Scientific Committee on Consumer Safety

SCCS

OPINION

on Homosalate

The SCCS adopted this document
at plenary meeting on 24-25 June 2021

ACKNOWLEDGMENTS

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

For the Ppeliminary and final version of the Opinion

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 6 November 2020 until 4 January 2021). Comments received during this time period are considered by the SCCS. For this Opinion, some changes occurred, in particular in sections 3.1.1.3, 3.1.6, SCCS general comment under 3.1.9, new study under 3.2.1.1, SCCS comment under 3.2.1.2, additional information under 3.2.2., SCCS overall comment on toxicokinetics under 3.2.2, calculation of SED under 3.3.2 including information on inhalation-oral and aggregated exposure, new information and SCCS comment under 3.4.1.1, new information under 3.4.4.1 including the SCCS comment, SCCS comment under 3.4.5, changes in section numbering 3.4.10.1 - 3.4.10.2.4, level 4 in the SCCS overall conclusion on endocrine disruption properties section 3.4.10.2.4, section 3.5, and relevant discussion parts. A large part of the conclusions remains unchanged except for a sentence on inhalation toxicity that was deleted and a paragraph on combined exposure to salicylic acid that was added. A last sentence on efficacy was also added under conclusion number 3.

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 homosalate, does the SCCS consider homosalate safe when used as a UV-filter in cosmetic products up to a maximum concentration of 10%?

On the basis of safety assessment of homosalate, and considering the concerns related to potential endocrine disrupting properties, the SCCS has concluded that homosalate is not safe when used as a UV-filter in cosmetic products at concentrations of up to 10%.

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

In the SCCS's opinion, the use of homosalate as a UV filter in cosmetic products is safe for the consumer up to a maximum concentration of 0.5% homosalate in the final product.

3 Does the SCCS have any further scientific concerns with regard to the use of homosalate in cosmetic products?

It needs to be noted that the SCCS has regarded the currently available evidence for endocrine disrupting properties of homosalate as inconclusive, and at best equivocal. This applies to all of the available data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, when considered individually or taken together. The SCCS considers that, whilst there are indications from some studies to suggest that homosalate may have endocrine effects, the evidence is not conclusive enough at present to enable deriving a specific endocrine-related toxicological point of departure for use in safety assessment.

Exposure to homosalate from other products than those in this Opinion has not been considered.

Combined exposure to salicylic acid either formed by metabolic transformation from homosalate, other salicylates (e.g. methylsalicylate) or directly from salicylic acid itself has not been considered in this opinion.

The use of Homosalate at the lower concentrations may have a bearing on efficacy as UV-filter, however this is outside the SCCS remit to assess the efficacy of cosmetic ingredients.

Keywords: SCCS, scientific opinion, homosalate, UV-filter, Regulation 1223/2009, CAS No 118-56-9, EC No 204-260-8

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on homosalate (CAS No 118-56-9, EC No 204-260-8), preliminary version of 27-28 October 2020, final version of 24-25 June 2021, SCCS/1622/20

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

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

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

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted a review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting 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 then organised a public call for data[2] from 16 May 2019 – 15 October 2019 on 14[3] of the 28 substances (to be treated with higher priority) in order to be able to prepare the safety assessment of these substances. Homosalate is one of the above-mentioned 14 substances for which the call for data took place.

Background on homosalate

In cosmetic products, the ingredient homosalate (CAS No 118-56-9, EC No 204-260-8) with the chemical names Benzoic acid, 2-hydroxy-, 3,3,5-trimethylcyclohexyl ester and (3,3,5-trimethylcyclohexyl) 2-hydroxybenzoate is currently regulated as a UV-filter in sunscreen products in a concentration up to 10% (Annex VI/3).

Homosalate has been subject to safety evaluations from SCCP in 2001[4] and 2007 (SCCP/1086/07). In particular, the SCCP Opinion from 2007 concluded that '*… the use of homosalate at a maximum concentration of 10% w/w in cosmetic sun screen product does not pose a risk to the health of the consumer. Uses of homosalate in other types of cosmetic products at concentrations up to 10.0% also does not pose a risk to the health of the consumer*'.

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

Terms of reference

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

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

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

[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_out145_en.htm

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Homosalate (INCI)
3,3,5-Trimethylcyclohexyl 2-hydroxybenzoate (IUPAC)

Ref.: 20, 37, 74, 75

3.1.1.2 Chemical names

Benzoic acid, 2-hydroxy-, 3,3,5-trimethylcyclohexyl ester (EC inventory)
Cyclohexanol, 3,3,5-trimethyl-, salicylate
Homomenthyl salicylate
m-Homomenthyl salicylate
Metahomomenthyl salicylate
Salicylic acid, 3,3,5-trimethylcyclohexyl ester
Salicylic acid, m-homomenthyl ester
3,3,5-Trimethylcyclohexyl salicylate

