Scientific Committee on Consumer Safety

SCCS

OPINION on

Opinion on
Ethylhexyl Methoxycinnamate (EHMC)

(CAS No. 5466-77-3/83834-59-7, EC No. 226-775-7/629-661-9)

The SCCS adopted this document
during the plenary meeting on 25 October 2024

ACKNOWLEDGMENTS

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

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1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Ethylhexyl Methoxycinnamate, does the SCCS consider Ethylhexyl Methoxycinnamate safe when used as UV-Filter in cosmetic products up to a maximum concentration of 10%?*

   Having considered the data provided, and the concerns relating to potential endocrine disrupting properties of EHMC, the SCCS cannot conclude on the safety of EHMC, because the information provided is insufficient to exclude genotoxicity.

   In addition, the available evidence also shows that EHMC is an endocrine-active substance due to clear demonstration of estrogenic activity and weak anti-androgenic activity both *in vitro* and *in vivo*.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Ethylhexyl Methoxycinnamate in cosmetic products?*

   /

3. *Does the SCCS have any further scientific concerns with regard to the use of Ethylhexyl Methoxycinnamate in cosmetic products?*

   The SCCS mandate does not address environmental aspects. Therefore, this assessment did not cover the safety of EHMC for the environment.

Keywords: SCCS, scientific opinion, Ethylhexyl Methoxycinnamate (EHMC), Octylmethoxycinnamate (OMC), Octinoxate, Regulation 1223/2009, CAS No. 5466-77-3/83834-59-7, EC No. 226-775-7/629-661-9.

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About the Scientific Committees

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

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

SCCS

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

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted the review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting (ED) properties. The review concluded that the Cosmetics Regulation provides the adequate tools to regulate the use of cosmetic substances that present a potential risk for human health, including when displaying ED properties.
The Cosmetics Regulation does not have explicit provisions on EDs. However, it provides a regulatory framework with a view to ensuring a high level of protection of human health. Environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 ('REACH Regulation').
In the review, the Commission commits to establishing a priority list of potential EDs not already covered by bans or restrictions in the Cosmetics Regulation for their subsequent safety assessment. A priority list of 28 potential EDs in cosmetics was consolidated in early 2019 based on input provided through a stakeholder consultation. The Commission carried out a public call for data in 2019[2] for 14 substances (Group A)[3] and a second call in 2021[4] for 10 substances (Group B)[5] in preparation of the safety assessment of these substances. Ethylhexyl Methoxycinnamate (EHMC) is one of the above-mentioned substances for which the call for data took place.

Background on Ethylhexyl Methoxycinnamate (EHMC)

Ethylhexyl Methoxycinnamate (EHMC) (CAS No. 5466-77-3/83834-59-7, EC No. 226-775-7/629-661-9) with the chemical name '2-ethylhexyl 4-methoxycinnamate' (also known as Octylmethoxycinnamate (OMC) and Octinoxate) is regulated as a UV-filter in sunscreen products in a concentration up to 10 % (Annex VI/12).

Ethylhexyl Methoxycinnamate absorbs only UVB radiation and, therefore, protects the skin only from damage caused by UVB light and not UVA. It has been used for decades as a UV filter in cosmetics, pharmaceuticals, intermediates and fine chemicals and it is also reported to be used as a UV stabiliser protecting cosmetic formulations against sunlight. Ethylhexyl Methoxycinnamate has been subject to a safety evaluation by SCC in 1991 and 1993[6] and by SCCNFP in 2001[7].

[1] https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-739-F1-EN-MAIN-PART-1.PDF
[2] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic%20products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, Homosalate, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein
[4] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic-products-0_en
[5] Butylparaben, Methylparaben, Ethylhexyl Methoxycinnamate (EHMC)/Octylmethoxycinnamate (OMC)/Octinoxate, Benzophenone-1 (BP-1), Benzophenone-2 (BP-2), Benzophenone-4 (BP-4), Benzophenone-5 (BP-5), BHA/Butylated hydroxyanisole/tert-butyl-4-hydroxyanisole, Triphenyl Phosphate and Salicylic Acid
[6] https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/scc_o_9.pdf
[7] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out145_en.htm

During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Ethylhexyl Methoxycinnamate as UV-filter in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Ethylhexyl Methoxycinnamate in view of the information provided.

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Ethylhexyl Methoxycinnamate, does the SCCS consider Ethylhexyl Methoxycinnamate safe when used as UV-Filter in cosmetic products up to a maximum concentration of 10%?*

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Ethylhexyl Methoxycinnamate in cosmetic products?*

3. *Does the SCCS have any further scientific concerns with regard to the use of Ethylhexyl Methoxycinnamate in cosmetic products?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

According to the Applicant, the substance Ethylhexyl Methoxycinnamate is a multi-constituent substance (organic) having the following characteristics and physical–chemical properties:

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Ethylhexyl Methoxycinnamate

For consistency, the term "EHMC" is used by the SCCS in this Opinion.

3.1.1.2 Chemical names

2-Ethylhexyl-4-methoxycinnamate (EHMC)
Octylmethoxycinnamate (OMC)
2-ethylhexyl-p-methoxycinnamate
Octyl methoxycinnamate
2-Ethylhexyl trans-4-methoxycinnamate
Octyl p-Methoxycinnamate
Octinoxate

SCCS comment
OMC and EHMC are both used interchangeably by the Applicant to describe 2-ethylhexyl-4 methoxycinnamate, with a branched 2-ethylhexyl chain group. The SCCS preference is to use the more widely used EHMC in this Opinion for consistency.

IUPAC name
2-Ethylhexyl (2E)-3-(4-methoxyphenyl)acrylate
(NTP, 2006; SCCS, 2000)

(2R)-2-ethylhexyl (2E)-3-(4-methoxyphenyl) prop-2-enoate; (2S)-2-ethylhexyl (2E)-3-(4-methoxyphenyl) prop-2-enoate
https://echa.europa.eu/el/registration-dossier/-/registered-dossier/15876/11/?documentUUID=89d57fa8-e0b2-458c-9ea7-87d727329695

3.1.1.3 Trade names and abbreviations

Parsol MCX
Neo Heliopan AV
Uvinul MC 80 N

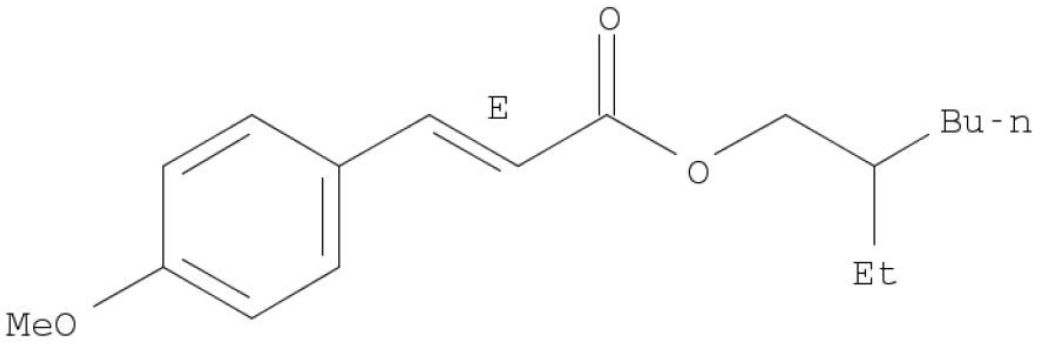
(NTP, 2006; SCC, 2000)

3.1.1.4 CAS / EC number

CAS: 5466-77-3, 83834-59-7
EC: 226-775-7, 629-661-9

3.1.1.5 Structural formula

EHMC



3.1.1.6 Empirical formula

$C_{18}H_{26}O_3$

(SCC, 2000)

3.1.2 Physical form

Pale yellow liquid

(ECHA,2021)

3.1.3 Molecular weight

290.4 g/mol

3.1.4 Purity, composition and substance codes

95-105%

Due to confidentiality agreements among EHMC suppliers, the data on the purity and impurities of the EHMC representative batches are not displayed in this opinion. For the assay and organic impurities, the appropriate method to be used is the USP method for Octinoxate.

3.1.5 Impurities / accompanying contaminants

Total impurities: max. 2%
Cis-isomer: max.0.5%
2-Ethylhexanol: max. 50 ppm

Heavy metals: Lead ≤5 ppm, Cadmium ≤1 ppm, Mercury ≤1 ppm, Cobalt ≤1 ppm, Chrome ≤1 ppm, Nickel ≤1 ppm, Total heavy metals ≤10 ppm, Arsenic ≤2 ppm

(DSM, 2016)

SCCS comment

Additional confident data on the purity and impurities of EHMC have been submitted to the SCCS. These data indicate that, in 7 batches, the trans-EHMC purity was greater than 98.4%. The cis-EHMC content ranged from 0.11% to 0.23%, with two other organic impurities present at levels ≤ 0.07%, and iso-octanol levels at ≤ 0.01%.

in 3 additional batches from another notifier, any individual impurity was reported to be ≤ 0.5%, with total impurities being ≤ 1.0%. The cis-EHMC content was ≤ 0.5%, 2-ethylhexanol was ≤ 5 ppm, 4,4-dimethoxystilbene was ≤ 900 ppm, aubeprine p-cresol was ≤ 30 ppm, and 2-ethylhexylacetate was ≤ 5 ppm. The 3-methyl-OMC (sum of isomers) was ≤ 0.4%.

According to Applicant's certificate of analysis, data on heavy metal impurities analysed by ICP-MS in these 3 batches show that heavy metal impurities (lead, cobalt, chrome, nickel, arsenic and antimony) are ≤ 1.0 ppm, while cadmium and mercury are ≤ 0.5 ppm and ≤ 0.1 ppm, respectively.

SCCS has also checked that at these levels these impurities are not of concern as they do not trigger any specific genotoxicity alerts and they are below the level considered safe under TTC.

3.1.6 Solubility

Water immiscible (0.051 – 0.0678 mg/L); completely miscible in ethanol, fats, oils and Isopropanol

(ECHA,2021)

In the Substance Evaluation Conclusion Document (2017):

The water solubility of EHMC was investigated according to OECD Guideline 105 and was found to be 0.22 - 0.75 mg/l at 21 °C using the flask method. It is considered to be slightly soluble (0.1-100 mg/L).

A second water solubility value, cited as supporting information is included in the registration dossier and is reported as being measured using the column elution method. The precise source is unclear but is noted as safety data sheet. OMC is a viscous liquid, so the column elution method for determining water solubility is not applicable. The eMSCA therefore considers that the value of 0.041 mg/L (for OMC) obtained in this supporting study is most likely not valid. In the opinion of the eMSCA the values for water solubility taken from the key study (0.22-0.75 mg/L) should be used.

3.1.7 Partition coefficient (Log Pow)

>6 at 23 °C

(ECHA,2021)

SCCS comment

The SCCS noticed that EHMC is very hydrophobic.

3.1.8 Additional physical and chemical specifications

Organoleptic properties: Slight odour
Melting point: -68.3°C
Boiling point: 382°C
Flash point: 204°C
Vapour pressure: 0.3 hPa at 154°C
Relative density (D 20/4): 1.01
Specific gravity (D 25/25): /
Viscosity: 99.8 mPa
UV peak absorbance: 311 nma
pKa: /
Acid value (potentiometric filtration, mg KOH/g): /
Refractive index (n 20/D, 20°C): /

(ECHA,2021)

3.1.9 Homogeneity and Stability

According to the applicant the normal pure synthetic material contains more than 98% of the trans-isomer. Upon exposure to UV radiation, it will undergo isomerization to the cis-form, which itself is a UV filter with a very similar shape of absorption curve. Hence, the cis-isomer will absorb UV radiation and isomerize back to the trans-form. Their back-and-forth isomerization will lead to a photostationary equilibrium (60% cis + 40% trans) (Köhnlein, M.).

The two isomers together form a stable system. This equilibrium is reached fast and accompanied by a drop in overall absorbance of around 20% but then stays stable under further irradiation. This decrease is due to the fact that although the two isomers have absorption curves very similar in shape, they differ in magnitude of their individual extinction coefficients.

SCCS comment
2-Ethylhexyl p-methoxycinnamate (EHMC; CASRN 5466-77-3) is a mixture of cis- and trans-isomers, with the trans-isomer (CASRN 83834-59-7) predominating. EHMC is a colourless to light-yellow highly hydrophobic viscous liquid that is insoluble in water (0.04 mg/L at 24ºC, pH 7.1) and is readily soluble in most organic solvents; EHMC absorbs ultraviolet (UV) A (320–400 nm) and UVB (290–320 nm) light and is photostable (Kockler *et al.*,2012).

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

In vitro

1st study: *In vitro* percutaneous absorption (human skin)

Guideline:	OECD Test Guideline 428 (2004)
Test system:	Human abdominal skin (Caucasian, age:34-61)
Test substance:	10% Ethylhexyl Methoxycinnamate and

	Radiolabelled compound [acrylate-3-14C]2-Ethylhexyl 4-methoxycinnamate
Test Formulation:	O/W emulsion
Batch:	BCBT6945
Purity:	98.9%
Route:	Topical application
TEWL:	0.7 - 5 g/m²/h (closed chamber)
Number of donors:	5
Number of cells per condition:	5 each at 30 min, 1, 2, 4, 8 hours and 12 at 24 hours
Total cells:	37
Thickness of skin:	350 - 450 µm
Washing of test formulation:	0.5 mL Tween 80® 5%; 1 half cotton bud 3.5 mL of UHQ water (0.5 mL, 7 times) 3 dried half cotton buds
Strips:	A maximum of 20 strips was taken.

The strips were pooled as follows: 1-2, 3-8, 9-14, 15-20.

Separation Epidermis/Dermis:	Yes (separation by heat)
Extraction solvent for RCD and RCR, tape strips and cotton-swabs:	Ethanol
Dose of test formulation:	2 mg/cm²
Exposure time:	30 min, 1, 2, 4, 8, 12 and 24 hours
GLP:	Yes
Study period:	2021

The *in vitro* absorption potential of Ethylhexyl Methoxycinnamate was determined on the surface of healthy human skin mounted on dynamic cells in an OECD Test Guideline 428 compliant study. The human skin samples used were obtained from abdominal surgery. The skin was dermatomed to a thickness of 350-450 µm. The integrity of the skin was determined by measuring the trans-epidermal water loss and skin samples exhibiting values between 0.7 and 5 g/m²/h were selected. The cosmetic formulation containing 10% Ethylhexyl Methoxycinnamate and the radiolabelled compound [acrylate-3-^{14}C]2-Ethylhexyl 4-methoxycinnamate was prepared and was applied (approximately 0.4 µCi) homogenously at 2 mg/cm^2 (i.e., 2 mg/cell). The study was designed to provide information on the kinetics of the absorption of Ethylhexyl Methoxycinnamate. The test formulation was applied for 24 h during which the skin absorption rates were determined at different time intervals by measuring the activity of the ^{14}C-labelled Ethylhexyl Methoxycinnamate. However, only the 24-h timepoint is relevant for this safety evaluation. After the exposure period of 24 h, the skin samples were washed with mild soap solution, rinsed and dried. The upper layers of the stratum corneum were removed by tape stripping. The remaining skin was heat-separated into epidermis and dermis. The stability of the test formulation was checked at the start and after 24 h, at 32°C, of the experiment.

Results

The mean results obtained for test formulation containing Ethylhexyl Methoxycinnamate and the radiolabelled compound [acrylate-3-^{14}C]2-Ethylhexyl 4-methoxycinnamate are presented in the following tables:

Table 1. In vitro percutaneous absorption ^{14}C- Ethylhexyl Methoxycinnamate through human split thickness skin

Conditions: Washing and dismantling at 24hrs:	Distribution % of applied dose [µg] mean ± SD		Distribution µg equiv./cm² mean ± SD	
	n=12		n=12	
	Mean	SD	Mean	SD
Total strips (1-20)	0.41	0.17	0.85	0.38
Skin Excess*	100.26	10.53	205.96	36.06
Epidermis	0.21	0.16	0.41	0.27
Dermis	0.02	0.01	0.03	0.01
Receptor fluid	0.06	0.08	0.13	0.18
Epidermis + dermis + receptor fluid **	0.28	0.17	0.57	0.31
Total Recovery	100.96	10.56	-	-

*Skin excess corresponds to: Washing + Donor compartment rinsing + surrounding skin
** absorbed fraction of the applied Ethylhexyl Methoxycinnamate according to SCCS NoG

The absorption results were presented according to the SCCS NoG with Receptor fluid + Rinsing Receptor compartment (RCR) + Epidermis + Dermis for 24 hours.

- test formulation in contact with the skin for 24 hours: 0.28% ± 0.17% of the applied dose corresponding to 0.57 µg eq/cm² ± 0.31 µg eq/cm²

The mean total recovery was within the SCCS acceptance criteria (i.e., 85-115%), validating the results obtained.

Conclusion

In conclusion, following topical application of 10% [^{14}C]-Ethylhexyl Methoxycinnamate in a representative O/W cosmetic formulation to human skin *in vitro*, the absorbed fraction of the applied test substance was less than 0.5% of the applied dose.

(Raynaud, 2021)

SCCS comments

The above GLP-OECD compliant *in vitro* dermal absorption study, meeting the SCCS Notes of Guidance (SCCS/1647/22) criteria, is considered scientifically acceptable and reveals a mean dermal absorption of 0.28 + 0.17% = 0.45% (mean + 1 SD) of the applied dose after 24 hours exposure.

2nd study: *In vitro* percutaneous absorption (rat skin)

Guideline:	Similar to OECD Test Guideline 428
Test system:	Naked rat skin
Test substance:	1, 3 and 10% Ethylhexyl Methoxycinnamate in Diethylene glycol monoethyl ether (Carbitol™)
Radiolabelling:	Yes, ^{14}C
Batch:	Not specified
Purity:	Not specified
Route:	Topical application
Dose:	120, 360 or 1200 µg/cm²
Exposure time:	1, 6, 16 and 24 hours
GLP:	No

Study period: 1979

The *in vitro* absorption potential of Ethylhexyl Methoxycinnamate was determined in a naked rat skin model similar to OECD Test Guideline 428 (non-GLP) under occlusive conditions. Three concentrations of test substance in Carbitol™ (i.e., 1, 3 and 10%) were applied for 1, 6, 16 and 24 hours during which the skin absorption rates were determined by measuring the activity of the ^{14}C-labelled Ethylhexyl Methoxycinnamate.

Results

The percentages of test substance absorbed (i.e., amount of test substance in stripped skin and chamber liquid) after 24 hours were 44.3% (1%), 35.6% (3%) and 22.7% (10%). The amount recovered from the stratum corneum was low and reached its maximum 24 hours after application. The portion of ^{14}C-labelled Ethylhexyl Methoxycinnamate found in the stripped skin increased to its maximum within 16 hours. Lower levels of Ethylhexyl Methoxycinnamate were found in the stripped skin 24 hours after application. A significant part of the applied dose was found in the chamber liquid (7-17%) after longer times of exposure.

Conclusion

Carbitol is a solvent known to have penetration enhancing properties. Under the experimental conditions of this *in vitro* study, it was observed that around 44.3, 35.6 and 22.7% of Ethylhexyl Methoxycinnamate, present at 1, 3 and 10% concentration in formulations respectively, penetrated the rat skin samples during 24 hours. However, it is well known that rat skin is not an adequate model for human skin in terms of dermal penetration. Systemic exposure of humans may be significantly overestimated if risk assessment is based on the results of rat skin because rat skin is more permeable than human skin, especially for lipophilic compounds (mean difference about 10-fold) (van Ravenzwaay & Leibold, 2004).

(ECHA, 2021)

3rd study: *In vitro* percutaneous absorption (Mini pig skin)

Guideline:	Similar to OECD Test Guideline 428
Test system:	Mini pig skin (Slovak large white)
Test substance:	7.5% Ethylhexyl Methoxycinnamate in a standard sunscreen formulation
Batch:	Not specified
Purity:	Not specified
Route:	Topical application
Dose: o/w lotion:	67.35 µg Ethylhexyl Methoxycinnamate /cm^2;
o/w cream:	58.9 µg Ethylhexyl Methoxycinnamate /cm^2
w/o cream:	58.9 µg Ethylhexyl Methoxycinnamate /cm^2
Number of cells/replicates:	Unknown
Number of donor:	Unknown
Exposure time:	6 hours
GLP:	No
Study period:	1982

The *in vitro* absorption potential of Ethylhexyl Methoxycinnamate was determined in mini pigs skin similar to OECD Test Guideline 428 (non-GLP). The effect of 3 cosmetic vehicles (i.e., o/w lotion, o/w cream, w/o lotion) on the penetration of Ethylhexyl Methoxycinnamate present at 7.5% in each vehicle through excised skin of mini pigs was evaluated. All three formulations were applied to the excised mini pig skins for 6 hours under occlusive conditions (doses of Ethylhexyl Methoxycinnamate in o/w lotion: 67.35 µg/cm^2; o/w cream: 58.9 µg/cm2 and w/o

cream: 58.9 µg/cm2) and skin absorption rates were determined by measuring the activity of the 14C-labelled Ethylhexyl Methoxycinnamate.

Results:

After an exposure time of 6 hours to the intact surface of the mini pig skin, much of the applied dose of Ethylhexyl Methoxycinnamate in all three vehicles remained on the skin surface. Based on the amount of Ethylhexyl Methoxycinnamate detected in the stripped skin and chamber liquid, the penetration rate of Ethylhexyl Methoxycinnamate in o/w lotion, o/w cream, w/o lotion cream were reported to be 2.8, 3.5 and 3.9% of the applied dose, respectively.

Conclusion:

Under the experimental conditions of this *in vitro* study, it was observed that around 2.8, 3.5 and 3.9% of Ethylhexyl Methoxycinnamate present in o/w lotion, o/w cream and w/o lotion formulations, penetrated the skin samples during 6 hours.

(ECHA, 2021)

4th study: *In vitro* percutaneous absorption (Pig-ear skin)

Guideline:	Similar to OECD Test Guideline 428
Test system:	Pig-ear skin (Slovak large white) Fresh and frozen stored thickness skin
Test substance:	10% Ethylhexyl Methoxycinnamate in a standard sunscreen formulation
Batch:	Not specified
Purity:	Not specified
Route:	Topical application
Dose of formulation:	2 or 0.5 mg/cm^2;
Skin preparation:	Dorsal skin of the upper half region of the ear
Thickness:	0.83-0.95 mm
Number of cells/replicates:	6
Number of donor:	Unknown
Exposure time:	6 or 24 hours
GLP:	No
Study period:	2015

In an *in vitro* study similar to OECD Test Guideline 428 (non-GLP), the percutaneous absorption of 10% Ethylhexyl Methoxycinnamate of 2 cosmetic formulations (i.e., o/w emulsion, w/o emulsion) was evaluated in pig-ear skin (0.83-0.95 mm) taken from full-thickness fresh ears of around 6 months old domestic pigs (Slovak large white). The two sunscreen formulations were applied separately at a dose of 2 or 0.5 mg/cm^2 to the stratum corneum of the full-thickness skin (FTS) disc outside the cell (2 cm^2). At the end of the experiment, the stratum corneum was not stripped out. The sunscreen remained on the skin surface for 6 or 24 hours under non-occluded conditions, mimicking normal human exposure to a sunscreen formulation. The test substance content was determined by HPLC.

Results:

The absorption rate of Ethylhexyl Methoxycinnamate was higher from w/o than from o/w emulsions. Distribution of the test substance throughout the skin after **24-hour** exposure to 2 and 0.5 mg/cm^2 of the test formulations (containing 10% of Ethylhexyl Methoxy-cinnamate) to the frozen-stored skin were as follows:

Table 2: Amounts of Ethylhexyl Methoxycinnamate measured at the end of the study (24-hour exposure) in different compartments (in µg/cm2)

Compartment	Amount of Ethylhexyl Methoxycinnamate (µg/cm² mean +/- 1SD)			
	Water-in-oil (w/o) emulsion		Oil-in-water (o/w) emulsion	
	2.0 mg emulsion/cm²	0.5 mg emulsion/cm²	2.0 mg emulsion/cm²	0.5 mg emulsion/cm²
Applied dose of Ethylhexyl Methoxycinnamate µg/cm²	200	50	200	50
Surface	135.1 ± 6.3	27.6 ± 2.2	137.8 ± 6.1	27.2 ± 1.5
Epidermis + stratum corneum	10.7 ± 1.2	10.3 ± 0.9	10.2 ± 1.5	8.8 ± 1.3
Dermis	24.1 ± 1.4	7.5 ± 0.5	24.3 ± 1.8	11.1 ± 0.3
Receptor fluid (RF)	3.2 ± 0.7	2.1 ± 0.3	1.9 ± 0.8	1.2 ± 0.07
Recovery (in % w/w)	87.6 ± 1.1	95.0 ± 4.0	88.1 ± 5.3	96.6 ± 2.4

Distribution of Ethylhexyl Methoxycinnamate through the skin from the sunscreen dose of 0.5 mg/cm² (containing 10% of Ethylhexyl Methoxycinnamate, 50 µg/cm²) after **6-hour** exposure and after following 18-hour permeation to the frozen-stored skin were as follows:

Table 3: Amounts of Ethylhexyl Methoxycinnamate measured at the end of the study (6-hour exposure) in different compartments (in µg/cm²)

Compartment	Amount of Ethylhexyl Methoxycinnamate (µg/cm² mean +/- 1SD)			
	Water-in-oil (w/o)		Oil-in-water (o/w)	
	Promptly after 6 hours of exposure	After 18 hours of permeation	Promptyl after 6 hours of exposure	After 18 hours of permeation
Applied dose of Ethylhexyl Methoxycinnamate µg/cm²	50	50	50	50
Surface	42.5 ± 5.3	41.2 ± 3.4	42.9 ± 1.3	41.9 ± 1.3
Epidermis + stratum corneum	4.8 ± 0.7	3.4 ± 0.6	2.7 ± 0.6	1.7 ± 0.2
Dermis	1.2 ± 0.08	2.1 ± 0.4	0.8 ± 0.07	2.3 ± 0.03
Receptor fluid (RF)	<LoQ	0.9 ± 0.06	<LoQ	<LoQ
Recovery (in % w/w)	97.0 ± 1.4	95.2 ± 1.7	92.8 ± 2.4	91.8 ± 2.2

From the results presented in Table 3, the study investigator derived a dermal absorption value of 1.77 µg/cm² for the w/o emulsion (equivalent to 3.54% of the applied dose), using the sum of Ethylhexyl Methoxycinnamate amount in the dermis and receptor fluid after 6

hours of exposure followed by 18- hour permeation to the frozen-stored skin, corrected by the fresh/frozen-stored skin permeability coefficient of 0.59 for Ethylhexyl Methoxy-cinnamate. Using similar calculations, dermal absorption of 1.36 µg/cm^2 (i.e., equivalent to 2.7% of the applied dose) was derived for the o/w emulsion.

Conclusion

Under the experimental conditions, application of 0.5 mg/cm^2 of a sunscreen containing 10% Ethylhexyl Methoxycinnamate resulted in dermal absorption of 1.77 µg/cm^2 (equivalent to 3.54% of Ethylhexyl Methoxycinnamate) and 1.36 µg/cm^2 (i.e., equivalent to 2.7% of the applied dose) in w/o and o/w emulsion respectively.

