Scientific Committee on Consumer Safety

SCCS

OPINION

on 4-Methylbenzylidene camphor

(4-MBC)

The SCCS adopted this document
by written procedure on 29 April 2022

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 22 December 2021 to 28 February 2022). Comments received during this period were considered by the SCCS. For this Opinion, main changes of the content occurred in sections 3.1, 3.2.4, 3.3.6, 3.3.10 and in the respective discussion sections.

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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 4-Methylbenzylidene camphor (4-MBC), does the SCCS consider 4-MBC safe when used as a UV-filter in cosmetic products up to a maximum concentration of 4%?

   The SCCS cannot conclude on the safety of 4-MBC, because the information provided is insufficient to fully evaluate potential genotoxicity.
   Moreover, there is sufficient evidence that 4-MBC may act as an endocrine disruptor and has effects on both the thyroid and estrogen systems. Effects on the androgen system are not so evident, as only *in vitro* evidence is available.
   Even if the genotoxic potential was excluded, the current re-evaluation of 4-MBC established a higher exposure level than in the previous Opinion. This would result in a lower MoS value, indicating that the use of 4-MBC at the maximum concentration of 4% in cosmetic ingredients would not be safe.

2. Alternatively, what is according to the SCCS the maximum concentration considered safe for use of 4-MBC as a UV-filter in cosmetic products?

   It is not possible to derive a maximum concentration for safe use of 4-MBC, because a genotoxicity potential cannot be excluded.

3. Does the SCCS have any further scientific concerns with regard to the use of 4-MBC in cosmetic products?

   The SCCS mandate does not address environmental aspects. Therefore, this assessment did not cover the safety of 4-MBC for the environment.

Keywords: SCCS, revision, scientific opinion, 4-Methylbenzylidene camphor (4-MBC), CAS No 36861-47-9/38102-62-4, EC No 253-242-6, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), scientific opinion on 4-Methylbenzylidene camphor (4-MBC), preliminary version of 22 December, final version of 29 April 2022, SCCS/1640/21.

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

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2. MANDATE FROM THE EUROPEAN COMMISSION

1. 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 specific 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[2] in 2019 on 14[3] of the 28 substances (to be treated with higher priority-Group A substances) in preparation of the safety assessment of these substances. 4-Methylbenzylidene camphor (hereinafter 4-MBC) is one of the above-mentioned 14 substances for which the call for data took place.

2. Background on 4-Methylbenzylidene camphor (4-MBC)

In cosmetic products, the ingredient 4-MBC (CAS No 36861-47-9/38102-62-4, EC No 253-242-6/-) with the chemical name 3-(4'-methylbenzylidene)-camphor is currently regulated as a UV-filter in sunscreen products in a concentration up to 4% (Annex VI/18).
The safety of 4-MBC was assessed several times: by the SCCNFP in 1998[4,] 2001[5] and 2004[6] and by SCCP in 2006[7] and 2008[8.] In particular, the SCCP Opinion from 2008 (SCCP/1184/08) concluded that '…4-MBC can be considered safe for use in finished cosmetic products (whole body application) at a concentration of up to 4%. It must be emphasized that this opinion is restricted to the safety evaluation of 4-MBC after dermal application of a cosmetic product containing this UV filter. Exposure scenarios via the inhalation route (through aerosols, sprays, etc.) or the oral route (through e.g. lip care products) are not covered. In these cases, risk cannot be excluded'.
During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of 4-MBC as a UV-filter in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on 4-MBC in view of the information provided.

[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 products_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/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out27_en.htm
[5] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out145_en.htm
[6] https://ec.europa.eu/health/ph_risk/committees/sccp/documents/out282_en.pdf
[7] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_075.pdf
[8] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_141.pdf

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of 4-Methylbenzylidene camphor (4-MBC), does the SCCS consider 4-MBC safe when used as a UV-filter in cosmetic products up to a maximum concentration of 4%?*

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of 4-MBC as a UV-filter in cosmetic products?*

3. *Does the SCCS have any further scientific concerns with regard to the use of 4-MBC in cosmetic products?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

4-Methylbenzylidene Camphor (INCI name)

3.1.1.2 Chemical names

Chemical name: 3-(4-Methylbenzylidene)-camphor;
3-(4-Methylbenzylidene) bornane-2-one;
3-(4-Methylbenzylidene)-dl-Camphor.
IUPAC name:
1,7,7-trimethyl-3-[(4-methylphenyl)methylene]bicyclo[2.2.1]heptan-2-one
Synonyms:4-MBC, 4-Methylbenzylidenecamphor, 1,7,7-Trimethyl-3-(4-methylbenzylidene)bicyclo(2.2.1)heptan-2-one, 3-(4-Methylbenzylidene)-dl camphor, 3-(4-Methylbenzylidene)-DL-camphor, 3-(4-Methylbenzylidene)camphor, 3-(p-Methylbenzylidene)-DL-camphor, 3-(p-Methylbenzylidene)bornan-2-one, 3-(p-Methylbenzylidene)camphor, Bicyclo(2.2.1)heptan-2-one, 1,7,7-trimethyl-3[(4-methylphenyl)methylene]-, p-Methylbenzylidenecamphor.

Ref: 36861-47-9_Data on 4-methylbenzylidene camphor_2019-09-23 + ED assay; ECHA ANNEX XV – IDENTIFICATION OF 4-MBC AS SVHC

3.1.1.3 Trade names and abbreviations

Trade name: Eusolex® 6300, Neo Heliopan®, Enzacamene®, Unival® MBC 95, Parsol®5000

COLIPA No: S 60

3.1.1.4 CAS / EC number

CAS:

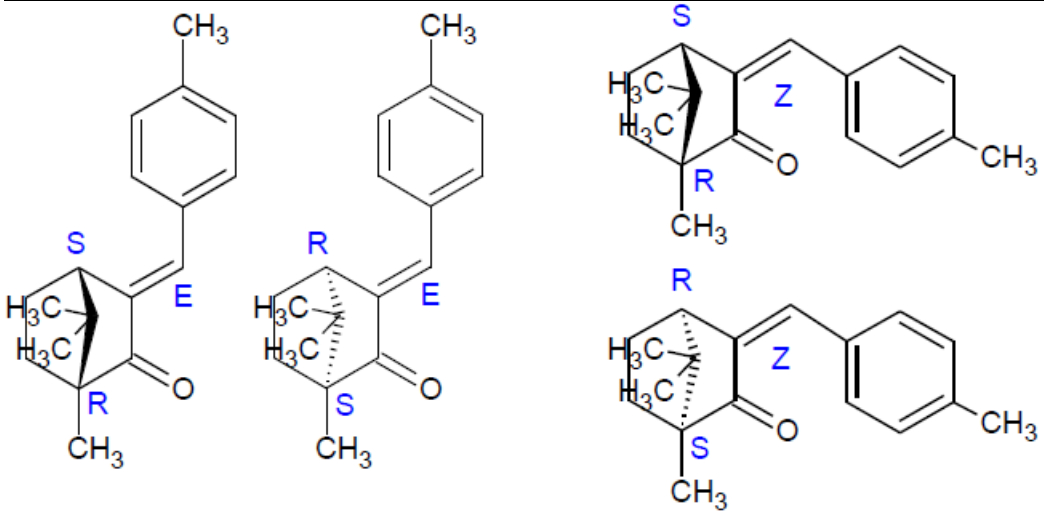
Table 1. CAS and EINECS numbers of non-exhaustive list of different isomeric forms of 4-MBC

Name	EINECS	CAS Number
(1*S*,3*E*,4*R*)-1,7,7-trimethyl-3-(4-methylbenzylidene)bicyclo[2.2.1]heptan-2-one	-	852541-30-1

(1*R*,3*E*,4*S*)-1,7,7-trimethyl-3-(4-methylbenzylidene)bicyclo[2.2.1]heptan-2-one	-	95342-41-9
(1*R*,3*Z*,4*S*)-1,7,7-trimethyl-3-(4-methylbenzylidene)bicyclo[2.2.1]heptan-2-one	-	852541-21-0
(1*S*,3*Z*,4*R*)-1,7,7-trimethyl-3-(4-methylbenzylidene)bicyclo[2.2.1]heptan-2-one	-	852541-25-4
(3*E*)-1,7,7-trimethyl-3-(4-methylbenzylidene)bicyclo[2.2.1]heptan-2-one	-	1782069-81-1
(1*R*,4*S*)-1,7,7-trimethyl-3-(4-methylbenzylidene)bicyclo[2.2.1]heptan-2-one	-	741687-98-9
(±)-1,7,7-trimethyl-3-[(4-methylphenyl)methylene] bicyclo[2.2.1]heptan- 2-one	253-242-6	36861-47-9
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-3-[(4-methylphenyl)methylene] -		38102-62-4

Ref: ECHA ANNEX XV – IDENTIFICATION OF 4-MBC AS SVHC

3.1.1.5 Structural formula



Ref: ECHA ANNEX XV – IDENTIFICATION OF 4-MBC AS SVHC

3.1.1.6 Empirical formula

Emp. Formula: $C_{18}H_{22}O$

3.1.2 Physical form

Pale white to white crystalline solid, weak camphor-like odour.

Ref: SCCP/1042/06; 36861-47-9_Data on 4-methylbenzylidene camphor_2019-09-23 + ED assay

3.1.3 Molecular weight

254.37 g/mol

3.1.4 Purity, composition and substance codes

The powder used in the tests is stated to be more than 99.5% pure. Other preparations, provided by the manufacturer, are assumed by the investigators to be equally pure; in some cases, analytical data were provided.

Table 1. Constituents (4-methylbenzylidene camphor)

Constituent	**Concentration range**
4-methylbenzylidene camphor	≥94.5 - ≤100 % (w/w)

New data submitted in March 2022

The chemical characterisation was performed by ^{1}H-NMR, ^{13}C-NMR spectra, GC-MS, FT-IR, and UV-VIS data and GC analysis. The results of the different analytical techniques are in agreement with the composition and the structure of Neo Heliopan MBC (a mixture of E and Z isomers). Specifically, this product is a mixture of 99.8 % E-isomer and 0.2 % Z-isomer, as calculated by a GC-FID method.
Data on purity of 4-MBC retrieved from the analytical certificates for 5 production batches of 4-MBC are presented in Table 2. The GC method used for product quality control purposes cannot separate the E- and Z-isomers so that the purity given represents their sum.

Table 2. Purity of Neo Heliopan MBC in five batches

Batch number	Purity (%)
10300093	99.9
10300099	99.9
10300108	99.7
10300109	99.9
10300111	99.9

Ref: SCCP/1042/06; 36861-47-9_Data on 4-methylbenzylidene camphor_2019-09-23 + ED assay; Strempel 2021

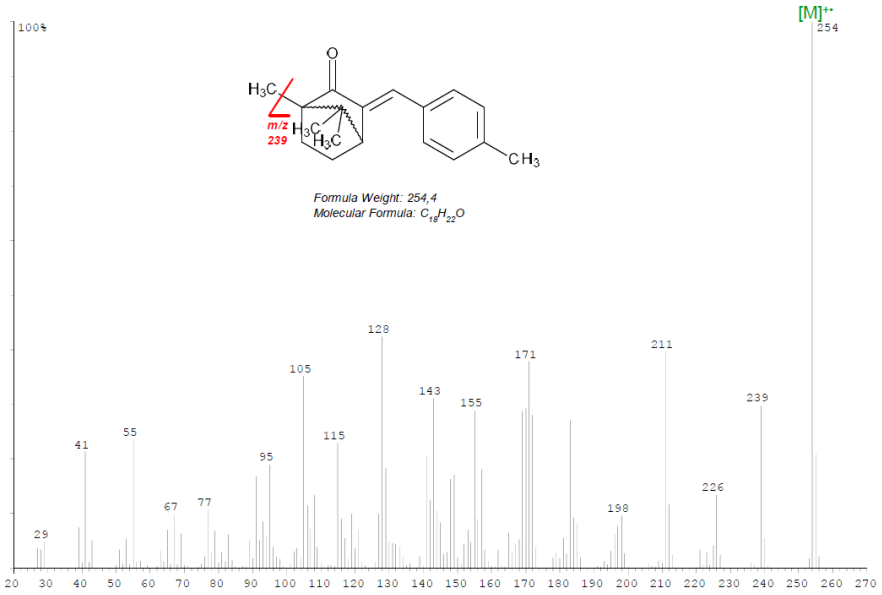
SCCS comment
The percentage of E- and Z-isomer was calculated based on one GC-FID chromatogram of one batch of Neo Heliopan MBC. The methodology used for the calculation of the stereoisomeric composition of Neo Heliopan MBC is unclear.