Ref.: 37, 74, 75

3.1.1.3 Trade names and abbreviations

Caswell No. 482B
CCRIS 4885
Eusolex HMS
Parsol HMS
COLIPA n° S12
Coppertone
Neo Heliopan® HMS
NSC 164918
Uniderm Homosal
Filtersol ''A'' (8CI)
Sunobel®HMS
Heliopan

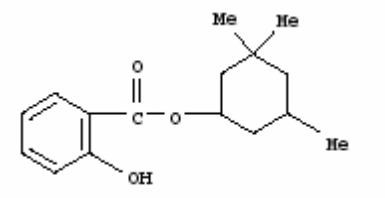
Ref.: 20, 37, 74, 75, 16

3.1.1.4 CAS / EC number

CAS No: 118-56-9
EC No: 204-260-8

Ref.: 37, 74, 75

3.1.1.5 Structural formula



Ref.: 64, 75

3.1.1.6 Empirical formula

Formula: $C_{16}H_{22}O_3$

Ref.: 74, 75

3.1.2 Physical form

Clear, colourless to pale yellow liquid

Ref.: 74, 75

3.1.3 Molecular weight

Molecular weight: 262.344 g/mol

Ref.: 22

3.1.4 Purity, composition and substance codes

Assay (GC):	98.0% min
UV absorbance (E 1%/1cm):	170-180 (at 305 nm)
Content (GLC, sum 2 isomers):	> 98.0 area %
Ash:	<0.05%
Sulphated ash:	<0.1%
Water:	0.01%
Additives:	no preservatives, no antioxidants, no solvents

Ref.: 74, 75

3.1.5 Impurities / accompanying contaminants

Metals:	Arsenic not detectable (<0.01 ppm) Lead not detectable (<0.50 ppm) Mercury: not detectable (<0.10 ppm) Cadmium: not detectable (<0.01 ppm) Nickel: not detectable (<0.50 ppm) Iron 1 ppm
Microbiological information:	<10/ml (detection limit)

Ref.: 74, 75

3.1.6 Solubility

Paraffin oil (at 20°C):	miscible
Isopropyl myristate (at 20°C):	miscible
Ethanol (at 20°C):	miscible
Water (at 25°C):	0.4 mg/L
Propylene glycol (at 20°C):	immiscible

Ref.: 21, 74, 75

3.1.7 Partition coefficient (Log Pow)

New Information
Log Kow (Log Pow): 6.34 at 40°C (experimental, HPLC method)

Ref.: 21

3.1.8 Additional physical and chemical specifications

Organoleptic properties:	slight mint odour
Melting point:	<-20°C at 101.3 kPa
Boiling point:	295.1°C at 101.3 kPa
Flash point:	171 ±2°C at 101.3 kPa
Vapour pressure:	0.015 Pa at 25°C
Relative density (D 20/4):	1.0512 (1.050-1.053)
Specific gravity (D 25/25):	1.049 –1.053
Viscosity:	85.1 mPa.s (dynamic) at 20°C and 21.0 mPa.s (dynamic) at 40°C
pKa:	8.1 ± 0.3 at 20°C (calculated value)
Acid value (potentiometric filtration, mg KOH/g):	0.0 - 1.0 max
Refractive index (n 20/D, 20°C):	1.516–1.519
Extinction	170 –180

UV/VIS spectrum in methanol (0.10 mg/ml cuvette 0.1 cm) λmax: 305 nm
UV spectrum in ethanol; λmax: 238.18 nm and 306.39 nm

Ref.: 21, 58, 74, 77

3.1.9 Homogeneity and Stability

Taken from SCCP/1086/07

Shelf life: at least 2 –3 years

Ref.: 74, 78

Photo-stability
The photo-stability of homosalate was examined in the presence of a photo-labile UV-A absorbing research material using the Suntest CPS Heraeus Xenon lamp (irradiance: 40 W/m² (24 min = 1 MED)). A 30 mg emulsion containing 5% homosalate was spread on a glass plate with an area of 10 cm², dried for 30 minutes and exposed to 5, 10, 15 and 20 MED under cooling (20°C). The samples were immersed in 25 ml ethanol and analyzed by UV spectrophotometry and by chromatography (HPLC). The decrease in homosalate content ranged between 0 – 2.7% and thus, homosalate was shown to be stable under these conditions.

Ref.: 41, 76, 79

In addition, dilute solutions in isopropanol and cyclohexane as well as in mineral oil and ethanol/water were shown to be photo-stable.

Ref.: 73

New information

Homosalate has been investigated for abiotic hydrolysis at pH 4, pH 7 and pH 9 (Method C7 of Regulation 440/2008/EC and Method 111 of the OECD Guidelines for Testing of Chemicals) and found to be rapidly degraded under environmentally relevant pH with an estimated half-life of 215 hours at neutral pH (pH 7) at 25°C. At the same temperature, at pH 4 and pH 9, the estimated half-lives were 210 hours and 69.7 hours respectively.