(Klimová *et al.*, 2015)

5th study: *In vitro* percutaneous absorption (Pig skin) flow-through system

The penetration of Ethylhexyl Methoxycinnamate, either alone or in mixture with Benzophenone-3, in sunscreen formulations (i.e., hydroalcoholic or di-isopropyl adipate formulation) through micro-Yucatan pig skin was determined *in vitro* using a flow-through system (250-300 µm skin thickness). In each experiment, a minimum of 4 replicates was used (number of donors unknown). The diffusion cells allowed 0.636 cm^2 skin to be exposed to 4 µL of the formulations containing Ethylhexyl Methoxycinnamate at 7% with/without 3% Benzophenone-3 for a period of 1, 2, 6 or 10 hours.

The following results were obtained:

(a) Ethylhexyl Methoxycinnamate alone in hydroalcoholic formulation: 0.48% in receptor fluid; 12.56% in viable skin; 58.13% retained inside stratum corneum
(b) Ethylhexyl Methoxycinnamate alone in di-isopropyl adipate formulation: 0.19% in receptor fluid; 2.55% in viable skin; 25.05% retained inside stratum corneum
(c) Ethylhexyl Methoxycinnamate with Benzophenone-3 in hydroalcoholic formulation: 0.36% in receptor fluid; 7.14% in viable skin; 55.15% retained inside stratum corneum
(d) Ethylhexyl Methoxycinnamate with Benzophenone-3 in di-isopropyl adipate formulation: 0.19% in receptor fluid; 3.52% in viable skin; 28.21% retained inside stratum corneum

Overall, the quantity of sunscreen reaching the receptor fluid within 10 hours was <1% of the applied dose. The per cent penetrated (i.e., receptor fluid + viable skin) was reported to be 2.74 and 13.04% in diisopropyl adipate and hydroalcoholic formulation respectively.

(Gupta *et al.*, 1999; NTP, 2006)

6th study: *In vitro* percutaneous absorption (Landras and Pietrain pig skin)-modified Franz diffusion cells

The skin penetration potential of Ethylhexyl Methoxycinnamate from sunscreen formulations (o/w nanocapsules (NC) emulsion with 5% Ethylhexyl Methoxycinnamate; water-in-oil (w/o) NC emulsion with 5% Ethylhexyl Methoxycinnamate; o/w emulsions with free 5% Ethylhexyl Methoxycinnamate; and w/o emulsions with free 5% Ethylhexyl Methoxycinnamate) through Landras and Pietrain pig skin was determined *in vitro* using modified Franz diffusion cells. Details on skin thickness were not provided by the study investigators. The number of replicates and donors is unknown. The formulations were applied at a finite dose of 8 mg/cm^2 on the skin for a period of 3 or 24 hours.

The following results were obtained:

(e) 5% Ethylhexyl Methoxycinnamate in o/w nano capsules emulsion: 0.016 and 0.053% in receptor fluid; 0.789 and 0.274% in viable skin; 8.321 and 15.572% retained inside stratum corneum
(f) 5% Ethylhexyl Methoxycinnamate in w/o NC emulsion: 0 and 0.087% in receptor fluid; 0.668 and 0.320% in viable skin; 16.338 and 17.555% retained inside stratum corneum
(g) Free 5% Ethylhexyl Methoxycinnamate in o/w emulsion: 0 and 0% in receptor fluid; 0.999 and 2.283% in viable skin; 40.497 and 36.591% retained inside stratum corneum
(h) Free 5% Ethylhexyl Methoxycinnamate in w/o emulsion: 0 and 0% in receptor fluid; 2.468 and 3.718% in viable skin; 45.812 and 46.393% retained inside stratum corneum

Overall, the quantity of Ethylhexyl Methoxycinnamate sunscreen reaching the receptor fluid over a period of up to 24 hours was <1% of the applied dose.

(Jiménez *et al.*, 2004; NTP, 2006)

7th study: *In vitro* percutaneous absorption (Human skin)- static diffusion Franz cells

The skin penetration of Ethylhexyl Methoxycinnamate from two vehicles (i.e., o/w emulsion, petrolatum jelly) through female human skin was determined *in vitro* using static diffusion Franz cells (600 µm thick skin). The cells allowed 1.76 cm^2 skin to be exposed to the formulation. 2.26±0.21 mg/cm^2 (o/w emulsion) and 2.52±0.4 mg/cm^2 (petrolatum) sunscreen product containing 7.5% Ethylhexyl Methoxycinnamate was applied to the skin discs for a period of 2 min; 0.5, 2 or 6 hours. Thereafter, the receptor fluid was removed and analysed. After a 2 minutes application time, Ethylhexyl Methoxycinnamate could be detected in the epidermis including the stratum corneum but values were below 1 and 2% for the o/w emulsion and petrolatum, respectively. Concentrations, in epidermis including the stratum corneum, at 6 hours, expressed as a percentage of the applied dose for the Ethylhexyl Methoxycinnamate were 8.62% for the o/w emulsion and 1.28% for the petroleum jelly. Dermis concentrations values at 6 hours, expressed as a percentage of the applied dose for the Ethylhexyl Methoxycinnamate were 0.78% for the emulsion and 0.43% for petroleum jelly. Ethylhexyl Methoxycinnamate could not be identified in the receptor fluid.

(Treffel and Gabard, 1996)

8th study: *In vitro* percutaneous absorption (Human abdominal skin and pig flank skin)

In vitro diffusion studies were conducted to compare the characteristics of human abdominal skin (HS; 1400 to 2200 µm skin thickness) with domestic female pig flank skin (PS; 2500 to 3500 µm skin thickness) with regard to the percutaneous absorption of Ethylhexyl Methoxycinnamate. The formulations containing 5% w/w Ethylhexyl Methoxycinnamate in o/w emulsions were applied for 6 hours. The receptor fluid was collected from the diffusion cell at 6 and/or 16 hours. At the end of the experiment, the excess formulation was removed from the skin surface with the aid of two dry cotton swabs followed by a cotton swab damped with water:ethanol (50:50). The Ethylhexyl Methoxycinnamate remained primarily on the skin surface after 16 hours of treatment. The amount recovered by washing was 81.2% (Pig Skin) and 87.7% (Human Skin) of the applied dose. The total skin content for the Ethylhexyl Methoxycinnamate in pig skin and human skin was 11.9 and 9.7%, respectively. Other skin part distributions were as follows: (a) pig skin: 7.43% in stratum corneum+ viable epidermis, 4.03% in the upper dermis and 0.49% in receptor fluid (b) human skin: 8.11% in stratum corneum+ viable epidermis, 1.15% in the upper dermis, and 0.42% in receptor fluid. The greater epidermal distribution observed for Ethylhexyl Methoxycinnamate confirmed its high affinity for the stratum corneum due to its capacity to form a reservoir within the lipid phases of this compartment. This reservoir effect was linked to its physicochemical properties and especially its log Kow > 6, indicating high lipophilicity.

(Benech-Kieffer *et al.*, 2000; NTP, 2006)

9th study: *In vitro* percutaneous absorption (Human skin)- Franz cells

The skin penetration of Ethylhexyl Methoxycinnamate from the two vehicles (i.e., o/w emulsion, petrolatum) through human skin was determined *in vitro* using Franz cells (details about skin thickness, number of donor and number of replicates not available). The cells allowed 1.76 cm^2 skin to be exposed to the formulation at room temperature (22°C). 3±0.4 mg/cm^2 sunscreen product containing 7.5% Ethylhexyl Methoxycinnamate was applied to the skin for either 30 min or 6 hours. At the end of the experiment, 1 mL of receptor fluid was removed from the cell and analysed. After 30 min and 6 hours, 0.1% of the applied dose of Ethylhexyl Methoxycinnamate in the o/w emulsion and 0.1-0.2% of the applied dose of Ethylhexyl Methoxycinnamate in petrolatum were detected in the dermis. After 30 min and 6 hours, 0.2% of the applied dose of Ethylhexyl Methoxycinnamate in o/w emulsion and 0.1-0.3% of the applied dose of Ethylhexyl Methoxycinnamate in petrolatum were detected in the epidermis including the stratum corneum.

(Chatelain *et al.*, 2003)

10th study: *In vitro* percutaneous absorption (Human abdominal skin)

In an *in vitro* study, the penetration and retention of Ethylhexyl Methoxycinnamate in mineral oil into epidermal membranes prepared from human female abdominal skin was evaluated. The epidermal membrane with a surface area of approximately 1.3 cm^2 was dosed by placing two drops of Ethylhexyl Methoxycinnamate containing mineral oil solutions onto the membrane surface. The average weights applied were 17.8 ± 0.24 mg for the 0.5% solution: 17.6 ± 0.24 mg for the 1% solution and 18.2 ± 0.20 mg for the 2% solution. After 24 hours, the epidermal membranes were removed from the diffusion cells, and samples were taken of tissue levels by one of two methods:

- Method A included the washing of the membrane surface with a mixture of DMSO/water; 2 tape strips of the epidermis; removal of the epidermis using the enzyme digestion technique, analyzing the remaining epidermal membrane following enzyme digestion, and the amount penetrating the receptor phase.
- Method B involved the same procedure without enzyme digestion of the viable epidermis but analysis of the entire epidermal membrane following surface washing drying and tape stripping.

The amount of Ethylhexyl Methoxycinnamate in each level was determined by HPLC with UV detection and expressed as a percent of the applied dose. Around 95–98% of the Ethylhexyl Methoxycinnamate was recovered on the surface of the epidermis. Recovery of 4% Ethylhexyl Methoxycinnamate in the receptor phase was reported.

(Hayden *et al.*, 2005)

11th study: *In vitro* percutaneous absorption (Human abdominal skin)

The *in vitro* human abdominal skin absorption potential of Ethylhexyl Methoxycinnamate was evaluated in a chamber experiment (details about skin thickness not available). At a dose of 7.5% Ethylhexyl Methoxycinnamate, approximately 0.03% was found in the chamber after 2 hours, 0.26% after 6 hours, and 2% after 18 hours. No further details of the experiment are available.

(SCC, 2000)

12th study: *In vitro* percutaneous absorption (Rat skin)

The *in vitro* rat skin absorption potential of Ethylhexyl Methoxycinnamate was evaluated in a chamber experiment (details about skin thickness are not available). Most of the Ethylhexyl Methoxycinnamate was found in the stripped skin, with less in the stratum corneum (SC) and the least in the chamber. The approximate amounts found in the chamber were 1.13% after 6 hours, 11.4% after 16 hours, and 17.9% at 24 hours. The figures for the horny layer and the stripping combined were 31.4, 44.4, and 45.7% (percentages of applied doses) respectively. Solutions of 3 and 20% of Ethylhexyl Methoxycinnamate provided similar results. No further details of the experiment are available.

(SCC, 2000)

Review publication

Jung *et al.*, in their review publication, showed that Rhesus/Squirrel monkey, domestic pig, and hairless guinea pig are more predictive of human skin absorption/penetration than common laboratory animals, such as rat, rabbit, guinea pig, generally overestimate human skin absorption/penetration.

(Jung and Maibach, 2015)

SCCS Overview of *in vitro* dermal absorption of EHMC

A range of *in vitro* skin absorption studies are available for assessing the skin penetration potential of EHMC applied in sunscreens or different types of representative sunscreen formulations (e.g., oil in water emulsions, water in oil emulsions) at concentrations up to 10% through human, pig or rat skin. Studies differed from each other not only in terms of the skin model or concentrations or amount being applied, but also in terms of exposure times.

In human and pig skin, the dermal penetration of EHMC was generally studied in non-Test Guideline compliant studies that present several limitations such as no separation of stratum corneum from the epidermis, no details on number of replicates and/or donors, missing information on mass balance recovery.

A new OECD Test Guideline 428 compliant *in vitro* dermal absorption study conducted with EHMC was provided. The study complies with the SCCS Basic Criteria for the *in vitro* Assessment of Dermal Absorption of Cosmetic Ingredients and revealed a mean dermal absorption of 0.28±0.17% (one SD) of the applied dose of EHMC present at 10% in a representative cosmetic formulation. The dermal absorption value of 0.45% (i.e., mean + 1SD) has therefore been used for Margin of Safety (MoS) calculations.

An overview of the dermal penetration studies available for EHMC is presented in Annex 1.

In vivo

***In vivo* human**

1st study: *in vivo* dermal absorption (human volunteers using a standardised tape-stripping method)

The human skin penetration of EHMC from two vehicles (i.e., oil in water emulsion(o/w) and petrolatum jelly) was determined *in vivo* using a standardised tape-stripping method. In the *in vivo* experiment, 2 mg/cm^2 sunscreen product containing 7.5% EHMC was applied to areas (10 × 10 cm) on the back side of 4 healthy volunteers (aged 22 - 31 years). After 0.5, 2 and

6 hours, the remaining product was removed with a paper towel and the skin was tape-stripped (10 × 20 mm) 15 times with Cellux tapes. The strips were pooled and the test substance was extracted with methanol, then quantified by HPLC. The amounts contained in the stratum corneum were 40-50% for the o/w emulsion and 10-15% for petrolatum. The maximal stratum corneum levels (15 strips) were obtained
at 0.5 hour, both other time points showed slightly lower values. The difference between both vehicles was higher in the superficial parts of the stratum corneum (strips 1-5) compared to the deeper parts (strips 11-15), demonstrating that the penetration-enhancing effect of the emulsion was more important in the upper layer of the stratum corneum.

(Treffel and Gabard, 1996)

2nd study: *In vivo* dermal absorption (human volunteers- standardized tape-stripping method)

The human skin penetration of EHMC from two vehicles, an o/w emulsion and petrolatum jelly, was determined in humans using a standardized tape-stripping method. In the experiment, 2 mg/cm² of sunscreen product containing 7.5% EHMC was applied to areas (2 × 2 cm) on the volar side of the forearm of 6 healthy volunteers (aged 25–53 years). Thirty minutes after application, the remaining product was removed from the skin with two dry cotton swabs and the skin was tape-stripped 16 times with D-Squames. The tapes were applied to skin with a constant pressure of 0.365 N/cm². Strip No. 1 was measured separately, strips No. 2–6, No. 7– 11 and No. 12–16 were pooled. The EHMC was extracted with methanol and subsequently quantified by HPLC.

The results showed a clear vehicle effect on penetration of EHMC into the stratum corneum. The effect of the emulsion formulation was more pronounced in the upper part (strips 2–6) than in the deeper parts (strips 7–11 and 12–16, respectively) of the stratum corneum. The study author speculated that the ingredients of the emulsion formulation that penetrated the stratum corneum increased the solubility of the EHMC. Further, the emulsion formulation may support an efficient partitioning of the UV filter into the stratum corneum. Both factors may be responsible for the higher amount of EHMC in the upper part of the stratum corneum (strips 2–6). The petrolatum jelly formulation possibly hampered these mechanisms. Additionally, different product spreadabilities, as well as changes in the formulation occurring after application of the emulsion gel formulation (e.g., water evaporation) which could possibly increase the thermodynamic activity of the EHMC, could also explain their efficient delivery to the upper part of the stratum corneum. The total amount of EHMC penetrating the stratum corneum (strips 2–16) from the o/w emulsion was significantly higher. The average penetrated percentage of the dose applied was 24.1% for the emulsion formulation and 10% for the petrolatum jelly

(Chatelain *et al.*, 2003)

3rd study: *In vivo* dermal absorption (human volunteers- 2 week)

In a 2-week percutaneous absorption study, a sunscreen formulation containing Ethylhexyl-Methoxycinnamate, BP-3 and 3-(4-methylbenzylidene) camphor at 10% each was applied topically at 2 mg/cm^2 to the whole body of 32 healthy volunteers (15 males, 17 postmenopausal females) daily, 4 days/week for 2 weeks. The controls used a basic cream formulation without UV filters. For EHMC, 3-4 hours after application, the maximum plasma concentration detected was 20 ng/mL for males and 10 ng/mL for females. 5 and 8 ng/mL of EHMC was found in female and male urine, respectively. It was concluded that, after whole body dermal application of the sunscreen formulation, the three UV filters were detected in the parent forms both in plasma and in urine, showing that there was skin penetration, systemic uptake and urinary excretion of the compounds.

(Janjua *et al.*, 2004; NTP, 2006)

4th study: *In vivo* dermal absorption (human volunteers- 4 days)

In a 4-day percutaneous absorption study, a sunscreen formulation containing the sunscreen actives EHMC, BP-3 and 3-(4-methylbenzylidene) camphor at 10% each were applied to the whole body topically at 2 mg/cm^2 to 32 healthy volunteers (15 young males, 17 postmenopausal females) daily for 4 days (corresponding to 40 g/day formulation, 4 g/day EHMC in males and 35 g/day formulation, 3.5 g/day EHMC in females).

Blood concentrations were measured at 0, 1, 2, 3, 4, 24 and 96 hours and urine concentrations at 0, 24, 48, 72 and 96 hours. One to 2 hours after the first application, all three UV filters were detectable in plasma. The maximum median plasma concentrations for the EHMC were 7 ng/mL for females and 16 ng/mL for males. Urine levels of 6 (females) and 3 (males) ng/mL were found, respectively. In plasma, the 96-hour median concentrations were higher compared with 24-hour concentrations for EHMC in men.

(Janjua *et al.*, 2008)

5th study: *In vivo* dermal absorption (human volunteers- Maximal Usage Trial (MUsT))

Guideline:	US FDA. Maximal usage trials for topical active ingredients being considered for inclusion in an over-the-counter monograph: study elements and considerations
Test system:	Human volunteers
Test substance:	Ethylhexyl Methoxycinnamate (formulation containing 7.5% Ethylhexyl Methoxycinnamate)
Route:	Topical
Dose:	2 mg formulation/cm²; applied to 75% of body surface area
Application:	0 hours on Day 1 and 4 times on Day 2 through Day 4 at 2-hour intervals
Number of applications:	13
Duration:	21 days
No. of participants:	12/formulation
Metabolite identified:	No
GCP:	Not specified
Study period:	2019

A clinical study determined whether EHMC was absorbed into the systemic circulation of 24 healthy participants after topical application of 2 sunscreen products (i.e., non-aerosol spray and pump spray).

The participants were randomized to use 1 of 2 sunscreens: non-aerosol spray (n = 12) and pump spray (n = 12). The concentration of EHMC was 7.5% in both products. Two milligrams of sunscreen per cm2 was applied to 75% of body surface area at 0 hours on day 1 and 4 times on day 2 through day 4 at 2 hours intervals (i.e., day 1: at 0 hours; day 2: at 24, 26, 28 and 30 hours; day 3: at 48,50, 52 and 54 hours; and day 4: at 72, 74, 76 and 78 hours). A total of 34 blood samples were collected over 21 days from each participant (i.e., day 1: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 9, 10, 12 and 14 hours; day 2: 23, 28 and 33 hours; day 3: 47, 52 and 57 hours; day 4: 71, 73, 74, 76, 78, 81, 82, 84 and 86 hours; day 5: 95 hours; day 6: 120 hours; day 7: 144 hours; day 10 at 216 hours; day 14 at 312 hours; and day 21 at 480 hours after the first sunscreen application). In addition, skin (stratum corneum) samples were collected by tape stripping (6 consecutive stripping) of the lower back (around 3.8 cm2) on day 7 and day 14. The amounts recovered after tape stripping and plasma concentrations were assessed with the validated liquid chromatography with tandem mass spectrometry methods. Maximum plasma concentrations of EHMC were analysed following a single application on day 1; maximum plasma
concentrations on day 4; area under the plasma concentration vs. time curve (AUC) on single (day 1) and multiple (day 4) applications; terminal half-life; and active ingredient

concentrations on days 7, 14 and 21 (last application was on day 4). All adverse events were recorded by clinic staff and adjudicated by the principal investigator. Post-hoc assessments included measurement of the amount of EHMC remaining in the skin on days 7 and 14.

Results

No serious adverse events were reported. Application site erythema and rashes were reported in 3 and 7 participants (out of the 24 total participants), respectively. The overall geometric mean maximum plasma concentration of EHMC was 7.9 ng/mL (CV: 86.5%) for non-aerosol spray and 5.2 ng/mL ng/mL (CV: 68.2%) for pump spray. EHMC was detectable in the skin following tape stripping, with greater amounts detectable at day 7 compared with day 14. The levels of EHMC in skin were 2373.6 ng/cm2 (CV, 149.7%) and 1675.2 ng/cm2 (CV, 132.7%) on day 7 and 284 ng/cm2 (CV, 353.3%) and 151.3 ng/cm2 (CV, 410.9%) on day 14 for non-aerosol spray
and pump spray respectively. A summary of the EHMC-specific findings from the study is presented in Table 4.

Table 4: Concentrations of EHMC in plasma and skin following exaggerated use of two products (n=12)

Compartment	Geometric mean maximum concentration in plasma (ng/ml) or skin concentration (ng/cm²) (CV%) [range]	
	Non-aerosol Spray	Pump spray
Plasma* (ng/mL)-Overall	7.9 (86.5) [2.6-30.6]	5.2 (68.2) [1.5-11.8]
Plasma (ng/mL) -Day 1	2.0 (96.0) [0.6-5]	1.1 (326.2) [0-4.1]
Plasma (ng/mL) - Day 4	7.9 (86.5) [2.6-30.6]	6.1 (53.8) [3.2-11.8]
Stratum corneum (Day 7)-ng/cm²	2373.6 (149.7) [493.2-12 200.5]	1675.2 (132.7) [470-5856.9]
Stratum corneum (Day 14)- ng/cm²	284.0 (353.3) [22.2-2977.6]	151.3 (410.9) [24-1809.8]

CV: coefficient of variation

*Maximum plasma concentration is the maximum ingredient concentration observed over the study duration. Maximum plasma concentration on day 1 (single application) was the maximum concentration over the interval of 0 to 23 hours and on day 4 was the maximum concentration over the interval of 73 to 95 hours. AUC on day 1 (single application) was the area under the curve over the interval 0 to 23 hours and on day 4 was the area under the curve from 73 to 95 hours.

Conclusion

In this study involving healthy volunteers, application of two commercially available sunscreen formulations containing EHMC at concentrations of 7.5% under exaggerated use conditions resulted in systemic absorption and associated plasma concentrations of between 5.2-7.9 ng/mL. The concentrations in the skin (stratum corneum) were in the range of 1675.2- 2373.6 ng/cm^2 and 151.3 - 284 ng/cm^2 on Days 7 and 14, respectively.

(Matta *et al.*, 2020)

6th study: *In vivo* dermal absorption

In a dermal absorption study in male volunteers, 2 g of an o/w cream containing 10% EHMC was applied to the interscapular area of 5 male subjects. The skin area covered was 25 x 30 cm. After application, the area was covered with 3 layers of gauze which was left in place for 12 hours. Blood was taken at times 0, 0.5, 1, 2, 3, 5, 7 and 24 hours. Urine was collected at 0, 1, 2, 3, 4, 5, 6,
7, 12, 24, 48, 72 and 96 hours. The control plasma samples showed a level equivalent to about 10 ng/mL EHMC before any application had been made. No increase in plasma levels of EHMC was observed. Urine showed a level of 100–300 ng/mL. The study authors concluded

that very little EHMC was dermally absorbed under the study conditions. This study is considered to be of limited use for the safety assessment of EHMC due to the absence of quantitative absorption data and limitation in reporting results.

(SCC, 2000)

Overview of *in vivo* dermal absorption studies of EHMC

The available human dermal pharmacokinetic studies predominantly focused on determining the dermal penetration of EHMC in human volunteers after topical application at various concentrations in sunscreens or different types of sunscreen similar vehicles (e.g., o/w or w/o emulsions, petrolatum) on various skin sites (e.g., back or forearm of patients). The application doses reflected normal, but also exaggerated, use of sunscreen products. The investigations measured the amounts of EHMC in the stratum corneum, the cumulative excretion of EHMC in urine or the concentrations EHMC in plasma. Individual investigations examined the impact of a different vehicle on the overall penetration profile of EHMC.
Under exaggerated exposure conditions such as those chosen in a human dermal maximal use absorption study (MUsT) conducted by Matta *et al.*, (2020) for EHMC present in sunscreen formulations applied in non-aerosol or pump sprays, the overall geometric mean-maximum plasma concentrations were in the range of 5.2–7.9 ng/mL. The concentrations of EHMC in the skin were in the range of 1657.2–2373.6 ng/cm^2 and 151.3–284 ng/cm^2 on days 7 and 14, respectively.

An overview of identified human dermal pharmacokinetic/bioavailability studies is presented in Annex 2.

Summary of dermal/percutaneous absorption

Based on the range of available *in vitro* and *in vivo* studies, the dermal absorption data from a new OECD Test Guideline 428 compliant study conducted with EHMC is considered as the key study. The study revealed a mean dermal absorption level of 0.28±0.17% of the applied dose of EHMC at a concentration of 10% in a representative cosmetic formulation. A dermal absorption of 0.45% (i.e., mean + 1SD) has been used for Margin of Safety (MoS) calculations.

SCCS comment
As described above, a dermal absorption of 0.45% (*i.e.,* Mean + 1SD) will be used for Margin of Safety (MoS) calculations based on the *in vitro* study in human skin performed according to OECD TG 428, which is also in line with SCCS basic criteria.

3.2.2 Other studies on toxicokinetics

3.2.2.1 Absorption, Distribution, Metabolism and Excretion (ADME)

1st study: *In vitro*-Human blood

The breakdown of EHMC at a concentration of 10 mg/mL in human blood was determined *in vitro*. EHMC is known to be cleaved slowly *in vitro* by esterases present in human blood plasma. In this study, the half-life of EHMC was determined to be approximately 10 hours. After 120 hours, the parent compound and 4-methoxycinnamate were found at 17.8 and 83.3%, respectively. No further details of the experiment are available.