3.1.5 Impurities / accompanying contaminants

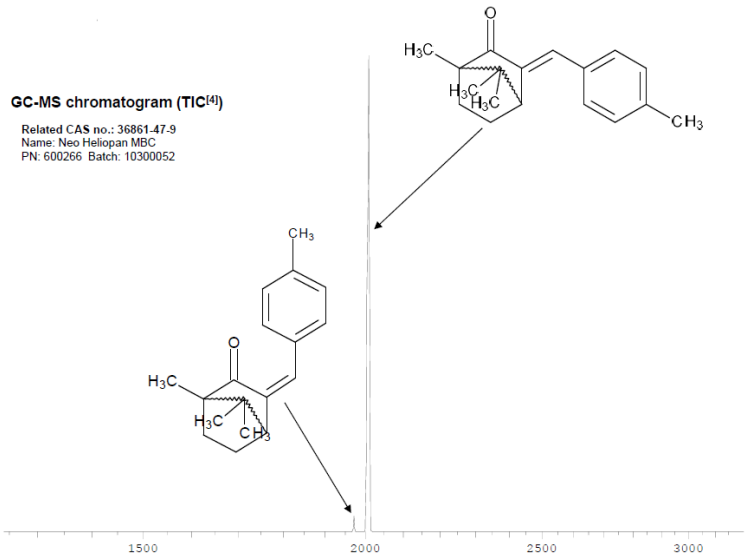
Impurities:
Camphor (GC): ≤ 0.02%
Methylbenzaldehyde (GC): ≤ 0.1%

New data submitted on March 2022

For the identification of possible impurities >1.0% GC, GC-MS and NMR was used. LC-MS was not performed, there are no additional information expected from this method. Higher molecular and non-volatile by-products are not present as a result of the production process and purification of the product.



GC-MS spectra of Neo Heliopan MBC.



GC-MS chromatogram of Neo Heliopan MBC.

There are no other impurities > 1.0% present, which could be detected by the techniques used. Each of the four unidentified impurities are only present at very low amounts of <0.03 %.

Ref: SCCP/1042/06; MEDA Pharma Dossier; Strempel 2021

SCCS comment
GC-MS spectra of Neo Heliopan MBC exhibited various ion peaks at significant abundance at m/z< 239, indicating the presence of possible impurities.

3.1.6 Solubility

Practically insoluble in water (1,3 mg/L; 20 °C); water solubility is 1.08 ± 0.15 mg/L at 20 °C (pH 5-6); (OECD Method 105, GLP-compliant).
Slightly soluble in ethanol (approximately 25%) and vegetable oils.
Very slightly soluble in chloroform.
Isopropanol: approximately 25%
Iso-propyl myristate: approximately 25%
Liquid paraffin: approximately 15%

Ref: SCCP/1042/06; 36861-47-9_Data on 4-methylbenzylidene camphor_2019-09-23 + ED assay

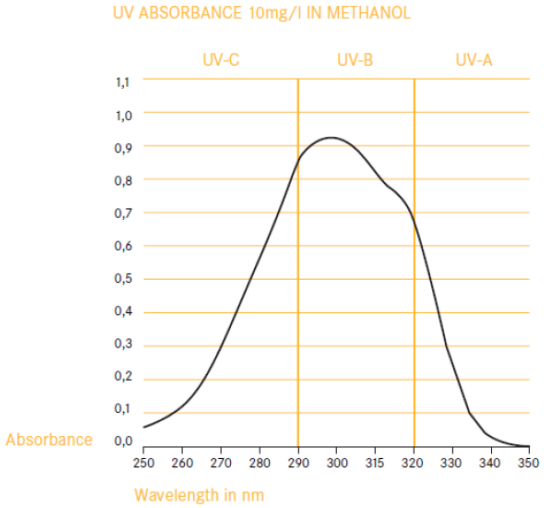
3.1.7 Partition coefficient (Log P_{ow})

Log K_{ow} (Log P_{ow}): 5.1 at 23°C (OECD Guideline 117; HPLC method).

Ref: SCCP/1042/06; 36861-47-9_Data on 4-methylbenzylidene camphor_2019-09-23 + ED assay

3.1.8 Additional physical and chemical specifications

- organoleptic properties (colour, odour, taste if relevant): pale white to white crystalline solid with faint characteristic odour; solid at 20°C and 101.3 kPa
- melting point: 68°C; 70.4°C at 101.3 kPa (EU Method A.1 and OECD 102)
- boiling point: 357°C at 101.3 kPa, (EU Method A.2 and OECD 103)
- flash point: flammability not classified (The test item could not be ignited by applying a flame as ignition source for at least 2 minutes).
- vapour pressure: 4.9E-4 Pa at 20°C, 1.0E-3 Pa at 25°C and 2.9E-2 at 50°C (OECD Guideline 104 and EU Method A.4).
- relative density compared to water at 4 °C: 1.108 at 20 ± 0.01°C (EU Method A.3 and OECD 109)
- viscosity:/
- pKa: /
- pH:/
- refractive index:/
- Loss on drying (50 oC): ≤ 0.2%
- Granulometry: The test item contains a coarse fraction >2000 µm of 24%. The fine fraction (76 %) of the test item has a median particle size D50 of 44 µm, which was determined by laser diffraction measurement. The D10 and D90 of the fine fraction were 6.5 and 142.3 µm, respectively (2017). The study was conducted according to OECD Guideline 110 (1981) and ISO 13320 (2009). The study was not GLP-compliant; however, the testing laboratory holds a GLP certificate.
- UV/visible light absorption spectrum:



Specific extinction $E^{1\%}/_{1cm}$, in methanol at λ_{max} 299 ± 2 nm: min. 930

New data submitted on March 2022

Extinction / Absorption maximum (10 mg/mL, in ethanol):
$E^{1\%}/_{1cm}$ 954 / λ_{max} 300 nm
$E^{1\%}/_{1cm}$ 328 / λ_{max} 226 nm

Specific optical rotation

$[\alpha]_D^{20}$ 2.70° (±0.17°)

The optical activity results from the starting material (Camphor).

Ref: 36861-47-9_Data on 4-methylbenzylidene camphor_2019-09-23 + ED assay; Symrise 2017; Strempel 2021

3.1.9 Homogeneity and Stability

Shelf life and storage conditions: 36 months in the original, closed container (protected from sunlight), dry, at 5 to 25°C.

New data submitted on March 2022

The function of UV filters is to absorb light energy. This light energy absorption changes the molecule energetically from a ground state to an excited state. In general, the excited state will regress to the ground state by dissipating the absorbed energy as kinetic energy (heat) or by emitting light of a lower wavelength (i.e. photons of lower energy). For 4-MBC, as for other UV filters with a non-ring C=C double bond, photoisomerization between the E- and the Z-isomeric forms can occur in the excited state. This constitutes another possibility how UV filters can dissipate the absorbed light energy and does not affect their intended function.
According to the Applicant, there is no indication that 4-MBC is photodegraded to other products. Tarras-Wahlberg et al. (1999) found 4-MBC to be UVA and UVB stable from a skin protection perspective since "the two isomers have very similar spectra and their protective power does not change significantly after photo-equilibrium has been established." Scalia et al. reported the occurrence of the Z-isomer (called cis-isomer in the paper) after exposing 4-MBC in an oil-in-water formulation spread on a tape strip to UV light of 750 W/m² for 1 hour. This corresponds to a very high irradiation dose of 270 J/cm² considering that the minimal erythema dose (MED) is about 0.025 J/cm². Rodil et al. (2009) reported on the photostability and photoisomerization of 4-MBC, by measuring the sum of the Z- and E-isomers of 4-MBC, the authors found "a high stability during the whole irradiation period of 72 h, …"

Ref: Symrise 2017; Tarras-Wahlberg 1999; Rodi 2009.

SCCS comment
No (photo-)stability data were provided, neither were the data on the solubility of 4-MBC in the receptor fluid of the *in vitro* dermal absorption study. A study from Scalia *et al*. (2007) suggests that 4-MBC undergoes significant degradation under sunlight exposure.

Overall SCCS comments on the chemical and physical specifications
All the information provided in the physicochemical part relates only to one substance composed of mixture of 2 isomers (99.8 % E-isomer and 0.2 % Z-isomer).
Recent information on the stability and photo-stability of 4-MBC and also its solubility in the receptor fluid is missing.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

4-MBC is used as an ultraviolet filter in sunscreen and other cosmetics at a maximum concentration of 4%. Maximum absorption at 300 nm.

Taken from SCCP/1184/08

Statement on the exposure pattern of 4-MBC

The Applicant stated that there is no interest in continuing to use 4-MBC in lip care products. Like almost all UV Filters, 4-MBC is also used as a UV filter for product protection. Typical use concentrations for that purpose are reported to be 0.5 % or lower. In addition, 4-MBC may be used to provide UV protection in daily skincare products (skin cream/lotions, anti-aging) which use UV protection as a product feature.

New data from literature
A survey based on 337 sunscreen products in the UK showed that there is a downward trend in the occurrence of 4-MBC. 4-MBC was used in 25% of the products in 2005, whereas it was used in only 1.2% of the products in 2010.

Ref: Kerr *et al*., 2011

According to older market surveys, 4-MBC belonged to the most frequently occurring UV filters in cosmetic products on the Swiss market (Hauri *et al*., 2003; Poiger *et al*., 2004). A study that assessed frequency of occurrence and concentrations of organic UV filters in 116 cosmetic products in Switzerland in 2013 showed that 4-MBC was not frequently present in these products. 4-MBC was detected in one lip care product. It was not detected in the sunscreens that were assessed.

Ref: Manova *et al*., 2013

SCCS comment
These studies show that 4-MBC is not frequently used anymore in sunscreens or other cosmetic products on the European market.

3.2.2 Dermal / percutaneous absorption

Taken from SCCNFP/0779/04 and SCCP/1042/06
A 5% of ^{14}C-labelled 4-MBC in an oil in water emulsion was applied at a dose of 1g over a shaved area of 200 cm² on the forearm of 6 volunteers. The amount of radioactivity measured in urine and faeces indicated a dermal absorption of about 1.9%. However, viewing the shortcomings in this and some other presented studies, a final conclusion on dermal absorption could not be drawn.

Taken from SCCP/1184/08
Skin penetration data from eight experiments (performed 1997-98) were summarised in the report provided by the Applicant. A typical cosmetic sunscreen formulation, containing 4% 4-MBC, was used. The experiments were performed according to OECD TG428 using pig skin.

Ref.: Diembeck *et al*., 2004

SCCP/1184/08 comments on dermal absorption:
- Since only a summary of eight studies is provided, the test descriptions are insufficient.
- The solubility of the test substance in the receptor fluid is declared to be sufficient, though no data have been provided to support this.
- For the lower values, the standard deviations are very high.
- Taking into account the values obtained in eight separate experiments, the mean dermal absorption value of 1.96 µg/cm² will be used for further calculations.
- In the dermal studies with the rat, 70 and 100 mg sunscreen/cm² were applied and the surplus was washed off after 24 hours. Therefore, the dosage mentioned are probably overestimated.

No new data was submitted in 2019.

Overall SCCS comments on dermal absorption
Several concerns regarding the submitted dermal absorption data were raised in the previous Opinions. Full study reports were not available, so there was no information on the methodologies used nor were the raw data available. The SCCS sent a request to the Applicant to obtain the full study reports but received only an Excel with raw data from four dermal absorption studies. The data provided could not be linked to the data earlier provided to the SCCP.
In absence of dermal absorption data, normally, a default dermal absorption value of 50% would be applied. Such a value was, however, considered not realistic for this UV-filter. Therefore, the SCCS considered it pragmatic to use the previous data of the eight studies and apply the SCCS basic criteria as described in the 11th Revision of the SCCS Notes of Guidance.
In the previous SCCP Opinion, the mean value was used without taking the standard deviation into consideration. Due to uncertainties regarding the data and the fact that standard deviations were high, the SCCS considers that for the dermal absorption, a mean value + 2SD will be used.
All values are shown in Table 3. It was noticed that 4-MBC was not detected in receptor fluid. Theoretically, a non-detectable amount of approx. 0.1 µg/cm² of the applied 4-MBC in the receptor fluid (resp. dermis) could be consistent with the analytical data (considering the detection limits). A value of 4.18 µg/cm^2 will therefore be used in the exposure calculation.

Table 3: Dermal absorption data

	Mean	**SD**	**Mean + 2SD**
Epidermis	1.5 µg/cm	0.7 µg/cm	2.9
Dermis	0.46 µg/cm	0.36 µg/cm	1.18
Receptor fluid	Not detected		0.1
			4.18 µg/cm²

3.2.3 Other studies on toxicokinetics

Taken from SCCNFP/0779/04 and SCCP/1042/06
As described in detail in SCCP/1042/06, a number of toxicokinetic and metabolism studies have been performed in rats and on human volunteers. The concentrations of 4-MBC and its two major metabolites (3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene)-camphor) were measured in plasma and/or urine after dermal or oral administration of 4-MBC, either dissolved in corn oil or incorporated in a sunscreen formulation.

The toxicokinetics of 4-MBC formed the major subject of SCCP/1184/08 Opinion. A brief description of the studies is presented below.

- The kinetics of 3-(4-Methylbenzylidene)camphor (Eusolex® 6300; 4-MBC) was investigated in humans after single dermal application of a sunscreen formulation: A single dermal application (3 males and 3 females volunteers) of a sunscreen formulation containing 4% 4-MBC was used to cover 90% of body surface leading to a mean dermal dose of 22.0 ± 1.3 mg /kg bw. 4-MBC and its metabolites were monitored over 96 h in plasma and urine. Maximum blood levels of 4-MBC of 200 pmol/ and 100 pmol/mL were determined in males in females, respectively, after 6 hours and

decreased afterwards to reach the limit of detection after 24 h (females) resp. 36 h (males). For metabolites detected, the maximum concentration was reached 12 h after start of topical application for 3-(4-carboxybenzylidene)-6-hydroxycamphor (50–80 pmol/mL) and 3-(4-carboxybenzylidene) camphor (100-200 pmol/mL). Only a small percentage of the dermally applied dose of 4-MBC was recovered as 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene) camphor in urine, partly as glucuronides. The observed low blood levels of 4-MBC and its metabolites and the low recovery of the applied dose in urine suggest a poor absorption of 4-MBC through human skin (< 0.5 % of dose).