Stability in organic solvents: Stable.

Ref.:21

Stability of homosalate in chlorinated water was studied in simulated swimming pool water samples. UV spectroscopy was used to follow the reaction of homosalate in the presence of

free chlorine. Water samples were filtered, acidified, and extracted by use of solid-phase extraction. Gas chromatography with mass spectrometry was used to identify the major transformation by-products. In the presence of free chlorine, after 24 hours, homosalate reacted with chlorine following zero order reaction giving the following derivatives: monochloro-homosalate, dichloro-homosalate and two diastereoisomers of monochloro-homosalate.

Ref.: 43

SCCS general comments to physico-chemical characterisation
The stability of the test substance in the marketed product (and in the test solutions) was not reported.
A full report of the chemical characterization of homosalate in terms of purity, identity and impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown. Identity and concentration of any impurities that may be present must also be stated.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

3.2.1.1 Percutaneous absorption *in vitro*

Taken from SCCP/1086/07

Human skin

Guideline:	OECD 428 (Draft, 2000); OECD Guidance Document 28 (2004); Basic criteria for *in vitro* assessment of cosmetic ingredients (SCCNFP/0750/03, October 2003); Diembeck et al., 1999
Test System:	Human skin
Substance:	10% homosalate in a standard sunscreen
Batch:	Non labelled: 4095213 (purity: 99.88% (GLC)) Radiolabelled: CFQ 14329, specific activity: 54 mCi/mmol
Purity:	Non labelled: 99.88% (GLC) Radiochemical purity: 99.8% (HPLC)
Dose:	approx. 3.4 mg dose formulation/0.64 cm^2 (corresponding to approx. 0.5 mg homosalate/cm^2)
Skin preparation:	Fresh dermatomed human skin from abdominal surgery from 3 female donors
Mean thickness (n=6):	Donor 1: 397±30 µm Donor 2: 357±13 µm Donor 3: 519±90 µm
Skin temperature:	32°C
Test chamber:	Flow-through automated diffusion cells (PermeGear Inc, Riegelsville, PA/USA)
Receptor fluid:	DMEM and Ham's F 12 culture medium (3:1) supplemented with hEGF, hydrocortisone, gentamycin, glutamine and 10% FCS
Solubility:	12 µg/ml in receptor fluid
Route:	Topical application
Exposure time:	24 h
GLP:	In compliance

Homosalate was investigated for its skin penetration *in vitro* as a 10% standard sunscreen formulation. Fresh dermatomed human skin from surgery was processed and put on the flow-through automated diffusion cells. The temperature was checked regularly and was about 32°C at ambient humidity. The receptor fluid was pumped at a speed of about 1.6 ml/h. The

complete formulation was prepared one day prior to the start. Homogeneity and concentration of radioactivity in the formulation were analyzed. A total amount of approx. 3.4 mg dose sunscreen formulation/0.64 cm^2 (corresponding to approx. 0.5 mg homosalate/cm^2) was applied. Exposure duration was 24 h. During exposure, receptor fluid samples were collected at regular intervals. After 24 h exposure, the skin surface was washed using a mild soap solution and cotton swabs. Each skin sample was tape stripped ten times using Dsquame. The tape strips containing pieces of epidermis were pooled. The mass balance was determined using receptor fluid, skin surface washes, receptor and donor compartment washes, tape strips and digested skin. Radioactivity was determined using LBK/Wallac S1414 scintillation counter.

Results
The results of dermal absorption in human skin were as follows:

Table 1: *In vitro* percutaneous penetration of homosalate in a standard sunscreen through viable human skin

Donor	**1 (n =6)**	**2 (n = 6)**	**3 (n = 6)**
Homosalate in formulation (%)	10.1	10.1	10.1
Dose (µg/cm^2)	544.9	548.0	541.2
N° of biopsies	6	6	6
Penetration into the receptor fluid after 24h	0.25% of dose	0.16% of dose	0.12% of dose
Flux constant (µg/cm^2x h)	0.077 ± 0.014	0.057 ± 0.004	0.039 ± 0.014
Lag time (h)	6.5 ± 0.4	7.0 ± 0.6	7.6 ± 1.0
Total absorption (% of dose) #	1.4 ± 0.4	0.9 ± 0.2	0.9 ± 0.3
# total absorption as amount in receptor fluid including wash and skin membrane excluding tape strips			

The mean flux constant for the absorption of homosalate after application of a 10% homosalate containing standard sunscreen formulation was 0.058 ± 0.019 µg/cm^2 x h. The mean total absorption was 1.1 ± 0.3% of the applied dose in human skin. The mean recovery was 92.4 ± 1.5%. The highest mean absorption was found in group A: 1.4 ± 0.4% with the highest absorption value 2.0%.

