, 5, 25, 50 mg/kg bw/day (once daily 1 mL/kg bw)
Route: Oral (gavage)
Exposure: 5 days/week
Exposure period: 90 days
Recovery: 4 weeks (control and high dose groups)
GLP: yes

Method: Mortality, clinical signs and body weights were recorded. Haematology and clinical chemistry parameters were assessed. Necropsy was conducted at the end of the treatment period or the recovery period. Organ weights were determined. Microscopic examinations were conducted on organs and tissue samples from the controls and high-dose group animals treated with 50 mg/kg.

Results: No mortality occurred. No treatment-related body weight changes were observed. Observation of liver toxicity included increases in absolute and relative liver weights/due to lipid contents at ≥ 25 mg/kg bw/day. A significant decrease in plasma cholinesterase activity (30 and 70% compared to controls) and lower plasma cholesterol levels were observed at 25 and 50 mg/kg bw/day in both sexes.

Effects on clinical chemistry were reversible in the recovery (high-dose) group. In females treated with 25 and 50 mg/kg bw/day, elevated weights of adrenal glands and hypertrophy of the zona fasciculata were observed. Compound-related testicular toxicity such as spermatocetes in the epididymides and testicular atrophy was observed at 50 mg/kg bw/day. Disturbances of spermatogenesis and spermatogenesis, testicular increases in Sertoli cell tubules and increased surface density in Leydig cells were described along with a decreased density of spermatozoa, nucleated cells and spermatocetes in the epididymides of the high dose animals. In the 4-week recovery group, the same testicular pathology was observed to a lesser extent. Based on these data, it was concluded that BMHCA led to treatment-related effects at ≥ 25 mg/kg bw/day. The NOAELs for testicular toxicity and systemic toxicity were suggested to be 25 mg/kg bw/day and 5 mg/kg bw/day, respectively.

Follow-up study (Givaudan, 1990f, RIFM#12145): 20 male and 20 female albino rats (Fü SPF) were administered BMHCA (purity: 97.4%) at 0 or 50 mg/kg bw/day in rape oil, 7 days a week for 24 or 52 days. Treatment-related effects again included the decrease of plasma cholesterol, cholinesterase and acetylcholinesterase levels.

2nd Study: Givaudan, 1990g, RIFM #12140
Guideline/Method: Subchronic study according to OECD 409
Species/strain: Dogs (Beagle)
Group size: 3 males and 3 females/group
Test substance: BMHCA
Batch: no data
Purity: 97.6%
Vehicle: Gelatine capsules
Doses: 0, 4.4, 22.3, 44.6 mg/kg bw/day (one capsule per day)
Route: Oral
Exposure: 7 days/week
Exposure period: 3 months
GLP: yes

Method: Body weights, clinical signs and mortality were recorded. Haematology and clinical chemistry assessments were performed at weeks 3, 6 and 13. Urine samples were collected for urinalysis at weeks 5 and 11. Necropsy and macroscopic and histopathological examination were conducted at the end of the study.

Results: No mortality occurred and no significant differences in body weight gains were observed. No significant differences in urinalysis, haematological and clinical chemistry parameters were observed. Gross pathology and histopathology revealed no specific substance-related findings, especially, no alterations on reproductive organs were observed in males or females. A subsequent study by the same authors (Givaudan, 1990d, RIFM#12147; 3 female beagle dogs treated with 200 mg/kg body weight/day orally by gelatine capsules for 90 consecutive days) again did not find treatment-related effects, and no alterations of clinical chemistry parameters or cholinesterase activity.

An explorative dose escalation study (Givaudan, 1990h, RIFM#12146; 2 male dogs treated with BMHCA at increasing doses of 50 µl/kg bw/day from day 1 to 7, 100 µl/kg bw/day from day 8 to 14, 200 µl/kg bw/day from day 15 to 21, 400 µl/kg bw/day from day 22 to 50 and 600 µl/kg bw/day from day 51 to 64 corresponding to 47 up to 564 mg/kg bw/day) revealed occasional vomiting in both animals, diarrhoea in one animal and body weight reduction together with an increase in glutamate dehydrogenase and alanine aminotransferase levels. Histological examinations showed multifocal inflammation in the liver and mild atrophy in seminiferous tubules (necrosis of germ cells, multinucleated giant cells in tubular lumen).

3.3.5.2.2. Dermal Route

Not available

3.3.5.2.3. Inhalation Route

Not available

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). Irrespective of the length of treatment period, in oral studies rats were found more sensitive than dogs to this compound. Clinical chemistry and histopathological examinations repeatedly revealed adverse effects on the liver and male reproductive system (testicular toxicity; cf. 3.3.8.). 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.

3.3.5.3. Chronic (> 12 months) toxicity

No data available.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

3.3.6.1.1. Bacterial reverse mutation test (Ames)

1st Study (Roche, 1984, RIFM#34333):

BMHCA was tested for mutagenicity with and without metabolic activation (S9-mix prepared from Aroclor 1254 induced male albino rat liver) according to the standard method (OECD 471; GLP). The *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, TA1537, TA1538 were exposed to the test substance (dissolved in ethanol) at concentrations ranging from 0.0078125 – 0.5 µL/plate. Controls: vehicle (ethanol) and positive controls (9-aminoacridine, 2-AAF, mitomycin C, NaN₃).

Results and conclusions: Toxic effects were noted in all strains at concentrations ≥0.125 µL/plate, with and without metabolic activation. The number of revertant colonies did not differ between plates containing the test substance and vehicle controls, with or without metabolic activation and thus BMHCA did not induce gene mutations. Positive controls induced revertant colonies.

2nd Study (Cocchiara et al., 2001, RIFM#37200):

BMHCA was tested for mutagenicity in the reverse mutation assay with and without metabolic activation (S9-mix prepared from Aroclor 1254 induced male Sprague-Dawley rat liver) according to the standard method (OECD 471; GLP). The *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA* were treated with the test substance (dissolved in DMSO) at concentrations ranging from 2.5 – 750 µg/plate (*S. typhimurium*) or from 2.5 – 5000 µg/plate (*E. coli*). Positive controls: 9-aminoacridine, 2-AAF, mitomycin C, NaN₃).

Results and conclusions: Toxicity was observed at ≥333 µg/plate (*S. typhimurium*) and ≥3333 µg/plate (*E. coli*). The number of revertant colonies did not differ between plates containing the test substance and negative controls either with or without metabolic activation and thus BMHCA did not induce gene mutations. By contrast, positive controls induced revertant colonies from each bacterial strain.

SCCS comment:

BMHCA was presumably negative in two studies using the bacterial Ames test. However, bacteriotoxicity was observed in both studies at low concentrations already starting at 0.125 µL/plate (background level of revertant reduced or no background growth). In addition, results from the first test showed a positive trend in the strain TA1538. Initially, results from the second experiment were considered unreliable since no real data were presented. Upon request, additional data were provided but showed only results from strains other than TA1538.