Ref: Dekant W & Schauer U (2005a)

- The toxicokinetics of Art. 105385 (Eusolex® 6300, 4 MBC) was investigated in rats after a single dermal application: Two formulations containing 4% or 20% of the test material Art. 105385 (Eusolex® 6300, 4-MBC) were tested for acute toxicity after dermal administration. Single dermal applications of 4-MBC doses of 400 and 2000 mg/kg bw were applied in formulations by occlusive patch for 24 h to 3 male and 3 female rats per group. Concentrations of 4-MBC and its two major metabolites were monitored over 96 h in plasma. Two formulations containing 4% or 20% of the test material Art. 105385 (Eusolex® 6300, 4-MBC) were tested for acute toxicity after dermal administration. Concentrations of 4-MBC and its two major metabolites were monitored over 96 h in plasma. Two major metabolites, 3-(4-carboxybenzylidene)-6-hydroxycamphor and 3-(4-carboxybenzylidene) camphor, were identified. Peak blood levels of 4-MBC of approx. 200 and 1,200 pmol/mL remained constant for up to 48 h after dermal application of 400 or 2,000 mg 4-MBC/kg bw, respectively. Peak blood levels of the metabolites exceeded the concentrations of the parent compound: Peak blood concentrations of the metabolites reached 18,000 pmol/mL [3-(4-carboxy-benzylidene)-6-hydroxycamphor] and 50,000 pmol/mL (14,217 ng/mL) [3-(4-carboxybenzylidene)-camphor] at 48 to 72 h after 4-MBC application in the high dose (2000 mg/kg bw). Based on the results of this study it can be concluded that 4-MBC is absorbed and metabolized in rats after single dermal application.

Ref: Dekant W & Schauer U (2005b)

- Biotransformation and kinetics of 3-(4-Methylbenzylidene)camphor (Eusolex®6300, 4 MBC) was investigated in rats after a single oral application: Male and female Sprague-Dawley rats (n = 3 per group) were administered single oral doses (via gavage) of 25 or 250 mg/kg bw of 4-MBC in corn oil. The results showed that absorbed 4-MBC undergoes extensive first-pass biotransformation in rat liver resulting in very low blood levels of the parent 4-MBC. Enterohepatic circulation of glucuronides derived from the two major 4-MBC metabolites may explain the slow excretion of 4-MBC metabolites with urine and the small percentage of the administered doses recovered in urine.

Ref: Dekant W & Völkel W (2005)

Taken from SCCP/1184/08

Summary of the SCCP discussion on the toxicokinetic-based MoS approach

- Reduction of the toxicokinetic factor of the MoS from 4 to 1:
  In first instance the SCCP did not accept to reduce the toxicokinetic factor from 4 to 1 due to following reasons: all available data (rat and human studies) indicate accumulation of 4-MBC and/or its metabolites after single and repeated exposure (see tables under section 3.4.1/ SCCP/1184/08), which makes the application of the toxicokinetic approach very difficult. It also renders the automatic extrapolation from data from a single application study to a repeated application situation inappropriate. Since the submitted studies were all performed under different conditions with diverging

dosage levels and forms of 4-MBC, the SCCP did not accept the reduction of 4 to 1 of the toxicokinetic factor of the MoS.

- Reduction of the toxicodynamic factor of the MoS from 2.5 to 1:
  A reduction of the toxicodynamic factor from 2.5 to 1 was not accepted either, although the SCCP acknowledged that rats might be more susceptible to thyroid perturbation than man. Thyroid hormone-related measurements were not included in the human dermal study and robust data supporting the hypothesis that 4-MBC or one of its metabolites would be the active compound with regard to human toxicity were not available. More detailed mechanistic data involving the use of pure metabolites were required to help clarify the toxicodynamic issue. To have more support in the decision-making process in this complex area, the SCCP invited an independent expert in toxicokinetics to express his opinion on the toxicokinetic approach proposed by the applicant.

In order to defend the reduction of the toxicokinetic factor from 4 to 1, upon request of the SCCP, an external expert in toxicokinetics studied the dossier.
Acknowledging the concerns of the Committee, the expert proposed to estimate the repeated dose plasma levels for 4-MBC and its metabolites, based upon the available single dose plasma levels and the amounts still present after 24 hours, out of which the "carryover" values (accumulation) could be calculated.
Comparing these levels with the plasma levels of 4-MBC and its metabolites in the rat at the NOEL instead of the NOAEL value (worst case), leads to the conclusion that the estimated human values are systematically lower than their rat counterparts, supporting approval of the requested reduction of the toxicokinetic factor from 4 to 1.
Nevertheless, this approach is based upon two assumptions, namely:
1) 100 mg/kg/day needs to be the actual NOEL-value for 4-MBC in the 90-day dermal study.
2) The presented human study needs to be considered as a worst-case situation.

The SCCP is of the opinion that:

- 100 mg/kg/day is the NOEL of the 90d dermal toxicity study, based upon thyroid effects occurring at higher levels.
- the application of 2mg/cm² of a sunscreen formulation can be considered as a worst-case scenario

As such, following the toxicokinetic expert's opinion and accepting that the toxicokinetic part of the MoS can be reduced from 4 to 1, a MoS of 25 needs to be achieved.

No new data was submitted in 2019.

SCCS comment
When comparing the human and rat toxicokinetic data, the AUC and C_{max} values in human are lower than in rats, supporting the reduction of the toxicokinetic factor from 4 to 1. The SCCS therefore agrees to reduce the toxicokinetic factor from 4 to 1 as proposed in the previous Opinion. The SCCS considers the use of a safety factor of 1 is appropriate for interspecies differences in toxicokinetics, whereas in the absence of substance specific data, a safety factor of 2.5 to account for interspecies differences in toxicodynamics will be used for MoS calculation (i.e. overall safety factor for interspecies differences is 2.5). Combining this with a factor of 10 to account for intra-human toxicokinetic and toxicodynamic differences leads to an overall MoS of 25 for use in safety calculations.

3.2.4 Calculation of SED/LED

Dermal exposure:

4-MBC at 4% in sunscreens:

Description	Parameter	Value	Unit
Maximum absorption through the skin	A	4.18	µg/cm²
Skin Surface Area (whole-body)	SSA	17500	cm²
Dermal absorption per treatment	SSA x A x 0.001	73.15	mg
Frequency	f	2	times per day
Bodyweight	BW	60	kg
Systemic exposure dose (SED)	SSA x A x 0.001 x f /BW	2.44	mg/kg bw/day

4-MBC at 4% in leave-on face cream:

Description	Parameter	Value	Unit
Maximum absorption through the skin	A	4.18	µg/cm²
Skin Surface Area (face & neck)	SSA	885	cm²
Dermal absorption per treatment	SSA x A x 0.001	3.70	mg
Frequency	f	2	times per day
Bodyweight	BW	60	kg
Systemic exposure dose (SED)	SSA x A x 0.001 x f /BW	0.123	mg/kg bw/day

SCCS comment
In the current Opinion, dermal absorption was calculated according to SCCS basic criteria and this resulted in a higher dermal absorption than that used in the previous Opinion (SCCP/1184/08). Also, the frequency of 2 applications per day was added in this calculation, which was not used in the previous Opinion. This has an impact on the SED, which is approximately 4 times higher than the SED reported for sunscreens in the previous Opinion (0.588 mg/kg bw/day for sunscreens).

3.3 TOXICOLOGICAL EVALUATION

3.3.1. Irritation and corrosivity

3.3.1.1 Skin irritation

From XXIV/1377/96 and SCCNFP/0779/04
No skin irritation was observed in the rabbit Draize test.
No skin irritation was observed in humans. Three tests were carried out on humans:

a) a 5% containing Duhring chamber was glued on the skin of 10 female subjects on the same site 5 days a week for 2 weeks. Exposure time was not stated. No skin irritation was observed.
b) 2 groups of 10 subjects were exposed in the same way as above but the skin was first scarified, and exposure was for 24h. The test was repeated on the same area of the skin 3 times. No irritation was observed.
c) 2 groups of 6 subjects had a stinging sensation after the application of lactic acid to the naso-labial fold. The test substance was applied in the same way. No reports of discomfort were reported.

No new data was submitted in 2019.

3.3.1.2 Mucous membrane irritation / eye irritation

From XXIV/1377/96 and SCCNFP/0779/04

No mucous membrane irritation was observed in the rabbit Draize test.

No new data was submitted in 2019.

3.3.2 Skin sensitisation

From XXIV/1377/96 and SCCNFP/0779/04
Concentrations of up to 3% 4-MBC in arachis oil or 0.5% aqueous carboxymethylcellulose did not cause any skin-sensitising effect in the guinea pig (Freund's Complete Adjuvans was not used).
5 men and 25 women were treated with 5% 4-MBC-containing w/o and o/w emulsions for 3 weeks at a rate of 3 applications/week. After 8-10 days, the applications were made again to a fresh area. No evidence of sensitisation was noted.
Tests in humans and animals for skin sensitisation were negative. The animal tests for sensitisation, however, were unsatisfactory, in as much as Freund's complete adjuvant has not been used. It was noted, however, that 4-MBC very rarely caused contact allergy in humans.

No new data was submitted in 2019.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

From XXIV/1377/96 and SCCNFP/0779/04
LD_{50}-oral-mouse: 10000 mg/kg
LD_{50}-oral-rat: 10000 mg/kg
LD_{50}-oral-dog: 5000 mg/kg

No new data was submitted in 2019.

3.3.3.2 Acute dermal toxicity

From XXIV/1377/96 and SCCNFP/0779/04
LD_{50}-dermal-rat: 10000 mg/kg

From SCCP/1042/06
LD_{50}-dermal-rat > 2000 mg/kg.
Based upon plasma measurements after dermal exposure to 4-MBC in the rat, it was concluded that the systemic exposure to the parent and its metabolites were dose-dependent.

No new data was submitted in 2019

3.3.3.3 Acute inhalation toxicity

No new data submitted in 2019.

3.3.4 Repeated dose toxicity

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

From SCCNFP/0779/04 and SCCP/1042/06

A 14-day oral study was performed in Beagle dogs with 20 (day 1), 100 (day 2), 500 (day 3 + days 5-14)) and 2500 (day 4) mg 4-MBC/kg bw/day.
The authors concluded that there were no treatment-related effects, although the T3 and T4 levels obtained after exposure were consistently higher than those before exposure. There also appeared to be a gradual increase over time. Therefore, the conclusion of the study was considered to be questionable.
21-day oral study was performed in Beagle dogs with 0 (day 1), 20 (day 4), 100 (day 8) and 500 (days 1121) mg 4-MBC/kg bw/day. Again, the conclusions of the test were questionable and again, T3 and T4 levels appeared to be slightly higher after exposure.

No new data was submitted in 2019.

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

From SCCNFP/0779/04 and SCCP/1042/06

In oral 28-day and 90-day studies, 4-MBC was administered daily to rats at dosage levels ranging from 25 to 312 mg/kg bw/day. The effects noted were mainly situated at the level of the thyroid axis, with deviations of normal thyroxine (T_4), triiodothyronine (T_3) and/or thyroid-stimulating hormone (TSH) levels, thyroid gland weight, etc. The oral NOAEL (90d - rat) based upon thyroid effects showed to be 25 mg/kg bw/day.

When dermally applied to the rat skin for 90 days at reported dosage levels of 0, 100, 400 and 2000 mg/kg bw/day, some slight thyroid effects were observed at 400 mg/kg bw/day, while the animals of the high-dosage group had to be sacrificed due to the severity of the local effects they experienced (epidermal lesions, wounds, necrosis).
The authors considered 400 mg/kg bw/day as the dermal NOAEL of 4-MBC and 100 mg/kg bw/day as its dermal NOEL.

No new data was submitted in 2019.

3.3.4.3 Chronic (> 12 months) toxicity

No new data was submitted in 2019.

3.3.5 Reproductive toxicity

3.3.5.1 Fertility and reproduction toxicity

SCCNFP/0779/04 and SCCP/1184/08

When tested in a one-generation reproduction toxicity study, 4-MBC displayed some minor thyroid effects at the highest dosage levels tested (25 and 50 mg/kg bw/day), though not at the lowest one (12.5 mg/kg bw/day). The study authors did not consider any of the observed effects relevant. SCCNFP concluded that levels up to 50 mg/kg bw/day of 4-MBC

did not affect the reproductive function of female rats or the development of offspring. This study points towards a reproduction toxicity NOAEL value of 50 mg/kg/day.

Data submitted 2019

Information taken from EFfCI

The results of reproduction toxicity studies with 4-MBC were reported in four separate publications; Durrer *et al*., 2005 and 2007 and Maerkel *et al*., 2005 and 2007. A treatment regime similar to that in one-generation reproductive toxicity studies with an extended period of treatment given to F1 pups which were raised to adulthood was used. Although the exposure duration was similar to that in a one-generation reproductive toxicity study, except for uterine weight in offspring, Durrer *et al*. (2005) did not include any apical or functional endpoints usually included in an extended one-generation reproduction study design, e.g. fertility of parental animals or postnatal developmental landmarks in offspring. Durrer *et al*. (2007) also did not report on endpoints related to the fertility of the parental animals but did investigate some postnatal developmental landmarks.