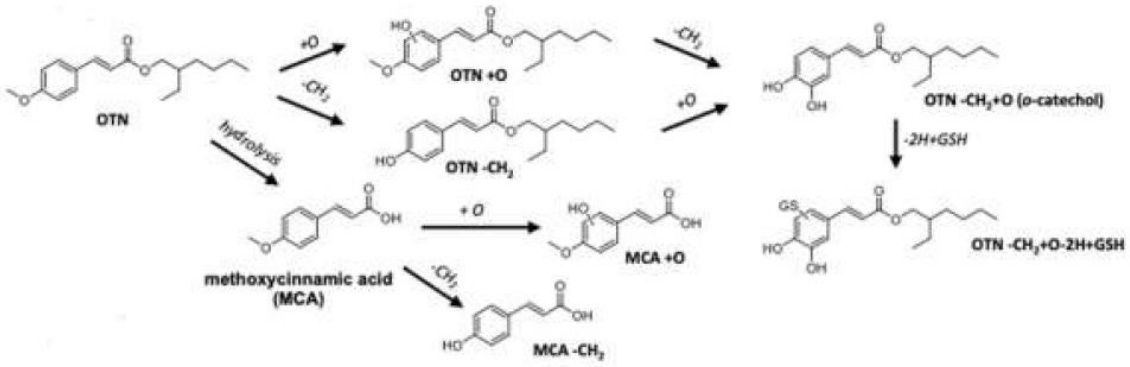
(NICNAS, 2017)

2nd study: *In vitro* - liver microsomes

An *in vitro* study was conducted to determine the oxidative metabolism of EHMC in rat and human liver microsomes with an emphasis on the potential formation of reactive metabolites, utilizing glutathione (GSH) as a trapping agent. Liquid chromatography coupled to high-resolution tandem mass spectrometry (LC/HRMS/MS) was performed on a quadrupole-time-of-flight hybrid mass spectrometer to characterize the metabolites and GSH adducts formed in *in vitro* incubations. EHMC was incubated at a final concentration of 10 µM with human liver microsomes (HLM) and rat liver microsomes (RLM) (1 mg/mL protein) containing 1 mM NADPH and 5 mM GSH in 100 mM potassium phosphate buffer (pH 7.4). Control samples were prepared with NADPH only, or without NADPH or GSH. The samples were placed in open tubes and protected from light for 20 min at 37°C, while mixing at 650 rpm. The reaction was then stopped with the addition of an equal volume of acetonitrile (1:1) to precipitate proteins, followed by centrifugation at 14,000 rpm for 8 min at 4°C. An equal volume of water was added to the supernatants to dilute samples to 25% acetonitrile prior to LC/MS/MS analysis.

Results

Oxidative metabolites and GSH adducts of EHMC were detected in both HLM and RLM incubations, with slight differences in relative abundances between rat and human microsome profiles. EHMC was found to be hydrolysed to 4-methoxycinnamic acid (MCA) and 2-ethylhexanol. A further range of metabolites was identified. Quantitative analysis of the metabolites was, however, not part of the study objectives. The following scheme presents the metabolism pathway of EHMC on the basis of the experiment proposed by the investigators:



(Guesmi *et al.*, 2020)

3rd study: *In vitro*- hepatocytes

EHMC metabolism was investigated *in vitro* in primary hepatocytes. Following incubation of [^{14}C]-EHMC at 10 µM with cryopreserved mouse, rat, and human hepatocytes for 5 hours, no parent EHMC was detectable at the end of the incubations anymore. Chromatograms contained a radioactive peak that co-migrated with p-methoxycinnamic acid, as well as two other major metabolite peaks. One of the two peaks was characterised as 2-ethylhexanoic acid by co-migration with a standard detected by UV absorbance at 220 nm, and by LC-MS. However, 2-ethylhexanol was not detectable by UV absorbance. Both 2-ethylhexanoic acid and 2- ethylhexanol were detected in hepatocyte incubations by GC-MS. These *in vitro* studies indicate rapid hydrolysis of EHMC to 2-ethylhexanol and p-methoxycinnamic acid following hepatocyte incubations. Further, Ethylhexyl Methoxy-cinnamate underwent more rapid clearance in rat and mouse hepatocytes compared with human hepatocytes.

(Fennell *et al.*, 2018)

4th study: *In vivo*

A human male volunteer received orally a single capsule containing 100 mg of Ethylhexyl Methoxy-cinnamate. Based on available *in vitro* information, EHMC is known to be slowly hydrolysed by plasma esterases. The cumulative excretion of 4- methoxy-cinnamate in urine over 24 hours was studied by GC/MS analysis of the methyl ester derivative (this method would also detect 4-hydroxycinnamic acid).
Over 24 hours, 13.2% of the ingested amount was recovered, equivalent to 21.5% of the amount that would be expected if EHMC was completely absorbed. The investigators did not specify whether the recovery measurements also included the excretion of non-metabolized EHMC.

(HSDB, 2014; SCC, 2000)

5th study: *In vivo*

Guideline:	Not available
Test system:	Rats/ Sprague-Dawley Mice/ B6C3F1/N
Number of animals:	3 animals/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Dose levels: Oral:	8, 80 and 800 mg/kg bw in male rats 8 mg/kg bw in female rats and male and female mice.
Intravenous (IV):	8 mg/kg bw in rats and mice
Dermal:	0.8, 8 and 80 mg/kg bw in rats and mice
Dose volume:	Oral: 5 mL/kg for rat; 10 mL/kg for mice
IV:	1 mL/kg for rats; 4 mL/kg for mice
Dermal:	0.5 mL/kg for rats; 1 mL/kg for mice
Route:	Oral, intravenous (IV) and dermal application
Vehicle:	Dermal: ethanol, acetone
Duration:	72 hours
Administration:	Oral: gavage; IV
Metabolites identified:	Yes
GLP:	No
Study period:	2012 (in-life portion completed in 2012; Article published in 2017)

The metabolism, distribution, and excretion of [^{14}C] EHMC was investigated *in vivo* following oral, intravenous, and dermal application in rats and mice. The radiolabel was incorporated in two positions in the parent compound to enable tracking of the hydrolysis products methoxycinnamate and 2-ethylhexanol of the ester. For the oral study, male rats were received doses of [14C] EHMC at 8, 80, and 800 mg/kg bw and females a dose of 8 mg/kg bw in female rats by gavage. Rats and mice received doses of [14C] EHMC at 8 mg/kg bw intravenously and 0.8,8, and 80 mg/kg bw by the dermal route. Urine samples were analysed by HPLC using a Waters 600 E controller pump, Rheodyne 7725i manual injector, and a β-RAM Model 2B radioactivity detector with a 250-µL LiGl solid scintillant cell.

Results

Rats: In males, at all dose levels, the radiolabel was rapidly excreted in the urine, with about 63–72% of the dose recovered in the first 24 hours post-dosing and a total of 78–82% excreted in urine through 72 hours post-dosing. About 3–4% of the lower doses and 8% of the high dose was recovered in faeces through 72 hours, suggesting either that the amount

of unabsorbed test substance increases with dose or that biliary excretion increases with dose. Recovery in the CO2 traps decreased with increasing doses, with 5.2, 4.1, and 0.6% of the dose recovered for the 8, 80, and 800 mg/kg doses, respectively.
Radioactivity in tissues at 72 hours post-dosing was very low, accounting for less than 1% of the dose in all groups. In female rats, disposition and excretion of radioactivity were similar to that in male rats at 8 mg/kg bw. The total radioactivity recovered for oral gavage administration ranged from 86-91%.
Excretion of [^{14}C]-EHMC derived radioactivity 72 hours following IV administration at 8 mg/kg bw was similar for both sexes to that following gavage administration. Approximately 75% was recovered in urine, 2–4% in faeces and 3% as CO2. Retention in tissues at 72 hours was about 1%, which was similar to the gavage dose groups. The concentrations of radioactivity in tissues were similar to those following gavage administrations and were highest in adipose, muscle, skin and liver.

The disposition of EHMC following dermal application was investigated at with two vehicles: ethanol and a lotion. With the ethanol as the vehicle, 34% (male) to 42% (female) of the dose was absorbed with 9 and 6% of the dose recovered at the skin dosing site. Approximately 1-2% was found in tissues in males and females. Elimination of the absorbed dose was primarily via urine, with a small portion of the absorbed dose excreted in faeces and exhaled as CO2.

Mice: Mice excreted the administered radioactivity mostly in urine, with about 57–73% of the dose recovered through 72 hours post-dosing. Recovery in faeces was higher (15–25%) in mice than in rats, but this may be due to faeces being contaminated with urine, a common occurrence in mouse disposition studies. Recovery in the CO2 traps was 2–4%. Approximately, 1% was recovered in volatile organics traps.
Less than 1% remained in adipose tissues and organs.

Following IV administration, distribution of [14C] EHMC-derived radioactivity after 72 hours in mice was similar to the results following gavage administration, with excretion of radioactivity mostly in urine. The excretion in faeces was in the range of 20-27%. Recovery in tissues was low (approximately 0.3%), and concentration in individual tissues was similar to those following oral administration.

Overall, recovery in urine and faeces accounted for 88 and 87% of the dose in male and female mice, respectively. Recovery as CO2 was about 2% and only 0.5% was recovered in volatile organics traps.

After dermal application, absorption of EHMC was moderate and was higher in mice (54–62%) than in rats (34–42%). The distribution and excretion followed a similar pattern to that after oral exposure. Identification of the metabolites of EHMC in urine indicated the extensive metabolism to 2-ethylhexanol and p-methoxycinnamate and their downstream metabolites. When a lotion vehicle was used (males only), only 11% of the dose was absorbed with 4% of the dose remaining at the dose site.

The metabolic pathway (Fig.1) and metabolites (Table 5) of EHMC are presented below.

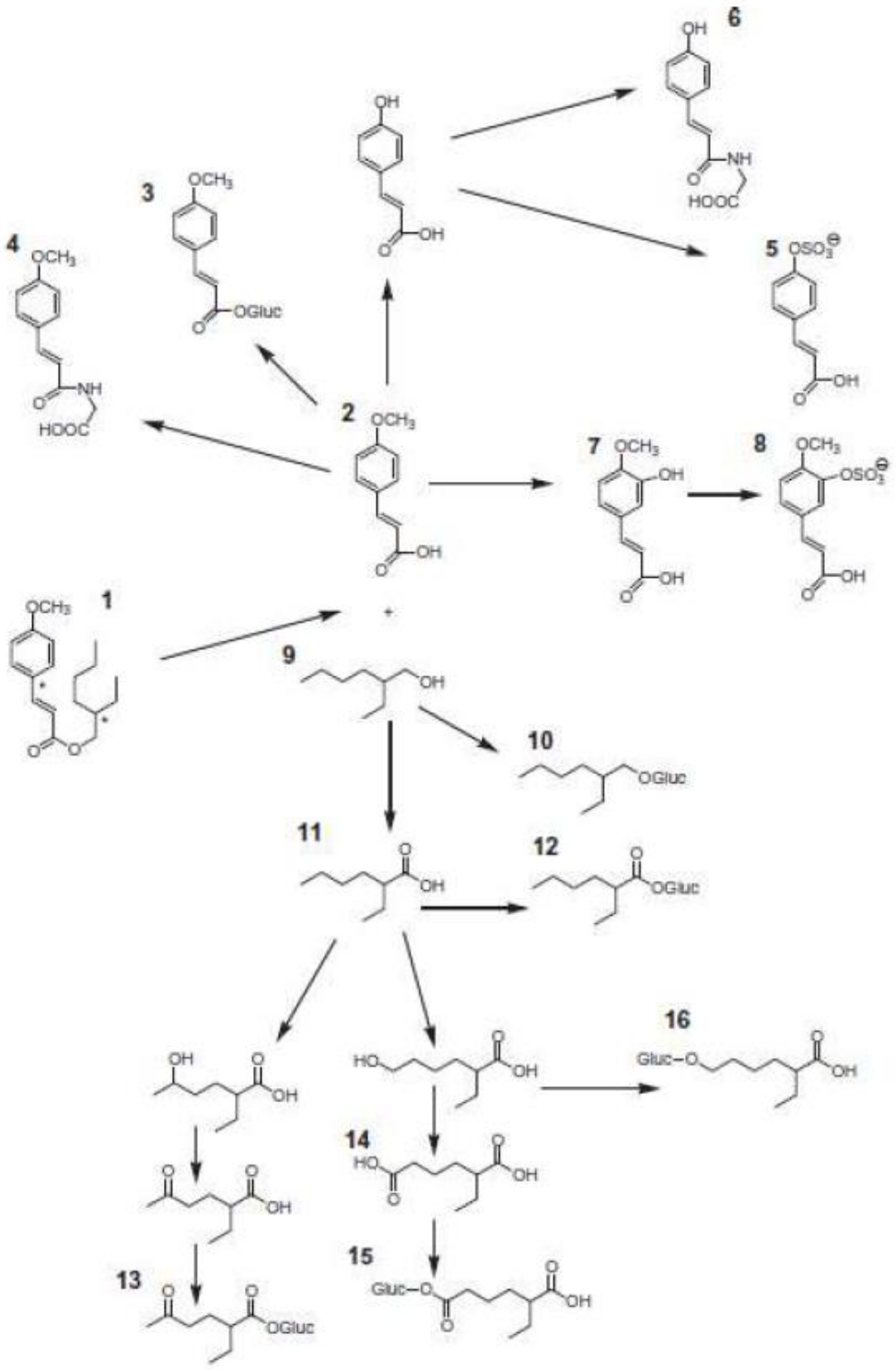


Figure 1: Metabolism of EHMC. Metabolites of EHMC identified are named in the following Table 5.

Table 5: Metabolites detected in urine from the administration of EHMC by gavage to male rats.

Number	Metabolite	Formula	Calculated exact mass	M–H⁻	Detected mass	Mass error (Da)	Mass error (ppm)
1	EHMC	$C_{18}H_{26}O_3$	290.1882	289.1804			
2	*p*-Methoxycinnamate	$C_{10}H_{10}O_3$	178.063	177.0552	177.0564	−0.00121	−6.8
3	*p*-Methoxycinnamate glucuronide conj	$C_{16}H_{18}O_9$	354.0951	353.0873	353.0881	−0.0008	−2.3
4	*p*-Methoxycinnamate glycine conj	$C_{12}H_{13}NO_4$	235.0847	234.0769	234.0777	−0.00084	−3.6
5	Hydroxycinnamate sulfate	$C_9H_7O_6S$-	242.9969	242.9969	242.9973	−0.00042	−1.7
6	Hydroxycinnamate glycine conj	$C_{11}H_{11}NO_4$	221.0688	220.061	220.062	−0.00099	−4.5
7	Hydroxy methoxycinnamate	$C_{10}H_{10}O_4$	194.0579	193.0501	193.0512	−0.00109	−5.6
8	Hydroxy methoxycinnamate sulfate	$C_{10}H_9O_7S$-	273.0075	273.0075	273.0440	−0.03655	−133.9
9[a]	Ethylhexanol	$C_8H_{18}O$	130.1358	129.128	–	–	–
10	Ethylhexanol glucuronide	$C_{14}H_{26}O_7$	306.1679	305.1601	305.1607	−0.00065	−2.1
11	2-Ethylhexanoic acid	$C_8H_{16}O_2$	144.115	143.1072	143.1084	−0.00117	−8.2
12	2-Ethylhexanoic acid glucuronide	$C_{14}H_{24}O_8$	320.1471	319.1393	319.1401	−0.00078	−2.4
13	2-Ethyl-5-ketohexanoic acid glucuronide	$C_{14}H_{22}O_9$	334.1264	333.1186	333.1189	−0.00032	−1.0
14	2-Ethyladipate	$C_8H_{14}O_4$	174.0892	173.0814	173.0824	−0.00099	−5.7
15	Ethyladipate glucuronide	$C_{14}H_{22}O_{10}$	350.1213	349.1135	349.1145	−0.001	−2.9
16	Hydroxyethylhexanoic acid glucuronide	$C_{14}H_{24}O_9$	336.142	335.1342	335.1351	−0.00087	−2.6
17	Methoxybenzoate	$C_8H_8O_3$	152.04734	151.0395	151.0407	−0.00116	−7.7
18	Methoxybenzoate glucuronide	$C_{14}H_{16}O_9$	328.0794	327.0716	327.0725	−0.0009	−2.8
19	Methoxybenzoyl glycine	$C_{10}H_{11}NO_4$	209.0688	208.0610	208.0618	−0.0008	−3.85
U1	Unassigned 1	–	–	–	157.0864	–	–
U2	Unassigned 2	–	–	–	129.0929	–	–

[a]Not detectable in urine by LC–MS

Conclusion

Under the conditions of the study, [^{14}C]-EHMC was extensively absorbed and excreted primarily in urine by 72 hours after oral administration in rats and mice and the distribution of [^{14}C]-EHMC-derived radioactivity after 72 hours was similar to the results following IV administration. This indicates a very high level of oral absorption. Identification of the metabolites of EHMC in urine indicated the extensive metabolism to 2-ethylhexanol and p-methoxycinnamate and their downstream metabolites.

(Fennell *et al.*, 2018)

Comment from the Applicant:

The comparison of the radioactivity distribution following oral and IV route administration of EHMC at the same dose trigger the following considerations:

- The presence of radioactivity in faeces and the GI tract after IV administration indicates excretion of EHMC-derived radioactivity into these two compartments and thus should be considered as bioavailable.
- As the total radioactivity amount in these two compartments is similar after oral and IV administration, the entire amount of radioactivity in the two compartments after oral administration should also be considered as bioavailable.
- Since the total bioavailability after oral dosing is equivalent to that after IV administration, EHMC should be considered to be completely bioavailable after oral administration.

Summary of ADME

The metabolism, distribution and excretion of EHMC was investigated *in vitro* in rat and human liver microsomes and rat, mouse and human hepatocytes. Overall, EHMC is extensively metabolised to a range of metabolites. It was shown to be slowly hydrolysed to 4-methoxycinnamic acid and 2-ethyl-hexanol but also oxidised and demethylated and combinations thereof.

The *in vivo* studies in rodents proposed a metabolic pathway indicating EHMC to be absorbed and metabolised rapidly and enzymatically converted to a range of metabolites. Metabolism paths have been proposed by Guesmi *et al.* (2019) and Fennel *et al.* (2018).
EHMC was excreted rapidly and primarily in urine, to a lesser extent in faeces and as CO2. Further, the (Fennell *et al.*, 2018) study indicated that distribution and excretion via the IV route are similar to that of oral gavage administration.
Overall, available data provide evidence that EHMC is rapidly and extensively absorbed across the gut and is hydrolysed to its primary metabolites 4-methoxycinnamic acid and 2-ethylhexanol. However, it was also shown to be oxidised and demethylated and combinations thereof and excreted rapidly and primarily in the urine. The metabolic profile of EHMC was qualitatively similar between humans and nonhuman species. Further, based on the evidence of similar bioavailability via oral and IV routes, EHMC can be expected to have complete absorption potential via the oral route suggesting an absorption value of 100%.

Based on the evidence of complete absorption of EHMC from the oral route, correction for oral bioavailability is not considered for the risk assessment/MoS calculations.

SCCS comment
Based on these results, it can be concluded that EHMC is extensively absorbed by oral route and therefore no correction factor needs to be applied in the MoS calculation to adjust the Point of Departure (POD) derived from the oral route.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

EHMC is approved to be used as a UV filter at concentrations of up to 10% in cosmetic products alone or in combination with other UV filters. EHMC may also be incorporated in cosmetic products for formula protection purposes and therefore it is used in several kinds of product types, such as but not limited to lotions, creams, sprays, and lip products.

3.3.2 Calculation of SED/LED

The systemic exposure dose (SED) for EHMC used as a UV filter in cosmetic products is calculated by multiplying the consumer's external sunscreen product exposure with the percentage of EHMC being dermally absorbed from the sunscreen product (i.e., 0.45%; mean + 1SD; see section 3.3.1.1).

Referring to (Biesterbos *et al.*, 2013), the SCCS NoG recommends for the safety assessment of sunscreen products a total daily product application of 18 g. This value considers applying the product to the whole body (i.e., 17500 cm^2; 1 mg of formulation/cm^2;) in two applications each day (i.e., 9 g/application) for the duration of a consumer's lifespan. Considering the design of the dermal absorption study using an amount of 2 mg of formulation applied per cm^2, calculating consumer exposure by multiplying the dermal absorption value (i.e., 0.88 µg/cm^2; mean + 1SD) with the totally exposed skin surface area (i.e., 17500 cm^2) and in addition considering two uses per day, would lead to an assumed sunscreen product exposure of 70 g/d. This is nearly 4 times higher than SCCS NoG recommended daily use value of 18 g and is therefore considered a gross overestimation of actual product use.

Likewise, the exposure scenario considers already a whole-body application, obsoleting the need to aggregate with additional use of face or hand care products. In this respect, (Biesterbos *et al.*, 2013) calculated the kappa coefficients for co-use of sunscreen with hand

cream and face cream which are 0.16 and 0.24 respectively. These numbers demonstrate a weak correlation and thus a low likelihood of co-use.

SEDs are also calculated for inhalation (Table 7) and oral exposure to product types containing 10% EHMC separately and as aggregate exposure (Table 8).

Dermal exposure

The SED by the dermal route was calculated using the details as described in the SCCS NoG 11th revision (SCCS, 2021).

Table 6. SED calculations after dermal exposure

Description and parameters	*Sunscreen product (lotions/creams)*
Amount of whole bodyproduct applied (A)	18 g/day
Concentration in the finished product (C)	10%
Absorption through the skin (DAp)	0.45%
Typical body weight of a human (BW)	60 kg
Systemic exposure dose (SED) (A x 1000 mg/kg x C/100 X DAp/100/60)	0.135 mg/kg bw/day

Inhalation

The systemic exposure dose by the inhalation route was calculated using an adapted deterministic 2-box model as described in the SCCS NoG 11th revision (SCCS, 2021).

For the calculations (see Table 6) it was assumed that for both pump and propellant sprays the same amount of sunscreen needs to reach the skin to ensure the necessary level of sun protection. For a propellant spray, this means that the additional amount of propellant gas needs to be added to the default value of 9 g/application, resulting in 15 g/application. By

applying a factor of 0.6 for the proportion of nonpropellant in the formulation, this results in an amount of 9 g/application on the skin.

Table 7. SED calculations after inhalation exposure

Description	Parameter	Propellent spray	Pump spray	Unit
Amount by application[1]	A	15000	9000	mg/application
Fraction of EHMC in nonpropellant	C	0.1	0.1	(w/w)
Proportion of non-propellant in formulation	P	0.6	1	-
Airborne fraction	AF	1	0.2	-
Potential amount to be inhaled	EA (A*C*P*AF)	900	180	mg
First step: Near-field, 1 m^3	V1	1000	1000	L
Breathing rate	BR	13	13	L/min
2 min in the near field	t_1	2	2	min
Potential amount inhaled during t_1	IA_1 (EA/V_1*BR*t_1)	23.4	4.68	mg
Second step: Far-field 10 m^3	V_2	10000	10000	L
Breathing rate	BR	13	13	L/min
10 min in far-field	t_2	10	10	min
Potential amount inhaled during t_2	IA_2 (EA/V_2*BR*t_2)	11.7	2.34	mg
Substance availability fraction	G	0.75	0.75	-
Respirable fraction	RF	0.2	0.01	-
Frequency of application	F	2	2	per/day
Default body weight	BW	60	60	kg
SED_{inhal}	(IA_1+IA_2) *G*RF* F/BW	0.176	0.002	mg/kg bw/day

[1]Adjusted for the proportion of propellant to achieve a final "on-body" amount of 9000 mg

- The airborne fraction AF was assumed according to the SCCS NoG 11th revision (SCCS, 2021).
- The near-field zone of the two-compartment model was assumed to have a volume V_1 of 1 m^3 and the duration of staying in the near-field zone t_1 as 2 min.
- For the far-field, a volume V_2 of 10 m^3 and a duration of 10 min (t_2) was assumed.
- The factor for substance availability G is based on Guidance from the European Commission, 1996. The respirable fraction (RF) of 0.2 and 0.01 is based on the internal CE survey.

The estimated systemic exposure dose (SED_{inh}) resulting from exposure to 10% w/w EHMC, when applied as sprays to the human skin, is calculated to be **0.176 mg/kg bw/day** for propellant spray and **0.002 mg/kg bw/day** for a pump spray.

Oral

The systemic exposure dose from lipstick (SEDoral) of EHMC is calculated as:

Relative daily exposure (Eproduct) = 0.9 mg/kg bw/day

Concentration of EHMC (C) = 10%

Retention factor (Fret)[1] = 100%

SEDoral Eproduct*(C/100)*(Fret/100) = **0.090 mg**/kg bw/day

[1]Potential amount available for oral exposure;

Aggregate

Aggregate exposure or total systemic exposure was calculated by adding up the exposures from the dermal (non-spray or spray product), inhalation (spray product) and oral (lip product) routes of exposure. This assumes that consumers may be using either a non-spray or a spray product in combination with a lip product.

Table 8. Calculation of total SED for aggregated exposures

SED_{dermal}	SED_{inhal}	SED_{oral}	SED_{total}
Sunscreen (lotion)		Lipstick	
0.135	-	-	0.135
0.135	-	0.090	0.225
Sunscreen (propellant spray)			
0.135	0.176	-	0.311
0.135	0.176	0.090	0.401
Sunscreen (pump spray)			
0.135	0.0018	-	0.137
0.135	0.0018	0.090	0.226

SCCS comments

For the aggregated exposure, the SCCS has considered the use of Face cream and Hand cream together with Sunscreen (propellant spray) and lipstick. Therefore, a revised table is presented below:

Table 9: Calculation of total SED for aggregated exposures

Products	Conc	SED Dermal	SED inhal	SED Oral	SED Total
	(%)	(mg/kg bw/day)	(mg/kg bw/day)	(mg/kg bw/day)	(mg/kg bw/day)
Sunscreen (dermal lotion)	10	0.135	0	0	0.135
Sunscreen (propellant spray)	10	0.135	0.176	0	0.311
Sunscreen (pump spray)	10	0.135	0.0018	0	0.137
Lipstick	10	0	0	0.09	0.09
Face cream	10	0.012	0	0	0.012
Hand cream	10	0.016	0	0	0.016
Aggregated exposure					0.429*

*aggregated exposure includes exposure via Sunscreen (propellant spray), Lipstick, Face cream and Hand cream

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

1st study:

Guideline:	Other Guideline (FED. REG. 38, NO. 187, SECTION 1500.41 P. 27019, SEPT. 27, 1973)
Species/strain:	Rabbits/ Vienna White
Number of animals:	6 (5 males, 1 female)
Test substance:	Ethylhexyl Methoxycinnamate
Vehicle:	No vehicle
Batch:	84/127
Purity:	Approx. 100%
Dose applied:	0.5 mL
Type of coverage:	Occlusive
Area of exposure:	2.5 x 2.5 cm
Duration of exposure:	24 hours
Observation:	15 days
GLP:	No
Study period:	1985

The skin irritation potential of EHMC was investigated in Vienna White rabbits. Approximately 0.5 mL of test substance was applied occlusively to the test site (over an area of 2.5 cm²) for 24 hours with observation period of 15 days. After the exposure period, the test substance was removed. All the animals were assessed daily for mortality and clinical signs of toxicity. The skin was examined for signs of erythema and oedema at 30 - 60 min, 24, 48, 72, 192 and 360 hours, after patch removal.

Results

Except for the scaling observed in one animal after eight days, there were no findings in any treated animal regarding mortality or clinical signs of toxicity during the study. The mean erythema and oedema scores over 24, 48 and 72 hours after the application of test substance

were 1.7 and 0.2, respectively. Erythema and oedema were reported to be fully reversible within 15 days and 48 hours, respectively.

Conclusion

Under the study conditions, EHMC was slightly irritating to rabbit skin.

(ECHA, 2021; NICNAS, 2017)

2nd study

EHMC was applied undiluted twice daily to 20 Guinea pig skin for 16 days. No signs of irritation were reported (no further details available) (SCC, 2000).

3rd study

Human data-Skin irritation
A repeated insult patch test (RIPT) was conducted in 53 human subjects using 2% EHMC. No skin irritation was reported (no more details available).

In another RIPT conducted in 54 human subjects, the application of 7.5% EHMC in petrolatum caused no irritation (no more details available).