3.3.6.1.2. Mammalian gene mutation test

Study (BASF SE, 2010a, RIFM# 60847):

Assay (OECD 476; GLP) conducted to investigate the induction of gene mutations at the *hprt* locus in Chinese hamster V79 cells. Three independent experiments: 1. BMHCA dissolved in acetone was added to cultures for 4 hours at dose levels of 0.3 – 16 µg/ml (without metabolic activation) or at 8 – 128 µg/ml (with metabolic activation). 2. BMHCA was added to cultures for 4 or 24 hours at dose levels of 0.5 – 24 µg/ml (24 hours, -S9-mix) or at 16 – 128 µg/ml (4 hours +S9-mix). 3. BMHCA preparation was added to cultures for 24 hours at dose levels of 4 – 40 µg/ml without metabolic activation. Metabolic activation system: phenobarbital/β-naphthoflavone induced rat liver S9-mix of male Wistar rats. Negative controls: solvent acetone; positive controls: EMS without S9-mix and DMBA

with S9-mix.

Results and conclusions: No reproducible increase in mutant frequency was observed in the main experiments up to the maximum concentration. In the first experiment the mutant frequency remained within the historical range of solvent controls. In the second experiment the range of the historical solvent control data was exceeded at 8.0 µg/mL in a culture without metabolic activation, at 16.0 µg/mL in one culture with metabolic activation, and at 64.0 µg/mL in both cultures with metabolic activation. However, the induction factor did not reach the threshold of 3.0. Further, the increased mutant frequency was not concentration-dependent. In the third experiment the mutant frequency was exceeded in one culture at the threshold of 16.0 and 24.0 µg/mL, respectively. Here, the range of the historical solvent control data was exceeded. But again, the increase observed was not dose-dependent and not reproducible. Reference mutagens, used as positive controls, induced the expected increases in mutant colonies. Overall, under the conditions applied, BMHCA did not induce gene mutations at the *hprt* locus in Chinese hamster V79 cells in the absence and presence of metabolic activation.

SCCS comment:

SCCS disagrees with the conclusion of the applicant that the mammalian gene mutation test was negative, as several data points from the experiments show significant increases in mutant frequencies induced by BMHCA when compared to the concurrent negative control. The increase was concentration-dependent at least at one sampling time (second experiment without S9-mix) when evaluated with an appropriate trend test. Furthermore, the increased mutant frequency was most pronounced in the third experiment. Here, the 3-fold increase of the mutant frequency over the concurrent control was outside the distribution of the historical negative control data.

3.3.6.1.3. Mammalian cell chromosomal aberration test

Study (Cocchiara et al., 2001, RIFM#37200):

Assay (OECD 473; GLP) conducted to investigate the induction of structural chromosome aberrations in Chinese hamster ovary (CHO) cells. BMHCA dissolved in DMSO was tested in the presence and absence of S9-mix prepared from Aroclor 1254 induced male Sprague-Dawley rat liver. CHO cells were exposed to BMHCA for 4 hours without S9-mix (30, 40 and 50 µg/mL), 4 hours with S9-mix (40, 60 and 75 µg/mL), and for 20 hours without S9-mix (5, 20 and 40 µg/mL). Cells were collected approximately 20 hours after starting the exposure. A total of 500 cell nuclei were counted and the mitotic index determined. Positive controls: mitomycin C (without S9) and cyclophosphamide (with S9). DMSO as negative control.

Results and conclusions: A concentration-dependent increase in cell growth inhibition was observed at higher test concentrations. Exposure to BMHCA for 4 hours without S9-mix led to statistically significant increases in the number of cells with numerical aberrations and structural aberrations when compared to solvent control. 20 hours of exposure without S9-mix resulted in statistically significant increases in the number of cells with structural but not numerical aberrations. In the presence of S9-mix, BMHCA induced a statistically significant increase in the number of cells with structural chromosome aberrations at the highest concentration tested. The values observed, however, were within the range of historical solvent controls. Positive controls showed statistically significant increases in chromosomal aberrations. Under the conditions of the assay, BMHCA therefore was shown to induce structural and numerical chromosomal aberrations in Chinese hamster ovary cells in the absence of S9 and when tested up to cytotoxic concentrations.

SCCS comment:

Upon treatment with BMHCA, an increase in CHO cells containing chromosomal aberrations was observed. However, no details are provided in the publication, thus being of only

limited value. Upon request, SCCS received additional data from the *in vitro* chromosomal aberration test in CHO cells that showed compound-induced numerical and structural chromosomal aberrations in the absence of S9-mix and a concentration-dependent increase of structural aberrations in the presence of S9-mix.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

3.3.6.2.1. *In vivo* mammalian erythrocytes micronucleus test

Study: Cocchiara et al., 2001, RIFM#37200

Guideline/Method: OECD 474

Species/strain: Mice (ICR)

Group size: 5 males and 5 females/group

Test substance: BMHCA

Positive control: Cyclophosphamide

Batch: 9000349505

Purity: no data

Vehicle: Corn oil

Doses: 0, 150, 300, 600 mg/kg bw

Route: Intraperitoneal (i.p.)

Exposure: Single application (volume: 20 mL/kg bw)

Exposure period: 24 and 48 hours

GLP: yes

Method: Prior to the main test, solubility was determined and a toxicity test was performed for dose selection of the main study. Control mice were dosed with the vehicle corn oil. Cyclophosphamide served as positive control and was administered at 50 mg/kg bw. Animals were sacrificed after 24 and 48 h following i.p. injection. Bone marrow was obtained, processed, and bone marrow cells were spread onto glass slides. The slides were fixed and stained. For each animal, 2000 polychromatic erythrocytes were scored for the presence of micronuclei. The portion of polychromatic erythrocytes (PCE) to total erythrocytes was recorded per 1000 erythrocytes.

Results and conclusions: No mortality occurred in any mouse at any dose level. Systemic toxicity in form of various clinical signs was observed in the mid- and high-dose group. A significant increase in the numbers of micronucleated PCE over all test animals in the high-dose group (600 mg/kg bw/day) was observed in males at 48 but not at 24 hours after treatment. In addition, the maximum of micronucleated PCE per animal observed were within the historical range of the solvent control. No significant and dose-related increases were observed in any other dose group. Under the conditions of the study, overall, BMHCA was shown to exhibit no clastogenic potential *in vivo*.

SCCS comment:

The *in vivo* micronucleus data show that there was a statistically significant increase of cells with micronuclei in males after 48 h and a non-significant increase in females after 24 h in the highest dose group (600 mg/kg bw/day). This highest dose is only one showing signs of systemic toxicity in each tested animal. Based on the *in vivo* micronucleus results presented, no firm conclusion can be drawn.