The study design used in the abovementioned studies was similar. Male and female Long Evans rats were administered the test item in feed at doses of 0.01, 0.1, 0.33, and 0.66 g/kg chow, corresponding to 0.7, 7, 24 and 47 mg/kg bw/day, for 10-weeks prior to mating Exposure of dams continued during pregnancy and lactation, and pups were weaned at postnatal day (PND) 28. Male and female littermates were then raised in separate groups until adulthood (Durrer *et al*., 2005 and 2007 and Maerkel *et al*., 2005 and 2007).

The following parameters and endpoints were assessed in these studies:

- Uterine weight, protein levels and gene expression were examined in the offspring (Durrer *et al*., 2005).
- Gene expression was examined in the medial preoptic area (MPO) and ventromedial hypothalamic nucleus (VMH) of the brain (Maerkel *et al*., 2005).
- Onset of puberty (preputial separation, vaginal opening) was investigated in males from PND41 and in females from PND30. In males, wet weights of the ventral prostate, testis, epididymis, seminal vesicles and liver were determined and the ventral lobe of prostate (ventral prostate) and the combined dorsal + lateral lobes (dorsolateral prostate) were dissected for molecular biological analysis (mRNA levels and protein levels) (Durrer *et al*., 2007). Male offspring used for molecular biological analysis of prostate were taken from the same litters as the females (Durrer *et al*., 2007).
- Thyroid toxicity and mRNA levels of oestrogen target genes in the medial preoptic area (MPO) and ventromedial hypothalamic nucleus (VMH) of the brain were investigated in Maerkel *et al*., 2007.

Results from Durrer *et al*., 2005

- Absolute and relative uterine weights and body weights of treated offspring did not differ significantly from untreated controls, except for the 24 mg/kg bw/day group, which showed a slight, but significant increase in absolute and relative uterine weight of around 15%.
- Uterine mRNA levels were significantly decreased at 24 and 47 mg/kg bw/day for progesterone receptor (PR) and estrogen receptor alpha (ERα) and at 24 mg/kg bw/day only for the androgen receptor (AR).
- Insulin-like growth factor 1 (IGF-1) mRNA expression was significantly increased at 7 mg/kg bw/day, however it was significantly decreased at 47 mg/kg bw/day.
- No significant effects were observed for ERβ mRNA levels.
- ERα protein levels were significantly decreased at 47 mg/kg bw/day
- PR-A protein levels were significantly decreased at 0.7 mg/kg bw/day.
- No significant effects were observed for ERβ and PR-B protein levels.

Results from Maerkel *et al*., 2005

- A significant decrease in PR mRNA levels in the VMH in F1 females and a significant increase in PR mRNA levels in the MPO in F1 males were observed at 7 mg/kg bw/day only.

Results from Durrer *et al*., 2007

- Body weight at onset of puberty was at control level in males, but significantly reduced in females at 7 to 47 mg/kg bw/day (0.7 mg/kg bw/day not assessed for body weight at onset of puberty).
- Adult body weights of treatment-exposed offspring were in the control range.
- The treatment delayed puberty onset in males (preputial separation) at and above 7 mg/kg in a dose-dependent manner. Onset was delayed 0.7, 2.84 and 3.26 days for 7, 24 and 47 mg/kg bw/day, respectively.
- No significant effects were observed in puberty onset in females (vaginal opening)
- Individual means and litter means for ventral prostate weight were significantly decreased in adult male offspring exposed to 7 to 47 mg/kg per day
- Absolute testis weight was significantly increased at 24 and 47 mg/kg per day. Individual means and litter means for epididymis and seminal vesicle weights were unaffected except for individual mean relative epididymis weights at 47 mg/kg per day.
- Liver weight remained unchanged.
- AR mRNA was reduced in dorsolateral prostate at 7, 24 and 48 mg/kg per day. R protein levels were reduced in dorsolateral prostate at 7 and 24 mg/kg per day. AR mRNA and protein levels in ventral prostate were reduced at 24 mg/kg per day.
- ER-α expression was lower in ventral than dorsolateral prostate; 4-MBC down-regulated ER-α mRNA in both tissues. A decrease in ER-α protein was observed in dorsolateral prostate; in ventral prostate, the protein was not detectable. ER-β mRNA was down-regulated in both prostate parts, but protein was increased in dorsolateral prostate at 7 mg/kg per day and unchanged in ventral prostate. The increase in ER-β protein coincided with the lowest level of AR protein. IGF-1 mRNA was downregulated in accordance to the dose. Effects of 4-MBC on all four genes were greater in dorsolateral prostate.

Results from Maerkel *et al*., 2007

- Statistically significant increases in absolute and relative thyroid weights in F1 offspring of both sexes at dose levels of 24 mg/kg bw/day and above. Relative thyroid weight of F1 males was significantly increased at the dose level of 7 mg/kg bw/day as well.
- Serum TSH was significantly elevated in F1 females at the highest dose of 47 mg/kg bw/day only, and serum T3 was elevated at dose levels of 24 mg/kg bw/day and above. In F1 males, only serum T3 levels were significantly elevated at 24 mg/kg bw/day, with no dose-response relationship observed.
- 4-MBC affected mRNA levels of ERα, PR, preproenkephalin (PPE), IGF-1 and steroid receptor coactivator (SRC-1) in the brain in a sex-specific and region-specific manner.

Ref: Durrer *et al*., 2005 and 2007; Maerkel *et al*., 2005 and 2007

In a special investigation designed to evaluate effects on female sexual behaviour following pre- and postnatal exposure, the same study design was used. Exposure to 4-MBC was 7 and 24 mg/kg bw/day. Female sexual behaviour was tested at 11–13 weeks of age on proestrus day. Treated and control females were mated with untreated normal males and the same group of untreated males was used for mating the control and treatment females. The number of events per 15 min was counted for the following female behaviours: (1) proceptive behaviour, (2) receptive behaviour, and (3) rejection behaviour. Oestrous cyclicity was examined by taking vaginal smears at 3 to 4 months of age by lavage for 21 days.

Results
- 4-MBC reduced proceptive behaviour and rejection behaviour towards the male for the treatment at 24 mg/kg bw/day
- Lordosis, a reflex action that causes many female mammals to adopt a body position that is often crucial to reproductive behavior, was reduced at both 7 and 24 mg/kg bw/day.
- Body weights and oestrous cycles of adult F1 female rat offspring were not affected by 4-MBC.

Ref: Faass *et al*., 2009

CEHOS, 2012
The Danish Centre on Endocrine Disrupters (CEHOS) described reproductive toxicity of 4-MBC. These effects were based on the same studies as described by EFfCI and will not be repeated.

Ref: Hass *et al*., 2012

SCCS comment
A series of publications from Schlumpf and co-workers describe the effects of 4-MBC on reproductive parameters. In these studies, exposure levels below the current NOAEL of 25 mg/kg bw/day were included. SCCS has evaluated the original publications and found them difficult to evaluate. For example, it is not fully clear how many original studies were performed and if data from different studies were investigated separately or together. These unclarities hampered the interpretation of the results of these studies by the SCCS and made it difficult to properly assess the weight of evidence from all the data and derive a justifiable NOAEL.

In these studies, many different molecular parameters were measured, and results were uneven and often not clearly dose-dependent. The studies showed that 4-MBC affected gene expression and protein levels of endocrine-related receptors and growth factors in the brain, prostate and uterus. Patterns were not always consistent between mRNA and protein levels. Gene expression patterns in the prostate differed between the dorsal and ventral sites, whereas the effects of 4-MBC were more pronounced in the dorsolateral prostate. Furthermore, the SCCS considers the effects observed as transient molecular events, since they do not lead to measurable significant effects on the reproductive functions in the studies. Due to the inconsistencies of the results, and the lack of agreement in the dose-response relationships to support the postulated mode of action, these molecular patterns cannot be used on their own to derive a point-of-departure for safety assessment.

The effects of 4-MBC observed on the thyroid were demonstrated in earlier studies as well, e.g. in the 90-day repeat toxicity study and the reproductive toxicity study described in previous Opinions. In these studies, effects on thyroid occurred at higher exposure levels than in the new reproduction studies. In the one-generation reproduction toxicity study described in SCCNFP/0779/04, only minor thyroid effects were induced at 25 and 50 mg/kg bw/day and not at 12.5 mg/kg bw/day. SCCNFP concluded that levels up to 50 mg/kg bw/day of 4-MBC did not affect the reproductive function of female rats or the development of offspring. In the 90-day oral study, 4-MBC induced significant thyroid effects at dose levels of 50 mg/kg bw/day and higher. At exposure levels of 25 mg/ kg bw/day only slight increases in T4 were seen, none of the other thyroid effects were observed at this dose (Hofmann, 1984). Hence, thyroid effects occur at oral exposure levels of 50 mg 4-MBC/kg bw/day. In the dermal-repeat dose-toxicity study, slight thyroid effects were observed at even higher exposure doses: e.g. 400 mg/kg bw /day. The NOAEL of 25 mg/kg bw/day was based on thyroid effects observed in the 90-day study (SCCNFP/0779/04 and SCCP/1042/06).
Taken these together, the SCCS is of the opinion that it is not biologically plausible that the molecular parameters measured in these studies at levels lower than the current NOAEL

could be associated with any adverse effects on reproduction that have not been demonstrated in earlier studies conducted under standardised and controlled protocols.

3.3.5.2 Developmental Toxicity

From XXIV/1377/96
A teratogenicity study revealed a NOAEL value for developmental effects of 10 mg/kg bw/day, based upon the observation of some retardation of ossification at 30 mg/kg bw/day. There was no evidence of teratogenesis. Based on this study, the NOAEL for developmental toxicity was determined as being 10 mg/kg bw/day.

From SCCP/1184/08
In the Opinion from 2008 the full developmental toxicity test report from 1988 was studied. This reveals that the effects on which the above-mentioned NOAEL is based are not clearly related to the test substance and the data obtained are not statistically significant. The study cannot be used to derive a NOAEL.

Data submitted 2019

Information taken from EFfCI
In a prenatal developmental toxicity study, groups of female rats were dosed with the test substance at dose levels of 0, 10, 30 and 100 mg/kg/day by oral gavage between days 6 to 15 of gestation (Gleich 1988). These females were killed on day 20 of gestation and the uterine contents examined in detail to evaluate any potential effects on reproduction and the embryo. Over this treatment regime, the dose levels of 10 and 30 mg/kg/day proved to be non-toxic to the pregnant female rat. However, the dose level of 100 mg/kg/day was shown to be minimally toxic to the dams as demonstrated by a slightly lower body weight gain by these females. There was no evidence of an effect of treatment on maternal reproductive parameters at any of the dose levels examined.
A small but statistically significant reduction in body weight was recorded for foetuses from Group 4 (100 mg/kg/day). Corresponding to these lower foetal weights in Group 4 was a lower degree of ossification of the sternum and the extremities seen in foetuses from this treatment group. Since a level of maternal toxicity was seen in this treatment group (slightly reduced weight gain), this incidence of reduced ossification was considered to be secondary to this effect on the dams. The dose-dependent increase of rudimentary lumbar ribs in both sexes of foetuses from Groups 3 and 4 (30 and 100 mg/kg/day) was also attributed to stress in the dams being sufficient to express the developmental instability inherent in the species.
Consequently, the NOAEL for both maternal and foetal toxicity in this study was determined to be 30 mg/kg bw/day.

Ref.: Gleich *et al*., 1988

A publication was submitted that described a developmental toxicity study with 4-MBC (Lichtensteiger *et al*., 2015). In this study, rats were exposed to mixtures of chemicals, including 4-MBC. There were no treatment groups that studied the effects of 4-MBC alone. This study on mixtures was therefore excluded.

Ref: Lichtensteiger *et al*., 2015

SCCS comment
The authors considered that the reduced ossification is due to reduced body weight gains in dams. From the SCCS point of view, this conclusion is questionable, since maternal toxicity does not necessarily lead to reduced ossification (Nitzsche, 2017).
The NOAEL for maternal and foetal toxicity from this developmental study is 30 mg/kg bw/day, which is higher than the previously reported NOAEL of 25 mg/kg bw/day for 4-MBC.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

Taken from SCCNFP/0779/04

A standard Ames test was carried out. There was no evidence of mutagenesis, with or without activation.

An *in vitro* chromosomal aberration test was carried out using a Chinese hamster V79 cell line. The test was evaluated as negative.

New data submitted on March 2022

Gene mutation study in Chinese hamster V79 cells (OECD TG 476; HPRT)

Guideline: OECD 476 (adopted on 29 July 2016)
Cells: Chinese hamster lung fibroblasts cell line V79
Replicates: duplicate cultures in two independent experiments
Test substance: 4-MBC, CAS 36861-47-9
Batch: 10300052
Purity: 99.8 % pure
Solvent: DMSO
Concentrations: Experiment 1 and 2 (4 hours of exposure)

- in the absence of S9 mix: 0.75*, 1.5*, 3*, 6*, 8*, 10, 12, 16 and 20 µg/mL (concentrations with * were analysed, relative adjusted cloning efficiency I at 8 µg/mL 5.6 % in Exp. 1 and 4.8 % in Exp. 2)
- in the presence of S9 mix: 0.78, 1.6*, 3.1*, 6.25*, 12.5*, 25*, 50* and 100 µg/mL (concentrations with * were analysed, relative adjusted cloning efficiency I at 50 µg/mL 73.8 % in Exp. 1 and 18.3 % in Exp. 2) Expression period 7 days.