Undiluted EHMC was occlusively applied to 60 human subjects (20 with sensitive skin) for 24 hours. Observations were made at 24, 48 and 72 hours after application. Under the test conditions, test substance showed no evidence of skin reactions.

(SCC, 2000)

SCCS comment on skin irritation
Under the experimental conditions reported, EHMC is considered slightly irritant to the skin.

3.4.1.2 Mucous membrane irritation / eye irritation

Guideline:	No guideline followed
Species/strain:	Rabbits/Albino
Number of animals:	3
Test substance:	Ethylhexyl Methoxycinnamate
Vehicle:	No vehicle
Batch:	Not available
Purity:	Not available
Dose applied:	100 mg
Concentration	100%
Duration of exposure:	Group I - 168 hours (not rinsed) Group II - 2 seconds (rinsed) Group III - 4 seconds (rinsed)
Observation:	168 hours (at 1, 24, 48, 72, 96 and 168 hours)
GLP:	No
Study period:	1971

The eye irritation potential of EHMC was investigated in Albino rabbits. 100 mg of undiluted test substance was placed into the conjunctival sac of the eye of three groups of three rabbits. Two groups of animals had eye rinsed after 2 and 4 seconds. The untreated eyes served as control. The observations for effects on the cornea, iris and conjunctivae were performed at

1, 24, 48, 72, 96 and 168 hours after instillation of the test substance and scored according to the Draize scale.

Results

Slight irritation of the conjunctivae was observed for a few hours after exposure to the test substance but were considered to be mechanically induced effects. No effects were observed after 24 hours. No other ocular reactions were noted in any of the animals during the 168 hours of the study. The mean Draize score for 1 hour for irritation was calculated to be 3.3 which was fully reversed within 24 hours.

Conclusion

Under the study conditions, EHMC was not irritating to rabbit eyes.

(ECHA, 2021 ; NICNAS, 2017)

SCCS comment on eye irritation

Under the experimental conditions reported, EHMC is considered not to be irritant to the eyes.

3.4.2. Skin sensitisation

Magnusson Kligman Maximisation test – Guinea pig

Guideline/method:	OECD Test Guideline 406
Species/strain:	Guinea pig/ Pirbright-Hartley
Group size:	20 female animals in the test groups
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	LJ 25607/20
Purity:	97.4-99.4%
Intradermal induction:	0.1 mL of 5% in olive oil DAB 9
Epicutaneous induction:	0.3 g undiluted
Challenge:	75% in olive oil DAB 9
Vehicle (for intradermal):	5% in olive oil DAB 9/0.9% aqueous NaCI solution (1:1)
Positive control:	1-chlor-2,4-dinitro-benzol
GLP:	Yes
Study period:	1993

EHMC was investigated for its potential to cause skin sensitisation in guinea pigs in an OECD Test Guideline 406 compliant study according to the Magnusson Kligman Maximisation test protocol.
During the induction phase, animals (n=20) in the test group received intradermal injections (0.1 mL of 5% test substance in olive oil DAB 9). Epicutaneous induction was carried out by applying a 0.3 g undiluted test substance to the skin.

During the challenge phase, control and test animals were exposed 21 days after intradermal induction on the flank to 75% test substance in olive oil DAB 9. Skin reactions were assessed according to the grading scale of Magnusson and Kligman.

A. Induction Exposure

In the main study, animals in the test group were intradermally injected with 0.1 mL of 5% test substance in the neck region (adjuvant/saline mixture 1:1 (v/v)). Control animals were treated in the same way, but without the test substance.

B. Challenge Exposure

Control and test animals were challenged 21 days after induction on the flank with 75% test substance. 24 and 48 hours after removing the dressings, the challenge reactions were graded according to the Draize scoring scale.

Results

Slight to well defined signs of irritation were observed in the test substance exposed groups of animals during the intradermal induction phase including well defined signs of irritation (grade 2 erythema) in one group where animals were exposed to the test substance during challenge phase. No effects were observed in all other treated animals.

Conclusion
Under the study conditions, EHMC did not trigger any skin reactions indicative of a skin sensitisation response.

(ECHA, 2021; NICNAS, 2017)

Local Lymph Node Assay (mice)

1st study:

Guideline/method:	Not specified
Species/strain:	Mice/BALB/c (female)
Group size:	Not specified
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Dosage level:	50, 25, 10, 5 and 0.25% (v/v)
Vehicle Acetone: olive oil	(4:1 v:v)
Route:	Dermal
Administration	Topical
GLP:	Not specified
Study period:	2012

The skin sensitisation potential of EHMC was investigated in a local lymph node (LLNA) assay and irritancy (IRR) assay in mice. Female mice were treated daily at concentrations of 50, 25, 10, 5 and 0.25% (v/v) in an acetone:olive oil vehicle (4:1 v:v) by topical application to the dorsum of each ear lobe (left and right). Control group of mice was treated with the acetone:olive oil vehicle (4:1 v:v) only. The mice were sacrificed, the draining lymph nodes excised and pooled.

During the treatment period all animals were assessed daily for mortality and clinical signs of toxicity as
well as for any treatment related effects during the observation period. The stimulation indices (SI) were calculated for each tested concentration.

Results

There were no statistically significant changes in lymph node cell proliferation in any of the treated groups compared to the vehicle group. Additionally, lymph node cell proliferation in each of the EHMC treatment groups was below the three-fold level of the vehicle response.
In the IRR assay, there were statistically significant increases in percent ear swelling following exposure to EHMC starting at 5%, with the greatest increase being observed at 25%, when compared to the vehicle control.

Conclusion

Under the conditions of this study, EHMC did not produce a skin sensitisation response.
(NTP, 2012)

2nd study

In a similar second LLNA study, mice were exposed to 5, 2.5, 1, 0.5 and 0.25% (v/v) of EHMC. There were no statistically significant changes in lymph node cell proliferation in any of the treated groups. In the IRR assay, there were statistically significant increases in percent ear swelling following exposure to test substance starting at 1%, with the greatest increase being observed at 5%, when compared to the vehicle control. No further study details are available.
(NTP, 2012)

Mouse ear swelling test

Guideline/method:	No guidelines followed
Species/strain:	Mice/ Not specified
Group size:	Not specified
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Epicutaneous induction:	1, 2.5 and 5% (First study)
0.25, 0.5 and 1%	(Second study)
Challenge: 5%	(First study)
0.5%	(Second study)
Vehicle:	Not specified
Positive control:	2,4-Dinitrofluorobenzene
Route:	Dermal
Administration:	Topical
GLP:	Not specified
Study period:	2012

The contact hypersensitivity potential of EHMC was investigated in two mouse ear swelling tests (MEST). In the first study, the test substance induction levels were 1, 2.5 and 5% and the challenge level was 5%. Control animals were treated in the same way, but without the test substance. The reactions were graded 24 and 48 hours post-challenge.
In the second MEST study, the test substance sensitization levels were 0.25, 0.5 and 1% at induction, with a 0.5% challenge concentration.

Results

There were no significant changes in percent ear swelling in any of the test substance exposed groups when compared to the vehicle control group at 24 hours post-challenge. At 48 hours post-challenge, a significant increase in percent ear swelling was observed in mice that had been both induced and challenged with 5% test substance. However, the increase in percent ear swelling in the positive control group was not significantly different from the positive control challenge only (PCCO) group at either 24- or 48 hour post-challenge, requiring a repeat of the study.

In the repeat MEST study, no significant changes in percent ear swelling were observed in any of the test substance exposed groups when compared to the vehicle control group at either the 24- or 48 hour post challenge. The positive control, 2,4-Dinitrofluorobenzene, significantly increased the percent ear swelling when compared to the PCCO group, as expected.

The results of the MEST were negative when mice were sensitised with 0.25 and 1.0% EHMC and challenged with 0.5% EHMC. A questionable, increase in the MEST was observed when mice were sensitised with 5% EHMC and challenged with 5% EHMC.

Conclusion

The results of the MEST were negative when mice were sensitised with 0.25 and 1.0% EHMC and challenged with 0.5% EHMC. A questionable, increase in the MEST was observed when mice were sensitised with 5% EHMC and challenged with 5% EHMC.

(NTP, 2012)

Human data- skin sensitisation

A repeat insult patch test (RIPT) was conducted in 53 human subjects using 2% EHMC. No skin sensitisation reactions were reported.

In another Draize RIPT conducted with 54 human subjects, 7.5% EHMC in petrolatum was applied for 48 hours under occlusive conditions for 11 applications. After a 14-day rest, a challenge application of a single dose was made. No skin sensitisation reactions were observed.

Another RIPT was conducted in 58 human subjects (12 males and 46 females, aged 18-63) using 10% EHMC in dimethyl phthalate. Of these, 6 subjects failed to complete the study for reasons not related to the experimental procedure. Induction applications were made on the skin of the back for 24 hours with occlusion, 3 times a week for 9 applications. Following a rest period of 2 weeks, a further patch was applied to a new site on the back for 24 hours under occlusive conditions. The exposed skin area was examined at 0, 24 and 48 hours after removal of the patch. No adverse reactions were noted at any stage of the study.

(SCC, 2000)

SCCS comment on skin sensitisation

The SCCS considers the HRIPT studies to be unethical.

The HRIPT and LLNA indicate absence of sensitisation potential of EHMC. The MEST (Mouse Ear Swelling Test) is considered outdated. In the open literature, sensitisation in humans is rarely reported, and if so, it is in conjunction with photosensitisation (see 3.4.8 Photo-induced toxicity). Therefore, the SCCS considers that the concern for skin sensitisation is negligible.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

1st study

Guideline:	Similar to OECD Test Guideline 401
Species/strain:	Rats/ Wistar
Number of animals:	5 animals/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Vehicle:	0.5 % preparation aqueous of carboxymethylcellulose
Batch:	2/4/83
Purity:	Approx. 100%
Dose levels:	5000 mg/kg bw
Dose volume:	10 mL/kg
Route:	Oral
Administration:	Gavage
Observation:	14 days

GLP: No
Study period: 1984

EHMC was investigated for acute toxicity in rats according to a protocol similar to OECD Test Guideline 401. Five male and female Wistar rats were administrated a single dose of 5000 mg/kg bw of test substance via oral gavage. Following exposure, the animals were observed for 14 days, and deaths were recorded.

Results
No clinical signs of toxicity or mortality were observed.

Conclusion
Under the conditions of the study, the LD50 of EHMC was considered to be equal or greater than >5000 mg/kg bw for male and female rats.

(ECHA, 2021)

2nd study

Guideline: No guideline followed
Species/strain: Mice/ Not specified
Number of animals: Not specified
Test substance: Ethylhexyl Methoxycinnamate
Vehicle: Gummi arabicum suspension
Batch: Not specified
Purity: Not specified
Dose levels: 6000 and 8000 mg/kg bw
Dose volume: Not specified
Route: Oral
Observation 10 days
GLP: No
Study period: 1968

EHMC was investigated for acute toxicity in mice. Mice were administrated doses of 6000 and 8000 mg/kg bw. Following exposure, the animals were observed for 10 days for signs of toxicity during the exposure period.

Results
No mortality was observed. Ataxia and respiratory depression were observed. Temporarily cramps were observed at 8000 mg/kg bw.

Conclusion
Under the conditions of the study, the LD50 of EHMC was considered to be greater than > 8000 mg/kg bw for mice. The oral toxicity of EHMC was considered to be very low.

(ECHA, 2021; NICNAS, 2017)

SCCS comment
EHMC is not acutely toxic by oral route.

3.4.3.2 Acute dermal toxicity

Guideline: Similar to OECD Test Guideline 402
Species/strain: Rat/CFY
Number of animals: 5 (sex not specified)
Test substance: Ethylhexyl Methoxycinnamate
Vehicle: Other: Sunscreen cream containing 2.5-7.5% of EMHC

Batch:	Not specified
Purity:	Not specified
Dose levels:	126.3 mg/kg bw
Dose volume:	5 mL/kg
Exposure:	24 hours
Observation	14 days
GLP:	No
Study period:	1977

The acute dermal toxicity of EHMC was evaluated according to a test similar to OECD Test Guideline 402 in rats. A sunscreen cream formulation containing up to 7.5% of test substance (equivalent to 126.3 mg/kg bw) was applied occlusively to skin of rats for 24 hours.

Animals were observed for mortality, body weights, and clinical signs for 14 days. Necropsy with gross pathological examinations were performed after sacrificing the animals at study Day 14.

Results

No mortalities, clinical signs of systemic toxicity or skin irritation were observed at 126.3 mg/kg bw. No significant gross findings were noted following necropsy and autopsy. Loss of bodyweight was seen in female rats in the first week, but it got restored in the second week.

Conclusion

Under the conditions of the study, the acute dermal LD50 of EHMC in rats was determined to be greater than 126.3 mg/kg bw.

(ECHA, 2021; NICNAS, 2017)

SCCS comment

Based on the above data, EHMC is considered to be of low acute toxicity by the dermal route at >126.5 mg/kg bw. However, higher doses were not tested.

3.4.3.3 Acute inhalation toxicity

Guideline:	OECD Test Guideline 403
Species/strain:	Rats/ Wistar
Number of animals:	5/sex
Test substance:	HR 92/660 523 (Ethylhexyl Methoxycinnamate)
Vehicle:	No vehicle
Batch:	2040059
Purity:	99.2%
Dose levels:	Sample I: 497 mg/m3; Sample II: 524 mg/m3
Type of exposure:	Head only
Duration of exposure:	4 hours
Observation period:	14 days
GLP:	Yes
Study period:	1993

EHMC was evaluated according to OECD Test Guideline 403 study for acute inhalation toxicity in rats. Wistar rats (5 males and 5 females) were exposed to the test substance contained in spray can at 2 and 5% in aerosol form for 4 hours at a concentration of 497 and 524 mg/m^3 (active ingredient) with a mean concentration of 511 mg/m^3. The animals were observed for signs of toxicity during the exposure period and 14 days thereafter.

Results

Except for the slight reduction of body weight gain (without statistical significance), there were no findings in any treated animal regarding mortality, clinical signs, changes in functional tests (reflexes and grip strength), or gross pathology.

Conclusion
The acute inhalation LC50 of EHMC in rats was determined to be greater than 511 mg/m^3.

(ECHA, 2021; NICNAS, 2017)

SCCS comment
Based on the above data, EHMC is considered to be of slight acute toxicity by inhalation at >511 mg/m^3. However, higher doses were not tested.

SCCS overall conclusion on acute toxicity
Acute toxicity of EHMC is not of major concern.

3.4.4 Repeated dose toxicity

3.4.4.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

Oral exposure

Guideline:	No guideline
Species/strain:	Rat/ Not specified
Group size:	5/sex /group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	Not specified
Dose levels:	0, 0.3, 0.9 or 2.7 mL/kg bw/day
Equivalent to	0, 300, 900 or 2700 mg/kg bw/day
Dose volume:	Not specified
Route:	Oral
Administration:	Gavage
Duration:	21 days
GLP:	Not specified
Study period:	Not specified (before 2000)

In a subacute repeated dose range finding study (compliance and species information not available), rats were administered EHMC by gavage at doses of 0, 0.3, 0.9 or 2.7 mL/kg bw/day which is equivalent to doses of 0, 300, 900 or 2700 mg/kg bw/day for 21 days.

Results
All animals of the highest tested dose groups exhibited loss of body weight and a reduced relative as well as absolute weight of the thymus. In the highest tested dose groups, male rats showed a decrease in absolute weight of the left kidney and female rats showed a decrease in the absolute weight of the heart.
Further, increases in the absolute weight of the pituitary were observed at the lower doses but these were not considered to be biologically significant.

Conclusion
The study investigators established the NOAEL for EHMC 900 mg/kg bw/day in rats.

(NICNAS, 2017; SCC, 2000)

SCCS comment
This study is considered of low reliability (not a guideline study, not a GLP study).

Dermal exposure

1st study

Guideline:	No guideline
Species/strain:	Rabbits/ New Zealand White
Group size:	5/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	No vehicle
Dose levels:	0, 500, 1500 or 5000 mg/kg bw/day
Dose volume:	Not specified
Route:	Dermal
Administration:	Occlusive
Duration:	21 days
GLP:	No
Study period:	1980

The dermal subacute toxicity of EHMC was investigated in New Zealand White rabbits (5/sex/group). EHMC was applied occlusively on the abraded skin of rabbits at doses of 0, 500, 1500 or 5000 mg/kg bw/day, 6 hours/day for 21 days. During the treatment period, animals were observed for clinical signs, dermal irritation, mortality, body weight and food consumption at defined intervals. Haematological parameters and clinical chemistry were also examined. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

Mortalities occurred in three animals of the high dose group. Two of these losses were associated with respiratory conditions and the third was assumed to be the result of enteric disturbances. Pathology findings in the surviving rabbits included diminished thymus, a low incidence of macroscopically observable focal liver necrosis, deleted liver glycogen and immature testes. These findings were related to the general debilitated condition of the rabbits rather than evidence of direct organ toxicity. At the highest treatment dose, lethargy, hunched posture, hair loss, soiled coats, emaciation, increased respiration, swelling of the conjunctivae and a retardation of testicular growth were observed. After three weeks, hematological changes in high dose animals included increased neutrophils and urea nitrogen, as well as decreased lymphocytes and alkaline phosphatase activity. Signs of irritation indicated by erythema, edema, desquamation, cracking and atonia (i.e., a decrease in normal elasticity or resilience of the skin) were observed at all doses but were more severe at the highest dose. Histopathology of the skin sites showed an epidermal proliferative response with low grade inflammatory reaction. This effect was dose-related and more pronounced in the rabbits of the highest dose. No evidence of systemic toxicity was found in intermediate or low dose group animals.

Conclusion

Under the conditions of the study, the study investigators established the NOAEL for EHMC at 1500 mg/kg bw/day.

(ECHA, 2021; NICNAS, 2017)

SCCS comment

This study is considered as reliable with restrictions as the top two doses exceeded the recommended limit dose of 1000 mg/kg/day.

2nd study

Guideline:	Similar to OECD Test Guideline 410
Species/strain:	Rats/Sprague-Dawley
Group size:	5/ sex/ group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	No vehicle
Dose levels:	0, 500, 1500 or 5000 mg/kg bw/day
Dose volume:	0, 0.5, 1.5 and 5 mL/kg bw/day
Route:	Dermal (intact and abraded skin)
Administration:	Occlusive
Duration:	28 days
GLP:	No
Study period:	1980

The dermal subacute toxicity of EHMC was investigated according to a protocol similar to OECD Test Guideline 410 in Sprague-Dawley rats (5/sex/group). Test substance was applied occlusively on the intact and abraded skin of rats at doses of 0, 500, 1500 or 5000 mg/kg bw/day, 6 hours/day for 28 days.

During the treatment period, animals were observed for clinical signs, dermal irritation, mortality, body weight and ophthalmoscopic examination at defined intervals. Haematological parameters and clinical chemistry were also examined. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No mortalities and no treatment related systemic effects were observed. No effects were noted at necropsy in any of the tissues or organs evaluated. All animals displayed low grade epidermal proliferation. This was dose dependent and appeared to be more prominent in males. Dermal inflammatory or fibrotic responses were not significant.

Conclusion

Under the conditions of the study, the NOAEL for EHMC was established by the study authors at 5000 mg/kg bw/day.

(ECHA, 2021; NICNAS, 2017)

SCCS comment
This study is considered as reliable with restrictions as the top two doses exceeded the recommended limit dose of 1000 mg/kg/day.

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

Oral exposure

Guideline:	OECD Test Guideline 408
Species/strain:	Rats/ Füllinsdorf Albino SPF
Group size:	12/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified

Purity:	Not specified
Vehicle:	No vehicle
Dose levels:	0, 200, 450 or 1000 mg/kg bw/day
Route:	Oral
Administration	Feed
Duration:	13 weeks
Recovery:	Yes, 6 rats for 5 weeks
GLP:	Yes
Study period:	1984

The oral subchronic toxicity of EHMC was investigated in an OECD Test Guideline 408 compliant feeding study. Füllinsdorf Albino SPF rats (12/sex/group) were dosed daily via the diet at 200, 450 and 1000 mg/kg bw/day of test substance for 13 weeks. Six rats/sex from controls and the high dose rats were kept for a recovery period of 5 weeks. The concentrations of the dietary test substance preparations were confirmed analytically. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Ophthalmoscopy and urine analysis were performed twice during the study. Blood chemical and haematological investigations were carried out at the beginning, during and at the end of the treatment period. An additional blood chemical investigation was performed after a recovery period. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No symptoms indicative of pathologic conditions, ophthalmological abnormalities or mortalities as consequence of the treatment with test substance were recorded during the study. The feed intake and body weight development of treated animals were similar to those of controls. Laboratory investigations in high-dose females (1000 mg/kg bw/day) revealed an increase of the plasma activity of glutamate dehydrogenase (GLDH) which was reversed after the recovery period. The absolute as well as the allometrically adjusted weights of the kidneys were slightly increased in males. No deviations of the weights were found after the recovery period, thus indicating an adaptive change.

The glycogen content of the livers was reduced in 5 of 12 animals, accompanied by slight shrinkage of the hepatocytes. In females the amount of iron positive material phagocytized by Kupffer cells was slightly increased. These conditions were reversed after the recovery period. There were no obvious effects related to the treatment, which were detectable by the hematological, blood chemical and urine parameters at the mid- (450 mg/kg bw/day) and low-dose (200 mg/kg bw/day) levels. A slight increase of the iron positive material phagocytized by the Kupffer cells was observed in mid-dose females. It was concluded that the treatment with test substance was well tolerated at all dose levels and only minor and reversible changes occurred at the dose level of 1000 mg/kg bw/day, whereas the dose of 450 mg/kg bw/day did not induce any adverse effects in the rats.

Conclusion

The study investigators established the NOAEL for EHMC at 450 mg/kg bw/day

(ECHA, 2021; NICNAS, 2017)

SCCS comment

The range of investigations was consistent with the version of the OECD TG at the time the study was conducted, including gross and histopathological examination of the thyroid gland. No treatment-related changes were reported for food consumption, body weight, body weight gain or mortalities.

This study is considered as reliable. A NOAEL of 450 mg/kg bw/day based on reduction of the glycogen content and shrinkage of hepatocytes at a dose of 1000 mg/kg/day, the highest dose tested, can be used as a POD for systemic effects after repeated oral exposure.

Dermal exposure

Guideline:	No Guideline
Species/strain:	Rats/Sprague-Dawley
Group size:	10/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	Mineral oil
Dose levels:	0, 55.5, 277 or 555 mg/kg bw/day
Dose volume:	Not specified
Route:	Dermal
Administration:	Topical
Duration:	13 weeks (5 days/week)
GLP:	Not specified
Study period:	Not specified, but before 2000

In a subchronic dermal repeated dose toxicity study, EHMC in mineral oil was applied on the shaved skin of rats at doses of 0, 55.5, 277 or 555 mg/kg bw/day, 5 days/week for 13 weeks.

Results

No mortalities were observed. Slight scaliness of the skin (attributed to the vehicle) was observed at the application sites for all animals. At the highest dose, elevated (but non-significant) serum alanine phosphatase (SAP) levels and increased relative liver weights were observed. Liver effects were not observable upon microscopic examination. There were no changes in haematological parameters.

Conclusion

The study investigators established the NOAEL for EHMC at 555 mg/kg bw/day.

(NICNAS, 2017; SCC, 2000)

SCCS comment

This study is considered of low reliability (not a guideline study, not a GLP study, exposure is not continuous…)

Inhalation route

No inhalation studies on EHMC could be identified.

SCCS overall conclusion on repeated dose toxicity

The oral repeated dose toxicity of EHMC has been investigated in rats in a standard 90-day oral dosing study at doses of up to 1000 mg/kg day and in a non-standard 35-day oral dosing study employing a single dose of 1000 mg/kg/day. In addition, two standard repeated dermal application studies are available (one each in the rat and rabbit). No study is available for the inhalation route.

The liver was found to be the principal target organ, following repeated oral dosing for 13 weeks with decreased hepatocyte glycogen content, accompanied by the shrinkage of hepatocytes in some males and females at the top dose. From the repeated oral exposure studies, a NOAEL of 450 mg/kg bw/day can be derived as a PoD for systemic effects.

3.4.4.3 Chronic (> 12 months) toxicity

/

3.4.5 Reproductive toxicity

3.4.5.1 Fertility and reproduction toxicity

Two-generation reproductive toxicity

Guideline/method:	OECD Test Guideline 416
Species/strain:	Rats/Wistar
Group size:	25/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Purity:	99.9 %
Batch:	uv2-01.019
Dose levels:	0, 150, 450 or 1000 mg/kg bw
Vehicle:	Unchanged, mixed with diet
Route:	Oral
Administration:	Feed
Exposure period:	Continuous administration until or up to about 16 hours before they were sacrificed (Feb 28-Jul 17 2002)
F1 generation:	After weaning, continuous administration of the test substance until or up to about 16 hours before they were sacrificed. (Jul 3 - Nov 11 2002)
F2 generation:	After weaning, continuous administration of the test substance until or up to about 16 hours before they were sacrificed (Nov 7 - Dec 3 2002)
Premating exposure:	F0 generation: 73 days
GLP:	Yes
Study period:	2005

The reproductive toxicity of EHMC was determined according to OECD Test Guideline 416 two-generation reproduction toxicity study in rats. Wistar rats (25/sex/group) were dosed daily via the diet at 0, 150, 450 or 1000 mg/kg bw for two successive generations. The calculated test substance intake for the premating phase was 153, 460 and 1015 mg/kg bw/day bw/day in males (mean of weeks 0–17) and 156, 468 and 1039 mg/kg bw/day for females (mean of weeks 0–10). For females, the test substance intake was 152, 451 and 1025 mg/kg bw/day during gestation (mean of days 0–20) and 137, 413 and 867 mg/kg bw/day during lactation (mean of days 1–14). The parental (F0) generation was exposed throughout premating period (73 days), mating (21 days), gestation (21 days) and up to weaning of the F1 offspring (21 days). The duration of exposure for the F1 generation was similar to F0.

Following pre-breed exposure, animals were paired within dose groups for 21 days to produce the F1 generation. At F1 weaning, pups were randomly selected to become parents of the next generation. The animals were paired to produce the F2 generation. Exposure to the test diets continued through mating, gestation, parturition and lactation. Endpoints evaluated in both generations of parental animals included clinical signs of toxicity, body weights and body weight changes, food consumption, reproductive parameters, necropsy findings for all animals and microscopic evaluation of reproductive organs from animals in the high dose and control groups. The dose formulations used in this study were analysed to confirm the final test substance concentration.

Results

F0 data: No mortality or treatment-related clinical signs of toxicity were observed for males and females during the study. Consistently decreased food consumption values were noted throughout the treatment period in the males, and females during the gestation period and lactational period. Differences in maternal weights or decreased weights were observed in high dose group animals throughout the gestational and lactational period. Fewer uterine implantation sites were observed in F0 dams, however, the number of implantation sites was abnormally high and considerably above the historical range in F0 female controls, whereas the number of implantations per dam in the high dose group was well within the historical range. This was considered to be an incidental finding and not directly related to treatment by study investigators. Pathological changes were observed at 1000 mg/kg bw/d in males and females. The eosinophilic homogeneous appearance of the liver cell cytoplasm indicative of enzyme induction was observed in males and females; an increased amount of haemosiderin in the spleen was observed in females and increased ulceration of the glandular stomach mucosa.