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.

3.3.7. 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.

3.3.8. Reproductive toxicity and teratogenicity**3.3.8.1. Reproduction toxicity**

Study: BASF SE, 2006a, RIFM# 53649

Guideline/Method: One-generation reproduction toxicity range-finding study

Species/strain: Rat (Wistar)

Group size: 10 males and 10 females/group

Test substance: BMHCA

Batch: no data

Purity: no data

Doses: 0, 400, 800, 1700, 3400 ppm, corresponding to 0, 14.5, 28.7, 62.6 and 119.7 mg/kg bw/day, respectively. Doses during gestation/lactation: 0, 200, 400, 850, 1700 ppm, corresponding to 12.9/10 and 25.8/18.3 mg/kg bw/day BMHCA for the two low dose groups. No doses were accounted for in the two high dose female groups due to lack of offspring (only 1 of 8 mated females became pregnant in the 1700 ppm group).

Route: Oral (microencapsulated in the diet)

Exposure period: 6 weeks prior to mating, during mating, gestation and lactation until weaning (14 weeks)

GLP: no

Method: The test substance was administered to groups of 10 male and 10 female young Wistar rats (F0 parental generation) via the diet (30.7% BMHCA, microencapsulated). About 6 weeks after the beginning of treatment, F0 animals were mated to produce a litter. The female F0 animals were allowed to deliver and rear their F1 pups until weaning (postnatal day 21). The study was terminated with the sacrifice of the F1 weanlings and F0 adult animals.

Results:

F0 generation: In the high-dose groups, males showed a dose-dependent decrease in body weights (5-30%) and body weight gains (10-40%) compared to controls. In this group the food consumption was reduced by 15%. Increases in relative liver weights were observed starting at 800 ppm (10-20% above control). Relative kidney weights (15% below control) were found in the high-dose group. Significantly increased levels of plasma alanine aminotransferase, alkaline phosphatase, glutamate dehydrogenase, and γ -glutamyltransferase were observed at ≥ 1700 ppm.

Testicular toxicity was observed at ≥ 1700 ppm: decreases in testes (30-45%) and cauda epididymis (30-40%) weights, diffuse testes degeneration and aspermia of the epididymis. In the high-dose group, weights seminal vesicle (10%) and prostate (20%) were observed, and also hyperplasia of Leydig cells.

Females (dams) showed decreases in body weights (5-10%) and body weight gains (10-30%) at ≥ 800 ppm. In the low-dose group, mean maternal body weights and body weight gain were below control during gestation and lactation. By contrast, no concurrent changes in mean relative liver or kidney weights were observed. Nevertheless, significant changes in clinical parameters, such as γ -glutamyltransferase and serum cholinesterase, were seen in all dose groups. Glutamate dehydrogenase was affected at ≥ 800 ppm.

F1 generation: No viable offspring was produced in animals treated with ≥ 1700 ppm BMHCA. In the 1700 ppm group, only one female had 1 implant which was finally resorbed. In the two lower dose groups mean implantation losses of 11% (400/200 ppm) and 16% (800/400 ppm) were observed in comparison to 5% in controls. Mean numbers of delivered pups were 9.4 (controls), 8.7 (400/200 ppm) and 7.9 (800/400 ppm). However, there were no stillborns in the treated groups with offspring. Postnatal survival: 99% (400/200 ppm) and 94% (800/400 ppm) between day 0 and 4. No pup mortality was observed between postnatal day 4 and 21. Birth weight reduction: 19% (400/200 ppm) and 22% (800/400 ppm). Reduction of pup weight at weaning and pup weight gain: 17%/16% (400/200 ppm) and 21%/21% (800/200 ppm).

Based on these data it can be concluded that dietary administration of BMHCA led to dose-dependent signs of systemic and reproductive toxicity (impaired fertility and pre-postnatal developmental toxicity). The dose of 400 ppm (~ 14.5 mg/kg bw/day) was suggested to be a NOAEL in males and a LOAEL in females (dams) with regard to systemic toxicity. By contrast, 800 ppm (~ 28.7 mg/kg bw/day) was suggested as NOAEL with regard to testicular (reproductive) toxicity.

SCCS comment:

The suggested NOAEL of ~ 29 mg/kg bw/day in rats with regard to testicular toxicity is in line with the observation from a 90-day study in rats (see section 3.3.5.2.1.). In this latter study a NOAEL of 25 mg/kg bw/day was derived for testicular toxicity. Similar as with this 1-generation study, in the 90-day study the NOAEL derived for systemic toxicity was also lower (*i.e.*, 5 mg/kg bw/day).

3.3.8.2. Teratogenicity

Study: BASF SE, 2004, RIFM# 52014

Guideline/Method: OECD 414, 87/302/EEC, US EPA OPPTS 870.3700

Species/strain: Rat (Wistar)

Group size: 25 pregnant rats/group

Test substance: BMHCA

Batch: 000STD77L0

Purity: 98.1%

Doses: 0, 5, 15, 45 mg/kg bw/day (effective doses: 0, 4.1, 12.7, 40.7 mg/kg bw/day)

Vehicle: Olive oil

Route: Oral (gavage)

Application volume: 5 mL/kg bw

Exposure period: gestation days 6 – 20

GLP: yes

Study period: 22/23/24 July 2003 – 5/6/7 August 2003 (3 replicates)

Method: BMHCA in olive oil was administered to 25 pregnant rats orally by gavage on gestational days 6 – 20. The control group was dosed with the vehicle only. At termination the dams were examined for mortality, clinical signs and abortion. Body weight was determined at regular intervals. On gestation day 20, blood was taken from all females,

which were subsequently sacrificed and assessed by gross pathology. For each dam, corpora lutea were counted and the number of implantation sites was determined. The fetuses were removed from the uterus and investigated for soft tissue or skeletal and cartilage findings.

Results:

Dams: No mortality but clear signs of maternal toxicity starting at the mid dose level: mean maternal weight gains significantly decreased on day 6 – 8 p.c to about 56% below control (recovery during the study period). Reduction of mean food consumption (18%) was observed in the high dose group followed by a statistically significant mean body weight loss (mean body weight gain ~25% below controls). In line, the corrected body weight gain was statistically significantly lower (about 32% below control). Mid-dose group: increases in mean alanine aminotransferase levels (20-30% above control) and decreases in serum cholinesterase levels (20-45% below control). In the high-dose group, mean glutamate dehydrogenase levels were 79% above controls. Further, reduced mean uterus weights (20% below controls) became apparent. Increases in absolute and relative liver weights were found at all dose levels, in the low-dose group, however, unaccompanied by clinical parameter alterations.