Positive controls: -S9mix: ethylmethane sulfonate, EMS, final concentration: 300 µg/mL (2.4 mM); +S9mix: 7,12-dimethylbenz(a)anthracene, DMBA, final concentration: 2.3 µg/mL (8.9 µM)
GLP: in compliance
Study period: July 2017 - August 2017
Reliability: Klimisch 1

Test Procedure
The main test was conducted in medium at test item concentrations of 0.75, 1.5, 3, 6, 8, 10, 12, 16 and 20 µg/mL in the absence of metabolic activation and 0.78, 1.6, 3.1, 6.25, 12.5, 25, 50 and 100 µg/mL in the presence of metabolic activation. A solvent control (DMSO) and positive controls (EMS, DMBA) were tested concurrently. Five replicates were assessed per test item treatment and control, and two parallel cultures were used throughout the assay. The treatment period was 4 hours, with and without metabolic activation, with a 24-hour pre-exposure period and 7-day expression (post-exposure) period in medium. Cells were fixed and stained with 10% methylene blue in 0.01% KOH solution 8 days after treatment.
Results
Precipitation and/ or severe cytotoxicity were observed in the four highest test concentrations without metabolic activation (10, 12, 16 and 20 µg/mL) and at the highest test concentration with metabolic activation (100 µg/mL), therefore mutation frequency was not assessed for these concentrations. Severe cytotoxicity also occurred at the

concentration of 8 µg/mL without metabolic activation, and this data were not taken into the consideration of the evaluation of this assay.
No relevant and reproducible increase in mutant colony numbers per 1 million cells was observed with and without metabolic activation.

Conclusion
The test item is considered to be non-mutagenic in the HPRT assay.

Ref: Chang (2017)

SCCS comment
In the opinion of the SCCS this study is valid and shows negative results of 4-MBC in V79 gene mutations endpoint.
The SCCS has noted a very slight increase in mutant frequency (MF) outside the 95% control limits of the solvent historical control data in culture I at 6 µg/mL (30.3 vs. 30.2 in historical control 95% CI). However, as this was a minor change, isolated, not associated with a statistical trend, it was considered biologically not meaningful.

SCCS comment on chromosomal aberration study
After re-evaluation of the chromosomal aberration study on 4-MBC from 1986 (Ref. 22 in SCCNFP/0779/04) the SCCS has noted that:

- one concentration of 3 µg/mL +S9, which was the lowest tested after 18 h of treatment, induced an increased number of aberrations; as the increase was only slight without any concentration-effect relationship, the result can be treated as not biologically meaningful,
- only 4 h exposure was used with and without S9 mix which is not in line with current OECD TG 473 (adopted on 29 July 2016) recommending using 4 h (-/+ S9) and 24 h (-S9) exposure.

Overall SCCS conclusion on genotoxicity
4-MBC was tested in one valid bacterial gene mutation study and one valid mammalian gene mutation study with negative results. The single available re-evaluated chromosomal aberration study does not meet the acceptance criteria of the current OECD TG 473. Therefore, the study was considered as inconclusive. Overall, the SCCS is not able to conclude on genotoxicity of 4-MBC with the currently available evidence.

3.3.6.2 Mutagenicity / genotoxicity *in vivo*

No new data was submitted in 2019.

3.3.7 Carcinogenicity

No data

3.3.8 Photo-induced toxicity

3.3.8.1 Phototoxicity / photo-irritation and photosensitisation

From SCCNFP/7799/04 and SCCP/1042/06
A mice study with 5% 4-MBC and a human study with 4% 4-MBC did not reveal any phototoxic effect. A guinea pig study with 5% 4-MBC and a human study with 4% 4-MBC did not reveal any photosensitising potential.

No new data were submitted in 2019.

SCCS Comment
4-MBC has occasionally been reported as being phototoxic to humans. Based on a European multi-center photo-patch test study on 1031 eligible patients, there were only 3 positive reactions to 4-MBC. Thus, the occurrence of phototoxicity from 4-MBC appears to be very low.

Ref: EMCPPTS (2012)

3.4.8.2 Photomutagenicity / photoclastogenicity

Taken from SCCNFP/0779/04

4-MBC did not increase the number of revertants in the presence of UVA/UVB light. The compound was not able to induce photomutagenic effect on *S. typhimurium* TA 102 and TA 1537 and on *E. coli* under the conditions of this study. The positive control induced a clear photomutagenic effect in *S. typhimurium* TA 102 and in *E. coli* WP2. It was weakly photomutagenic in *S. typhimurium* TA 1537.

4-MBC has been tested for photoclastogenicity potential on mammalian cells (CHO-K5) exposed to UVA/UVB light. The CHO cells were treated with concentrations of 4-MBC ranging from 1 to 6.6 µg/ml (3 doses) and concomitantly exposed to solar simulated irradiation at UV doses ranging from 200 to 2000 mJ/cm² of UVA and from 4 to 25 mJ/cm² for UVB. Concomitant controls were made in the absence of UV light and with positive control exposed to UV light (chloropromazine). Under the experimental conditions, there was no indication of photoclastogenicity induced by 4-MBC.

No new data was submitted in 2019.

3.3.9 Human data

/

3.3.10 Special investigations

Endocrine activity

During the call for data, SCCS received reports from EFfCI and CEHOS (Hass *et al*., 2012) that describe the available studies on the endocrine activity of 4-MBC. In addition, literature was provided by Italy, the United Kingdom and MEDA pharma. These studies were described in the dossiers from EFfCI and CEHOS.
Recently, the ECHA Member States Committee unanimously identified 4-MBC covering any of the individual isomers and/or combinations, as SVHC, due to their endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health (ECHA, 2021). This decision is based on evaluations of the same *in vitro* and *in vivo* studies that were used by EFfCI and CEHOS in their assessments. On 17 January 2022 4-MBC was added in the Candidate List of substances of very high concern (ECHA decision, 2022).

The assessment done for ECHA is used as a lead for the evaluation of endocrine activity of 4-MBC by the SCCS and relevant parts are included in this Opinion. The full report can be found online (ECHA, 2021).

1) Non-test information, *in silico*, read across, *in chemico*

No data submitted.

2) In vitro and other assays

From SCCNFP/0483/01
A general screening assay (E-screen) with a human breast cancer cell line, MCF-7 cells, was carried out. A positive test was based upon the binding of the test compound with the estrogen receptor leading to cell proliferation.
As a positive control, 17 b-estradiol, was used and it was, as expected, positive in the assay. 4-MBC was found to be positive in the assay and caused cell proliferation. EC_{50} values for 17 b-estradiol and 4-MBC were found to be 1.22 pM and 3.02 µM, respectively.
The SCCNFP concluded that the potency of the positive control is in the order of picomoles; the *in vitro* potency of the UV-filters tested lays in the range of micromoles, which means a difference of 1 million units.

Data submitted 2019
Information taken from CEHOS (2012)
Several *in vitro* studies with 4-MBC have been performed. The compound has been shown to act as an estrogen by altering gene transcription in MCF-7 cells (Heneweer *et al*., 2005) and causing their proliferation (Tinwell *et al.,* 2002; Schlumpf *et al*., 2001; Schlumpf *et al*., 2004; Matsuomo *et al*., 2005). It has been shown to bind to the ER (Minh *et al*., 2008; Matsuomo *et al*., 2005; Seidlowa-Vuttke *et al.,* 2006a; Gomez *et al*., 2005; Schreurs *et al*., 2005) and it has been reported to be a preferential ER-β ligand with limited ER-α binding capacity *in vitro* (Mueller *et al.,* 2003; Schlumpf *et al*., 2004a). In other studies, no clear-cut difference in the effect of 4-MBC on ERα and ERβ was evident (Schreurs *et al*., 2002). Morohoshi *et al.,* 2005 also found no ER binding in the two-yeast receptor assay. No androgenic or antiandrogenic effect *in vitro* was seen in a study by Ma *et al*., 2003, while the presence of weak anti-androgenic activity and strong progesterone activity was seen by Schreurs *et al*., 2005. Furthermore, 4-MBC can also affect the thyroid system *in vitro*, by binding to the thyroid receptor (Hoffmann *et al.,* 2009). These results show that 4-MBC probably has multiples endocrine disrupting modes of action, including estrogenic action.

Conclusion from CEHOS:
In vitro, there is strong evidence of estrogenic activity, as 4-MBC has been shown to bind to the ER, alter gene transcription and cause proliferation of MCF-7 cells. No androgenic or anti-androgenic effects *in vitro* were seen in one study, while anti-androgenic activity and strong progesterone activity was seen in another study. 4-MBC can also affect the thyroid system *in vitro*, by binding to the thyroid receptor.

Information taken from EFfCI
Only studies that are not reported in CEHOS (2012).

Gomez E *et al.,* 2005 investigated the oestrogenic activity of 4-MBC in a reporter gene assay, using ER negative, ERα and ERβ cell lines (HELN, HELN ERα and HELN ERβ, respectively). 4-MBC resulted in activation of the ERα cell line, with approximately 40% transactivation at concentrations of 3×10^{-6}M and above relative to treatment with 1×10^{-8}M E2. A slight (<30%) activation of the ERβ receptor was observed in the same concentration range.

Morohoshi K. *et al*., 2005 investigated the oestrogenic and anti-oestrogenic activity of 4-MBC, using two *in vitro* assays: (1) an ELISA-based estrogen receptor competitive binding assay (ER-ELISA) and (2) a modified yeast two-hybrid estrogen assay, with and without addition of rat liver S9 mix. No estrogenic activity of 4-MBC.

Kunz PY, *et al.,* 2006 investigated the oestrogenic activity of 4-MBC (0.06-2.7 µg/L), in the YES assay using *S. cerevisae* yeast transfected with the human oestrogen receptor α (hERα), among others. 4-MBC did not result in any effects on hERα activation, indicating that it did not have any agonistic estrogenic activity in this yeast transactivational assay.
Kunz PY and Fent K 2006 investigated the endocrine activity of 4-MBC (10^{-7} to 10^{-2} M), in the YES assay using yeast *S. cerevisae* stably transfected with either the human oestrogen receptor α (hERα) or the human androgen receptor (hAR) in order to test either the estrogenic/anti-estrogenic activity of 4-MBC, or its androgenic/anti-androgenic activity. In the ECHA Annex XV report (2021), it is stated that 4-MBC did not cause any cytotoxicity in any assay up to 10^{-2} M, the highest tested concentration. No estrogenic transactivation was measurable in the estrogen agonism assay, but clear anti-estrogenic activity was observed. Complete inhibition of E2-induced activity was measured at the highest concentration tested and a full dose-response curve was obtained, demonstrating the anti-estrogenic potency of 4-MBC. The IC_{50} was determined as 8.73 x 10^{-5} M (22 mg/L). No androgenic transactivation was measured, but clear anti-androgenic activity was observed. A full dose-response curve with complete inhibition of dihydrotestosterone (DHT), proving the anti-androgenic potency of 4-MBC. The IC_{50} was determined as 1.18 x 10^{-5} M (3 mg/L) 4-MBC did not show any agonist activity on hERa or AR. 4-MBC did show anti-oestrogenic and anti-androgenic activity, but only at test concentrations that were above the water-solubility limit for the substance.

Schlumpf M *et al.,* 2006, investigated the oestrogenic activity in the E Screen cell proliferation assay in MCF-7 cells, and in recombinant ERα and ERβ ligand-binding assays, and the binding of 4-MBC to proteins in cytosolic porcine extracts of uteri was also tested. Weak oestrogenic activity with an EC50 of 3.99 µM (1.01 mg/L) for cell proliferation was observed in the E-Screen. Some test concentrations were above the water-solubility limit for the substance. Some evidence for weak oestrogenic activity was observed with the ER beta recepter but not with the ERalpha.

Schmitt *et al*., 2008, investigated the oestrogenic activity of 4-MBC in a yeast assay transfected with the human oestrogen receptor α (hERα). A significant increase in β-galactosidase activity was observed, which followed the activation of hERα and was therefore associated with an oestrogenic response, but only at test concentrations above the water-solubility limit of 4-MBC.

Schiffer C *et al.,* 2014 investigated the effects of 4-MBC on the activation of the sperm-specific CatSper channel. The study highlights potential concerns for sperm *in vitro*, through CatSper activation.

Rehfeld A *et al.*, in 2016, investigated the effects of 4-MBC on CatSper channel activated Ca^{2+} influx. Suboptimal Ca^{2+} influx is associated with reduced male fertility, and correct CatSper function is absolutely essential for fertilisation. The study was conducted with human sperm cells and highlights potential concerns for sperm *in vitro*, through CatSper activation, at exposure concentrations between 10^{-3} and 10^{3} µM (0.00025 and 254 mg/L) 4-MBC for up to 232 seconds. 4-MBC was among the most potent of all 29 tested UV filters, and at 10 µM the mean relative maximal induction of Ca^{2+} signal was 97% of progesterone response at 5 µM. The EC_{50} for 4-MBC was 0.52 ± 0.31 µM. The Ca^{2+} signals induced by 4-MBC were fast and transient and resembled the Ca^{2+} signal induced by progesterone, suggesting a similar mode of action between this UV filter and progesterone.