Ref.: 15

Rat skin

Guideline:	OECD 428 (Draft, 2000); OECD Guidance Document 28 (2004); Basic criteria for *in vitro* assessment of cosmetic ingredients (SCCNFP/0750/03, October 2003); Diembeck et al., 1999
Test System:	Rat skin (Sprague-Dawley)
Substance:	10% homosalate in a standard sun screen
Batch:	Non labelled: 4095213 (purity: 99.88% (GLC)) Radiolabelled: CFQ 14329, specific activity: 54 mCi/mmol
Purity:	Non labelled: 99.88% (GLC) Radiochemical purity: 99.8% (HPLC)

Dose:	approx. 3.4 mg dose formulation/0.64 cm^2 (corresponding to approx. 0.5 mg homosalate/cm^2)
Skin preparation:	Fresh punched-out rat skin from 3 female Sprague-Dawley rats
Mean thickness (n=6):	Rat 1: 669±47 µm Rat 2: 755±73 µm Rat 3: 763±89 µm
Skin temperature:	32°C
Test chamber:	Flow-through automated diffusion cells (PermeGear Inc, Riegelsville, PA/USA)
Route:	Topical application
Receptor fluid:	MEM (Minimal Essential Medium) supplemented with gentamycin, glutamine and 10% FCS
Solubility:	12 µg/ml in receptor fluid
Exposure time:	24 h
GLP:	In compliance

The same 10% homosalate containing standard sunscreen formulation was also tested in rats. Freshly punched-out skin samples from 3 female Sprague-Dawley rats were investigated according to the same procedure as described above for human skin with the exception that the receptor fluid consisted of MEM (Minimal Essential Medium) supplemented with gentamycin, glutamine and 10% FCS.

Results
The results of dermal absorption in viable rat skin were as follows:

Table 2: *In vitro* percutaneous penetration of homosalate in a standard sunscreen through viable rat skin

Animal	**Rat 1 (n = 6)**	**Rat 2 (n = 6)**	**Rat 3 (n = 6)**
Homosalate in formulation (%)	10.1	10.1	10.1
Dose (pg/cm^2)	535.9	535.9	535.9
N° of biopsies	6	6	6
Penetration into the receptor fluid after24h	1.33% of dose	3.62% of dose	3.45% of dose
Flux constant (pg µg / x cm^2/ x h)	0.412	0.997	1.012
Lag time (h)	6.8 ± 0.3	4.6 ± 0.9	5.7 ± 0.2
Total absorption (% of dose) #	7.4 ± 2.7	7.7 ± 1.3	11.0 ± 1.8

total absorption as amount in receptor fluid including wash and skin membrane excluding tape strips

Conclusion from the authors
The results of dermal absorption in viable rat skin were as follows:
The mean flux constant for the absorption of homosalate after application of a 10% homosalate containing standard sunscreen formulation was 0.807 ± 0.342 µg/cm^2 x h. The mean total absorption was 8.7 ± 2.0% of the applied dose in rat skin. The mean recovery was 93.1 ± 1.3%.

Ref.: 15

Skin penetration *in vitro* was also determined with two sunscreen formulations containing 5% homosalate and other sunscreens prepared as an O/W emulsion gel or petrolatum jelly preparation. Human full thickness skin obtained from 3 female breast or abdominal surgery donors was mounted on static Franz diffusion cells. An amount of 3.0 mg/cm^2 of each sunscreen formulation was applied for 30 min or 6 h and penetration in the epidermis and dermis was determined.

The amount of homosalate measured after 30 min in the epidermis was 0.4 µg/cm^2 (0.2% of dose) independent from formulation and amounted to 0.3 µg/cm^2 (0.2% of dose) tested as an emulsion gel or 0.6 µg/cm^2 (0.3% of dose) when applied in petrolatum. No homosalate could be determined after 30 min or 6 h in the dermis. Thus, only adsorption in the epidermis was noted and no penetration through the skin.

Ref.: 12

A published study showed that pre-treatment of freshly excised full-thickness dorsal skin from female hairless mice with an ethanol (80%) solution containing 5% homosalate led to enhanced transdermal penetration of a pesticide (2,4-dichlorophenoxyacetic acid). However, this study was considered as not valid and of no relevance for the assessment of the percutaneous absorption of homosalate *in vitro*.