Pregnancy was confirmed for 22-23 rats/group. Mean post-implantation losses were increased in the high-dose group (45 mg/kg bw/day): resorptions were found at 15.1% per dam compared to 4.4%, 4.7%, and 4.9% at 0, 5 and 15 mg/kg bw/day, respectively. Sex distribution and placental weights remained unaffected by the test substance. No dead fetuses, abortions or premature births were observed in any group.

Foetuses: Signs of prenatal developmental toxicity were observed at the mid- and high-dose group (reduced foetal weights and an increased rate of skeletal variations). Malformations were observed in 3 out of 170 high dose group fetuses (1.8%; 3 out of 23 litters affected). Findings reported: anasarca, polydactyly, cervical hemivertebra.

Soft tissue variations (dilated renal pelvis, ureters, cerebral ventricles) occurred in all groups including controls (no statistical difference between treatment groups and controls). Despite also being observed in controls and low dose groups, a significant increase in mean percentages of skeletal variations per litter were found in mid and high dose animals (mainly delays and disturbances in ossification of the skull, sternebra and pubic girdle). Delays and disturbances of ossification occurred in conjunction with decreased foetal weights, thus suggesting that it is an indicator for adverse effects on foetal maturation (fetotoxicity) rather than a sign of specific teratogenicity.

In addition, discoloration of foetal livers was noted in 1.7% and 15.5% of the mid- and high- dose animals, respectively. This sign corresponded to the liver changes observed in the respective dams.

Conclusion:

The oral administration of BMHCA to pregnant Wistar rats from implantation to day 20 of gestation resulted in overt (systemic) maternal toxicity at 15 and 45 mg/kg bw/day, but not at 5 mg/kg bw/day (body weight gain and impaired liver function). Gestation became affected at 45 mg/kg bw/day (increased number of post-implantation losses). Prenatal developmental toxicity with regard to reduced foetal body weights was observed in the mid- and high-dose groups, thereby corresponding to significant maternal toxicity at the same dose levels. Further, the overall incidence of skeletal variations was significantly increased in mid- and high-dose animals. The NOAEL for both maternal and prenatal developmental toxicity was 5 mg/kg bw/day (or 4.1 mg/kg bw/day, effective dose).

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 (cf. 3.3.5.1. and 3.3.5.2.). 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 (cf. 3.3.5.2.). 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.

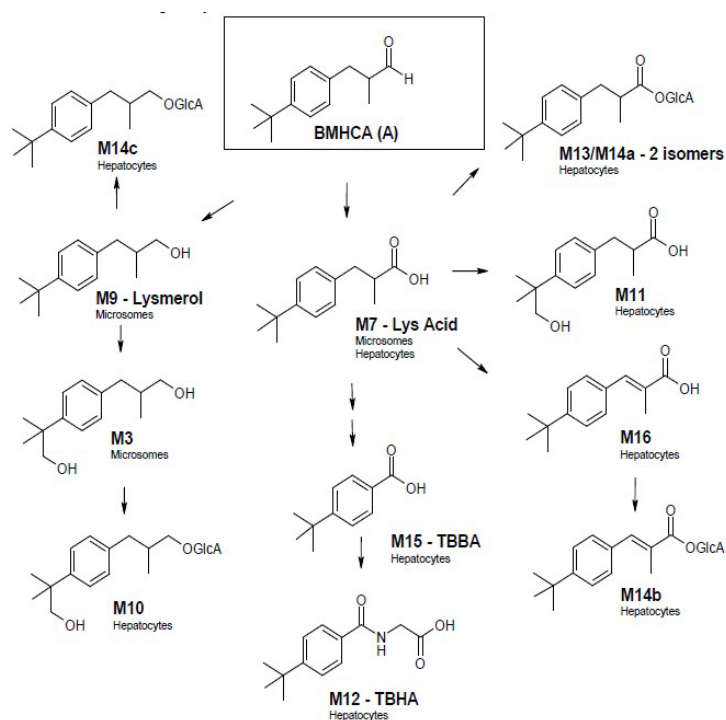
3.3.9. Toxicokinetics

3.3.9.1. Metabolism *in vitro*

Study (BASF SE 2010; GLP): Investigative comparative *in vitro* metabolism study applying liver microsomes or hepatocytes from rats, mice, rabbits, and humans, respectively.

Liver microsomes and hepatocytes of male Han-Wistar rats, male CD1-mice, male white New Zealand rabbits and male humans were incubated with [¹⁴C]-BMHCA at BMHCA concentrations of 10, 50 and 100 µM. (Note: The highest concentration is equal to plasma levels occurring after orally applied BMHCA doses that were shown to induce testicular toxicity.) Metabolic profiles were analysed and quantified by HPLC. Structure elucidation of metabolites was performed from 100 µM BMHCA incubates of liver microsomes and hepatocytes of rats and humans by LC/MS analyses. Positive control incubations were carried out with testosterone (200 µM).

Results: [¹⁴C]-BMHCA was intensively converted into 9 main metabolites by microsomes (8 by hepatocytes). All metabolites separated by HPLC were more hydrophilic than the parent compound. Metabolism can be summarised as follows (according to CLH report, BASF 2013):



In liver microsomes, oxidation or reduction of BMHCA to the corresponding acid (lysmerylic acid, M7) or alcohol (lysmersol, M9), the latter being further oxidised at its *tert*-butyl group (to yield M3), was observed. In hepatocytes, as dehydrogenation follow-up product of M7 (E)-3-(4-*t*-butyl-phenyl)-2-methyl-acrylic acid (M16) was observed. Further, *p*-*t*-butylbenzoic acid (TBBA, M15) and its glycine conjugate (*p*-*t*-butyl-hippuric acid; TBHA, M12) occurred in rodents. In addition to these metabolites, glucuronic acid conjugates of M3, M7, M9 and M16 were detected. Lysmerylic acid (M7) was the main metabolite in hepatocytes of all species. Significant species differences in metabolic profiles were noted for TBHA (M12; 4.9 – 27.1% in mice, 3.5 – 3.6% in rats, not in rabbits or humans) and TBBA (M15). Among the species tested, rat hepatocytes formed the highest amounts of TBBA (M15; 8.3 – 29.3% in rats, 1.9 – 7.5% in humans, ≤0.5% in mice, ≤2.0% in rabbits).

3.3.9.2. Toxicokinetics *in vivo*

1st Study: BASF SE, 2006b, RIFM#53648

Guideline/Method: Explorative comparative oral screening study on plasma kinetics

Species/strain: Rats (Wistar), Mice (C57BL/6NCrl)

Group size: 5 males/group

Test substance: BMHCA and lysmerylic acid (M7)

Batch: no data

Purity: no data

Vehicle: Olive oil

Doses: 0, 50 mg/kg bw

Route: Oral (gavage)

Exposure: Single application of each compound (volume: 5 mL/kg bw)

Exposure period: 24 hours

GLP: no

Method: Blood was taken retro-orbitally, 3 days prior to gavage, directly after the first oral application (i.e. 20 minutes for mice and 10 minutes for rats), as well as 2, 4, 8, and 24 hours after application and blood plasma was analysed for BMHCA and lysmerylic acid by HPLC/MS.