F1 generation: There were no treatment-related effects on F1 pup viability or survival. No treatment related clinical signs of toxicity were observed in F1 pups. No treatment-related mortality or clinical signs of toxicity were observed in any dose group. No adverse effects were observed on the reproductive performance (oestrous cycles, sperm and follicle parameters, mating, fertility), sperm morphology and motility, gestation and parturition. A slight delay of preputial separation in males and vaginal patency in females were seen. Decreased implantation was observed in mid and high dose groups (10.7 and 10.3 implants/dam).
There was a statistically significantly reduction in the number of implantation sites at the high dose in both parental generations, and also at 450 mg/kg bw/day in F1 parents, compared to controls. The number of implantation sites in the F0 and F1 female control animals was particularly high, in fact exceeding the historical range. The number of implantation sites in the F0 females at 1000 mg/kg bw/day (10.0 ± 2.0) was very close to the historical control range (10.2–11.5). In the F1 generation females, the number of implantation sites at 450 mg/kg bw/day (10.7 ± 2.8) and 1000 mg/kg bw/day (10.3 ± 1.8) was fully within the historical range. The fact that subsequent follicle counts were normal in all F1 parents indicates that if the marginal reduction in implantation rate was truly related to treatment, it was not related to egg maturation. Moreover, in both generations, the post-implantation loss was normal for all groups, again indicating the absence of a treatment-related effect on this parameter. Overall, there was a small reduction in parental food consumption and body weight. Slight transient decreases in offspring body weight were observed at 1000 mg/kg bw/day. Continuous exposure for two generations did not result in parental toxicity or adverse effects on reproduction or reproductive tissues.

Conclusion
Under the study conditions, the NOAEL for parental animals and offspring was set at 450 mg/kg bw/day based on the decreased body weights, increased liver weight and hepatic cytoplasmic eosinophilia in the parental animals at 1000 mg/kg bw/day as well as a secondary reduction in implantation rate and reduced body weights and delayed sexual maturation of the pups.

(Schneider *et al.*, 2005)

SCCS comments
This study is considered reliable without restriction and a NOAEL of 450 mg/kg bw/day (male/female) can be derived for systemic parental toxicity (P0/F1) and also for offspring toxicity (F1/F2) based on effects on pup weights.

3.4.5.2 Developmental Toxicity

1st study: Prenatal, Oral – Rat

Guideline/method:	US FDA guidelines (1966)
Species/strain:	Rats/Albino
Group size:	36/female/group
Test substance:	Ethylhexyl Methoxycinnamate
Purity:	Not specified
Batch:	Not specified
Dose levels:	0, 250, 500 or 1000 mg/kg bw
Vehicle:	Not specified
Route:	Oral
Administration:	Gavage
Exposure period:	From gestation day (GD) 6 to GD14
GLP:	Not specified
Study period:	Not specified, but pre 2000

The prenatal developmental toxicity of EHMC was determined according to US FDA guidelines (1966) in pregnant female albino rats. The test substance was administered orally to 36 pregnant females at doses of 0, 250, 500 or 1000 mg/kg bw/day during Days 6 to 14 of gestation. During the study, all animals were monitored daily for clinical signs, abortions and mortality. The foetuses were delivered via caesarean section and subjected to teratological evaluations (external and skeletal examinations).

Results
There was no increase in the number of malformed foetuses in any of the treated groups compared to the control group. No mortality or treatment-related clinical signs of toxicity were observed for females during the study. Slight reduction in the body weight was observed at the highest dose. Skeletal variation was seen to be increased.

Conclusion
Under the study conditions, the test substance was not teratogenic up to highest tested dose of 1000 mg/kg bw/day.

(SCC, 2000)

SCCS comment
In the Evaluation Conclusion Document (2017) this study is described in more detail (Vehicle: 5% Carboxymethylcellulose, 0.5% Benzyl-EtOH, 0.4% TWEEN 80, 0.9% NaC1), which better demonstrates the reliability of the results. A NOAEL of 1000 mg/kg bw/day can be derived for maternal toxicity and for developmental toxicity, as no effects were observed at the highest dose.

2nd study - Prenatal, Dermal - Rabbit

Guideline/method:	US FDA guidelines (1966)
Species/strain:	Rabbits/ Swiss Hare
Group size:	20/female/group
Test substance:	Ethylhexyl Methoxycinnamate
Purity:	Not specified
Batch:	Not specified
Dose levels:	80, 200 or 500 mg/kg bw
Vehicle:	SSV: 0.5 % Carboxymethylcellulose, 0.5 % Benzyl-EtOH, 0.4 % TWEEN 80, 0.9 % NaCl

Route:	Oral
Administration:	Gavage
Exposure period:	From gestation days (GD) 7 to GD20
GLP:	Yes
Study period:	1983

The prenatal developmental toxicity of EHMC was determined according to US FDA guidelines (1966) in pregnant female Swiss Hare Rabbits. The test substance was administered orally to 20 pregnant females at doses of 80, 200 or 500 mg/kg bw/day during Days 7 to 20 of gestation. Fetuses were removed on GD 20 by ovariohysterectomy, tested for viability (24 hours). During the study, all animals were monitored daily for clinical signs, abortions and mortality. The foetuses were delivered via caesarean section and subjected to teratological evaluations (external and skeletal examinations).

Results

There was no increase in the number of malformed foetuses in any of the treated groups compared to the control group. No mortality or treatment-related clinical signs of toxicity were observed for females during the study. A slight reduction in the body weight and increase in the frequency of constipation and anorexia were observed at the highest dose. Reproductive parameters were not affected. The foetuses did not show any skeletal or visceral abnormalities. The median individual body weight of foetuses was decreased at 500 mg/kg bw/day but was within the range of other doses and the controls. It was not clear if this effect was due to direct intrauterine drug action or to a reduced body weight gain of the dams. The 24 hours survival rate of the foetuses was not affected by the treatment of the dams.

Conclusion

Under the study conditions, the NOAEL for maternal and developmental toxicity was set at 500 mg/kg bw/day.

(ECHA, 2021; NICNAS, 2017)

SCCS comment

This study can be considered as reliable. A NOAEL of 500 mg/kg bw/day can be derived for maternal toxicity and for developmental toxicity as there were no adverse effects on the dams or foetuses at doses of up to 500 mg/kg/day, the highest dose tested.

3rd study

In a pilot prenatal developmental toxicity study according to OECD Test Guideline 414 (no information regarding GLP compliance; study period not specified- but pre-2000), female albino rats were orally administered (gavage) EHMC at a single dose of 1000 mg/kg bw/day on GD 7–16.
No maternal, embryotoxic or teratogenic effects were observed.

(ECHA, 2021; NICNAS, 2017)

SCCS comment

This study is considered as a low reliable study which can only provide supportive information.

Overall conclusion from SCCS on reproductive toxicity

SCCS concurs with NTP/NIEHS conclusion that:

- Under the conditions of this modified one-generation (MOG) study, there was no evidence of **reproductive toxicity** of 2-ethylhexyl p-methoxycinnamate (EHMC) in

Hsd:Sprague Dawley® SD® rats at exposure concentrations of 1,000, 3,000, or 6,000 ppm. Mating and littering were not affected significantly by EHMC exposure.

- Under the conditions of this MOG study, there was equivocal evidence of **developmental toxicity** of EHMC in Hsd:Sprague Dawley® SD® rats based on the observed postnatal effects on body weight that showed some indication of recovery by study end, delays in postnatal day 28 adjusted vaginal opening and balanopreputial separation, which could have been influenced by the apparent transient effects on body weight, and time in oestrus was slightly longer in EHMC-exposed females relative to that of the control group. No other signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action were observed. EHMC exposure did not induce any specific foetal malformations.

https://ntp.niehs.nih.gov/sites/default/files/ntp/htdocs/dart/dart06_508.pdf

Based on the two-generation reproductive toxicity study, a NOAEL of 450 mg/kg bw/day (male/female) can be derived for systemic parental toxicity (P0/F1) and also for offspring toxicity (F1/F2) based on effects on pup weights.

3.4.6 Mutagenicity / genotoxicity

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

Bacterial reverse mutation test (Ames)

Guideline:	OECD Guideline 471
Test system:	Salmonella typhimurium strains TA 1535, TA1537, TA 98, TA 100 and TA 102
Replicates:	3
Test substance:	Ethylhexyl Methoxycinnamate
Solvent:	DMSO
Batch:	Not specified
Purity:	98.5%
Test concentrations:	0, 50, 150, 500, 1500, 5000 µg/plate
Treatment:	With and without S9-mix
Negative control:	Not specified
Positive control:	sodium azide; 2-nitrofluorene; 9-aminoacridine; Mytomycin C; 2-aminoanthracene
GLP:	Yes
Study period:	1999

The mutagenic potential of EHMC was evaluated in an OECD Test Guideline 471 complaint study in Salmonella typhimurium strains TA 1535, TA 100, TA 1537, TA 98 and TA 102 with and without metabolic activation (S9-mix). The concentrations of the test substance ranged from 0 to 5000 µg/plate. Negative solvent control and appropriate positive controls were used in the experiments.

Results
The test substance, EHMC, did not show any mutagenic activity up to the highest concentration in the presence or absence of S9-mix. The positive controls induced an increase in revertant colonies in the expected range. Total bacteria count remained unchanged, and no inhibition of growth was observed. Substance precipitation occurred at the dose of 1500 and 5000 µg/plate.

Conclusion
Under the conditions of the study, the EHMC was not mutagenic in the bacterial reverse mutation test (Ames test), neither in the presence nor absence of metabolic activation.

(ECHA, 2021)

SCCS comment
The results indicate no induction of gene mutations in the Ames test by EHMC.

Bacterial reverse mutation test (Ames) - NTP Study Number: G20239

Guideline:	OECD Guideline 471
Test system:	Salmonella typhimurium strains TA98, TA100, and E. coli WP2 uvrA pKM101
Replicates:	3, unless samples marked toxic or contaminated were excluded from mean and SEM calculations
Test substance:	Ethylhexyl Methoxycinnamate
Solvent:	DMSO
Batch:	Not specified
Purity:	Not specified
Test concentrations:	10, 12.5, 50, 100, 125, 500, 1000, 1500, 6000 µg/plate (precipitation observed at 1500 and 6000 µg/plate)
Treatment:	With and without S9-mix
Negative control:	DMSO
Positive control:	sodium azide; 2-aminoanthracene, 2-aminoanthracene,9-aminoacridine, 4-nitro-O-phenylenediamine
GLP:	Yes
Study period:	2018 (request)

(NTP, 2020)

Bacterial reverse mutation test (Ames) - NTP Study Number: 201557

Guideline:	OECD Guideline 471
Test system:	Salmonella typhimurium strains **TA1535**, **TA1537**, **TA98**, **TA100**
Replicates:	3, unless samples marked toxic or contaminated were excluded from mean and SEM calculations
Test substance:	Ethylhexyl Methoxycinnamate
Solvent:	DMSO
Batch:	Not specified
Purity:	Not specified
Test concentrations:	0, 100, 333, 1000, 3333, 10000 µg/plate (precipitation at the higher concentration)
Treatment:	With and without S9-mix
Negative control:	DMSO

Positive control: sodium azide; 2-aminoanthracene, 2-aminoanthracene,9-aminoacridine, 4-nitro-O-phenylenediamine

GLP:	Yes
Study period:	2018 (request)

Study Result: Negative

(NTP, 2020)

SCCS comment on the two NTP reports (NTP Study Number: G20239 and 201557)
The full protocols of the studies are not available. These two Ames tests analysed together gather the appropriate strains of bacteria. However, both have limitations: neither one indicates purity of the test item; statistical analysis is not provided; raw data is not presented. Therefore, the 2 NTP reports were considered of limited relevance.

Mammalian Cell Gene Mutation Test in Chinese hamster lung fibroblasts (HPRT locus)

Guideline:	Similar to OECD Test Guideline 476
Test system:	Chinese hamster lung fibroblasts (V79), HPRT locus
Replicates:	Duplicates
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Concentrations:	5, 10 and 20 µg/mL
Exposure duration:	2 hours
Expression time:	7 days
Vehicle:	Methanol
Positive controls:	With S9-mix: 7,12-dimethylbenzanthracene, N-dimethylnitrosamine Without S9-mix: Ethylmethanesulphonate
GLP:	Yes (reported on ECHA database, but no certificate available)
Study period:	1983

EHMC was tested in a study similar to OECD Test Guideline 476 to investigate the mutagenic potential at the HPRT locus (6-thioguanine resistance) in V79 Chinese hamster lung fibroblasts.
The study consisted of a cytotoxicity range finder followed by the main experiment, each conducted in the presence and absence of metabolic activation (S9-mix).
A preliminary cytotoxicity experiment was performed on cell cultures with the dose levels ranging from 5-20 µg/mL in the presence and absence of an S9-mix. Results from the preliminary cytotoxicity test were used to select the test substance dose levels for the mutagenicity experiments.
Test substance treatments were performed for 2 hours exposure period both with and without S9-mix at 3 dose levels (5, 10 and 20 µg/mL), vehicle and positive controls. All doses were plated to determine viability and 6-thioguanine resistance 7 days after treatment.

Results

Precipitation and cloudy precipitate of the test substance was seen at the end of the exposure period at 20 µg/mL. Mutant frequencies (MF) in-vehicle control cultures fell within acceptable ranges and clear increases in mutation were induced by the positive control treatment with and without S9-mix. Therefore, the study was considered valid. No statistically significant increases in mutant frequency were observed following treatment with test substance at any concentration tested in the presence or absence of S9-mix in both independent experiments.

Conclusion:
Under the conditions of the study, EHMC did not induce mutations at the HPRT locus of V79 cells in the presence or absence of S9-mix.

(ECHA, 2021)

SCCS comment
Since the exposure conditions are not according to OECD TG 476 (2 hours of exposure, instead of recommended 3-6 hrs; only 3 concentrations tested, instead of recommended minimum 4 concentrations), the study was considered as not reliable.

Chromosome aberration study in mammalian cells

Guideline:	Similar to OECD Test Guideline 473
Test system:	human peripheral blood lymphocytes
Replicates:	3
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Concentrations:	5, 25, 50.0 µg/mL with S9-mix
	2, 10 and 20.0 µg/mL without S9-mix
Vehicle:	DMSO
Positive controls:	With S9-mix: Cyclophosphamide and
	Without S9-mix: Bleomycin
GLP:	Yes
Study period:	1984

EHMC was investigated in a study similar to OECD Test Guideline 473 to investigate the chromosome aberration potential in human peripheral blood lymphocytes cell line *in vitro*. The test substance dissolved in DMSO was tested in the presence and absence of S9-mix. The concentration range to be evaluated was selected based on a range-finding study. The cell cultures were exposed to the test substance for 24 hours at concentrations levels of 2, 10 and 20 µg/mL in the absence of S9-mix. Cultures were exposed to 2 hours of treatment at concentrations of 5, 25 and 50 µg/mL in the presence of S9-mix. Bleomycin and Cyclophosphamide were used as positive control substances. A solvent control (DMSO) was also included in the test.

Results

None of the cultures treated with test substance in the presence and absence of S9-mix exhibited biologically relevant or statistically increased numbers of aberrant metaphases. The positive controls induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the used S9-mix. The test substance did not show any chromosomal aberration in the presence or absence of S9-mix.

Conclusion

Under the conditions of the study, EHMC did not cause chromosomal aberrations in human peripheral blood lymphocytes in the absence or presence of metabolic activation.

(ECHA, 2021)

SCCS comment

Since the purity of the test item is not provided and the exposure conditions are not according to OECD TG 473 (2016) (2 hours of exposure in the presence of S9-mix, instead of recommended 3-6 hrs without or with metabolic activation; 200 metaphases scored, instead of recommended minimum 300 metaphases), the study was considered as not reliable.

DNA damage and/or repair study (UDS assay)

Guideline:	Similar to OECD Test Guideline 482
Test System:	Rat hepatocytes
Replicates:	Not specified
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Concentrations:	2.5, 5, 7.5, 10, 15, 20 µg/mL
Vehicle:	DMSO
Positive controls:	2-acetylaminofluorene

GLP:	Yes
Study period:	1986

EHMC was investigated in a study similar to OECD Test Guideline 482 to evaluate its DNA damage and/or repair potential in unscheduled DNA synthesis in rat hepatocytes. The concentrations of the test substance ranged from 2.5 to 20.0 µg/mL. 2-acetylaminofluorene was used as a positive control substance. A solvent control (DMSO) was also included in the test. The viability of the cells after treatment was determined by in situ trypan blue exclusion. 50 -100 nuclei were counted in the assay.

Results

The test substance, EHMC did not induce DNA damage resulting in unscheduled DNA synthesis in freshly prepared rat hepatocytes. Neither 5 nor 18 hours of treatment of cultured rat hepatocytes with 2.5 to 20 µg/mL test substance-induced significant changes in the nuclear labelling of the cells. The test substance was seen to be slightly cytotoxic in this study. Test substance exposure at 5- and 18-hours treatment with 20 µg/mL reduced cell viability to 71 and 86% respectively.

Conclusion
Under the study conditions, EHMC was not genotoxic in a DNA damage and repair study (UDS assay) in rat hepatocytes.

(ECHA, 2021 ; NICNAS, 2017)

SCCS comment
Following the OECD Council decision, the Test Guideline 482 'Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *in vitro*' was deleted on 2nd April 2014. Hence, the results can only be regarded as supportive in Weight Of Evidence (WoE).

Mammalian cell transformation assay

Guideline:	EU Method B.21
Test system:	Balb/c 3T3 clone A31-11
Replicates:	8 or 15 Petri dishes per dose
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Concentrations:	1.25, 2.5, 5, 7.5, 10 µg/mL
Preincubation time:	48 hours
Exposure duration:	3 days
Expression time:	4 weeks
Vehicle:	DMSO
Positive controls:	20-Methylcholanthrene
GLP:	Yes
Study period:	1985

EHMC was investigated for mammalian cell transformation potential in Balb/c 3T3 clone A31-11 cell line *in vitro*. The concentrations of the test substance ranged from 1.25 to 10 µL/mL. 20-Methylcholanthrene was used as positive control substance. A solvent control (DMSO) was also included in the test. The cell cultures were exposed to the test substance for 3 days at concentrations levels of 1.25, 2.5, 5, 7.5, 10 µg/mL. The cell transformation of the mammalian cells after treatment was determined by 10% Giemsa and 50% May-Grunwald.

Results

Test substance exposure for 3 days did not induce cell transformation in Balb/c 3T3 clone A31-11. The concentration of 10 µg/mL was taken as the highest dose tested throughout the study because at this concentration survival of Balb/c 3T3 cells was reduced to 50% related to the concurrent control cultures.

Conclusion
Under the study conditions, EHMC did not induce mammalian cell transformation *in vitro* and was not considered genotoxic.

(ECHA, 2021 ; NICNAS, 2017)

SCCS comment
According to the "Guidance document on the *in vitro* Bhas-42 cell transformation assay" [ENV/JM/MONO(2016) No. 231] the protocol used in this study with 3 days exposure corresponded to the initiation test component of the cell transformation assay. The promotion test component should have included 10 days exposure to EHMC. After analysis of the results, and as it provides no investigation of potential promotion effects, the SCCS considers the study of limited relevance.

Additional studies published in the scientific literature other than those provided in the initial dossier submitted by Applicants in response to the call from the Commission were identified by the SCCS during the preparation of this opinion. Therefore, SCCS asked the applicants to update their assessment by including this additional information. However, due to certain shortcomings some of these studies were considered to be of limited usefulness for the assessment of the genotoxicity/mutagenicity of EHMC. These studies have been summarised below with the SCCS comment.

Bonin et al, 1982

The mutagenic potential of EHMC was investigated in the Ames assay using 5 strains TA100, TA98, TA1535, TA1537 and TA1538 in the presence and absence of metabolic activation. EHMC was found positive exclusively in the TA 1538 only without metabolic activation. The authors questioned the potential impact of a trace contaminant because the positivity in all tested batches was not reproducible.

It should also be noted that the studies have not been performed according to OECD TG 471, e.g. only 4 concentrations and duplicate plates were used, and were not performed according to GLP. The purity or any analytics of the batches tested was not reported although the positive findings were considered to result from an impurity. These results have to be regarded as historic and not relevant for the high purity material that is in use today.

SCCS comment to Bonin *et al.*, 1982
The findings reported in the paper present a matter for concern, however their relevance is not clear. Seven out of 10 different samples were positive, and in one strain without S9-mix. This may result from the presence of an unknown impurity. It could be expected that if EHMC is truly positive, it would give consistently positive results with all samples tested. The purity of EHMC is not specified which is a significant limitation of the study.

Ashwood-Smith *et al.*, 1993

In this study, Ashwood-Smith *et al.* (1993) found EHMC to be negative in the AMES assay. Otherwise Ashord-Smith observed cytotoxicity when EHMC and UV irradiation were combined.

Notably, no difference was found between cis- and trans-EHMC, considering the fast photoisomerization of EHMC, which was, however, not discussed in the paper.

SCCS comment to the study by Ashwood-Smith *et al.,* 1993

In the study both, cis and trans isomers of EHMC yielded negative results, with or without S9. The results are of limited reliability as only two S. typhimurium strains were used.

Necasova *et al.*, 2016 and Sharma *et al.*, 2017

Based on the possible isomerization of the trans EHMC into cis EHMC upon some UV-light exposure conditions, these studies aimed to explore and compare the genotoxic potential of trans and cis EHMC in three *in vitro* assays, the SOS Chromotest and UmuC test (Necasova *et al.*, 2016) and *in vitro* Comet assay in the human liver stem (HL1-hT1) and the lymphoblastoid (TK6) cell lines (Sharma *et al.*, 2017).

Trans and cis EHMC isomers were found positive in the UmuC test and the *in vitro* Comet assay using the HL1-hT1 and TK6 cell lines. The positivity appeared at lower concentration for the cis isomer in the UmuC test and HL1-hT1 comet assay. The two chemicals showed opposite outcome in the SOS Chromotest, with positivity reported for the Cis isomer only.
Sharma *et al.* (2017) claim to have observed DNA damage in an in-vitro Comet test using TK-6 and HL1-hT1 cells (no UV irradiation was used in this study other than for preparation of cis-EHMC). In the latter positive findings with cis- and trans-EHMC occurred only at 25 µg/mL, the highest concentration tested. In TK-6 cells trans-EHMC caused positive effects at 25 and slight effects at 12.5 µg/mL, while cis-EHMC was found positive at all concentrations tested with a non-monotonic dose response relationship. The authors claim to have performed each experiment at least three times independently. If Fig. 1 summarizes the result of all three or more experiments, which has not been explicitly stated, it is notable, that the response at 3.13 µg/mL seemed to have been higher in ALL EXPERIMENTS than at 6.25 µg/mL. This has been concluded from the very narrow confidence intervals given in Fig. 1. This casts some doubts with regard to the reliability of the experiments. Also, it is notable that consistent positive effects were only seen at 25 µg/mL. This concentration has proven to be too toxic to mammalian cells in the previously reported assays, i.e., the V79 HPRT assay, the human lymphocyte chromosomal aberration test, the UDS rat hepatocyte assay). In all assays 20 µg/mL was used as the highest concentration, indicating already a considerable (and just not too high) cytotoxicity level. Therefore, it may be assumed that the positive findings of Sharma *et al.* (2017) were seen at a concentration causing extensive cytotoxicity. Sharma *et al.* did not test the viability of the cells alongside in the genotoxicity studies and they did not report the level of cytotoxicity that 25 µg/mL caused. Therefore, there is some serious doubt about the reliability of the findings and this study can only be attributed a low weight of evidence.

The findings from Necasova *et al.* (2016) in two bacterial mutagenicity screening tests, not performed under GLP, have limited weight of evidence because three producers of EHMC tested their materials in AMES tests and found no evidence that EHMC causes mutations in bacteria:

1. **Symrise 1995: OECD TG 471, GLP, study is part of the 2021 submission;**

A Confidential full study report was provided to the SCCS for Photo-Ames test following OECD TG 471

2. **BASF 2005: OECD TG 471, GLP, study report provided with this submission;**

Ames test, BASF, 2005

Guideline:	OECD 471
Test system:	TA 1535, TA 100, TA 1537, TA 98 and E. coli WP2 uvrA
Test substance:	Uvinul MC 80
Batch:	Betriebs-Ch. UV2-04.093 PBG-Ch. 00021 377L0
Purity:	99.8%
Test Conditions:	Standard plate test (SPT) and preincubation test (PIT) both with and without metabolic activation (Aroclor-induced rat liver S-9 mix).
Dose Range:	20 µg - 5 000 µg/plate (SPT); 4 µg – 2 500 µg/plate (PIT); Precipitation of the test substance was found from about 2 500 µg/plate onward.
Exposure duration:	48-72h
Vehicle:	DMSO
Positive controls:	with S9-mix : 2-aminoanthracene (TA 1535, TA 100, TA 1537, TA 98 and E.coli WP2 uvrA; without S9-mix: N-methyl-N'-nitro-N-nitrosoguanidine (TA1535, TA100); 4- N-methyl-N'-nitro-N-nitrosoguanidine (TA98); 9-aminoacridine (TA1537) ; 4-nitroquinoline-N-oxide (E.coli WP2 uvrA)
Replicates:	2 experiments and 3 test plates per dose or per control
GLP:	Yes
Study period:	2005

The substance Uvinul MC 80 was tested for mutagenicity in the Salmonella typhimurium / Escherichia coli reverse mutation assay both in the standard plate test and in the preincubation test with and without the addition of a metabolizing system (S-9 mix) obtained from rat liver using the Salmonella strains TA 1535, TA 100, TA 1537, TA 98 and Escherichia coli WP2 uvrA.

Results:

An increase in the number of his^+ or trp^+ revertants was not observed in the standard plate test or in the preincubation test either without S-9 mix or after the addition of a metabolizing system.

Conclusion from the authors:
According to the results of the present study, the test substance Uvinul MC 80 is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay under the experimental conditions chosen here.

SCCS comment
The results of the valid study on Uvinul MC80 by BASF (report #40M0026/044151, 2005) are considered negative.

(BASF 2005)

3. **dsm-firmenich: Schüpbach (1985) and Albertini (1991);** similar to OECD TG 471, GLP study reports provided with this submission.

SCCS comment to the study by Necasova *et al.*, 2016
In the study from **Necasova *et al.*, 2016,** both isomers were tested in SOS Chromotest and UmuC test. Trans-EHMC induced significant genotoxicity in both bioassays at the highest concentrations (0.5 - 4 mg/ mL), while cis-EHMC induced significant genotoxicity only in UmuC test at concentrations of 0.25 - 1 mg/mL. In the opinion of the SCCS, the results can be treated only as supplementary in the WoE.