Ref.: 59

New information

Topical formulations of Vaseline, an oily solution, lotion and gel containing 10% of homosalate, were evaluated *in vitro* by Kim et al. (2014) for the dermal permeation potential of homosalate using excised rat skin mounted on Franz diffusion cells. Dermal tissues were obtained from male Sprague-Dawley rats, stored at −20°C and used within 1 week after dermal harvest. The receptor medium consisted of Tween 80 and 0.02 *M* phosphate buffer (5:95 % (v/v)). After the rat skins were equilibrated with the receptor phase, each topical preparation was applied to the skin in the donor compartment. The amount of homosalate applied was 20 mg and the volume of the receptor compartment was 10 ml. The receptor temperature was maintained at 37±0.5°C in a water bath. When tested in the receptor medium at 37°C, homosalate was found to be stable over the 48 h period.
Concentrations of homosalate in the receptor fluids and *in vitro* skin samples were determined by a validated HPLC/ UV detection (UVD) assay method.
Among tested topical preparations, gel showed the highest permeation of homosalate into the skin. The percentage of applied homosalate found in the stratum corneum determined at the end of the diffusion experiment (48 h) was significantly higher for gel (14.7 ±3.6%) than for Vaseline (2.4 ±2.5%), lotion (2.1 ± 1.4%), and the oily solution (1.5 ± 0.7%). Similarly, the percentage of applied homosalate noted in combined viable epidermis and dermis was significantly higher for gel (6.9 ± 2.9%) than for Vaseline (2.1 ± 0.9%), lotion (1.8 ± 0.2%), and the oily solution (1.5 ± 0.3%). The percentage of applied dose observed in receptor medium was minimal for all preparations (<0.005%).
Authors concluded that the higher amount of homosalate retained in stratum corneum compared to viable skin suggests that the partitioning of homosalate into the skin layers is a slow process. They attributed the greater partitioning of homosalate into the stratum corneum either to the ingredients of the gel formulation (Carbomer 940 and Poloxamer 188) migrating into the stratum corneum and thereby increasing the solubility of homosalate, or to enhanced hydration of stratum corneum due to a high water content (64.3%).

Ref.: 49

Additional information submitted during/after commenting period

***In vitro* percutaneous absorption**

Guideline:	OECD TG 428, OECD Series on Testing and Assessment No. 156, Guidance Notes on Dermal absorption (2011), SCCS/1358/10,
Test system:	Split thickness human abdominal skin samples (300-400 µm) in flow-through cell system
Number of donors:	13 samples from 7 donors (41 to 61 years)
Membrane integrity:	permeation of tritiated water, samples were rejected if the tritiated water absorption was >1.6%
Radiolabeled test substance:	[phenyl-U-^{14}C]homosalate
Batch:	5577SXD005-5

Specific activity:	1142 MBq/mmol, 4.34 MBq/mg
Radiochemical purity:	97.7% (HPLC)
Chemical purity:	98.6% (HPLC)
Non-labeled test substance:	Neo Heliopan® HMS
Batch:	50100965
Chemical purity:	99.8% (GLC)
Test item:	30/CCSKN 5727/00 Light Soft Body Lotion, batch 'January 2019', containing a final concentration of homosalate 1.0% (w/w) consisting of 1.04 mg [phenyl-U-^{14}C]homosalate per gram test item and 9.06 mg Neo Heliopan® HMS per gram test item, with an activity of 4.50 MBq/g test item
Dose applied:	10 µL/cm² of the test item
Exposed area:	1 cm²
Exposure period:	24 hours
Sampling period:	24 hours (aliquots collected 0-1, 1-2, 2-4, 4-6, 6-8, 8-10, 10-12, 12-16, 16-20, 20-24 hours post dose)
Receptor fluid:	phosphate buffered saline containing polyoxyethylene-20 oleyl ether
Solubility in receptorfluid:	>10 µL test item in 1.5 mL receptor fluid (assuming 100% absorption in 1 hour post dose)
Mass balance analysis:	Provided (see table)
Tape stripping:	Yes (20 tape strips, digested and measured individually)
Method of Analysis:	LSC
GLP:	In compliance
Study period:	January – November 2018

Split-thickness human skin (13 samples from 7 individual donors) was mounted into flow-through diffusion cells (1 cm²). Phosphate buffered saline (PBS) containing polyoxyethylene-20 oleyl ether (PEG; 6%(w/v)), sodium azide (0.01%(w/v)), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL) with the pH adjusted to 7.4 ± 0.1 was used as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C throughout the experiment. The integrity of all human skin samples was within the acceptance criteria (≤1.6% of the applied dose of tritiated water). The test preparation was prepared by incorporating [phenyl-U-^{14}C]homosalate into a cream formulation at a final concentration of 1.0%(w/w) homosalate. The test item was applied (10 µL/cm²) to human split-thickness skin samples mounted into flow-through diffusion cells *in vitro*.
Absorption was assessed by collecting receptor fluid in hourly fractions from 0 to 2 hours post dose, then in 2-hourly fractions from 2 to 12 hours post dose and then in 4-hourly fractions from 16 to 24 hours post dose. The exposure was terminated at 24 h post dose by washing the skin surface with five cotton swabs dampened with 3% Teepol in water. The skin samples and cells were dried with a cotton swab. The skin was divided into exposed and unexposed sections. The exposed epidermis was separated from the dermis. All samples were analyzed by liquid scintillation counting.