Results: Neither mortality nor clinical signs occurred. Upon application of BMHCA, no parent compound was detectable in any plasma sample of both rodent species afterwards. In the male rat, lysmerylic acid (M7) was detected in all plasma samples and highest plasma concentration was observed 4 hours after application of BMHCA or directly after application of M7. In mice, the highest plasma levels of M7 were observed directly after application of both BMHCA and M7. Toxicokinetics parameters for lysmerylic acid in rodents show no species difference, whereas some difference in the toxicokinetics after oral application of BMHCA between rat and mouse can be observed (Table from CLH report, BASF 2013):

Test substance	Species	T _{max}	C _{max} (µg/g)	AUC ₀₋₂₄ (µg x h/g)	T _{1/2}
BMHCA	Rat	4 h	8.8	81.4	5.8 h
	Mouse	Directly after application, i.e. 20 minutes.	18.4	85.1	3.3 h
Lysmerylic acid	Rat	Directly after application i.e. 10 minutes.	29.4	89.3	3.6 h
	Mouse	Directly after application, i.e. 20 minutes.	22.1	106.7	4.0 h

2nd Study: Huntingdon Research Center, 1995, RIFM #23697 (cf. 3.3.4.2.2.)
Guideline: Comparable to OECD TG 417 and 427. Comparative oral and dermal absorption study according to an approved study protocol following SOPs.
Species/strain: Rats (SPF RORO)
Group size: 8 males
Test substance: [¹⁴C]-BMHCA
Batch: Labelled: 19154-162-40 (215.7 µCi/mg, radiochemical purity: >98%); Unlabelled: 6252-91
Purity: no data
Dose levels: 25, 100 mg/kg bw
Vehicle: Rape oil
Application volume: 2 mL/rat (single application)
Route: Oral (gavage)
GLP: yes
Method: Plasma was collected and counted at 0, 0.5, 1, 2, 4, 6, 8, 24 and 48 hours after BMHCA application. The samples were processed and radioactivity was measured using an LKB 1410 liquid scintillation counter.

Results: Following administration of 25 and 100 mg/kg bw, mean C_{max} were 14.3 and 52 µg/mL at mean T_{max} of about 3.5 and 1.8 hours, respectively. Thus, C_{max} was linearly related to oral dose levels. Thus, oral administration of [¹⁴C]-BMHCA led to rapid absorption for both doses applied and proportionate plasma maximum concentration levels (C_{max}). By contrast, the AUC was found to increase disproportionately to the dose applied (AUC₀₋₄₈ values of 122 and 937 µg × h/mL after 25 and 100 mg/kg bw). The latter finding was interpreted to be due to a saturation of renal clearance.

3rd Study (Roche, 1985, RIFM#56760): Comparative elimination study after oral application (no guideline study).
Excretion patterns of the BMHCA metabolite *p-t*-butyl benzoic acid (TBBA, M15) and its glycine conjugate (TBHA, M12) were examined in rats, mice, guinea pigs, dogs and monkeys (no data on strains). Urine was collected for 24 hours after the last bolus of oral administration (gavage) of BMHCA for 5 consecutive days. BMHCA doses applied: 50 – 400 mg/kg bw/day (rats); 100 mg/kg bw/day (mice, guinea pigs, monkeys); 45 mg/kg bw/day (dogs). Urinalysis of conjugates was performed by GC/MS.

Results: The main urinary metabolite in orally treated rats, dogs and monkeys was TBBA, in the guinea pigs and mice TBHA. The urinary TBBA levels in rats were detected in the range of 7 – 19% of the initial BMHCA dose applied. Corresponding levels in the two monkeys treated were 3 and 11% of the initial BMHCA dose applied.

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 (cf. section 3.3.4.2.), 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 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 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*.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation

Study: Roche 1983, RIFM#35334

Guideline/Method: Cosmetics Toiletry and Fragrance Association (CTFA) testing guidelines

Species/strain: Guinea pigs (Himalayan)

Group size: 10 males and females

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: Ethanol

Doses: 3%, 10%

Route: Epicutaneous application

Positive control: 8-MOP (8-methoxypsoralen, 0.1%)

Light source: Westinghouse FS 40 "Black Lamp" (320 - 400 nm)

Irradiation: 20 J/cm²

Readings: 4, 24, 48 hrs

GLP: no

Method: Guinea pigs received open epicutaneous applications of 0.025 mL of 3% or 10% BMHCA to both shaved flanks at maximum 5 test sites of an area of 2 cm². In addition, 2% DMSO was added to enhance skin penetration. Thirty minutes later, the left flanks were exposed to 20 J/cm² of UV irradiation. The right flanks were not exposed and served as the non-irradiated controls. The sites on both flanks were evaluated 4, 24, and 48 hours after application.

Results: No skin reactions were observed at any time point on the irradiated and non-irradiated skin sites after application of 3% BMHCA. Five out of 10 animals exposed to 10% BMHCA showed slight skin reactions at the irradiated and non-irradiated testing sites. 8-MOP controls showed the expected skin reactions. Under the conditions applied 10% ethanolic BMHCA led to slight skin irritation, which was not enhanced by irradiation.

3.3.10.2. Photosensitisation

Study: Roche, 1986b, RIFM#56766

Guideline/Method: Cosmetics Toiletry and Fragrance Association (CTFA) testing guidelines

Species/strain: Guinea pigs (Himalayan)

Group size: 10 males and females

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: Ethanol

Doses: 10% (for induction), 1% (for challenge)

Route: Open epicutaneous application

Positive control: Tetrachlorosalicylanilide (TCSA, 3% for induction, 0.1% for challenge)

Light source: UV-A: Westinghouse FS 40 Black lamp or a Philips Actinic TL lamp; UV-B:

Philips UV-B Sunlamp TL/12

Irradiation: 10 J/cm² (UV-A) and 1.8 J/cm² (UV-B)

Readings: 24, 48 hrs

GLP: no

Method: Prior to the first induction application, Freund's Complete Adjuvant (FCA) was injected into the test site. For induction, a 0.1 mL aliquot of 10% BMHCA in ethanol was applied on the shaved skin area (6 – 8 cm²). Thirty minutes later the test site was exposed to 10 J/cm² UV-A and then exposed to 1.8 J/cm² UV-B. Five induction applications were performed over a 2-week period. After a 1-week resting period, a 0.025 mL/cm² aliquot of 1% BMHCA was applied to both flanks. Thirty minutes later, the left flank was exposed to 10 J/cm² UV-A. Reactions were read 24 and 48 hours after irradiation.