Rehfeld *et al*., in 2018, investigated the *in vitro* effects on sperm of 4-MBC. The study highlights potential concerns for sperm *in vitro*, through CatSper activation.

SCCS comment
Kunz *et al. (*2006) study investigating oestrogenic effect of 4-MBH was conducted at concentrations higher than the water solubility limit.

Information taken from ECHA Annex XV proposal 2021
Yin *et al.,* 2015: investigated the anti-progestogenic activity of 4-MBC (test concentrations from 10^{-8} to 10^{-5} M) by using human endometrial epithelial adenocarcinoma Ishikawa cells. This was compared with the effect of three selective PR modulators. Effects of 4-MBC in combination with progesterone on the progesterone-sensitive target gene estrogen sulfotransferase (SULT1E1) were obtained by RT-qPCR. The SULT1E1, which plays a critical role in the inactivation of estrogens and in the pathogenesis of estrogen dependent tumours, was identified as the most responsive marker in Ishikawa cells to anti-progestogenic effects by gene expression profiling in the research group's previous work. The induction of progesterone on SULT1E1 mRNA levels by progesterone was concentration-dependently antagonised by RU486, UPA and ZK137316, whereas 4-MBC had no effect on SULT1E1 mRNA levels, indicating no anti-progestogenic effect in this test system.

Nashev *et al.,* 2010 worked on several 17β-hydroxysteroid dehydrogenase activities in Human Embryonic Kidney HEK-293 cells transfected with plasmids expressing human 17β-HSD1, 2, 3 and 5 playing an important role in the testosterone production and conversion, as well as AR. These experiments showed that 4-MBC concentration dependently inhibited 17β-HSD3 mediated conversion of AD to testosterone, with an estimated IC_{50} of 10.7 µM. 4-MBC did not inhibit 17β-HSD5, but did inhibit 17β-HSD1 and 17β-HSD2 activity. 17β-HSD2 was inhibited with an IC_{50} of 5.9 µM. 17β-HSD1 was weakly inhibited by 4-MBC (IC_{50} of 70 µM).
MBC did not activate AR at concentrations up to 20 µM, but did act in an anti-androgenic manner by inhibiting testosterone-dependent AR activation (84 % AR activation compared to testosterone)

Jimenez-Diaz, I. *et al*. (2013) worked on interactions of 4-MBC with the hERα and hAR using two *in vitro* bioassays based on reporter gene expression (in PALM prostatic cells) and cell proliferation assessment (E-Screen bioassay using MCF-7 cells). In the E-screen, 4-MBC (10 µM) increased the number of cells by approximately 2.8-fold (EC_{50}= 24.14 µM). 4-MBC did not antagonise E2-induced proliferation in MCF-7 cells. 4-MBC did not show agonistic AR activity (tested in the concentration range of 0.01–10 µM). When the AR antagonistic activity was tested, 4-MBC proved to be potent hAR antagonist at 10 µM concentration (IC_{50} = 9.12 µM, respectively), strongly inhibiting the luciferase activity induced by R1881. In conclusion, 4-MBC showed estrogenic activity in the E-Screen bioassay and potent hAR antagonism

Jocsak G *et al*., 2016 investigated if 4-MBC affects ERβ mRNA expression in primary cerebellar cell cultures. They showed that 4-MBC affected ERβ mRNA expression in these cell cultures.

3) In vivo assays

From SCCNFP/0483/01
An uterotrophic assay was carried out using two different exposure routes, namely oral and dermal exposure.
For the oral exposure, a dose-dependent increase of uterine weights was observed for 4-MBC, but no maximal effect was seen, as was the case for the positive control.
ED50 values were found to be 0.818 µg/kg/day and 309 mg/kg/day for the positive control and 4-MBC, respectively.
For the dermal exposure assay, 4-MBC exhibited a dose-dependent increase in uterine weight, with a significant effect at a concentration of 5% and 7.5% in olive oil**.**

The SCCNFP noticed that the assay deviated from the OECD guideline proposal and several methodological shortcomings were noticed. Furthermore, *in vivo* potency of the UV-filters is importantly lower than the one observed for the positive control.
According to data of Schlumpf *et al*. (2001) The NOEL (estrogenic activity) of 4-MBC in the *in vivo* is 66 mg/kg bw/day. The SCCNFP concluded that, based on the actual scientific knowledge, organic UV-filters used in cosmetic sunscreen products, including 4-MBC, which are allowed on the EU market today have no estrogenic effects that could potentially affect human health.

Human data

From SCCP/1042/06
A number of human tests for the effect on thyroid and pituitary hormones following cutaneous application were described.
In the largest (double-blind) study, 24 volunteers (12 males and 12 females) were dermally exposed twice per day to 5 grams of an oil-in-water formulation containing 6% of 4-MBC, for 14 days. No significant change in thyroid-related hormones was noted. The thyroid volume was found to be reduced by 1.7% in the treated group and increased by 3.11% in the placebo group (also consisting of 12 males and 12 females). Although these findings were found to be statistically significant, the authors attribute them to the inaccuracy of the method used rather than to any substance-related effect.

Weight-of-evidence assessment on endocrine activity relevant for human health - ECHA (2021)

Please note that all references can be found in the ECHA (2021).

<u>Thyroid mode of action</u>

Endocrine activity via T modality:
Integrated lines of evidence led to the conclusion that there is **strong evidence of endocrine activity via T modality.** Few studies investigated the same endpoints in relation to thyroid disruption, but the collected **mechanistic data show some evidence for thyroid disrupting properties *in vitro***. Table 4 summarizes the studies, showing the lines of evidence for endocrine activity via T modality.

Table 4: Lines of evidence for endocrine activity via T modality*

Table 4.4 Lines of evidence for **endocrine activity** via **T modality** (*in vitro* mechanistic). All reported changes are statistically significant with p<0.05, unless specified otherwise.

Reference	Modality	Effect classification	Effect target	Observed effect	Assessment of each line of evidence
Hofmann *et al.* 2009 (Klimisch 2)	T	*In vitro* mechanistic	Disruption of the Thyroid Hormone system	Both agonist & antagonist activity on the TRa	**Some evidence for thyroid hormone (TH) disrupting properties** Few studies have investigated the same endpoints. However, the data indicates that 4-MBC can act as both an agonist and antagonist on the thyroid receptor and increase deiodinase gene expression. K3/K4 studies: Thyroid peroxidase inhibition was not observed. One study showed decrease iodine uptake.
Hofmann *et al.* 2009 (Klimisch 2)	T	*In vitro* mechanistic	Disruption of the Thyroid Hormone system	↑ DIO1 expression	
Song M *et al.* 2013 (Klimisch 2)	T	*In vitro* mechanistic	Disruption of the Thyroid Hormone system	Upregulated expression of genes associated with deiodinase activity	
Schmutzler *et al.* 2004 (Klimisch 3)	T	*In vitro* mechanistic	Disruption of the Thyroid Hormone system	No TPO inhibition	
Schmutzler *et al.* 2007 (Klimisch 4)	T	*In vitro* mechanistic	Disruption of the Thyroid Hormone system	Decreased iodine uptake into FTRL-5 cells	

*Table taken from the ECHA (2021)

The *in vivo* mechanistic data show **moderate-strong evidence of effect on thyroid hormone levels** in rats exposed in adulthood with an overall pattern of increased TSH and T3 and, in some studies, decreased or unaffected T4 levels. One study investigating perinatal exposure found decreased TSH and T3 in 55-day old offspring that were no longer exposed after PND 22, where increased TSH and T3 levels were seen in adult offspring after continued exposure after weaning (Maerkel *et al*., 2007). The perinatal studies did not see effects on T4. Two studies reported no effects on thyroid hormone levels – a dermal study in rats and a dermal experimental study in humans.
Two published studies of lower reliability found evidence of altered TSH, T4 (Seidlova-Wuttke *et al.,* 2006a) and T3 levels (Schmutzler *et al.,* 2004) and another study found no evidence of such effects in dogs (n=2/sex) exposed orally.

Adverse effect related to T modality:
The lines of evidence showed **strong evidence to conclude that 4-MBC has adverse effects via T modality *in vivo*** (Table 5).

Table 5: Lines of evidence for endocrine activity via T modality (*in vivo* mechanistic)*

Table 4.5: Lines of evidence for **endocrine activity** via **T modality** (*in vivo* mechanistic). All reported changes are statistically significant with p<0.05, unless specified otherwise. NS: Non-statistically significant

Reference	Modality	Effect classifi-cation	Effect target	Species	Exposure	Route	Dose (mg/kg bw/day)	General toxicity	Observed effect on thyroid hormone level	Assessment of each line of evidence
OECD TG 408 Unpublished, 1984a (Klimisch 1)	T	*In vivo* mecha-nistic	Thyroid hormon e (TH) levels	Rat	90 day study (n=20/ sex)	Feed	50, 125, 312. A dose of 25 was included with its own control group.	Decreased bw gain in females at 312 mg/kg (transient in 125 mg/kg). No effect on male bw. Relative liver weights were increased in females at 125 and males at 312 mg/kg bw/day.	All doses from 50 mg/kg and above caused stimulatory effect on the thyroid gland in males and females, including increases in circulating T3 and TSH levels. T4 levels were normal except from a few isolated cases.	**Moderate-Strong evidence** of effect on TH levels. All rodent studies using oral exposure report effects on THs after exposure. There is a very consistent pattern of increased TSH and T3. In studies examining higher doses, T4 was typically decreased, whereas studies investigating lower doses showed unaltered T4 levels. A dermal study in rats showed no effects on TH levels, and also no effects were seen in a dermal study in humans.
Unpublished, 1983a (Klimisch 1)	T	*In vivo* mecha-nistic	TH levels	Rat	17 day repeated dose (n=10/ sex)	Oral	30, 300	No effect on bw or food consumption	Increased serum TSH (1.9 fold in males and 7.5 fold in females) at 300 mg/kg bw/day	
Unpublished, 1983b (Klimisch 2)	T	*In vivo* mecha-nistic	TH levels	Rat	28 day repeated dose (n=10/ sex)	Oral	1000	Marked systemic toxicity, seen as clinical signs and markedly reduced bw.	T3 serum levels were increased by 96% in males and 28% in females, and T4 serum levels were decreased by 30% in males and 23% in females, compared to the controls.	
OECD TG 421 Unpublished, 2004 (Klimisch 2)	T	*In vivo* mecha-nistic	TH levels	Rat	21 days exposure in the pre-mating period (n =3-5)	Oral	12.5, 25, 50	In parental females there were no treatment-related clinical signs, no mortality and no effects on bw gain or food consumption in any dose. Water consumption was increased in the high dose group.	Females: T4 levels unaffected. TSH marginally increased by 15-25% in mid and high dose group (NS. Likely due to high variation). T3 dose-dependently increased with a 25% increase in the high dose group (NS. Likely due to high variation).	
OECD TG 421 Unpublished, 2004 (Klimisch 2)	T	*In vivo* mecha-nistic	TH levels	Rat	Premating, gestation, lactation (84 days exposure. n=10 parental females/ exposure group)	Oral	12.5, 25, 50	4-MBC exposure caused no effect on dam bw after 84 days of exposure, but water consumption was increased in the high dose group.	Dams on PND22 (after 84 days of exposure): No effect on T4 and TSH. Small (8-11%), increase in T3 in all dose groups (8-11%) (NS. Likely due to high variation.	
OECD TG 421 Unpublished, 2004 (Klimisch 2)	T	*In vivo* mecha-nistic	TH levels	Rat	Gestation, lactation until PND 22 (n=30)	Oral	12.5, 25, 50	No decreases in offspring bw were observed during the course of the study (including on PND 55) in 4-MBC exposed animals. Liver weights were not assessed.	Offspring PND55 Males: No effects on T4. T3 (16%) and TSH (16%) levels were decreased in high dose group. Females: No effects on T4 and TSH. T3 levels were NS decreased in high dose group (11%).	K3/K4 studies: Two studies in rats point in the same direction as the other rats studies whereas one study in dogs showed no effects on TH levels
Maerkel *et al.* 2007 (Klimisch 2)	T	*In vivo* mecha-nistic	TH levels	Rat	Pre-mating to PND 12.	Feed	7, 24, 47	Bw were unaffected at 12 weeks of age (PN 84). Liver weights were not reported here but were reported as unaltered in adult male offspring in the Durrer *et al.* 2007.	Adult offspring: Increased levels of TSH in females in the high dose group and a slight increase in T3 (middle and high dose group). No effects on male TSH and T3. Serum T4 levels were unaffected in both sexes.	
OECD 411 Unpublished, 2005 (Klimisch 1)	T	*In vivo* mecha-nistic	TH levels	Rat	90 days (n=20/sex)	Dermal	100, 400, 2000	No effect on body or liver weight seen at 100 and 400 mg/kg. Highest dose terminated after 11 days due to severe local effects.	No treatment-related changes on T3, T4 or TSH levels were observed.	
Unpublished, 1995 (Klimisch 2)	T	*In vivo* mecha-nistic	TH levels	Human (male and female)	14 days, twice daily	Dermal	6% on 1200 cm2 body surface	No systemic toxicity observed	There was no effect on thyroid hormone levels (T3, T4, TSH).	
Schmutzler *et al.* 2004 (Klimisch 3)	T	*In vivo* mecha-nistic	TH levels	Rat	12 weeks (adult, ovariecto mized rats) (n=8-11)	Feed	66, 310 mg/ animal/day	Clinical symptoms, body and liver weight were not reported in this publication	At both doses TSH and T3 levels were elevated, and T4 serum levels were decreased. In the liver, type I 5-deiodinase was decreased in both treatment groups (NS)	
Seidlova-Wuttke *et al.* 2006a (Klimisch 3)	T	*In vivo* mecha-nistic	TH levels	Rat	12 weeks (adult ovariecto mized rats) (n=12)	Feed	230, 1000	Animals exposed the to both doses of 4-MBC gained less bw than controls	Both doses caused decreased T4, increased TSH and NS increase in T3 levels.	
Unnamed, 2003 (Klimisch 3)	T	*In vivo* mecha-nistic	TH levels	Dog	21 day study (n = 2/sex)	Oral	0 mg/kg on day 1, 20 mg/kg on day 4, 100 mg/kg on day 8, 500 mg/kg on days 11 - 21.	No general toxicity was observed	No effect on thyroid hormone levels (T3, T4, TSH).	