Results
The distribution of [phenyl-U-^{14}C]homosalate (% applied dose) at 24 hours post dose is shown in Table 3. The majority of the applied dose (89.19%) was removed at 24 hours post application during washing which is referred as dislodgeable dose. A further 0.27% of the applied dose was removed with the donor skin wash. Therefore, the total dislodgeable dose was 89.46% of the applied dose. The mean total unabsorbed dose was 93.55% of the applied dose. This consisted of the total dislodgeable dose, unexposed skin (0.18%) and the test item associated with the stratum corneum (3.92%). The first 2 tape strips contained 1.64% of the applied dose. Tape strips 3-20 contained a further 2.28%. The absorbed dose (0.34%) was the sum of the receptor fluid (0.30%), receptor wash (<0.01%) and receptor rinse (0.05%). The exposed epidermis and exposed dermis contained 0.39% and 0.24% of the applied dose, respectively.

Table 3: Dermal distribution and absorption of [phenyl-U-^{14}C]homosalate 24 hours after application to split-thickness human abdominal skin *in vitro*.

	Fraction of applied dose % (mean ± SD, n = 13)	Flux µg equiv./cm² (mean ± SD, n = 13)
Dislodgeable dose	89.19 ± 8.29	80.28 ± 7.46
Skin wash	0.27 ± 0.41	0.25 ± 0.37
Total dislodgeable dose	89.46 ± 8.12	80.52 ± 7.31
Stratum corneum (tape strips 1-20)	3.92 ± 3.02	3.53 ± 2.71
Unexposed skin	0.18 ± 0.23	0.15 ± 0.21
Total unabsorbed dose	93.55 ± 6.21	84.20 ± 5.59
Exposed epidermis	0.39 ± 0.60	0.34 ± 0.54
Exposed dermis	0.24 ± 0.39	0.20 ± 0.35
Receptor fluid	0.30 ± 0.13	0.27 ± 0.12
Receptor rinse	0.05 ± 0.03	0.04 ± 0.03
Total absorbed dose	0.34 ± 0.15	0.31 ± 0.13
Dermal delivery	0.97 ± 0.83	0.85 ± 0.74
Mass balance	94.53 ± 5.98	85.05 ± 5.39

Dislodgeable Dose = Wet Cotton Swabs + Dry Cotton Swab; Total Dislodgeable Dose = Dislodgeable Dose + Skin Wash; Total Unabsorbed Dose = Total Dislodgeable Dose + Stratum Corneum + Unexposed Skin; Total Absorbed Dose = Cumulative Receptor fluid + Receptor Rinse; Exposed Epidermis = Exposed area of Skin (Epidermis) after tape stripping; Exposed Dermis = Exposed area of Skin (Dermis) after tape stripping; Dermal Delivery = Total Absorbed Dose + Exposed Skin; Mass Balance = Total Unabsorbed Dose + Dermal Delivery

The dermal delivery (amount in receptor fluid plus amount in skin after tape-stripping) of [phenyl-U-^{14}C]homosalate was 0.97 ± 0.83% (0.85 ± 0.74 µg equiv./cm^2). The mass balance for [phenyl-U-^{14}C]homosalate was 94.53% (85.05 µg equiv./cm^2) of the applied dose.

Ref.: 11

SCCS comment
The concentration of homoslate used in this study was 1%, 10 times lower than the maximum homosalate intended-use concentration in cosmetic products. Therefore, it is considered of limited value for the determination of dermal absorption value.
The SCCS further notes that the composition of the cream formulation (light soft body lotion) in which homosalate was present is not given, neither how representative it is for sunscreen products.

Human skin

Guideline:	OECD Test Guideline 428 (2004)
Test system:	Human split thickness skin membranes mounted into static diffusion cells
Number of donors:	12 skin samples from 4 female donors (aged between 45 and 63 years)
Membrane integrity:	Yes, measurement of electrical resistance barrier integrity assessment
Test substance:	Homosalate/ [phenyl-U-^{14}C]-Homosalate
Batch:	50101266 (non-radiolabelled) / 11312DZA002-2 (labelled)
Test item:	radiolabelled Homosalate in an oil/water-based formulation at 10% (w/w)
Purity:	non labelled: 99.5% Radiochemical purity: 98.8% Specific activity: 217 µCi/mg
Skin preparation:	Split thickness skin membranes

Dose applied:	ca. 2 mg/cm^2
Exposed area:	3 cm^2
Exposure period:	24 hours
Sampling period:	0.5, 1, 2, 4, 8, 12 and 24 hours post dose
Receptor fluid:	Phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG; 6%, w/v), sodium azide (0.01%, w/v), streptomycin (ca 0.1 mg/mL) and penicillin (ca 100 units/mL).
Solubility in receptor fluid:	13.4 mg/L
Samples assessed:	Tapes (20 stripping); epidermis, dermis, receptor fluid, washing solution
Analysis:	Liquid scintillation counting
Tape stripping:	Yes (20 strips in total)
Method of analysis:	Liquid scintillation counting (LSC) and/or HPLC
GLP:	Yes
Study period:	2020-2021