Results: After challenge, no irritative or a photosensitising reaction was noted in any of the treated animals either with or without UV-A/B. The positive controls showed the expected skin reactions after irradiation. Therefore, under the conditions applied, 10% BMHCA for induction and 1% BMHCA for challenge revealed with no photosensitising potential in guinea pigs.

Confirming study (RIFM 1980e, RIFM #56767): 0.1 ml of an aliquot of 10% BMHCA in rectified alcohol applied to an 8 cm² site on the upper dorsal area of 8 guinea pigs. The animals were then exposed to UV-B for 15 minutes and then exposed to UV-A for 4 hours. Nine induction applications followed by irradiation were performed over an 18-day period. After a 10-day rest, 0.025 ml of 10% alcoholic BMHCA was applied to a 2 cm² area on both flanks. The left flank was then irradiated, the right flank not. Again, 10% BMHCA showed no photo-irritative or photosensitising potential in guinea pigs after 24 or 48 hours.

SCCS conclusion on photo-induced toxicity:

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

3.3.11. Additional Human data

For dermal /percutaneous absorption see section 3.3.4.2., and section 3.3.9.1. for human metabolism *in vitro*.

3.3.11.1. Irritation

Study: RIFM 1998, RIFM #34404

Guideline/Method: Dermal irritation test according to approved study protocol and SOPs

Group size: 25 human volunteers (3 males, 22 females, age: 23-64)

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: 75% ethanol, 25% diethyl phthalate

Doses: 0, 10, 15, 20, 25% (0.3 ml aliquots)

Route: Occlusive epicutaneous application

Duration: 24 hrs

GLP: Yes

Study period: 3 August 1998 – 6 August 1998

Method: Aliquots of BMHCA samples were applied for 24 hours using the 25 mm Hill Top Chamber[®] system on the back of the volunteers. Patches were removed and the skin reactions were graded 20 min after patch removal based on a 7 units scoring system.

Results: At 10 and 20% BMHCA, 1/25 and 2/25 showed faint, minimal erythema (grade 0.5 out of 4), while at 15 and 25% as well as at the vehicle control site no skin reaction was

noted in any of the 25 volunteers. Therefore only some light skin reactions were observed in certain individuals without concentration dependency. No skin irritation was seen at the highest concentration.

SCCS comment:

In rabbit experiments, undiluted BMHCA is a proven skin irritant (cf. section 3.3.2.1.). In addition, weak signs of skin irritancy were occasionally observed in the human repeated insult patch test (HRIPT) as well (cf. 3.3.11.2., below).

3.3.11.2. Sensitization

Study: RIFM 1999a, RIFM #34405; Cocchiara, Api, 2003, RIFM #41754

Guideline/Method: HRIPT according to approved study protocol and SOPs

Group size: 121 human volunteers (30 males 89 females completed the study)

Test substance: BMHCA

Batch: no data

Purity: no data

Vehicle: 75% ethanol, 25% diethyl phthalate

Dose: 25% (0.3 ml aliquots)

Negative control: Saline

Route: Occlusive epicutaneous application

GLP: Yes

Study period: 4 November 1998 – 11 December 1998

Method: An approved study protocol was performed with 25% BMHCA (corresponding to about 30 mg/cm²) under GLP conditions. During induction, approximately 0.3 ml was applied on a Webril/adhesive patch (25 mm Hill Top Chamber System[®]) to the dry wiped skin on the left side of the back of each volunteer. The patch was occlusively covered and remained on the skin for 24 hours. Then patches were removed and the skin was scored. Patch removal was followed by a rest period of 24 hours. A series of 9 induction patches was completed over a period of 3 weeks. The last induction patch was followed by a rest period of two weeks. Challenge: a webril/adhesive patch was applied with 0.3 ml 25% BMHCA solution and fixed semi-occlusively on the right side of the back of each volunteer for 24 hours. The application site was scored at 24, 48, 72 and 96 hrs after removal of the patch.

Results: During induction, slight signs of irritation were noted in 7 volunteers. Individual volunteers also showed skin reactions after treatment with vehicle or saline. At challenge, 1/119 exhibited a low-level skin reaction attributable to BMHCA, while two other volunteers showed skin reaction against controls.

Follow-up studies:

1. HRIPT under GLP conditions with 25% BMHCA in 25% ethanol/75% diethyl phthalate (corresponding to 30 mg/cm²) in 119 volunteers (106 completed). None of the participants showed signs of sensitisation (RIFM 2002, RIFM #40923; Cocchiara, Api, 2003, RIFM #41754).

2. HRIPT with 5.5% alcoholic BMHCA (corresponding to 4.2 mg/cm²) or 4% BMHCA in petrolatum (corresponding to 3.1 mg/cm²) applied 64 and 43 human volunteers, respectively. None of the volunteers showed signs of sensitisation (RIFM 1972a, RIFM #6112; RIFM 1980b, RIFM #54702; RIFM 1980f, RIFM #15029).

3. HRIPT with 5% BMHCA in diethyl phthalate (corresponding to 3.7 mg/cm²) in 103 human volunteers. None of the volunteers showed signs of sensitisation (RIFM 1988a, RIFM #9360).

4. Two human maximisation tests were conducted on 25 volunteers with BMHCA at 4% (corresponding to 3.8 mg/cm²) or 5% (corresponding to 3.5 mg/cm²) in petrolatum. BMHCA was applied under occlusion to the same sites of volar forearms for 48 hours (with a total of five 48-hrs applications). Patch sites were pre-treated for 24 hours with 5% aqueous SDS

under occlusion. Following a 10-day rest period, challenge patches were applied under occlusion to fresh sites for 48 hours, preceded by 1-hr applications of 10% aqueous SDS under occlusion. Under these conditions, sensitisation was not observed with 4% BMHCA, but 9 out of 25 volunteers displayed skin sensitisation at 5% BMHCA (RIFM 1971b, RIFM #1805; RIFM 1972b, RIFM #1804).

Two older HRIPT tests showed the absence of sensitising potential of BMHCA (RIFM 1964, RIFM #6186; RIFM 1971a, RIFM #2730 and RIFM 1965, RIFM #6187).

SCCS comment:

The SCCS considers the HRIPT unethical.

3.3.11.3. Other clinical data

Published data on positive patch-test reactions in various clinical studies (mostly patients attending a dermatology clinic) have been summarised (SCCS 2012: SCCS/1459/11). BMHCA scored ++, indicating that between 11 and 100 positive reactions have been recorded. The frequency of the positive reactions against 5% BMHCA varied from 0% to 1.2% (Larsen et al. 1996; 2 out of 167 individuals); in most studies it was below 0.6%.