*Table taken from the ECHA (2021)

Table 6: Lines of evidence for endocrine activity via T modality*

Table 4.6: Lines of evidence for **adverse effects** *in vivo* via **T modality**. All reported changes are statistically significant with p<0.05, unless specified otherwise. NS: Non-statistically significant.

Reference	Modality	Effect target	Species	Exposure	Route	Dose (mg/kg bw/day)	General toxicity	Observed effects	Assessment of each line of evidence
OECD TG 408, Unpublished 1984a (Klimisch 1)	T	Thyroid gland	Rat	90 days (n=20 animals /sex)	Feed	0, 50, 125, 312. A dose of 25 was included with its own control group.	Decreased bw gain in females at 312 mg/kg (transient at 125 mg/kg). No effect on male bw. Relative liver weights were increased in females at 125 and males at 312 mg/kg bw/day.	Doses of 50, 125 & 312 mg/kg bw/day induced increases in thyroid weights and follicular hyperplasia, hypertrophy etc. in both sexes in a dose related manner. The changes in the thyroid were still apparent at the end of the follow-up recovery period albeit less pronounced. No effects seen at 25 mg/kg bw/day.	**Strong evidence for effect on the thyroid gland** All oral rat studies investigating thyroid weight, report an increase, and almost all report adverse histopathology findings on the thyroid gland. A rat study using dermal exposure route did not see effects on thyroid gland histopathology. K3/K4 studies: Two studies were performed in dog. In one of them indications of adverse effects on histopathology were seen, in the other one there were no effects.
Unpublished, 1983a (Klimisch 1)	T	Thyroid gland	Rat	17-day study (n=10/sex)	Oral	0, 30, 300	No effect on bw or food consumption	At 300 mg/kg bw/day the thyroid gland weight was increased (140% in males and 160% in females). Hypertrophy of the follicular epithelium with occasional incisures of the thyroid gland was also observed pathologically (4/20 in controls, 8/20 at 30 mg/kg and 16/20 at 300 mg/kg).	
Unpublished, 1983b (Klimisch 2)	T	Thyroid gland	Rat	28-day study (n=10/sex)	Oral	0, 1000	Marked systemic toxicity, seen as clinical signs and markedly reduced bw (20% lower in males and 10 % lower in females). Liver weights were increased and thymus and adrenal weights reduced.	Thyroid weights increased approximately 1.9 fold in both sexes. Males displayed mild (1/10 animals), moderate (5/10) to marked (4/10) stimulation of the thyroid, whereas mainly mild stimulation (7/19 animals. 2 animals were moderately-marked) were observed in the females.	
OECD TG 421 Unpublished, 2004 (Klimisch 2)	T	Thyroid gland	Rat	Pre-mating, mating and lactation (n=10)	Oral	12.5, 25, 50	No effect on dam bw after 84 days of exposure, i.e. at the time of weaning, but water consumption was increased in the high dose group. Liver weight was not assessed.	Dams on PND22 (after 84 days of exposure): Marked increase of 19% in thyroid weight in the high dose group (NS. Likely due to high variation)	
OECD TG 421 Unpublished, 2004 (Klimisch 2)	T	Thyroid gland	Rat	Gestation, lactation until PND 22 (n=10)	Oral	12.5, 25, 50	No effect on offspring bw on PND 22. Liver weights not assessed.	Offspring PND22: Marked increase (33%) in thyroid gland weights in male offspring at weaning (NS. Likely due to high variation). No effect in females.	
OECD TG 421 Unpublished, 2004 (Klimisch 2)	T	Thyroid gland	Rat	Gestation, lactation until PND 22 (n=10)	Oral	12.5, 25, 50	No effect on offspring bw on PND 55. Liver weights not assessed.	Offspring PND55: No effect on male or female thyroid gland weight or histology.	
Maerkel *et al.* 2007 (Klimisch 2)	T	Thyroid gland	Rat	Fetal, postnatal, pubertal and adult exposure	Feed	7, 24, 47	Offspring bw were unaffected at 12 weeks of age (PN 84). Liver weights were not reported here but were reported as unaltered in the Durrer *et al.* 2007	Adult offspring: 24 and 72 mg/kg bw/day markedly increased thyroid weights (abs. and rel.) in a dose related manner in males and females (24 mg/kg: 40-53 %; 72 mg/kg: 25-32 %). No effect was seen at 7 mg/kg bw/day.	
OECD TG 411 Unpublished, 2005 (Klimisch 1)	T	Thyroid gland	Rat	90-day study (n=20/sex). Only 11 days in 2000 mg/kg bw/day dose.	Dermal	100, 400, 2000	No effect on body or liver weight seen at 100 and 400 mg/kg. Highest dose terminated after 11 days due to severe local effects.	No effect on thyroid gland was seen at any dose level.	
Unpublished, 2003 (Klimisch 3)	T	Thyroid gland	Dog	14-day study (n=1/sex)	Oral	20 mg/kg on day 1, 100 mg/kg on day 2, 500 mg/kg on day 3, 2500 mg/kg on day 4 and 500 mg/kg on days 5–14	No effect on food consumption, bw, clinical chemistry or gross pathology. Vomiting seen after treatment with 2500 mg/kg in males.	The male dog showed minimal activation of the thyroid gland, characterized by small and middle-sized follicles. No effects were seen in the female dog.	
Unnamed, 2003 (Klimisch 3)	T	Thyroid gland	Dog	21 day study (n=2/sex)	Oral	0 mg/kg on day 1, 20 mg/kg on day 4, 100 mg/kg on day 8 and 500 mg/kg on days 11-21.	No general toxicity was observed	There was no effect on thyroid gland histopathology. No general toxicity was observed	
TG 421 Unpublished, 2004 (Klimisch 2)	Sensitive to, but not diagnostic of T	Nervous system	Rat	Gestation, lactation until PND 22 (n=10)	Oral	12.5, 25, 50	No effect in offspring bw were observed. Offspring liver weights were not assessed.	Offspring: No effect on learning and memory in male or female offspring.	**Not sufficient evidence to determine whether learning and memory are affected by developmental exposure to 4-MBC** Only investigated in one study using relatively low doses, a group size of 10 and a rather insensitive scoring method for the behavioural results, is not in itself robust enough to exclude effects on neurodevelopment.

*Table taken from the ECHA (2021)

There is **strong evidence for adverse effect on the thyroid system** with several studies reporting increased thyroid weight after oral exposure (Maerkel *et al*. 2007).

In the 90-day oral study (Hoffmann, 1984) thyroid gland weights were elevated and histopathology was adversely affected in both male and female rats (n=20/sex) in a dose-dependent manner. The effects were statistically significant at high doses of 50, 125 and 312 mg/kg. In a developmental toxicity screening study, using only gestational and lactational exposure to 4-MBC, thyroid gland weight in the dams at weaning (PND 22) showed a (non-statistically significant) 20% increase, and in the male offspring, a non-statistically significant 33% increase. No adverse thyroid effects were seen in the offspring on PND 55, suggesting that the adverse effects on the gland were transient if exposure was discontinued. Doses of 24 and 72 mg/kg bw/day markedly and dose-dependently increased thyroid weights (absolute and relative) in adult male and female offspring exposed during development, when exposure continued into adulthood (Maerkel *et al.,* 2007).
A dermal study in rats showed no effects on the thyroid glands, at doses which in an oral study would likely have affected the animals (Unpublished, 2005). It is possible that the lack of toxicological effects was related to differences in toxicokinetics, due to the different route of exposure. In two oral studies in dogs, which due to the low number of animals were assigned a lower reliability score (K3), no clear thyroid effects were seen.
In an oral developmental toxicity study, no exposure-related effects on learning and memory were observed. Since only one study was performed and the employed methods for the assessment of learning behaviour had some important limitations, there is not sufficient evidence to determine whether learning and memory are affected by developmental exposure to 4-MBC.

Estrogenic mode of action

Endocrine activity
There is **weak-moderate evidence of endocrine activity *in vivo*** from effects on **gonadotropin levels;** reliable studies measuring these hormones found changes in LH and FSH levels (Carou *et al*., 2008) and these results are corroborated by results from studies with lower reliability that are pointing in the same direction (Seidlova-Wuttke *et al*., 2006a; Carou *et al*., 2009a; Carou *et al*., 2009b). In adult females exposed prior to mating, quite low doses of 4-MBC caused LH levels to decrease by 60% (non-statistically significant effect), while FSH levels were unaffected. In adult male rats receiving low dose s.c. injections for 2 or 5 days, statistically significantly decreased serum concentrations of LH and FSH and decreases in hypothalamic GnRH release were seen (Carou *et al.,* 2008).
GnRH levels were also affected in dams and offspring in the extended reproductive toxicity screening study (Unpublished, 2004). Here 4-MBC exposure stopped at weaning on PND 22 and the highest investigated dose was 50 mg/kg bw/day. In dams on PND22, FSH concentrations were increased by 260% in the high-dose group (3.6 times higher than control) and LH was increased by 1500% and 3300% in mid- and high-dose groups, respectively (corresponding to 16 and 34 times higher than the control group). The effects were non-statistically significant, likely due to high variation in the data. In male offspring on PND55, a statistically significant decrease in FSH levels was seen in mid- (27%) and high-dose (56%) groups. LH was dose-dependently decreased in mid- (20%) and high-dose (34%) groups (non-statistically significant). In the female PND 55 offspring, a non-statistically significant decrease of 61% in FSH was seen, whereas LH was unaffected. In a developmental study with lower reliability, using a dose of 100 mg/kg every other day during pregnancy, 40-80% decreases in LH, FSH and GnRH levels were seen in the adult male offspring and 90-400% increases in FSH and LH concentrations were seen in the adult female offspring (Carou *et al.,* 2009b). In another developmental study with lower reliability, doses of 100 and 500 mg/kg bw/day during pregnancy altered LH and FSH concentrations in pre- and peripubertal male offspring, but in opposite directions on PND 15 (decrease) and 30 (increase) (Carou *et al.,* 2009a). An unreliable study in adult OXV

females exposed for 12 weeks showed statistically significant increases in the circulating levels of LH (Seidlova-Wuttke *et al.,* 2006a).
One experimental dermal study conducted in humans reported no effects on gonadotropins, which may be due to the exposure route as well as the relative short exposure period of 4 days (Janjua *et al*., 2004).

Adverse effect related to EAS modalities:
There is **moderate-strong evidence for adverse effect related to EAS modalities,** as **altered female sexual behaviour was seen in offspring exposed during fetal, postnatal, pubertal and adult life** (Faass *et al*., 2009). The exposures very markedly and in a dose-related-manner altered the sexual behaviour of adult female offspring. The effects were reduced proceptive and receptive behaviour, and increased rejection behaviour towards the male. The effects were statistically significant both when analysed on an individual basis (n=12-14) and on a litter basis (n=6-7) and were statistically significant in both examined dose groups (7 & 24 mg/kg). The behavioural results were closely correlated to findings of altered gene expression in sexually dimorphic areas of the brain in two studies with lower reliability (Maerkel *et al.,* 2005, 2007).

There is **moderate evidence for other adverse effects on female reproduction, including changes in reproductive organ weights, ano-genital distance (AGD), vaginal opening (VO) and estrous cycling** (Faass *et al*., 2009). One study using a high dose that also induced systemic toxicity, resulted in reduced ovary weights in adult females. In the TG 421 reproductive toxicity screening study, where exposure stopped at weaning, no effects on ovary or uterus weight were seen in dams on PND 22 (after 84 days of exposure). 4-MBC did however statistically and significantly increase ovary weights in the high dose offspring, when measured at weaning. The effects were no longer seen on PND55. This study also found statistically significant reduced uterus weights in offspring's at PND 22, but not at PND 55. None of the studies found any effects on ovary or uterus histology.
AGD was statistically and significantly increased in the female offspring from low- and high-dose groups in the TG421 reproductive screening study. No consistent pattern was seen for VO. Indications of delayed VO was seen in the TG421 study. A statistically significant effect was seen in the low dose group, but not at higher exposures. As exposure in this study stopped at weaning, the females were no longer exposed to 4-MBC at the time of the assessment. In another developmental study, where exposure continued throughout puberty, no effect on female sexual maturation was seen (Durrer *et al.,* 2007). Estrous cyclicity was not affected in females exposed during adulthood (Unpublished, 2004) whereas a non-statistically significant increase in irregular estrous cycling was seen in a study with developmental and continued exposure (Faass *et al.,* 2009). A developmental study with lower reliability showed no effect on uterus weight in adult females exposed during fetal development, while VO was shown to be 3 days advanced (Carou *et al.,* 2009b). The different exposure period and markedly higher doses used in this study may have affected the hormonal system differently that the lower perinatal exposure employed in the TG421 study and the study by Durrer *et al*., 2007. However, the study is assessed to be not reliable, and therefore relatively little emphasis is put on its findings.
No effects of 4-MBC were reported on other female reproductive parameters (number of resorptions, implantations, corpora lutea). Absence of effect on these parameters alone cannot lead to a conclusion that 4-MBC have no endocrine-disrupting effects, as the endpoints investigated are only sensitive but not diagnostic to EATS.