SCCS comment to the study by Sharma *et al.*, 2017
In Sharma *et al.*, 2017, in TK-6 cells trans-EHMC induced positive effects at 25 and slight effects at 12.5 µg/mL, while cis-EHMC was positive at all concentrations tested with a non-

monotonic dose response relationship. In HL1-hT1 cells, cis-EHMC and trans-EHMC increased DNA damage detected at the concentration 25 µg/mL. According to the methodology description, cytotoxicity of both isomers was apparently measured but results not provided, hence the results were considered of limited reliability.

Schüpbach M., 1983

In addition, a Drosophila mutagenicity testing of the PARSOL MCX has been conducted (**Schüpbach M., 1983**), in a sex-linked recessive lethal assay in Drosophila melanogaster. In the experiments no significant difference in the mutation frequencies of treated and untreated groups was observed.

SCCS comment to the study by Schüpbach M., 1983
Results on Drosophila melanogaster can be treated only as supplementary in the WoE.

SCCS comment to the study by Schüpbach M., 1985
The results of the study are acceptable and considered negative, however purity of the test item was not provided.

Conclusion from the Applicant on the additional published genotoxicity/mutagenicity studies.

In summary, EHMC (unspecified and trans isoforms) was described positive in the Drosophila melanogaster test, the sister chromatid exchange test, the UmuC test, the SOS Chromotest and the high throughput Comet assay using two cell lines. It should be noted that the cytotoxicity level was not reported in all the assays when positivity was reached. Additionally, all the reported tests are not part of the currently recommended testing strategy to determine the genotoxic potential of new chemicals and the OECD test guidelines on the Drosophila melanogaster and the sister chromatid exchange were withdrawn in 2014.

The mutagenic potential of EHMC observed in the TA1538 without metabolic activation in the Ames assays may be attributed to a trace contaminant. Moreover, the positivity observed exclusively in the TA1538 seems quite unusual as it was not associated with positivity in the TA 98. The TA98 strain was derived from the TA1538 strain by introducing a plasmid which leads to greater sensitivity (McCann et al, Proc. Nat. Acad. Sci. USA Vol. 72, No. 3, pp. 979-983, 1975).

Finally, there's no evidence from these assays that the cis isomer behaves differently to the trans isomer with respect to genotoxic potential. Therefore, the results reported in these additional articles do not call into question the conclusions on the evaluation of genotoxic potential of EHMC presented in the submitted dossier.

SCCS overall comment on *in vitro* genotoxicity/mutagenicity
The full set of available information on *in vitro* genotoxicity is summariszed in Table 10, including also the datasets described in photogenotoxicity section (controls without UV irradiation);

Table 10. Summary of the analysis of data on in vitro genotoxicity/mutagenicity of EHMC available to the SCCS.

Publication/study report	Endpoint	Test organism	Isomer tested Ratio cis/trans	Reliability/relevance Result
	***In vitro* gene mutations:**			
ECHA, 2021	Bacterial reverse mutation test (Ames test)	TA 1535, TA1537, TA 98, TA 100 and TA 102	NA	Valid, negative

NTP #G20239	Bacterial reverse mutation test (Ames test)	TA98, TA100, and E. coli WP2 uvrA pKM101	NA	Limited reliability, negative
NTP #201557	Bacterial reverse mutation test (Ames test)	TA1535, TA1537, TA98, TA100	NA	Limited reliability, negative
Bonin *et al.*, 1982	Bacterial reverse mutation test (Ames test)	TA100, TA98, TA1535, TA1537 and TA1538.	NA	Limited reliability, positive 7/10 samples in TA1538 –S9
Ashwood-Smith et al, 1993	Bacterial reverse mutation test (Ames test)	S. typhimurium TA TA98 and TA100 used	Both, cis and trans tested negative -/+S9	Limited reliability, negative, but 2 S. typhimurium strains used
Symrise (by Bayer 1995)	Bacterial reverse mutation test (Ames test and photomutagenicity)	S. typhimurium TA 102 and TA 1537	NA	Acceptable, negative, -S9-mix, 2 S. typhimurium strains used
BASF # 40M0026/044151, 2005	Bacterial reverse mutation test (Ames test) – Uvinul MC80	TA 1535, TA 100, TA 1537, TA 98 and E. coli WP2 uvrA	NA	Valid, negative
Schuepbach, 1985	Bacterial reverse mutation test (Ames test) – Parsol MCX	TA 1535, TA 1537, TA 1538, TA 98, TA 100, TA 97 and TA 102		Limited reliability, negative
BASF, 2001b	Bacterial reverse mutation test (Ames test and photomutagenicity) – Uvinul MC80	TA 1537, TA 98, TA 100, and TA 102	NA	Acceptable, negative, -S9-mix; 4 S. typhimurium strains used
ECHA, 2021	Mammalian Cell Gene Mutation Test (HPRT locus)	V79	NA	Not reliable
	***In vitro* chromosomal aberrations:**			
ECHA, 2021	Chromosomal aberrations	Human lymphocytes	NA	Not reliable
BASF, 2001c	Chromosomal aberrations photomutagenicity	V79	NA	Limited reliability Only –S9 tested; 3h+18h, 3h+24h, 100 metaphases scored, low concentrations tested ≤1µg/mL, mitotic index >82%
Roche, 1993	Chromosomal aberrations photomutagenicity	CHO	NA	Limited reliability Only –S9 tested; time of exposure to EHMC +/- UVA/UVB not clear (most probably 10-60 min. of exposure + 18h post-incubation), 100 metaphases scored
	Other endpoints *in vitro*:			
ECHA, 2021	DNA damage and/or repair study, UDS assay	Isolated rat hepatocytes	NA	Limited relevance, negative
ECHA, 2021	Mammalian cell transformation assay	Balb/c 3T3 clone A31-11	NA	Limited relevance, negative
Necasova *et al.* 2016	SOS Chromotest and UmuC test		trans-EHMC: significant genotoxicity in both bioassays at the highest concentrations (0.5 - 4 mg/ mL)	Limited relevance, positive

			cis-EHMC: significant genotoxicity only in UmuC test at concentrations of 0.25 - 1 mg/mL	
Struve *et al.*, 2007	Comet assay – photogenotoxicity	L5178Y cells	NA	Negative -/+ UVA/UVB
Sharma *et al.*, 2017	Comet assay	HL1-hT1 and TK6 cells	In TK-6 cells trans-EHMC: positive effects at 25 and slight effects at 12.5 µg/mL cis-EHMC: positive at all concentrations tested with a non-monotonic dose response relationship In HL1-hT1 cells cis-EHMC and trans-EHMC increased DNA damage detected at the concentration 25 µg/mL	Limited reliability, positive Cytotoxicity was apparently measured but results not provided.

The results of all analysed studies in the Ames test repeatedly indicate lack of gene mutation potential of EHMC. One study on Ames test (Bonin *et al.*, 1982) may indicate a mutagenicity hazard, however, as was already explained, the relevance of the study is limited due to unknown purity of the 10 tested samples.
One study on mammalian cell gene mutations in Chinese hamster lung fibroblasts was considered not reliable.
One study on chromosomal aberrations on human peripheral blood lymphocytes was considered not reliable. After the SCCS request for additional data evaluation, the Applicant provided 2 more studies on chromosomal aberration tests on EHMC which were part of the photomutagenicity studies: i) chromosomal aberrations in photomutagenicity testing (BASF, 2001c) on V79 cells and ii) Chromosomal aberrations in photomutagenicity testing by Roche (1993) on CHO cells. The results of both studies were considered by the SCCS of limited reliability. In conclusion, no valid data on chromosomal damage *in vitro* were available.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

The applicants informed SCCS that the CE OMC consortium did not use in the safety assessment presented the *in vivo* mammalian erythrocytes Micronucleus assay performed by NTP. Even if this study was deemed to be in compliance with the provisions on animal testing ban in the Cosmetics Products regulation, the test was not used in the safety assessment presented as it was considered to give only collateral and confirmatory evidence of the safety of the ingredient.

The studies available to the SCCS on *in vivo* micronucleus tests with EHMC are summarised below.

***In vivo* mammalian erythrocytes Micronucleus assay**

Guideline/method:	Similar to OECD Test Guideline 474
Species/strain:	Mice/ Fullinsdorf Albino SPF
Group size:	3/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Dose level:	1000, 2500 and 5000 mg/kg bw
Route:	Oral
Administration:	Gavage
Frequency of Treatment:	30 and 6 hours prior to sacrifice
Post-exposure period:	30 hours
Vehicle:	Rape oil
Positive controls:	Procarbazine hydrochloride administered at 50 mg/kg bw
GLP:	Yes
Study period:	1983

EHMC was investigated in a test similar to OECD Test Guideline 474 study for the induction of micronucleated polychromatic erythrocytes in the bone marrow of male and female mice after two-fold oral dose administration. A preliminary experiment was performed, to select the test substance dose levels for the main micronucleus assay.
Groups of 3 animals/sex/dose level received the test substance at 1000, 2500 or 5000 mg/kg bw by oral gavage. A concurrent control group of 3 mice/sex was dosed similarly with the vehicle only and a positive control group received a single oral gavage administration of Procarbazine hydrochloride at 50 mg/kg bw.
Animals were sacrificed 30 hours of post-exposure to test the substance.

During the in-life period, mortality and clinical signs were assessed. Following necropsy and preparation of bone marrow smears, 2000 polychromatic erythrocytes from each of the male and female animals of every test group were evaluated and investigated for micronuclei. The parameters included number of polychromatic/normochromatic erythrocytes ratio and occurrence of micronuclei.

Results

Test substance did not induce chromosome breaks or mitotic non-disjunctions in mouse bone-marrow cells. There was no test substance related increase in micronuclei in bone marrow polychromatic erythrocytes at any dose level.

Conclusion
Under the conditions of the study, EHMC was negative in the bone marrow micronucleus test in mice.

(ECHA, 2021 NICNAS, 2017)

SCCS comment
According to OECD TG 474, five animals/group should be tested (not only 3) and the proportion of immature erythrocytes (PCE) among total erythrocytes should be presented. No data on the result of the solvent control (rape oil) were provided. Hence, the results are considered of limited reliability.

***In vivo* mammalian erythrocytes Micronucleus assay (NTP)**

Guideline/method:	OECD Test Guideline 474
Species/strain:	Rat/Harlan Sprague Dawley
Group size:	5/sex/group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Dose level:	1000, 3000 and 6000 ppm
Route:	Oral in diet
Administration:	Dosed-Feed
Frequency of Treatment:	16 weeks (number of treatments: 112)
Post-exposure period:	0 hours
Vehicle:	Feed
Positive controls:	/
GLP:	Yes
Study period:	Date Report Requested: 09/23/2018?

Methodology

Blood was sampled and micronuclei polychromatic (PCE) and normochromatic erythrocytes (NCE) were measured using flow cytometry.

Results

Tissue: Blood; Sex: Male; Number of Treatments: 112; Time interval between final treatment and cell sampling: 0 h

	MN PCE/1000			MN NCE/1000			% PCE	
Dose (ppm)	N	Mean ± SEM	p-Value	N	Mean ± SEM	p-Value	Mean ± SEM	p-Value
Vehicle Control[1]	5	0.880 ± 0.227		5	0.045 ± 0.012		0.874 ± 0.046	
1000.0	5	0.830 ± 0.034	0.7795	5	0.029 ± 0.007	0.9133	0.925 ± 0.037	0.4911
3000.0	5	0.738 ± 0.087	1.0000	5	0.027 ± 0.006	0.9568	1.036 ± 0.089	0.1481
6000.0	5	0.480 ± 0.108	1.0000	5	0.015 ± 0.003	0.9690	0.977 ± 0.045	0.1549
Trend p-Value		0.9118			0.9905		0.1755	

Trial Summary: Negative

Tissue: Blood; Sex: Female; Number of Treatments: 112; Time interval between final treatment and cell sampling: 0 h

	MN PCE/1000			MN NCE/1000			% PCE	
Dose (ppm)	N	Mean ± SEM	p-Value	N	Mean ± SEM	p-Value	Mean ± SEM	p-Value
Vehicle Control[1]	5	0.670 ± 0.133		5	0.040 ± 0.009		1.132 ± 0.122	
1000.0	5	0.510 ± 0.073	0.8709	5	0.018 ± 0.004	1.0000	0.954 ± 0.072	0.7850
3000.0	5	0.490 ± 0.033	0.9270	5	0.010 ± 0.001	1.0000	0.778 ± 0.169	0.2615
6000.0	5	0.430 ± 0.082	0.9451	5	0.009 ± 0.002	1.0000	0.913 ± 0.081	0.5443
Trend p-Value		0.9523			0.9998		0.1217	

Trial Summary: Negative

(NTP, 2020)

SCCS comment

The study results indicate no potential of EHMC to induce chromosomal damage in the rat *in vivo* after repeated oral exposure. However, considering that the full protocols of the studies are not available on the website, and the purity of the test item is not provided, the SCCS considers the study of limited reliability.

Overall SCCS comment on genotoxicity/mutagenicity

When submitting additional data on genotoxicity/mutagenicity of EHMC, the Applicant provided study reports which were unavailable to the SCCS. These included reports on testing Uvinul MC80 product in the Ames test and additional photomutagencity tests. All available documents have been analysed by the SCCS and the summary of the analysis is presented in Table 11.

Table 11. Summary of the analysis of in vivo data on genotoxicity/mutagenicity of EHMC available to the SCCS.

Publication/study report	Endpoint	Test organism	Isomer tested Ratio cis/trans	Reliability/relevance Result
ECHA, 2021	*In vivo* mammalian erythrocytes Micronucleus assay –	Mouse, 6 and 30 h post-exposure	NA	Limited reliability, negative
NTP, 2020, #G20239B	*In vivo* mammalian erythrocytes Micronucleus assay	Rat, 14 days of exposure, flow cytometry	NA	Limited reliability, negative

In summary, the results of the Ames tests consistently indicate no gene mutation potential of EHMC. One study on Ames test (Bonin *et al.*, 1982) may indicate a mutagenic hazard, however, the relevance of the study is limited due to unknown purity of the 10 tested samples. One study on mammalian cell gene mutations in Chinese hamster lung fibroblasts was considered not reliable.
One study on chromosomal aberrations on human peripheral blood lymphocytes was considered not reliable. Two studies on chromosomal aberrations on V79 and CHO cells were considered of limited reliability.
Some of the studies on DNA damage, and/or repair (UDS assay), cell transformation and SOS Chromotest and UmuC tests were positive, but the results are regarded as supportive in WoE.

Two *in vivo* mammalian erythrocytes micronucleus tests were both negative, however, given different limitations in methodology and reporting insufficiencies the studies were considered of limited reliability.

Overall, based on the collective view of the available data, the SCCS is of the opinion that EHMC is likely to have no gene mutation potential. However, the currently available evidence is not sufficient to exclude potential to induce chromosomal damage.

3.4.7 Carcinogenicity

Guideline compliant dermal or oral carcinogenicity studies are not available for EHMC. However, EHMC has been evaluated for tumour promotion and tumour protective effects in various dermal photocarcinogenicity studies in mice. The studies are summarised below, in section 3.4.8.3.
As supportive information in the WoE, EHMC was tested and shown to be negative in the initiation protocol in the cell transformation assay on Balb/c fibroblasts (paragraph 3.4.6.1 Mutagenicity / genotoxicity *in vitro*).

SCCS comments
Although there are no indications for carcinogenicity from the available repeated dose studies, EHMC genotoxicity cannot be excluded. Therefore, the SCCS considers that the concerns for carcinogenicity cannot be ruled out.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

1st Study: *In vitro*, OECD Test Guideline 432

Guideline:	OECD Test Guideline 432
Test system:	BALB/c mice fibroblast cell line 3T3 and human keratinocyte cell line (HaCaT)
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	96%
Vehicle:	Ethanol
Exposure duration:	24 hours
Irradiation:	1.7 mW/cm2 for 10 minutes (UVA)
Study period:	2010

EHMC was tested in an *in vitro* phototoxicity test conducted according to OECD Test Guideline 432 both in the presence (Irr+) or in the absence (Irr-) of irradiation (1.7 mW/cm2 for 10 minutes) using BALB/c mice fibroblast cell line 3T3 and human keratinocyte cell line (HaCaT) at 3-690 µM and 3-700 µM concentrations, respectively. Known positive (5-methoxypsoralen, chlorpromazine, and quinine) and negative (acetyl salicylic acid, hexachlorophene, and sodium lauryl sulphate) controls were tested together.

Results
The cytotoxicity of the solvent did not show a statistically significant difference relative to the negative controls, both in the presence or in the absence of irradiation. Negative controls were confirmed to be non-phototoxic in the keratinocytes and 3T3 fibroblasts. The IC50 values of EHMC for HaCaT keratinocytes, were 635.6±47.9 µM (Irr-) and 437.8±129.5 µM (Irr+), with a corresponding Photo Irritation Factor (PIF) value of 1.58±0.45. The IC50 value of EHMC for the 3T3 fibroblasts was calculated to be 606.1±29.5 µM (Irr+) with a corresponding PIF value of >1.15. The positive and negative controls gave the expected responses and fulfilled the requirements for a valid test.

Conclusion
Under the test conditions, EHMC was assessed to be non-phototoxic in the HaCaT (photoirritation factor – 1.58) and 3T3 (photoirritation factor – >1.15) models.

Ref.: Maciel *et al.*, 2019

2nd study

No photoinduced skin reactions were observed in a guinea pig dermal phototoxicity study conducted with EHMC (further study details not available) (study period-1982).

(DSM, 2016)

3rd study

In a guinea pig dermal photosensitization test conducted with Ethylhexyl Methoxy-cinnamate, no photoallergenic skin reactions were reported (no details available) (study period-1982).

(DSM, 2016)

Additional studies published in the scientific literature other than those provided in the initial dossier submitted by Applicants in response to the call from the Commission were identified

by the SCCS during the preparation of this opinion. Therefore, SCCS asked the applicants to update their assessment by including this additional information. These studies have been summarised below with the SCCS comment.

Phototoxicity Test

Guideline:	Draft OECD *in vitro* 3T3NRU photoxicity test, Feb. 2000 EEC 2000/33 (B.41), L 136, 2000
Test system:	Balb/c 3T3 cells
Test substance:	UVINUL MC 80 N
Batch:	UV2-01.019
Purity:	99.9%
Vehicle:	acetone
Concentrations :	up to 100 pg/ml
Exposure duration:	1h
Replicates:	/
Irradiation:	5 J/cm^2 (UVA) for 50 minutes
Positive Control :	Chlorpromazine
GLP:	yes
Study period:	2001

In this study the toxicity of the test substance UVINUL MC 80 N at simultaneous irradiation with artificial sunlight was determined. Cytotoxicity was measured using the Neutral Red (NR) assay and Balb/c 313 cells clone 31.

For the determination of a phototoxic potential the cells were treated with the test substance in the absence and presence of artificial sunlight (wavelength >320 nm) at concentrations up to 100 pg/ml. After 1 h pre-incubation with 8 concentrations of the test substance or the positive control, the cells were irradiated with artificial sunlight for 50 minutes with 1.7 mW/cm^2 UVA, resulting in a radiation dose of 5 J/cm^2 UVA. Parallel cultures were kept in the dark for 50 minutes. The cytotoxic response curves of the test groups were compared. The EC_{50}-values were determined and compared to calculate a photo-irritancy factor (PIF) and to measure a possible phototoxicity.

Results:

In the absence and presence of artificial light the test substance did not induce, up to the highest tested concentration, any strong cytotoxic effects leading to a reduced neutral red uptake below 50% of the negative control. Therefore, the EC_{50} values could not be calculated and 100 µg/ml was used as C_{max} for both the irradiated and non-irradiated cultures. The PIF of the test substance was *1.

Conclusion:
It can be stated that in the study described and under the experimental conditions reported treatment of Balb/c 3T3 cells with UVINUL MC 80 N did not show any phototoxic effects.

(BASF, 2001a)

In human volunteers

1st study

Guideline:	Not specified
Test system:	Human
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified

Vehicle:	Not specified
Exposure duration:	24 hours
Replicates:	Duplicates
Irradiation:	10 J/cm2 (UVA)
Study period:	2010

The photoallergic potential of EHMC was investigated in 10 females and 1 male in duplicates to the back of the patients for 24 hours. Patches containing 7.5% test substance in petrolatum was applied via Finn Chambers. One application site was irradiated with 10 J/cm2 UVA. Immediately after UVA exposure, the UV treated skin sites were examined to determine immediate skin reactions. Following the examination, the patch areas were covered with an opaque tape material and the skin was examined for reaction after 24 hours (day 3), then at 5 to 7 days of exposure.
Results
No skin reactions were seen in any patients at any application site throughout the study.

Conclusion
Under the conditions of the study, there is no indication for a photoallergic potential in male and female patients after EHMC exposure.

(Shaw *et al.*, 2010)

2nd study

Guideline:	Not specified
Test system:	Human
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	Not specified
Exposure duration:	48 hours
Replicates:	Duplicates
Irradiation:	10 J/cm2 (UVA)
Study period:	1994

The photoallergic potential of EHMC was investigated in 62 photosensitive patients in duplicates for 24 hours. Patches containing 2% test substance in petrolatum was applied via Finn Chambers. Patches were removed after 48 hours and patients were assessed for skin reactions. One set of patch sites were irradiated with 10 J/cm2 UVA. The irradiated and non-irradiated skin sites were examined for reactions after 48 hours of exposure. Across all photoallergens, 14 out of 62 patients showed 27 positive reactions (22.6%). Out of the 27 positive reactions, only one photoallergic response was produced by EHMC.
Results
Out of 62 patients, 14 patients showed 27 positive reactions (22.6%). Out of the 27 positive reactions, only one photoallergic response was produced by EHMC.

(Kerr and Ferguson, 2010; Leow *et al.*, 1994)

3rd study

The phototoxic potential of EHMC was investigated in 10 patients for 24 hours. EHMC was tested in the form of patches containing EHMC. The application site was exposed to a sub-erythematous dose of UV irradiation. The skin was examined for the reaction after 24 hours of exposure. No evidence of phototoxicity was reported in the study.
Under the conditions of the study, there is no indication for a phototoxic potential in human subjects after EHMC exposure.

(SCC, 2000)

4th study

Guideline:	Not specified
Test system:	Human
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	Not specified
Replicates:	Duplicates
Exposure duration:	24 and 48 hours
Irradiation:	5 J/cm2 (UVA)
GLP:	Not specified
Study period:	2012

A prospective, multicentre photopatch test study investigated suspected photoallergic contact dermatitis (PACD) in 1031 patients (715 females, 316 males) in 30 centres across 12 European countries. EHMC was tested in the form of patches contained 19 organic UV absorbers, including 10% EHMC in petrolatum and five topical NSAIDs, were applied in duplicates to the back of the patients for 24 or 48 hours. One application site was covered with a UV-impermeable material and the other side was irradiated with 5 J/cm2 UVA. The skin was examined for reaction at five different time points: pre-irradiation, immediately post-irradiation, 24, 48 and 72 hours post-irradiation according to standard scoring systems (grade 0-4). All photopatch test reactions were graded using the International Contact Dermatitis Research Group (ICDRG) grading system. Investigators were asked to assign relevance to any positive reactions whenever possible using the COADEX system.

Results
A total of 346 photoallergic contact dermatitis reactions (PACD) reactions in 200 patients were recorded. There were 7 PACD reactions reported for 10% EHMC in petrolatum. In comparison to PACD, allergic contact dermatitis (ACD) was much less frequent, with a total of 55 reactions recorded in 47 subjects. There were 2 ACD reactions reported for 10% EHMC in petrolatum.

Conclusion
Under the conditions of the study, there were 7 PACD and 2 ACD reactions reported for 10% EHMC in petrolatum in male and female patients.

(Kerr *et al.*, 2012)

SCCS comment
Although, EHMC has been reported as phototoxic and photosensitising in humans (Gonçalo 2021, Kerr 2012), these studies indicate that only a small fraction of the cases can be attributed to EHMC. Therefore, the SCCS considers that the risk of photo induced effects of EHMC can be considered low.

3.4.8.2 Photomutagenicity / photoclastogenicity

Gene mutations

1st study: Bacterial reverse mutation test (Ames test and photomutagenicity) – Uvinul MC80;

Summary by authors of the report:

This study was performed to investigate the potential of UVINUL MC 80 to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test

(experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1537, TA 98, TA 100, and TA 102. These strains were chosen since they tolerate relatively high doses of UV irradiation used to assess the possible photomutagenic potential of sunblockers.

The assay was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate. The test substance was tested at the following concentrations: 33; 100; 333; 1000; 2500; and 5000 µg/plate
No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation.
The plates incubated with the test substance showed normal background growth up to 5000 µg/plate in all strains used.
No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with UVINUL MC 80 at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Conclusion
In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test substance did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, UVINUL MC 80 is considered to be non-mutagenic in this Salmonella typhimurium photomutagenicity assay.

SCCS comment
The study is considered valid with negative results. Four S. typhimurium strains were used (TA1537, TA98, TA100, and TA102).

(BASF, 2001b)

2nd study: Bacterial reverse mutation test (Ames test and photomutagenicity) - Symrise (by Bayer 1995)

A Confidential full study report was provided to the SCCS for Photo-Ames test and photomutagenicity.

SCCS comment
The GLP study is considered valid with negative results. Only two S. typhimurium strains were used (TA 1537 and TA 102).

Chromosomal aberrations

3rd study: Photoclastogenicity in Chinese hamster ovary (CHO) cells

Guideline:	Not specified
Test system:	Chinese hamster ovary (CHO) cells
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	215687, Givaudan
Purity:	98.7%
Vehicle:	DMSO
Test concentrations:	5 to 25 µg/mL
Irradiation:	200 – 2000 mJ/cm^2 UVA and 4 to 25 mJ/cm^2 UVB
GLP:	Compliant
Study period:	1993

EHMC was tested in a photoclastogenicity test to evaluate its clastogenic potential in CHO cells. The CHO cells were exposed to 5 to 25 µg/mL EHMC and radiation 200 – 2000 mJ/cm^2 UVA and 4 to 25 mJ/cm^2 UVB.

Results
The UV irradiation was clastogenic in CHO cells at the top dose, but the EHMC exhibited a protective effect.

Conclusion
Under the study conditions, EHMC was not photoclastogenic in CHO cells.

(Roche 1993, NICNAS, 2017; SCC, 2000)

SCCS comment
The mutagenic activity of the UVB sunscreen Ro 05—8640 (Parsol MCX) was evaluated in the chromosomal aberration test with Chinese Hamster Ovary cells (clone CHO—K5) in two independent experiments. Based on the methodology description, the times of incubations of the cells with test substance and/or UVA/UBV are not clear (most probably it was 10-60 min. of exposure + 18h post-incubation period). Only 100 metaphases were scored for aberrations. Due to these limitations, the SCCS considers the study of limited reliability.