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. So, reactions were just occasionally observed at concentrations of <5%. Overall, mainly based on clinical studies, and already since 2012, the SCCS considers BMHCA as an “established contact allergen in humans” (SCCS, 2012).

3.3.12. Special investigations

See 3.3.2.3.

3.3.13. Safety evaluation (including calculation of the MoS)

According to the mandate/ToR (see pages 5-6), there are the following different categories to be considered in the exposure assessment (values in brackets: relative daily exposure [mg/kg bw/day] or frequency x surface area treated per day [times x cm²]; numbers according to SCCS's NoG 2012):

1. Lip products: 0.1% BMHCA (0.90)
2. Deodorants/antiperspirants: 0.2% BMHCA (22.08)
3. Hydroalcoholic products for shaved skin: 0.6% BMHCA (1 x 305)
4. Hydroalcoholic products for unshaved skin: 1.9% BMHCA (1 x 305)
5. Facial cream, facial make-up, hand cream: 1.0% BMHCA (24.14, 7.90, 32.70)
6. Mouthwash, toothpaste: 3.0% BMHCA (32.54, 2.16)
8. Make-up remover, nail care: 2% BMHCA (8.33, -/-: no data on nail care in NoG)
9. Shampoo, rinse-off conditioner, bar soap: 2.5% BMHCA (1.51, 0.60, 3.33)

An *in vivo* study with 3 volunteers demonstrated only a mean value of 1.4% (range 0.8 – 2.4%) absorption through human skin (dose applied: 12% BMHCA dissolved in 70% ethanol). By contrast, and depending on the kind of formulation (ethanolic vs. creamy), the numbers obtained with mini pig skin *in vitro* were higher, in particular when BMHCA was applied as part of a cream formulation: 4.9% (ethanolic solution of 1% BMHCA) and 24.7% (cream formulation containing 0.6% BMHCA, not further specified). Thus worst case estimations of the SED outlined below were performed with absorption values of 5% (ethanolic solution) and 25% (cream formulation) (see also section 3.3.4. for further explanation).

By considering the percentage of BMHCA suggested permissible in the product, the application of these numbers would result in the following SED values (values in brackets: relative daily exposure in µg/kg bw/day x percent of BMHCA in the product x fraction absorbed; *oral exposure: 100% absorption):

1. Lip products: 0.225 µg/kg bw/day (900 x 0.001 x 0.25)
2. Deodorants/antiperspirants: 2.208 µg/kg bw/day (22080 x 0.002 x 0.05)
5. Facial cream, facial make-up, hand cream: 60.35 µg/kg bw/day (24140 x 0.01 x 0.25), 19.75 µg/kg bw/day (7900 x 0.01 x 0.25), 81.75 µg/kg bw/day (32700 x 0.01 x 0.25)
6. Mouthwash, toothpaste*: 976.2 µg/kg bw/day (32540 x 0.03 x 1), 64.8 µg/kg bw/day (2160 x 0.03 x 1)
8. Make-up remover, nail care: 41.65 µg/kg bw/day (8330 x 0.02 x 0.25), since no data are available for nail care products at all (i.e., penetration of BMHCA through the nail plate), the SED for these kinds of products cannot be assessed.
9. Shampoo, rinse-off conditioner, bar soap: 9.44 µg/kg bw/day (1510 x 0.025 x 0.25), 3.75 µg/kg bw/day (600 x 0.025 x 0.25), 20.81 µg/kg bw/day (3330 x 0.025 x 0.25)

The SED values for products in product categories 3 and 4 can be estimated as follows: Total skin area treated per day x dermal absorption in µg/cm² / 60 kg bw. Based on the amount of absorption obtained with mini-pig skin *in vitro* (ethanolic solution of 1% BMHCA: 6 µg/cm² without and 11 µg/cm² including the fraction detected in the *stratum corneum* layer; see also section 3.3.4. for further explanation) the SED values [in µg/kg bw/day] were calculated as follows:

3. Hydroalcoholic products for *shaved* skin: 55.9 (1 x 305 x 11 / 60)
4. Hydroalcoholic products for *unshaved* skin: 30.5 (1 x 305 x 6 / 60)

Adding all of the numbers derived above leads to the total SED level of: **1368 µg/kg bw/day.**

CALCULATION OF THE MARGIN OF SAFETY (aggregate exposure)

Total systemic exposure dose (SED)	= 1.4 mg/kg bw
No observed adverse effect level NOAEL (90-day, oral, rat)	= 5 mg/kg bw/d

Margin of Safety	NOAEL/SED	= 3.6
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Individual MoS values for the respective product groups:

1. Lip products: 220000 (possible oral exposure not considered)
2. Deodorants/antiperspirants: 2300
3. Hydroalcoholic products for shaved skin: 89
4. Hydroalcoholic products for unshaved skin: 160
5. Facial cream, facial make-up, hand cream: 31
6. Mouthwash, toothpaste: 4.8
8. Make-up remover, nail care: <120 (no data on nail plate penetration available)
9. Shampoo, rinse-off conditioner, bar soap: 150

SCCS comment:

The original dossier delivered by the applicant in April 2013 suggested a MoS value of 359 based on the following assumptions:

1. The percentage of *fragrance mixture* being present in the product lies in the range of 0.2 – 1.2%. According to the dossier, this range is expected to encompass all different kinds of product categories available at the market.
2. The maximum concentration of BMHCA present in such *fragrance mixtures* is assumed to be no higher than 7.89%. The applicant explains that this number results from the examination of several thousands of different commercial formulations, but omitted to deliver specific data that would support this claim.
3. The maximum absorption rate is set at 6%, based on the only human study available (see 3.3.4.2.1.) and the following reasoning: Considering that an amount of 1.4% was excreted in the urine within 24 hours and no radioactivity has been detected thereafter, a mean value of 2% was taken for the fraction systemically bioavailable after dermal exposure. Further, with regards to possible intra-species variation, an additional assessment factor of 3 has been used leading to an overall default value of 6% for dermal absorption.
4. A NOAEL of 5 mg/kg bw/day.

Based on assumptions 1 and 2, the percentage of BMHCA reported to be present in the different types of cosmetic products was as low as 0.0158 – 0.1184%. This means that the applicant claimed that no more than 0.1184% of BMHCA will be found in any of the finished products available at the market. Based on these numbers and the additional suggestion that the dermal absorption may not exceed a fraction of only 6%, the applicant derived the above mentioned MoS value of 359 (total SED of 6.963 µg/kg bw/day).