In females, there is moderate to strong scientific evidence that combined perinatal and adult exposure to 4-MBC can lead to adverse effects on sexual behaviour (reduced proceptive and receptive behaviour, and increased rejection behaviour towards the male) as well as a moderate degree of evidence for other adverse effects on female reproductive development (changes in ovary weight, uterine weight and AGD, VO. In addition, there is weak-moderate evidence of alterations in circulating follicle-stimulating hormone (FSH), luteinising hormone (LH) and gonadotropin releasing hormone (GnRH) levels *in vivo*. The

performed MoA analysis shows a biologically plausible link between the estrogenic endocrine mechanism and the reported adverse effects. The molecular initiating event (MIE) is the activation of the ER(s), which can result in increased ER activity in specific tissues, including specific areas of the brain. If such changes occur during the first two weeks of postnatal life, the female brain is not organised properly. This can lead to disrupted regulation of LH and FSH in adulthood and may as a consequence adversely affect sexual behaviour. Additionally, altered ER signalling has previously been shown to alter female AGD, ovary and uterus development and timing of sexual maturation.
In males, there is moderate to strong scientific evidence of persistent reductions in prostate weight in several studies in adult animals, whereas the results are less clear from the available developmental toxicity studies. The Mode of Action analysis shows that estrogens are important regulators of adult prostate growth and function, and that increased ER signalling may affect prostate growth during early prostate development. Although patterns of effects of estrogenic substances may vary, it is biologically plausible that the observed effects of 4-MBC are related to an increase in estrogen signalling. In addition to the role of estrogens, it has been shown that dysregulation of the FSH system plays a significant role in prostate growth. Although the evidence is currently limited, it is biologically plausible that altered gonadotropin secretion may contribute to the observed changes in adult prostate growth.

Other potential modes of action
In addition, there is some supportive *in vitro* evidence showing androgen receptor (AR) antagonistic activity. This endocrine activity could also plausibly contribute to the adverse effects on both the male and female reproductive system in rodents.

Summary of the ED assessment done by ECHA (2021)
There is scientific evidence to conclude that **4-MBC is an endocrine disruptor via T and E modalities**, according to a mode of action analysis including an evaluation of biological plausibility.

SCCS Overall Conclusions on the ED Properties
When taking all lines of evidence into account, the SCCS concurs with ECHA that there is sufficient evidence that 4-MBC may act as an endocrine disruptor and have effects on both the thyroid and estrogen system. Effects on the androgen system are not so evident, as only *in vitro* evidence is available. The *in vivo* studies that investigated effects of 4-MBC on the estrogen system found effects at exposure levels of 100 mg/kg bw/day and higher. Hence, these effects occur at exposures that are much higher than the NOAEL for 4-MBC. Effects observed in more recent reproduction studies of Schlumpf and co-workers (see section 3.3.5 Reproductive toxicity) were considered not to be biologically plausible to support adverse endocrine effects and as such cannot be used to derive a NOAEL or LOAEL.

The effects of 4-MBC observed on the thyroid were consistently demonstrated in several studies. The majority of these studies show that oral exposure levels of 50 mg/kg bw/day of 4-MBC and higher induced significant thyroid effects. Based on the 90-day exposure study, the NOAEL of 4-MBC was determined to be 25 mg/kg bw/day. From dermal exposure, thyroid effects occur at relatively high exposure doses: e.g. 400 mg/kg bw/day.

3.4 SAFETY EVALUATION (including calculation of the MoS)

Based on an evaluation of the data provided, the SCCS is not able to conclude on the genotoxic potential of 4-MBC and therefore a safety evaluation was not performed.

It is important to note that the current re-evaluation of 4-MBC also resulted in a change in the SED, which was calculated to be approximately 4-fold higher than in the previous Opinion. This value would lead to a different MoS as compared to the previous Opinion, and

to a different conclusion on safety, e.g. that a maximum concentration of 4% 4-MBC in sunscreens would not be safe.

3.5 DISCUSSION

Physicochemical properties
All the information provided in the physicochemical part relates only to one substance composed of mixture of 2 isomers (99.8 % E-isomer and 0.2 % Z-isomer).
Information on the stability and the photo-stability of the test substance must be provided.

Exposure assessment & Toxicokinetics
Dermal absorption for 4-MBC was 4.18 µg/cm^2. This value was higher than in the previous Opinions. Due to uncertainties regarding the data and the fact that standard deviations were high, the mean value + 2SD was used, instead of the mean value that was used in earlier assessment by the SCCP. This resulted in a SED of 2.43 mg/kg bw for 4-MBC at 4% in sunscreens.

When comparing the human and rat toxicokinetic data, the AUC and Cmax values in humans are lower than in rat, supporting the reduction of the toxicokinetic factor from 4 to 1. The SCCS therefore agrees to reduce the toxicokinetic factor from 4 to 1 as proposed in the previous Opinion. The SCCS considers the use of a safety factor of 1 appropriate for interspecies differences in toxicokinetics, whereas in the absence of substance specific data, a safety factor of 2.5 to account for interspecies differences in toxicodynamics will be used for MoS calculation (i.e. overall safety factor for interspecies differences is 2.5). Combined with a factor of 10 to account for intra-human toxicokinetic and toxicodynamic differences, this leads to an overall MoS of 25 that will be used for safety calculations.

Toxicological Evaluation

Irritation and corrosivity
4-MBC induced no skin irritation in experimental animals and humans. No mucous membrane irritation was observed in experimental animals.

Skin sensitisation
Tests in humans and animals for skin sensitisation were negative. The animal tests for sensitisation, however, were unsatisfactory, in as much as Freund's complete adjuvant has not been used. It was noted, however, that 4-MBC very rarely caused contact allergies in humans.

Acute toxicity
LD50-oral-mouse: 10000 mg/kg
LD50-oral-rat: 10000 mg/kg
LD50-oral-dog: 5000 mg/kg

Repeated dose toxicity
In 28-day and 90-day oral studies, 4-MBC was administered daily to rats at dosage levels ranging from 25 to 312 mg/kg bw/day. The effects noted were mainly situated at the level of the thyroid axis, with deviations of normal thyroxine (T4), triiodothyronine (T3) and/or thyroid-stimulating hormone (TSH) levels, thyroid gland weight, etc. The oral NOAEL (90d - rat) based upon thyroid effects was derived at 25 mg/kg bw/day.

When dermally applied to the rat skin for 90 days at reported dosage levels of 0, 100, 400 and 2000 mg/kg bw/day, some slight thyroid effects were observed at 400 mg/kg bw/day, while the animals of the high-dosage group had to be sacrificed due to the severity of the local effects they experienced (epidermal lesions, wounds, necrosis). The authors

considered 400 mg/kg bw/day as the dermal NOAEL of 4-MBC and 100 mg/kg bw/day as its dermal NOEL.

Reproductive toxicity
A series of publications from Schlumpf and co-workers describe the effects of 4-MBC on reproductive parameters. In these studies, exposure levels below the current NOAEL of 25 mg/kg bw/day were included. It is not fully clear how many original studies have been performed and if data from different studies have been investigated separately or together. These unclarities hampered the interpretation of the results of these studies by the SCCS and made it difficult to properly assess the weight of evidence of all data and derive a justifiable NOAEL.
In these studies, many different molecular parameters were measured and results were uneven and often not clearly dose-dependent. The studies show that 4-MBC affected gene expression and protein levels of endocrine-related receptors and growth factors in the brain, prostate, and uterus. Patterns were not always consistent between mRNA and protein levels. Gene expression patterns in the prostate differed between the dorsal and ventral sites, whereas the effects of 4-MBC were more pronounced in the dorsolateral prostate.
Furthermore, the SCCS considers the effects observed as transient molecular events, since they do not lead to measurable significant effects on the reproductive functions in the studies. Due to the inconsistencies of the results and the lack of agreement in the dose-response relationships to support the postulated mode of action, these molecular patterns cannot be used on their own to derive a point-of-departure for safety assessment.
The effects of 4-MBC observed on the thyroid were demonstrated in earlier studies as well, e.g., in the 90-day repeat toxicity study and the reproductive toxicity study described in previous Opinions. In these studies, effects on thyroid occurred at higher exposure levels than in the new reproduction studies. In the one-generation reproduction toxicity study described in SCCNFP/0779/04, only minor thyroid effects were induced at 25 and 50 mg/kg bw/day and not at 12.5 mg/kg bw/day. SCCNFP concluded that levels up to 50 mg/kg bw/day of 4-MBC did not affect the reproductive function of female rats or the development of offspring. In the 90-day oral study, 4-MBC induced significant thyroid effects at dose levels of 50 mg/kg bw/day and higher. At exposure levels of 25 mg/ kg bw/day only slight increases in T4 were seen, none of the other thyroid effects were observed at this dose (Hofmann, 1984). Hence, thyroid effects occur at oral exposure levels of 50 mg 4-MBC/kg bw/day. In the dermal repeat dose toxicity study, slight thyroid effects were observed at even higher exposure doses: e.g., 400 mg/kg bw/day. The NOAEL of 25 mg/kg bw/day was based on thyroid effects observed in the 90-day study (SCCNFP/0779/04 and SCCP/1042/06).
Taken these facts together, the SCCS is of the opinion that it is not biologically plausible that the molecular parameters measured in these studies can be associated with any adverse effects on reproduction that have not been demonstrated in earlier conducted studies with standardised and controlled protocols.

A prenatal developmental toxicity study with 4-MBC resulted in a NOAEL for both maternal and foetal toxicity of 30 mg/kg bw/day.

Mutagenicity / genotoxicity
4-MBC was tested in one valid bacterial gene mutation study and one valid mammalian gene mutation study with negative results. After re-evaluation of the chromosomal aberration study on 4-MBC from 1986, the SCCS has noted that only 4 h exposure was used with and without S9 mix, which is not in line with current OECD TG 473 recommending using 4 h (-/+ S9) and 24 h (-S9) exposure. Therefore, the study was considered as inconclusive. Overall, the SCCS is not able to conclude on the genotoxicity of 4-MBC.

Carcinogenicity
No data provided

Photo-induced toxicity
4-MBC has occasionally been reported as being phototoxic for humans. The occurrence of phototoxicity from 4-MBC, based on a European multi-center photo-patch test study, appears to be very low.

There are no indications that 4-MBC is photomutagenic nor photoclastogenic.

Human data
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Special investigation: assessment of endocrine disrupting potential (including human data)

Recently, ECHA has identified 4-MBC as a SVHC. The assessment to underpin this is described by ECHA (2021) and is used as a lead for the evaluation of endocrine activity of 4-MBC by the SCCS.

When taking all lines of evidence into account, the SCCS concurs with the ECHA, that there is sufficient evidence that 4-MBC may act as an endocrine disruptor and has effects on both the thyroid and estrogen systems. Effects on the androgen system are not so evident, as only *in vitro* evidence is available.
The *in vivo* studies that investigated effects of 4-MBC on the estrogen system, found effects at exposure levels of 100 mg/kg bw/day and higher. Hence, these effects occur at exposures that are much higher than the NOAEL for 4-MBC.
The effects of 4-MBC observed on the thyroid were demonstrated in several studies. The majority of these studies show that oral exposure levels of 50 mg/kg bw/day of 4-MBC and higher induced significant thyroid effects. Based on the 90-day exposure study, the NOAEL of 4-MBC was determined to be 25 mg/kg bw/day. After dermal exposure, thyroid effects occur at higher exposure doses: e.g. 400 mg/kg bw /day.

This Opinion did not address the potential impact of 4-MBC on the environment.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of 4-Methylbenzylidene camphor (4-MBC), does the SCCS consider 4-MBC safe when used as a UV-filter in cosmetic products up to a maximum concentration of 4%?*

   The SCCS cannot conclude on the safety of 4-MBC, because the information provided is insufficient to fully evaluate potential genotoxicity.
   Moreover, there is sufficient evidence that 4-MBC may act as an endocrine disruptor and has effects on both the thyroid and estrogen systems. Effects on the androgen system are not so evident, as only *in vitro* evidence is available.
   Even if the genotoxic potential was excluded, the current re-evaluation of 4-MBC established a higher exposure level than in the previous Opinion. This would result in a lower MoS value, indicating that the use of 4-MBC at the maximum concentration of 4% in cosmetic ingredients would not be safe.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of 4-MBC as a UV-filter in cosmetic products?*

   It is not possible to derive a maximum concentration for safe use of 4-MBC, because a genotoxicity potential cannot be excluded.

3. *Does the SCCS have any further scientific concerns with regard to the use of 4-MBC in cosmetic products?*

   The SCCS mandate does not address environmental aspects. Therefore, this assessment did not cover the safety of 4-MBC for the environment.

5. MINORITY OPINION

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7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181