4th study: Chromosomal aberrations photomutagenicity test on V79 cells

Summary by authors of the report:

The test substance Uvinul MC 80 dissolved in DMSO was assessed for its potential to induce structural chromosomal aberrations in V79 Chinese Hamster cells in the absence and presence of artificial sunlight in two independent experiments.
The Atlas Suntest CPS, a xenon burner with an additional special filter glass, emitting visible and UVA/UVB light > 290 nm was used as light source. In this study, the cultures were pre-incubated with the test substance for 30 min. After pre-incubation, the cultures were exposed to 225/8.7 mJ/cm2 UVA/UVB (experiment I), 225/7.8 mJ/cm2 UVA/UVB (experiment II) or 375/12.9 mJ/cm UVA/UVB (experiment II). Three hours after start treatment, the cultures were washed twice. Corresponding cultures with the test substance were kept in the dark for the 3 hrs exposure period. 18 hrs (experiment I) and 28 hrs (experiment II) after start of treatment, the cultures were prepared for cytogenetic evaluation.
In the cytogenetic experiments for each experimental group two parallel cultures were set up. Per culture 100 metaphases were scored for structural chromosome aberrations.
The top dose in the range finding experiment (3000 µg/ml ~10 mM) was chosen with regard to the molecular weight of the test item with respect to the current OECD Guideline 473. The applied concentrations for the cytogenetic experiment were chosen based on the toxicity of the test substance observed in the pre-test.
In the cytogenetic experiments, toxic effects indicated by reduced mitotic indices below 50 % of control were observed in the presence of irradiation after 3 hrs treatment in experiment I only.
In both independent experiments, a statistically significant increase in the number of cells carrying structural chromosomal aberrations was observed, neither in the absence nor in the presence of artificial sunlight. No increase in the frequencies of polyploid metaphases was found after treatment with the test substance as compared to the frequencies of the controls. Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations.

Conclusion
It can be stated that under the experimental conditions reported, the test substance Uvinul MC 80 did not induce structural chromosome aberrations in the absence and presence of artificial sunlight as determined by the chromosomal aberration test in V79 cells (cell line

from the lung of the Chinese Hamster). Therefore, Uvinul MC 80 is considered to be non-photoclastogenic in this chromosomal aberration test.

SCCS comment

The SCCS considers the study of limited reliability because only 100 metaphases were scored for aberrations and low concentrations of EHMC were tested ≤1 µg/mL, at which mitotic index was >82%.

(BASF, 2001c)

Other studies

5th study: Photo-comet assay

Guideline:	Not specified
Test system:	L5178Y cells
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	DMSO
Test concentrations:	500, 625, 1000 and 1250 µg/mL
Irradiation:	600 mJ/cm^2 UV-A and 30 mJ/cm^2 UV-B
Positive control:	Chlorpromazine, 1.5 µg/mL
GLP:	Not specified
Study period:	2007

EHMC was tested to determine the photogenotoxicity in an *in vitro* photo-Comet assay in L5178Y cells. Alamar Blue assay and Trypan Blue test were used for the determination of the cytotoxicity limits in the standard photo comet assay. The L5178Y cells were incubated with the EHMC (500, 625, 1000 and 1250 µg/mL in DMSO) for 20 min and irradiated with simulated sunlight in the wavelength range from 280 to 800 nm. The applied UV dose was 600 mJ/cm^2 UV-A and 30 mJ/cm^2 UV-B. After a post-incubation of 10 min, the Alamar Blue assay as well as the Trypan Blue test and the alkaline comet assay were performed.

Results

Based on the cell viability test results (100% cell viability), the EHMC was not considered to be cytotoxic with or without UV irradiation. Positive control (1.5 µg/mL chlorpromazine) increased the tail moment of the cells in all experiments more than three-fold compared with the solvent control. The EHMC did not induce a significant change of the tail moment at any of the concentrations tested, either with or without irradiation.

Conclusion

Under the study conditions, the EHMC was neither cytotoxic nor genotoxic with or without UV–vis irradiation.

(Struwe *et al.*, 2007)

SCCS comment

The results of the study indicate no DNA damaging effect of EHMC in the absence of presence of UVA/UVB irradiation. The results are treated as supportive in the WoE.

6th study: Photomutagenicity in *Saccharomyces cerevisiae*

Guideline:	Not specified
Test system:	Saccharomyces cerevisiae
Test substance:	Ethylhexyl Methoxycinnamate

Batch:	Not specified
Purity:	Not specified
Vehicle:	DMSO
Test concentrations:	0.06 to 625 µg/mL
Irradiation:	50 J/cm² (50000 mJ/cm^2) UVA and 1.2 J/cm² (1200 mJ/cm^2) UVB
GLP:	Not specified
Study period:	Not specified

EHMC was tested in a photomutagenicity test to evaluate its mutagenic potential in *Saccharomyces cerevisiae*. The cells of *Saccharomyces cerevisiae* were exposed to 0.06 to 625 µg/mL EHMC dissolved in DMSO and radiation up to 50 J/cm^2 (50000 mJ/cm^2) UVA and controls were also employed.

Results

EHMC did not show any mutagenic activity. UVA and UVB (more markedly) were mutagenic.

Conclusion

Under the conditions of the study, EHMC was not photomutagenic for *S. cerevisiae*.

(NICNAS, 2017; SCC, 2000)

SCCS comment

The results of the study indicate no DNA damaging effect of EHMC in the absence of presence of UVA/UVB irradiation. The results are treated as supportive in the WoE.

Overall SCCS comment on photogenotoxicity/photomutagenicity

EHMC was tested in 2 bacterial photomutagenicity tests with negative results, however, the studies do not cover for all test strains required by OECD TG 471.
The two chromosomal aberration photomutagenicity tests, one on V79 cells and the other on CHO cells, were considered negative, however of limited reliability.
EHMC was tested in one Comet assay on L5178Y cells with negative result and one photomutagenicity test on *Saccharomyces cerevisiae* with negative result. Both tests are regarded as supportive in WoE.

Overall, the available evidence is not sufficient for excluding photomutagenic effect of EHMC.

3.4.8.3 Photocarcinogenicity

1st Study

Guideline:	Not available
Species/strain:	Mice/ HRA/Skh
Group size:	5 /males/ group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	Ethanol
Dose levels:	50% v/v Ethylhexyl Methoxycinnamate
Dose volume:	Not specified
Route:	Dermal
Administration:	Topical
Duration:	200-300 days
Irradiation	UV A and UV B
GLP:	Not specified
Study period:	Not specified but pre 1984

EHMC was evaluated for its tumour initiating potential in a dermal UV carcinogenicity study in hairless mice. In Experiment 1, groups of 20-22 HRA/Skh mice were painted daily for 9 weeks on the dorsum with 50% v/v EHMC in ethanol followed by exposure to one of the artificial UV lights sources, treated with EHMC or UV light alone.

The mice were examined for skin pathology and tumour production until day 200 from the study start. Representative tumours from affected mice were excised for histological classification and comparison with normal epidermis. Subsequently, in Experiment 2, all of the surviving EHMC protected UV-irradiated mice from Experiment 1, together with the mice treated with EHMC alone and a group of previously untreated mice, were treated over 8 weeks (2x per week) to the dorsal skin with 0.05% croton oil in acetone for 4 weeks commencing at day 200.

Results

Tumours began appearing on the unprotected UV-irradiated mice 19 days after completion of the treatment regime. Histologically, a variety of benign and malignant tumours were identified. No signs of an erythemal response were seen at any time. Although EHMC was applied only to the dorsum posterior to the shoulders, no erythema of the ears, head or neck were observed.

EHMC also gave protection against the development of skin tumours. Only 4 mice of the 160 treated with EHMC and UV irradiation produced tumours within the 200 days of Experiment 1. Following the eight bi-weekly applications of croton oil to EHMC treated mice, tumours began to appear immediately and continued to do so until the animals were sacrificed at day 300. Mice developed multiple tumours including pre-malignant, especially on UV 1 exposed mice. Croton oil did not promote any tumours on previously untreated control mice.
However, tumours were promoted on 3 of the 16 surviving mice previously treated with the EHMC alone. Statistical analysis showed that the promotion of these tumours was significant when compared with the previously untreated croton oil mice which did not respond. Exposure of EHMC protected mice to UV seems, by inspection of the tumour incidence, to have initiated tumours on more mice than either EHMC alone. However, statistical analysis did not reveal any significant difference between either EHMC alone or with UV.

Conclusion

Overall, EHMC-treated mice were protected against gross pathology and histopathology from the repeated sub-erythemal or erythemal doses of UV but subsequent treatment with the tumour promoter croton oil produced tumours on a significant number of animals. Statistical analysis of the incidence of promoted tumours indicated that prior UV irradiation may not have been responsible and indicated that EHMC may initiate tumours in this strain of mice. However, limitations in the experimental conditions applied in this study, e.g., lack of appropriate controls and insufficient quality of EHMC samples, hampered reliable and robust data interpretation

(Gallagher *et al.*, 1984; IARC, 1992)

2d study

Guideline:	Not available
Species/strain:	Mice
Group size:	5 /males/ group
Test substance:	Ethylhexyl Methoxycinnamate
Batch:	Not specified
Purity:	Not specified
Vehicle:	Sunscreen preparation
Dose levels:	5 or 10% Ethylhexyl Methoxycinnamate

Dose volume:	Not specified
Route:	Dermal
Administration:	Topical
Duration:	40 weeks; 5 days/week
Irradiation:	UV A and UV B
GLP:	Not specified
Study period:	Not specified but pre 1996

In a photocarcinogenicity study, mice were exposed to UV radiation (UVR) 5 days/week for 40 weeks. Two different weekly doses of 960 (high dose) and 480 (low dose) mJ/cm^2 of UV B per week were given. Two control groups were irradiated without topical application. Two groups received a topical application of either 5 or 10% EHMC in an oil in water emulsion ('sunscreen preparation') on a skin surface of approximately 40 cm^2. The sunscreen was applied 30 minutes prior to UV exposure 3 days per week and 30 minutes after UV exposure for two further days, consistent with the design of a standard photo-carcinogenesis study. Animals were examined for tumours by accepted morphological criteria. One chart was established for each animal to record (narrative and drawing) the number and size of all tumours. In addition, body weights were recorded weekly and a viability check was performed twice a day. The two UVR control groups demonstrated a UVR-dependent response for cumulative tumour prevalence, tumour yield and median latent period.

Results

Neither concentration of EHMC increased the probability of tumour development. Topical application of EHMC at both concentrations resulted in a 6-week delay in the median latent period compared to high UVR controls. Tumour protection factors were calculated from the results and to be equal to 2.4 for the two preparations containing EHMC.

Conclusion

Under the conditions of the study, the study investigator concluded that the study provides evidence that EHMC is safe for use in sunlight.

(Fourtanier, 1996; NICNAS, 2017)

3rd study

A study was conducted to determine the inhibition of UV-induced tumours by EHMC in mice (species details not provided). Hairless mice were exposed to doses of the UV stimulating solar energy spectrum (duration and radiation not stated). After a rest period (duration not stated), tumour promotor 12-O-tetradecanoyl phorbol-13-acetate, was applied to the skin 3 times per week. Suitable controls were used. The treated mice were observed to be completely protected by, 50% EHMC, and 7.5% EHMC was observed to be equivalent to reducing the solar exposure four-fold. So EHMC showed protection from UV induced tumours. There was no evidence of the chemical being a promoter of carcinogenicity. No other study details are available.

(NICNAS, 2017; SCC, 2000)

SCCS comments

Although there are no indications of EHMC (photo)mutagencity from the available repeated dose studies on the photocarcinogenicity of EHMC, EHMC (photo)mutagenicity cannot be excluded. Therefore, the SCCS considers that the evidence for excluding photocarcinogenicity of EHMC is not sufficient.

3.4.9 Human data

Human biomonitoring

EHMC was examined in Chinese students for the formation and excretion of the test substance and its metabolites in urine using an ultrahigh performance liquid chromatography (UHPLC) system hyphenated with Agilent 6540 series quadrupole-time of flight mass spectrometry (Q-TOF-MS) and to understand the potential influential demographic factors. In total 108 urine samples were collected from Chinese children and adolescents, aged 6 to 18, from a suburban district in Shanghai, which nested in the cohort of the national Puberty Timing and Health Effects in Chinese Children (PTHEC). This method included anthropometric measurement, sexual maturation assessment and a questionnaire.
interview.

EHMC, 4-methoxycinnamic acid (4-MCA) and 4' methoxy acetophenone (4'-MAP) were found in 50.9%, 66.7%, and 91.7% of urine samples, respectively. The detected concentration ranges were highest for 4-MCA, namely, up to 41.14 ng/mL. 4'-MAP was detected with the median concentration of 2.74 ng/mL, ranging from below LOD to 27.19 ng/mL. EHMC showed both the lowest detection rate and the lowest urinary concentration, namely, with the highest concentration as 19.21 ng/mL.

Table 12: Concentration of EHMC and two of its metabolites in the urine samples of participants in a pilot study (n = 108). Corrected by specific gravity.

n = 108	>LOD	>LOQ	Minimum (ng/mL)	Percentile (ng/mL)			Maximum (ng/mL)
				25th	50th	75th	
4'-MAP	91.7%	51.9%	LOD	LOD	2.74	7.87	27.19
4-MCA	66.7%	31.5%	LOD	LOD	LOQ	7.35	41.14
EHMC	50.9%	8.3%	LOD	LOD	LOD	LOQ	19.21

Overall, quantitative results revealed that their excretion concentrations were much higher than the parent compound. The results indicated wide exposure to EHMC, 4-MCA and 4'-MAP. The correlation between the urinary concentration of EHMC and 4-MCA as well as 4-MCA and 4'- MAP provided important clues as to the sources and metabolic pathways among these three compounds.
Among EHMC and its two metabolites, significantly unequal distribution of 4-MCA concentration was observed on family's social and economic status, with slightly higher geometric means on lower education and economics.

Under the conditions of the study, significant correlations were found between the urinary concentration of EHMC and 4-MCA as well as 4-MCA and 4'-MAP for both genders. Also, levels of EHMC were found to be positively associated with age while 4-MCA negatively related to the father's education level and family economics.

(Huang *et al.*, 2020)

3.4.10 Special investigations

Endocrine disruption properties

***In vitro* studies**

Gomez *et al.*, 2005

Estrogenic effects of three classes of substances included in cosmetic formulations parabens, ultraviolet (UV) screens, and musk fragrances—were studied. Their estrogenic activity was measured using three reporter cell lines: HELN, HELN ERα, and HELN ERβ. These three cell lines allowed for the measurement of estrogenic activity toward estrogen receptors α and β (ERα and ERβ), while taking non-specific interactions into account. Eight of the 15 substances tested showed specific estrogenic activity with the following degree of potency on ERα: butylparaben > propylparaben > homosalate = octyl-dimethyl-PABA = 4-methyl-benzylidenecamphor = **octyl-methoxycinnamate** (OMC) > ethylparaben = galaxolide.

Among these active substances, parabens activated ERα and ERβ similarly, UV screens activated ERα moderately and had almost no effect on ERβ, and fragrances did not activate ERβ. OMC activated ERα at concentration higher than 10^{-6}M (1µM).

Schlumpf *et al.* 2001

The authors performed the E-SCREEN assay as recommended by Soto and co-workers, who developed the assay on MCF-7 cells. Cell proliferation was dose-dependently increased by most of the UV screens, tested including OMC, with a bell-shaped dose–response curve, with a maximum effect at around 10 µM and a EC50 of 2.37 µM. According to their maximum effects on cell proliferation in relation to the positive control E2, OMC acted as partial agonist on estrogen receptor.

Ma *et al.* 2003

The study focuses on potential actions on androgen receptors (AR) in the human breast carcinoma cell line MDA-kb2, which expresses functional endogenous androgen receptor (AR) and glucocorticoid receptors (GR). The cell line is stably transfected with a luciferase reporter plasmid coupled to the receptors, meaning that compounds acting through AR or GR can therefore induce luciferase expression. These cells were used for screening of several UV filters: benzophenone–3 (Bp-3), benzophenone–4, 3-benzylidene camphor, 4-methylbenzylidene camphor, butyl-methoxy-dibenzoylmethane, homosalate (HMS), octyldimethyl-PABA, and octyl-methoxycinnamate. OMC, tested from 1 nM to 10 µM, exhibited neither androgenic activity nor anti-androgenic activity (when tested in co-exposure with 0.1 or 0.5 nM dihydrotestosterone).

Morohoshi *et al.* 2005

In this study, 37 chemicals including OMC were selected based on their usage in sunscreen lotions (and not from their structure) and were evaluated for their estrogenic activities using an enzyme-linked immunosorbent assay (ELISA)-based estrogen receptor competitive binding assay (ER-ELISA), and a modified yeast two-hybrid-estrogen assay. In addition, the authors reported the results of a two-hybrid assay to detect the estrogen antagonistic activity of the compounds. Both two-hybrid-estrogen assays were conducted with and without treatment with a rat liver S9 mix preparation to better understand the effects of possible mammalian metabolic activation/deactivation of the compounds. No estrogenic activity for

OMC was detected in either ER-ELISA or yeast two-hybrid assay, which is in contradiction with other authors, probably because the concentrations tested in this study are lower compared to the other papers testing the endocrine disruptor activity of OMC (37.5 µM for ER-ELISA and 10 µM for the yeast two-hybrid assay).

Schreurs *et al.*, 2002

A sensitive *in vitro* reporter gene assay was used to assess the (anti-)estrogenic activity of OMC in the stably transfected HEK293 reporter cells (ERα and ER β). OMC (10^{-7} to 10^{-4}M) did induce neither estrogenic activity towards ERα and ERβ, nor antagonistic effect towards ERα and ERβ.

Schreurs *et al.*, 2004

The authors used 1) the 293HEK cells, stably transfected with either hERa or hERb (estrognicity and anti-estrogenicity testing), and a 3xERE-tata-Luc-reporter gene construct, 2) the AR Calux® assay on U2-OS cells, that stably contain a 3xARE-TATA-Luc-reporter construct in combination with a hAR expression plasmid (for androgenicity and anti-androgenicity) and 3) the PR-calux® assay on U2-OS cells containing a 3xPRE-TATA-Luc-reporter). They showed weak ERa agonism (dose-response curve of EHMC (10^{-7}, 10^{-6} and 10^{-5}M) on hERa reaching its plateau level at 42%, no EC50 calculated), but potent PR antagonism induced by OMC (IC50 = 0.5 µM).

Strajhar *et al.*, 2017

This study is based on the validated OECD test guideline 456 based on human adrenal H295R cells that promotes measurement of testosterone and estradiol production as read-out to identify potential endocrine disrupting chemicals. The authors demonstrated that steroid profile changes induced by 10 µM OMC with increased corticosteroids could be explained by elevated expression of CYP11B2 and 3bHSD2 mRNA levels. This suggests that OMC, among other, does not directly modulate the activity of these enzymes but rather alter their expression levels.

***In vivo* studies**

NTP 2021 report

In the report are summarized the studies and conclusions on the modified one-generation study of 2-Ethylhexyl p-Methoxycinnamate (CASRN 5466-77-3) administered in feed to Sprague Dawley rats with prenatal, reproductive performance, and subchronic assessments in F1 Offspring (see also section….). The scope of EHMC studies includes the assessment of potential endocrine activity as outlined in the U.S. EPA Endocrine Disruptor Screening Program Tier 1 studies (estrogen- and androgen-receptor binding and activation, Hershberger and uterotrophic assays, aromatase inhibition, and steroid synthesis inhibition), metabolism and disposition following oral gavage and dermal exposure, and characterization of the potential effects of continuous EHMC exposure over multiple generations using the NTP modified one-generation study design.
In this study, exposure to EHMC in feed began on gestation day (GD) 6. At weaning, 1 and 2 pups/sex/litter were allocated to prenatal and reproductive performance cohorts, respectively; one pup/sex from 10 litters was allocated to the subchronic cohort and an additional one pup/sex/litter was allocated to the biological sampling cohort. In addition to an assessment of reproductive performance, F2 fetal outcomes (GD 21 fetal examinations) were assessed in the prenatal cohort, the potential effects on parturition and early growth of the

F2 generation were assessed in the reproductive performance cohort, and the potential effects on adult F1 organ systems were evaluated in the subchronic cohort. Apical indicators sensitive to endocrine modulation were measured.
A diet low in phytoestrogen was chosen and exposure concentration through the diet was 1000, 3000 and 6000 ppm. Mechanistic screening studies have indicated that EHMC is capable of transactivation of the estrogen receptor (ER), inducing uterotrophic responses, and attenuating progesterone receptor transactivation. EHMC exposure did not appear to induce any substantial effects on androgen receptor (AR)-dependent endpoints. Although F1 male rats exposed to 6,000 ppm displayed a slight but significant delay in attainment of balanopreputial separation (when adjusted for body weight on postnatal day 28) and F1 male rats in the subchronic cohort displayed a slight but significant decrease in absolute ventral prostate gland weight, no concomitant effects were observed in anogenital distance or male areolae/nipple retention in F1 or F2 male rats.
No malformations in AR-dependent tissues or histopathological findings consistent with alterations in androgen action or apparent effects of EHMC exposure on F1 male reproductive performance in either mating cohort. This indicates a normal functioning male reproductive system. The absence of reproductive effects in male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats in the current study are inconsistent with previously reported decreased sperm counts in Wistar Han rats following gestational and lactational EHMC exposure (NTP, 2020). The different study results could reflect different sensitivities of the two rat strains or the different dosing paradigms (gavage *vs* dietary).

Schlumpf et al 2001

After administration in powdered feed for 4 days, OMC (and 2 other UV filters 4-MBC and Bp-3) and the positive control, ethinylestradiol, elicited dose-dependent increases in uterine weight of immature Long Evans rats. The rank order of potency, 4-MBC > OMC > Bp-3 at a dose of 119 mg/kg bw/day and an ED50 of 309 mg/kg bw/day.

Comment: **The *in vitro* (proliferation) and *in vivo* dose–response curves of OMC suggest partial agonism.**

Danish EPA report from 2012

DNEL of 1667 µg/kg bw/d is based on a LOAEL of 500 mg/kg bw/d for changed sex hormone levels and reduced sperm count in offspring dosed during fetal development and in the postnatal period (Axelstad *et al.*, 2011). Furthermore, at higher doses the substance induces increased uterine weight, changed uterine weight and histology, and changed gene expression in uterus in screening studies for estrogenic effect (Klammer *et al.*, 2005; Seidlova-Wuttke *et al.*, 2006). Estrogenic receptor activity has also been observed in cell-based studies (Seidlova-Wuttke *et al.*, 2006).
DNEL of 1000 µg/kg bw/day is based on a NOAEL of 100 mg/kg bw/day in a study showing a decrease in T4 level in male rats dosed by gavage for 5 days (Klammer *et al.*, 2007). Other rat studies show a corresponding effect on T4 levels after OMC dosing of pregnant (Axelstad 2011) and ovariectomized female rats, respectively (Seidlova-Wuttke *et al.*, 2006). Furthermore, OMC has been shown to affect the deiodinase enzyme activity in the liver. This mechanism is one of the ways in which thyroid disrupting chemical substances may affect the thyroid hormone system. The data showing endocrine disrupting effects on both the reproduction system and the thyroid hormone system of OMC is considered to be robust.

Schmultzer *et al.*, 2004

Ovariectomized female rats were treated for 12 weeks by oral application of octyl-methoxycinnamate (OMC) by specially prepared rat chow (*n* = 8–11 animals per group) at 2.5 (low) or 12.5 g/kg bw (high). Diet was carefully taken into consideration and food containing or completely free from soy was also compared in this experiment. The increase of malic enzyme, a well-characterized endpoint of thyroid hormone action, caused by high and low concentrations of OMC, by soy combined with E2 in the kidney and by soy in the liver as compared to the respective untreated controls were significant. Hepatic 5'deiodinase expression is known to be stimulated by T3 and high carbohydrate diet. In this study, it was significantly reduced by OMC at both doses. T4 was decreased in the low doses OMC as well as in all soy-fed animals. Nevertheless, the authors concluded that there was no consistent pattern in the effects of the substances used, and each compound, including OMC elicited its own spectrum of alterations, arguing for multiple targets of interference with the complex network of thyroid hormone action and metabolism.

Seidlova-Wuttke *et al.* 2006

Female Sprague–Dawley rats were allocated in group of 11 ovariectomized (ovx) animals. Immediately following ovariectomy, rats were substituted with E2-, OMC- or 4MBC-containing food, while control rats received soy-free pelleted food only. OMC doses were 57.5 mg per 20 mg of food intake for the low dose or 275 mg for the high dose tested. OMC stimulated uterine weight only slightly at the higher dose. The thickness of the whole endometrium and of the endometrial epithelium was slightly increased while endometrial thickness was slightly reduced, and myometrial thickness remained unaltered. Slight effects on the 3 estrogen-regulated genes in the uterus (PR, IGF1 and ERh) were observed: OMC stimulated thickness of the epithelium and IGF1 and PR gene expression slightly, which is clearly an estrogenic effect.

Klammer *et al.* 2005

This pharmacodynamic study was performed to quantify the multi-organic estrogenic effects of OMC on various estrogen modulated endpoints and to assess no-risk threshold value for the most sensitive parameter, followed by an extrapolation to humans via the acceptable daily intake value and the margin of safety value. Ovariectomized female offspring of Sprague–Dawley rats was maintained on soy-free food, water *ad libitum*. Seventeen days after surgery, animals (N=12) were treated orally per gavage once per day (between 5:30 and 6:30 a.m.) for 5 days with 1 mL containing either pure olive oil (control), 600 µg/kg bw estradiol-valerate (E2) or 10, 33, 100, 333 and 1000 mg OMC /kg. The uterine weight increased significantly upon E2, as well as upon OMC treatment. The expression level of ER gene, which is significantly decreased under E2 treatment, is up regulated under OMC treatment. Metabolic effects were also observed: OMC application resulted in a decrease in IGF1 gene expression, cholesterol and LDL serum levels, as well as triglyceride serum levels. Leptin and HDL serum levels remained unaffected. No significant differences were seen in the glucose serum levels. Except for the uterine ER gene expression, where a hill model was used, all parameters were fitted using the power model. The BMD values derived from the fitted models range from 11.0 (uterus, ER expression) to 914.0 mg/kg bodyweight per day (serum cholesterol levels). Depending on the parameter, the endocrine activity of OMC was estrogenic (uterine weight, C3 expression, TERP1 expression, IGF1 expression, cholesterol and LDL serum levels) while other parameters such as leptin and HDL serum levels remained unaffected by OMC treatment in contrast to E2 treatment. OMC must be considered as a selective estrogen receptor modulator and not a "pure" estrogen.

Lorigo *et al.*, 2018

The table underneath gives an overview with the conclusions of the systematic review performed by the authors of the effects observed after OMC exposure.