The 2013 IFRA dossier was intended to demonstrate the safety of BMHCA when used as fragrance ingredient in cosmetic leave-on and rinse-off type products. In the accompanying request IFRA suggested safe concentration limits for BMHCA in the finished products in the range between 0.1% (category 1, lip products) and 3.0% (category 6, mouthwash, toothpaste) (see numbers above and section 1, "Background"). In light of the maximum level of BMHCA that allegedly would be present in the finished products (i.e. 0.1184%), the suggested numbers seem inapprehensibly high. Moreover, taking these numbers into

Revision of opinion on Butylphenyl methylpropional

account, the total systemic exposure dose (SED) calculated turned out to be no less than 1400 µg/kg bw/day thus leading to a MoS of only 3.6. For this calculation dermal bioavailability values of 5% (ethanolic solution) and 25% (cream formulation) were used based on the reasoning as outlined in section 3.3.4.1 of this opinion.

In February 2015, IFRA submitted additional information on the actual use levels of BMHCA in BMHCA-containing products currently present on the European market. Based on these survey data, IFRA calculated an overall SED for all products of 14.906 µg/kg bw/day and—in addition—this time was seeking support (approval) for the following use levels:

Product types	Finished product concentration
Hydroalcoholic products	1.9%
Deodorants	0.12
Make up	0.05
Liquid foundation	0.04
Face cream	0.05
Body lotion	0.1
Shampoo /Conditioner	0.05
Hair styling	0.04
Shower gel / bath products	0.1

As can be seen by comparing the numbers presented in the table above with the originally proposed limits in the finished products (cf. section 1, "Background"), the newly suggested values were completely different (with the exception of hydroalcoholic products). Further, some of the old categories disappeared (e.g., lip products, mouthwash, toothpaste), while others such as 'body lotion' newly emerged.

Calculation of the total SED of the products and its respective contents as listed in the revised table:

Application of the suggested numbers will result in to the following SED values in µg/kg bw/day (values in brackets: relative daily exposure in µg/kg bw/day x percent of BMHCA in the product x fraction absorbed):

1. Hydroalcoholic products (for *shaved* skin): 55.9 (1 x 305 x 11 / 60)
2. Deodorants: 1.325 (22080 x 0.0012 x 0.05)
3. Make up: 0.988 (7900 x 0.0005 x 0.25)
4. Liquid foundation: 0.833 (8330 x 0.0004 x 0.25)
5. Face cream: 3.018 (24140 x 0.0005 x 0.25)
6. Body lotion: 30.8 (123200 x 0.001 x 0.25)
7. Shampoo/Conditioner: 0.189 (1510 x 0.0005 x 0.25) / 0.075 (600 x 0.0005 x 0.25)
8. Hair styling: 0.574 (5740 x 0.0004 x 0.25)
9. Shower gel/bath products: 0.698 (2790 x 0.001 x 0.25) / 0.833 (3330 x 0.001 x 0.25)

Adding of all of these numbers leads to a total SED level of: 95.2 µg/kg bw/day. Based on the NOAEL of 5 mg/kg bw/d, the MoS derived for aggregate exposure would be no higher than **53**, thus still much lower than 100.

Individual MoS values for the respective product groups:

1. Hydroalcoholic products (for *shaved* skin): 89
2. Deodorants: 3800
3. Make up: 5100

4. Liquid foundation: 6000
5. Face cream: 1700
6. Body lotion: 160
7. Shampoo/Conditioner: 19000
8. Hair styling: 8700
9. Shower gel/bath products: 3300

Compared to the value derived by the applicant (MoS of 108 for aggregate exposure), the much lower MoS levels derived here are mainly due to the different dermal absorption rate used by SCCS when BMHCA is part of “cream” formulations. While industry always set the percentage of dermal penetration at 6%—irrespective of the kinds of products to be considered—based on the studies provided SCCS concluded a penetration rate of 5% for BMHCA-containing ethanolic solutions and 25% for BMHCA-containing creamy formulations, respectively (cf. above and section 3.3.4. for further explanation).

3.3.14. Discussion

Physicochemical properties

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 $\geq 97.5\%$ (w/w). According to the applicant the degree of purity can be as high as $\geq 99.5\%$ (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

The acute toxicity after all relevant routes of application of BHMCA was investigated in rats and rabbits. Based on the LD₅₀ 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 signs of systemic toxicity after exposure to a saturated atmosphere.

Irritation / sensitisation

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 sensitizer”. 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 sensitizing 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. BMHCA poses a risk of inducing skin sensitisation in humans.

Dermal absorption

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 which has been found in excised mini pig skin. Since the absorption of BMHCA in mini pig skin was much higher in real cream formulations, it becomes likely that also human skin might be much better penetrable for BMHCA when being present in cream formulations. The SCCS therefore concludes that the maximum fraction of BMHCA being absorbed by human skin will be in the range of 5% (ethanolic solution) and 25% (cream formulation).

Mutagenicity / genotoxicity

The genotoxicity of BMHCA was investigated for the three endpoints of genotoxicity, that is, gene mutations, chromosomal aberrations and aneuploidy. Neither mutagenicity nor chromosomal damage can be excluded based on the *in vitro* data provided. Due to the lack of sufficient and detailed information, no firm conclusion can be drawn from the *in vivo* micronucleus data provided.

Toxicokinetics

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 (cf. below).

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.

Repeated dose toxicity

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 can be derived

for systemic effects and reproductive effects, respectively. This result has been further supported by reproductive toxicity studies (cf. below).

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 an 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 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 (cf. above). 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 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. So, the data available do not support the conclusion that this metabolite would be mainly responsible for the testicular effects observed with BMHCA in rats.

Carcinogenicity

No specific investigations available. There is no evidence from repeated dose studies in animals that BMHCA is capable of inducing cancer.

4. CONCLUSION

The applicant was neither able to submit reliable data on the actual usage of BMHCA in the different product groups placed on the European market, nor clearly defined unambiguous limits in the finished products to be assessed by SCCS.

1. Does the SCCS consider 2-(4-tert-butylbenzyl)propionaldehyde (BMHCA) safe for use as fragrance ingredient in cosmetic leave-on and rinse-off type products in a concentration limit(s) according the ones set up by IFRA?

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.

2. Does the SCCS have any further scientific concerns with regard to the use of 2-(4-tert-butylbenzyl)propionaldehyde (BMHCA) as fragrance ingredient in cosmetic leave-on and rinse-off type products?

BMHCA poses a risk of inducing skin sensitisation in humans.

During the commenting period the applicant commented on the maximum use levels of BMHCA in the finished cosmetic product types. Also further information on genotoxicity was provided. It was also proposed to initiate an in vitro study on dermal penetration of 14C-BMHCA through human skin (according OECD TG 428).

A reassessment of 2-(4-tert-butylbenzyl)propionaldehyde (BMHCA) based on the new data is foreseen.

5. MINORITY OPINION

None

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