Split-thickness human skin (12 samples from abdomen of 4 female donors) was mounted into static diffusion cells containing receptor fluid (phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG; 6%, w/v), sodium azide (0.01%, w/v), streptomycin (ca 0.1 mg/mL) and penicillin (ca 100 units/mL)). The skin surface temperature was maintained at 32 ± 1°C throughout the experiment. Membrane integrity assessment was performed by checking the electrical resistance barrier. All skin samples exhibited a resistance greater than 4 kΩ. The test preparation was applied at a dose of 2 mg/cm^2 to all samples. The cells were not occluded. The test substance stability during dosing was confirmed by high performance liquid chromatography (HPLC).
The exposure duration was 24 hours. Absorption was assessed by collecting the receptor fluid at 0.5, 1, 2, 4, 8, 12 and 24 hours post dosing. After 24 hours exposure, the skin surface was washed with a concentrated commercial hand wash soap followed by rinsing with a dilute soap solution (2%, v/v) and drying the surface with a tissue swab. The cells were dismantled, and the donor chamber retained for analysis. The skin was removed, and the underside of the skin was dried with a tissue paper swab. The stratum corneum was tape stripped and the skin divided into exposed and unexposed skin. The exposed skin was separated into dermis and epidermis samples. The receptor chambers were rinsed with acetonitrile and the samples retained for analysis. The skin samples were dissolved with Solvable™ tissue solubilizer. All samples were analysed by liquid scintillation counting (LSC).

Results
The results of dermal absorption in human skin are presented in Table 4.

Table 4: *In vitro* percutaneous absorption of [^{14}C] Homosalate through human split-thickness skin

3.3 Dose group / human		
Test substance		[^{14}C]-homosalate
Number of Samples		11
Analysis Type		Liquid Scintillation Counting
Application Rate (mg/cm^2)		1.99
	Distribution % of applied dose [µg] mean ± SD	**Distribution µg equiv./cm^2 mean ± SD**
Total Dislodgeable Dose	72.32±5.28	140.64±10.47
Stratum Corneum	11.87±5.42	23.08±10.55
Unexposed Skin	0.03±0.02	0.06±0.04
Total Unabsorbed Dose	84.22±1.94	163.77±3.78
Epidermis	3.39±1.38	6.60±2.69

Dermis	0.25±0.15	0.48±0.29
Total Absorbed Dose	0.22±0.13	0.42±0.25
Dermal Delivery	Dermal Delivery 3.86±1.43	7.50±2.79
Mass Balance	Mass Balance 88.07±1.27	171.27±2.46

Total dislodgeable dose = skin wash 24 hours + tissue swab 24 hours + pipette tip 24 hours + donor chamber wash
Stratum corneum = tape strips 1 to 20.
Total unabsorbed dose = total dislodgeable dose 24 hours + stratum corneum + unexposed skin.
Epidermis = epidermis + clingfilm.
Total absorbed dose = cumulative receptor fluid + receptor chamber wash.
Dermal delivery = total absorbed dose + epidermis + clingfilm + dermis.
Mass balance = total unabsorbed dose + dermal delivery.

Conclusion from the authors

Following topical application of [^{14}C]-Homosalate to human skin *in vitro*, the total absorbed dose, dermal delivery and mass balance were 0.22±0.13% (0.42±0.25 µg equiv./cm^2), 3.86±1.43% (7.50±2.79 µg equiv./cm^2) and 88.07±1.27% (171.27±2.46 µg equiv./cm^2), respectively.
The above *in vitro* dermal absorption study was considered scientifically acceptable revealing a mean dermal delivery of 3.86 ± 1.43% of the applied dose.

Ref.: 30

SCCS comment

The SCCS will use mean +1SD from this properly performed skin penetration study for MoS calculation.

3.2.1.2 Percutaneous absorption *in vivo*

Taken from SCCP/1086/07

Only few studies with homosalate were published and are available in the open literature. The tape stripping methodology was applied by Chatelain et al. (2003) and Sarveiya et al. (2004). In both studies it was shown that penetration through the skin was minimal and the vast majority was retained by the stratum corneum. In addition, Chatelain et al. (2003) observed a difference in respect to the applied formulation. The total amount penetrating into the stratum corneum was higher from the O/W emulsion gel than from the petrolatum jelly formulation.
Finally, no quantitative conclusion for skin penetration is possible but qualitatively, it can be stated that, as for the *in vitro* results, -the stratum corneum adsorbed the greatest fraction and only small amounts can be considered as absorbed and systemically bioavailable. In addition, the type of preparation/formulation had an influence on the proportion of adsorption.