Endocrine activity	**Effects observed**
Estrogenicity	**Effects**
	No estrogenic activity in zebrafish
	↑ uterine weight, in immature Long Evans and ovariectomized Sprague-Dawley female rats
	↑ endometrial thickness and uterine myometrial, and uterine and vaginal epithelial thickness
	↑ in PR and IGF-1 expression levels in the uterus and vagina
	No changes in bone density, but ↓ levels of osteocalcin (OMC at the highest dose)
	↑ serum concentrations (LH), in ovariectomized Sprague-Dawley female rats
	↑ C3 and TERP1 expression levels in the uterus and pituitary, respectively; ↓ triglyceride, serum cholesterol, and LDL levels; No changes in serum levels of leptin and HDL, in ovariectomized Sprague-Dawley female rats
	↓ IGF-1 expression levels in the liver; ↑ expression ERβ;
	↓ (significant) body weight and adipose tissue deposits, ↓ triglyceride levels, ↓ serum cholesterol, leptin, HDL and LDL levels, in ovariectomized Sprague-Dawley female rats
	↑ plasma concentration of vitellogenin (VTG) Change vitellogenin and choriogenin mRNA expression, and ERα, in the liver of medaka fish
	↑ ecdysone receptor (EcR) and heat shock protein 70 (hsp70) genes expression levels
Anti-androgenic activity	↓ the serum Testosterone levels in immature offspring rats
	Earlier reproductive senescence in the female offspring
	In male offspring, ↓ epididymal sperm count, and ↑ prostate atypical hyperplasia

	In both sexes, ↑ incidence of pituitary tumors, in developmental rats
Anti-progestenic activity	↑ PR transcription in the uterus and vagina, in ovariectomized Sprague-Dawley female rats (3 mouths)
	↓ Concentration (progesterone) in plasma, in Wistar rats in developing
Anti-thyroid activity	Change T3 and TSH levels; ↓ T4 levels; ↓ activity Dio1 in the liver, in ovariectomized Sprague-Dawley female rats
	No change T3 and TSH levels, but ↓ T4 levels, in ovariectomized Sprague-Dawley female rats
	↓ activity Dio1 in the kidney and liver, in ovariectomized Sprague-Dawley female rats
	No changes in pro-TRH expression; ↓ (dose-dependent) T3, T4, and TSH levels
	No changes NIS and TPO expression levels
	↓ activity Dio1 in the liver, in ovariectomized Sprague-Dawley female rats
	↓ T4 levels in pregnant female rats and young male offspring (No effects of female offspring)
	↑ thyroid weight in young rats of both sexes, in Wistar rats in developing

Schmutzler *et al.*, 2004

To assess the effect of OMC on thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney, female Sprague Dawley rats (n = 8–11 animals per group) were ovariectomised at 14 weeks of age and treated afterwards for 12 weeks by oral application in a specially prepared rat chow containing OMC, 2.5 (low) or 12.5 g/kg (high) and 17β-estradiol benzoate (E2, 34.2 mg/kg) as a positive control. Food containing or completely free from soy was also compared in this experiment as soy and, especially, its flavonoid compound genistein have been shown to have major impact on thyroid function. OMC surprisingly did not inhibit TPO *in vitro*, but reduced T4 levels although estrogenic properties are known for this compound. This interference with the thyroid axis needs to be shown in further experiments. The malic enzyme activity in the liver was slightly higher at the high dose of OMC. The increase caused by low concentrations of OMC, by soy combined with E2 in the kidney and by soy in the liver as compared to the respective untreated controls were significant.
Deiodase activity was decreased by OMC (both doses) alone and in combination with soy-containing food. No effect of OMC was observed on TPO activity. T4 was decreased in the low doses OMC-treated group, as well as in all soy-fed animals. OMC here does not clearly act as an estrogenic agonist in this context.

Schreurs *et al.*, 2002

In this study combining *in vitro* (see above) and *in vivo* experiments, the authors performed experiments using zebrafish, in which an estrogen responsive luciferase reporter gene has been stably introduced. In this transgenic zebrafish assay, none of the tested compounds, including OMC at 10 µM showed estrogenic activity.

Szwarcfarb *et al.*, 2008

The authors have studied the *in vitro* effects on the hypothalamic release of LHRH as well as as well as on the amino acid neurotransmitter system in immature rats of 15 (prepubertal) and 30 (peripubertal) days of age. A stock solution of OMC in ethanol was diluted in the cell medium at the final concentration of 2.63×10^{-7}M, so that ethanol did not exceed 0.001% v/v. OMC decreased the LH-RH release significantly in male and female rats of both age. In male rats, OMC diminished the excitatory amino acid aspartate (ASP) and Glutamate (GLU) without modifications in the hypothalamic GABA release while it increased the release of GABA in females. These results suggest that the inhibitory effect of OMC on LHRH release appears to be related to its action on the inhibitory and excitatory amino acid neurotransmitters in male and female rats during sexual maturation.

Human studies

Huang *et al.*, 2020

This study included 521 elementary and high school students from a suburban area of Shanghai, with one step done in October to November 2011, and the follow-up study in April to May 2013. Twelve urinary organic UV filters were quantified. The pubertal development was assessed at each study period by trained physicians using Tanner staging. EHMC and its metabolite 4'-methoxyacetophenone (4'-MAP), benzophenone 2 and 3 (BP-2, BP-3) and Ethylhexyl dimethyl PABA (OD-PABA) were the most extensively detected UV filters in urine. EHMC and its metabolite were negatively correlated with stages of testicular volume and genital development. EHMC was associated with later pubertal onset of pubic hair and testicular volumes in boy.

SCCS comment
The available evidence suggests that OMC is likely an endocrine disruptor, as it can alter normal functioning of the exposed organisms. Specifically, it has been shown that OMC has an estrogenic, anti-progesterone in rats and in human cells, and an anti-androgenic activity has been observed in rats.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

Having considered the data provided, and the concerns relating to potential endocrine disrupting properties of EHMC, the SCCS is of the view that assessment of safety of EHMC cannot be carried out because the genotoxicity potential cannot be excluded on the basis of the available evidence.

3.6 DISCUSSION

EHMC is approved to be used as a UV filter at concentrations of up to 10% in cosmetic products alone or in combination with other UV filters. EHMC may also be incorporated in cosmetic products for formula protection purposes and therefore it is used in several kinds of product types, such as but not limited to lotions, creams, sprays, and lip products.

Physicochemical properties

2-Ethylhexylp-methoxycinnamate (EHMC; CASRN 5466-77-3) is a mixture of cis- and trans-isomers, with the trans-isomer (CASRN 83834-59-7) predominating. EHMC is a colorless to light-yellow viscous liquid that is relatively insoluble in water (0.04 mg/L at 24ºC, pH 7.1) and is readily soluble in most organic solvents.2; 3 EHMC absorbs ultraviolet (UV) A (320–400 nm) and UVB (290–320 nm) light and is photostable.

The Applicant reports that cis-EHMC is considered as an impurity in EHMC, (at levels < 0.5%).

Additional confident data on the purity and impurities of EHMC have been submitted to the SCCS. These data indicate that, in 7 batches, the trans-EHMC purity was greater than 98.4%. The cis-EHMC content ranged from 0.11% to 0.23%, with two other organic impurities present at levels ≤ 0.07%, and iso-octanol levels at ≤ 0.01%.

in 3 additional batches from another notifier, any individual impurity was reported to be ≤ 0.5%, with total impurities being ≤ 1.0%. The cis-EHMC content was ≤ 0.5%, 2-ethylhexanol was ≤ 5 ppm, 4,4-dimethoxystilbene was ≤ 900 ppm, aubeprine p-cresol was ≤ 30 ppm, and 2-ethylhexylacetate was ≤ 5 ppm. The 3-methyl-OMC (sum of isomers) was ≤ 0.4%.

According to Applicant's certificate of analysis, data on heavy metal impurities analysed by ICP-MS in these 3 batches show that heavy metal impurities (lead, cobalt, chrome, nickel, arsenic and antimony) are ≤ 1.0 ppm, while cadmium and mercury are ≤ 0.5 ppm and ≤ 0.1 ppm, respectively.

SCCS has also checked that at these levels these impurities are not of concern as they are below the TTC thresholds and do not trigger any specific genotoxicity alerts.

Exposure

Dermal/percutaneous absorption

A GLP-OECD compliant *in vitro* dermal absorption study, meeting the SCCS Notes of Guidance (2021) criteria was provided and considered scientifically acceptable. Following topical application of 10% [^{14}C]-Ethylhexyl Methoxycinnamate in a representative O/W cosmetic formulation to human skin *in vitro* reveals a mean dermal absorption of 0.28 + 0.17% = 0.45% (mean + 1 SD) of the applied dose after 24 hours exposure.

Toxicokinetic

The metabolism, distribution and excretion of EHMC was investigated *in vitro* in rat and human liver microsomes and rat, mouse and human hepatocytes. Overall, EHMC is extensively

metabolised to a range of metabolites. It was shown to be slowly hydrolysed to 4-methoxycinnamic acid and 2-ethyl-hexanol but also oxidised and demethylated and combinations thereof.

The i*n vivo* studies in rodents proposed a metabolic pathway indicating EHMC to be absorbed and metabolised rapidly and enzymatically converted to a range of metabolites. Based on these results, EHMC is considered as extensively absorbed by oral route and therefore no correction factor should be applied in the MoS calculation to adjust an oral Point of Departure (POD).

Systemic Exposure

The systemic exposure dose (SED) for EHMC used as a UV filter in cosmetic products is calculated by multiplying the consumer's external sunscreen product exposure with the percentage of EHMC being dermally absorbed from the sunscreen (Table 6).

SEDs are also calculated for inhalation (Table 7) and oral exposure to product types containing 10% EHMC separately and as aggregate exposure (Table 8).

Aggregate exposure or total systemic exposure was calculated by adding up the exposures from the dermal (non-spray or spray product), inhalation (spray product) and oral (lip product) routes of exposure (Table 8).

Toxicological Evaluation

Irritation and corrosivity

Under the experimental conditions reported, EHMC is considered slightly irritant to the skin.
Under the experimental conditions reported, EHMC is considered not to be irritant to the eyes.

Skin sensitisation

The SCCS considers the HRIPT studies to be unethical.

The HRIPT and LLNA indicate absence of sensitisation potential. The MEST is considered outdated. In the open literature, sensitisation in humans is rarely reported, often in conjunction with photosensitisation (see 3.4.8 Photo-induced toxicity). The SCCS considers the concern for skin sensitisation as negligible.

Acute toxicity

EHMC is of slight acute toxicity by any routes.

Repeated dose toxicity

The oral repeated dose toxicity of EHMC has been investigated in rats in a standard 90-days oral dosing study at doses of up to 1000 mg/kg day and in a nonstandard 35-days oral dosing study employing a single dose of 1000 mg/kg/day. In addition, two standard repeated dermal application studies are available (one each in the rat and rabbit). No studies are available for the inhalation route.

The liver was found to be the principal target organ, following repeated oral dosing for 13 weeks with decreased hepatocyte glycogen content, accompanied by the shrinkage of hepatocytes in some males and females at the top dose. The NOAEL of 450 mg/kg bw/day can be used as a PoD for systemic effects after repeated oral exposure.

Reproductive Toxicity

There is no evidence of reproductive toxicity of EHMC in Hsd:Sprague Dawley® SD® rats at exposure concentrations of 1,000, 3,000, or 6,000 ppm. Under the conditions of a modified one-generation (, there is equivocal evidence of developmental toxicity of EHMC in Hsd:Sprague Dawley® SD® rats based on the observed postnatal effects on body weight that showed some indication of recovery by study end, delays in postnatal day 28 adjusted vaginal opening and balanopreputial separation, which could have been influenced by the apparent transient effects on body weight, and time in estrus was slightly longer in EHMC-exposed females relative to that of the control group. No other signals consistent with alterations in estrogenic, androgenic, or antiandrogenic action were observed. EHMC exposure did not induce any specific fetal malformations.

Based on the two-generation reproductive toxicity study, a NOAEL of 450 mg/kg bw/day (male/female) can be derived for systemic parental toxicity (P0/F1) and also for offspring toxicity (F1/F2) based on effects on pup weights.

genotoxicity/mutagenicity

The results of the Ames tests consistently indicate no gene mutation potential of EHMC. One study on Ames test (Bonin *et al.*, 1982) may indicate a mutagenic hazard, however, the relevance of the study is limited due to unknown purity of the 10 tested samples. One study on mammalian cell gene mutations in Chinese hamster lung fibroblasts was considered not reliable.
One study on chromosomal aberrations on human peripheral blood lymphocytes was considered not reliable. Two studies on chromosomal aberrations on V79 and CHO cells were considered of limited reliability.
Some of the studies on DNA damage, and/or repair (UDS assay), cell transformation and SOS Chromotest and UmuC tests were positive, but the results are regarded as supportive in WoE. Two *in vivo* mammalian erythrocytes micronucleus tests were both negative, however, given different limitations in methodology and reporting insufficiencies, the studies were considered of limited reliability.

Overall, based on the collective view of the available data, the SCCS is of the opinion that EHMC is likely to have no gene mutation potential. However, the currently available evidence is not sufficient to exclude potential to induce chromosomal damage.

Carcinogenicity

Although there are no indications for carcinogenicity from the available repeated dose studies, EHMC genotoxicity cannot be excluded. Therefore, the SCCS considers that the concerns for carcinogenicity cannot be ruled out.

Photo-induced toxicity

EHMC has phototoxic and photosensitising properties in humans. (Gonçalo 2021, Kerr 2012). These studies indicate that among patients with phototoxic or photoallergic dermatitis, only a small fraction of cases can be attributed to this compound; therefore, the risk can be considered low.

Photogenotoxicity/photomutagenicity

EHMC was tested in 2 bacterial photo mutagenicity tests with negative results, however, the studies do not cover for all test strains required by OECD TG 471.

The two chromosomal aberration photomutagenicity tests, one on V79 cells and the other on CHO cells, were considered negative, are of limited reliability.

EHMC was tested in one Comet assay on L5178Y cells with negative result and one photomutagenicity test on Saccharomyces cerevisiae with negative result. Both tests are regarded as supportive in WoE.

Overall, the available evidence is not sufficient for excluding a photomutagenic effect of EHMC.

Photocarcinogenicity

Although there are no indications of EHMC (photo) mutagencity from the available repeated dose studies on the photocarcinogenicity of EHMC, EHMC (photo)mutagenicity cannot be excluded. Therefore, the, the SCCS considers that the evidence for excluding photocarcinogenicity of EHMC is not sufficient.

Special investigation: endocrine disrupting effects

The available evidence suggests that OMC is likely an endocrine disruptor, as it can alter normal functioning of the exposed organisms. Specifically, it has been shown that OMC has an estrogenic, anti-progesterone in rats and in human cells, and an anti-androgenic activity has been observed in rats.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Ethylhexyl Methoxycinnamate, does the SCCS consider Ethylhexyl Methoxycinnamate safe when used as UV-Filter in cosmetic products up to a maximum concentration of 10%?*

   Having considered the data provided, and the concerns relating to potential endocrine disrupting properties of EHMC, the SCCS cannot conclude on the safety of EHMC, because the information provided is insufficient to exclude genotoxicity.

   In addition, the available evidence also shows that EHMC is an endocrine-active substance due to clear demonstration of estrogenic activity and weak anti-androgenic activity both *in vitro* and *in vivo*.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Ethylhexyl Methoxycinnamate in cosmetic products?*

   /

3. *Does the SCCS have any further scientific concerns with regard to the use of Ethylhexyl Methoxycinnamate in cosmetic products?*

   The SCCS mandate does not address environmental aspects. Therefore, this assessment did not cover the safety of EHMC for the environment.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

Annex 1: Overview of available in vitro dermal absorption studies

Study type	Study details	Key results	Reference
In vitro human skin absorption study-GLP-OECD compliant	10% Ethylhexyl Methoxycinnamate with a [acrylate-3-14C]2-Ethylhexyl 4-methoxycinnamate tracer in a representative cosmetic O/W formulation applied at 2 mg/cm² for 24 hours – 5 donors, 12 replicates in total	The absorption – amount recovered in the receptor chamber and the viable skin - was 0.28% ± 0.17% (0.45 %; mean+1 SD) of the applied dose corresponding to 0.57 µgeq/cm² ± 0.31 µgeq/cm² at 24 hours – mass balance within acceptance criteria	(Raynaud, 2021)
In vitro naked rat skin absorption study (Similar to OECD TG 428; non-GLP)	1, 3 and 10% Ethylhexyl Methoxycinnamate in Diethylene glycol monoethyl ether applied for 1, 6, 16 and 24 hours	Percent (stripped skin+ chamber liquid) Ethylhexyl Methoxycinnamate absorbed after 24 hours: 44.3% (for formulation containing 1% Ethylhexyl Methoxycinnamate); 35.6% (for formulation containing 3% Ethylhexyl Methoxycinnamate) and 22.7% (for formulation containing 10% Ethylhexyl Methoxycinnamate). 7 - 17% of applied dose found in the chamber liquid after longer times of exposure	(ECHA, 2021)
In vitro pig skin absorption study (Similar to OECD TG 428; non-GLP)	7.5% Ethylhexyl Methoxycinnamate o/w lotion, o/w cream, w/o lotion (o/w lotion: 67.35 µg/cm²; o/w cream: 58.9 µg/cm² and w/o cream: 58.9 µg/cm²) applied for 6 hours under occlusive conditions – number of replicates and donors not available	Absorption based on the amount of Ethylhexyl Methoxycinnamate in o/w lotion, o/w cream, w/o lotion cream recovered in the stripped skin and the receptor chamber is reported to be 2.8, 3.5 and 3.9% of the applied dose	(ECHA, 2021)

In vitro pig ear skin absorption study	10% Ethylhexyl Methoxycinnamate in o/w emulsion and w/o emulsion applied on skin surface at a rate of 0.5 or 2 mg/cm² for 6 or 24 hours under non-occluded conditions (six replicates)(number of donors unknown) Epidermis included stratum corneum	Skin distribution of Ethylhexyl Methoxycinnamate from sunscreen dose of 0.5 mg/cm² (containing 10% of Ethylhexyl Methoxycinnamate, 50 µg/cm²) after 6 hour exposure and 18 hour permeation to the frozen-stored skin: • w/o: surface: 42.5 and 41.2 µg/cm², epidermis: 4.8 and 3.4 µg/cm², dermis: 1.2 and 2.1 µg/cm², receptor fluid: below limit of quantification and 0.9 µg/cm² • o/w: surface: 42.9 and 41.9 µg/cm², epidermis: 2.7 and 1.7 µg/cm², dermis: 0.8 and 2.3 µg/cm², receptor fluid: below limit of quantification. Dermal absorption value of 1.77 µg/cm² (equivalent to 3.54% of the applied dose)	(Klimová *et al.*, 2015)

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In vitro pig skin absorption study (non GLP)	Radiolabelled 7% Ethylhexyl Methoxycinnamate alone or in combination with 3% Benzophenone-3 in hydroalcoholic or diisopropyl adipate formulations, at a rate of 6.3 µL/cm² for a period of 1, 2, 6 or 10 hours (donors-not specified, replicates= 4)	• Ethylhexyl Methoxycinnamate alone in hydroalcoholic formulation: 0.48% in receptor fluid; 12.56% in viable skin; 58.13% retained inside *stratum corneum* • Ethylhexyl Methoxycinnamate alone in di-isopropyl adipate formulation: 0.19% in receptor fluid; 2.55% in viable skin; 25.05% retained inside *stratum corneum* • Ethylhexyl Methoxycinnamate with Oxybenzone in hydroalcoholic formulation: 0.36% in receptor fluid; 7.14% in viable skin; 55.15% retained inside *stratum corneum* • Ethylhexyl Methoxycinnamate with Oxybenzone in di-isopropyl adipate formulation: 0.19% in receptor fluid; 3.52% in viable skin; 28.21% retained inside *stratum corneum*	(Gupta *et al.*, 1999; NTP, 2006)
In vitro pig skin absorption study	o/w nano-capsules (NC) emulsion with 5% Ethylhexyl Methoxycinnamate, w/o NC emulsion with 5% Ethylhexyl Methoxycinnamate, o/w emulsions with free 5% Ethylhexyl Methoxycinnamate and w/o emulsions with free 5% Ethylhexyl Methoxycinnamate on the skin for a period of 3 or 24 hours (number of replicates and donor unknown)	• 5% Ethylhexyl Methoxycinnamate in o/w nano capsules emulsion: 0.016 and 0.053% in receptor fluid; 0.789 and 0.274% in viable skin; 8.321 and 15.572% retained inside *stratum corneum* • 5% Ethylhexyl Methoxycinnamate in water-in-oil (w/o) NC emulsion: 0 and 0.087% in receptor fluid; 0.668 and 0.320% in viable skin; 16.338 and 17.555% retained inside *stratum corneum* • Free 5% Ethylhexyl Methoxycinnamate in o/w	(Jiménez *et al.*, 2004; NTP, 2006)

		emulsion: 0 and 0% in receptor fluid; 0.999 and 2.283% in viable skin; 40.497 and 36.591% retained inside *stratum corneum* Free 5% Ethylhexyl Methoxycinnamate in w/o emulsion: 0 and 0% in receptor fluid; 2.468 and 3.718% in viable skin; 45.812 and 46.393% retained inside *stratum corneum*	
In vitro human skin absorption study	7.5% Ethylhexyl Methoxycinnamate in oil in water emulsion and petrolatum jelly applied to the skin discs for a period of 2 min; 0.5, 2 or 6 hours (number of replicates and donor unknown)	After 2 minutes - Ethylhexyl Methoxycinnamate in epidermis including stratum corneum <1 and <2% for the o/w emulsion and petrolatum, respectively. At 6 hours - 8.62% for o/w emulsion and 1.28% for petroleum jelly. Dermis concentrations values at 6 hours - 0.78% for the emulsion and 0.43% for petroleum jelly. Ethylhexyl Methoxycinnamate not identified in receptor fluid	(Treffel and Gabard, 1996)
In vitro human and pig skin absorption studies	5% w/w Ethylhexyl Methoxycinnamate in o/w emulsion applied for 6 hours. (Epidermis includes stratum corneum)	• Ethylhexyl Methoxycinnamate content in pig skin: 7.43% in epidermis, 4.03% in upper dermis, 4.52% in transepidermal penetration and 0.49% in receptor fluid • Ethylhexyl Methoxycinnamate content in human skin: 8.11% in epidermis, 1.15% in upper dermis, 1.57% in transepidermal penetration and 0.42% in receptor fluid	(Benech-Kieffer *et al.*, 2000; NTP, 2006)
In vitro human skin	7.5% Ethylhexyl Methoxycinnamate in oil in water emulsion and	After 30 min and 6 hours, 0.1% of the applied dose of Ethylhexyl Methoxycinnamate o/w	(Chatelain *et al.*, 2003)

absorption study	petrolatum jelly applied to the skin for a period of 30 min and 6 hours (number of replicates, donor unknown) (Epidermis includes stratum corneum)	emulsion and 0.1-0.2% of the applied dose of Ethylhexyl Methoxycinnamate in petrolatum were detected in dermis. After 30 min and 6 hours, 0.2% of the applied dose of Ethylhexyl Methoxycinnamate in o/w emulsion and 0.1-0.3% of the applied dose of Ethylhexyl Methoxycinnamate in petrolatum were detected in epidermis	
In vitro human skin absorption study	17.8 ± 0.24 mg for the 0.5% Ethylhexyl Methoxycinnamate in mineral oil solution; 17.6 ± 0.24 mg for the 1% Ethylhexyl Methoxycinnamate in mineral oil solution and 18.2 ± 0.20 mg for the 2% Ethylhexyl Methoxycinnamate in mineral oil solution applied to skin for 24 hours (number of replicates and donors unknown)	Around 95–98% of the Ethylhexyl Methoxycinnamate recovered on the surface of the epidermis. A recovery of 4% Ethylhexyl Methoxycinnamate in the receptor phase was reported	(Hayden *et al.*, 2005)
In vitro human skin absorption study	7.5% Ethylhexyl Methoxycinnamate applied for 2, 6 and 18 hours (No further details available)	Amount found in chamber - 0.03% after 2 hours, 0.26% after 6 hours and 2% after 18 hours	(SCC, 2000)
In vitro rat skin absorption study	3% and 20% Ethylhexyl Methoxycinnamate applied for 6, 16 and 24 hours (No further details available)	Amount found in chamber – 1.13% after 6 hours, 11.4% after 16 hours and 17.9% after 24 hours	(SCC, 2000)

Annex 2: Overview of available human dermal harmacokinetic/bioavailability studies

Study type	Study details	Key results	Reference
Systemic absorption of Ethylhexyl Methoxycinnamate	2 mg/cm^2 sunscreen product containing 7.5% Ethylhexyl Methoxycinnamate	Amounts contained in the *stratum corneum* were 40-50% for the o/w emulsion and 10-15% for petrolatum.	(Treffel and Gabard, 1996)
from two vehicles *in vivo* using a standardized tape-stripping method	applied to the back side of 4 healthy volunteers. 0.5, 2 and 6 hours later, product removed and skin tape-stripped (10 × 20 mm) 15 times	Maximal *stratum corneum* levels (15 strips) obtained at 0.5 h. Difference between vehicles higher in the superficial parts of the *stratum corneum*, demonstrating that penetration enhancing effect of the emulsion was more important in the upper layer of the *stratum corneum*	
Penetration of Ethylhexyl Methoxycinnamate from two vehicles (i.e., o/w emulsion, petrolatum) *in vivo* using a standardized tape-stripping method	2 mg/cm² sunscreen product containing 7.5% Ethylhexyl Methoxycinnamate applied on the volar side of the forearm of 6 healthy volunteers. 30 min after application, product removed and skin tape-stripped 16 times	Total amount of Ethylhexyl Methoxycinnamate penetrating the *stratum corneum* from the emulsion gel formulation significantly higher. Average penetrated percentage of the dose applied: 24.1% for o/w and 10% for petrolatum	(Chatelain *et al.*, 2003)

Systemic absorption of the sunscreens after repeated whole-body topical application	2 mg/cm^2 sunscreen product containing 10% Ethylhexyl Methoxycinnamate applied to whole body surface area daily for 4 days/week, 2 weeks	Maximum median plasma Ethylhexyl Methoxycinnamate concentrations: 10 ng/mL for females and 20 ng/mL for males. .	(Janjua *et al.*, 2004; NTP, 2006)
Sunscreens in human plasma and urine after repeated whole-body topical application	2 mg/cm^2 sunscreen product containing 10% Ethylhexyl Methoxycinnamate applied to whole body surface area daily for 4 days	Maximum median plasma Ethylhexyl Methoxycinnamate concentrations: 7 ng/mL for females and 16 ng/mL for males.	(Janjua *et al.*, 2008)
Human dermal maximal usage trial (MUsT)	2 mg/cm² non-aerosol spray and pump spray sunscreen products containing 7.5% Ethylhexyl Methoxycinnamate applied to 75% of body surface area during 4 days, total of 13 applications	Maximum plasma Ethylhexyl Methoxycinnamate concentrations: 7.9 ng/mL for non-aerosol spray and 6.1 ng/mL for pump spray.	(Matta *et al.*, 2020)
Systemic absorption of Ethylhexyl Methoxycinnamate from cream after dermal application	2 g o/w cream containing 10% Ethylhexyl Methoxycinnamate applied to an interscapular area of 5 male subjects (25x30 cm). Area covered with 3 layers of gauze left in place for 12 hours	No increase in plasma levels of Ethylhexyl Methoxycinnamate. Urine showed levels of 100–300 ng/mL. Little Ethylhexyl Methoxycinnamate dermally absorbed under the study conditions	(SCC, 2000)