Ref.: 12, 29, 65

New information

In vivo percutaneous absorption studies were conducted (Kim et al. 2014) in rats after topical application of 100 and 200 mg gel preparation containing 10 and 20 mg (n = 5 per dose) of homosalate on a 3x3 cm application site. At 0, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, and 120 h after application, blood samples were collected and stored at −20°C until analysis. At 12 h following the application of gel, the applied area was rinsed with alcohol swabs to remove any unabsorbed homosalate, with the percentage of applied homosalate found in the 12 h skin wash being 93.68 ± 7.11 and 94.49 ± 7.31% for low and high doses, respectively.
Homosalate concentrations in plasma and skin samples obtained from the *in vivo* i.v. injection and topical application studies were determined by liquid chromatography (LC)–mass spectroscopy (MS)/MS.

After i.v. injection (0.5, 2, or 5 mg/kg), the pharmacokinetics of homosalate was linear and was characterized by a large Volume of distribution (Vd_{ss}) (13.2–17 L/kg), high Clearance (Cl_s) (4.5–6.1 L/h/kg), and a half-life ($t_{1/2}$) between 6.1 and 8.4 h. After topical application of gel, the bioavailability of homosalate was 5.4 ± 1.1 and 4.2 ± 0.6% for low and high doses (10 and 20 mg), respectively. At the end of the sampling time (120 h) only a small fraction (<0.17%) of applied homosalate remained on the skin. Consistent with the prolonged absorption (t_{max} 11.2 ± 1.8 and 12 ±0 h for low and high doses, respectively), the terminal $t_{1/2}$ was longer after topical application (23.6–26.1 h) compared to i.v. injection.

Ref.: 49

Commercially available sunscreen lotion (SPF 30) containing 8% (w/v) homosalate, 7.5% (w/v) octyl methoxycinnamate, 6% (w/v) benzophenone-3, and 5% (w/v) octyl salicylate was applied at a rate of 2 mg/cm^2 to an equal-sized area (112 cm^2) on the face or back of volunteers (5 male, 7 female, age range 22–61 years in the main study). The study was of crossover design with sunscreen application on the face or back on day 1, followed by application on the other site on day 8 of the study. Authors explain that this was the application rate used by the volunteers when in a preliminary study they were instructed to "apply the sunscreen liberally as if they were on the beach". The sunscreen lotion remained unoccluded for 8 hours before being removed by washing the site. An area of the skin was immediately tape stripped by application and removal of Scotch crystal clear tape (3 cm × 1.9 cm). The tapes were applied to the treated areas by application of a consistent pressure along the tape. The stratum corneum was sequentially stripped 16 times on the back and 6 times on the face, and the strips were grouped for analysis by HPLC with UV absorption detection (group 1, strip 1; group 2, strips 2–6; group 3, strips 7–11; group 4, strips 12–16). Blood samples were taken from all volunteers at pre-application baseline and at a suitable steady-state time after application (7.5 hours), and the urine obtained over 48 hours after application was collected. Sunscreen content in all samples was analyzed by HPLC. Lower limit of detection and quantitation was 2 and 4 ng for homosalate. Extraction procedures from bovine serum albumin receptor fluids (0.5 mg/mL), plasma (0.5 mg/mL), urine (5 mg/mL), and skin were validated at > 98%, 97%, 86%, and 98% recovery, respectively.
The amount of homosalate in the stratum corneum of the back after 8 hours was approximately 17 ± 2.5, 24 ± 6, 7 ± 0.05 and 5 ± 1 µg for groups 1, 2, 3 and 4, respectively (n=12). A comparison of the distribution of sunscreens in the stratum corneum of the face and back of the volunteers was made. The difference in absorption between the anatomical sites was statistically significant for homosalate. The amount of homosalate present in the superficial stratum corneum layers of the face was approximately two to three times more than that in the back. The percentage of applied dose of sunscreen in the six superficial layers of the stratum corneum was approximately 10%.
Homosalate was not detected in the plasma or urine samples of the volunteers.

Ref.: 10

SCCS comment
A full report on a new skin penetration study using human skin was submitted to the SCCS in February 2021 [Finlayson (2021)]. The study was designed according to the SCCS Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCS/1358/10) and the OECD Test Guideline No. 428 for skin penetration studies. Human samples were derived only from 4 females (not both genders). Based on Finlayson (2021) a dermal absorption of 5.3% (mean + 1SD: 3.86±1.43%) was used in the calculation of SED.
A further study using a 1% solution of homosalate was also provided during the public commenting period. However, due to the shortcomings discussed above, this study was not used for MoS calculation.

3.2.2 Other studies on toxicokinetics

New Information

Systemic bioavailability in humans after dermal exposure

Human milk samples were collected from mothers of three different cohorts in 2004, 2005 and 2006, who gave birth to a singleton child in Basel, Switzerland. Human milk samples were recruited from summer to late fall, when sunscreens were used in addition to other cosmetics. 54 samples of human breast milk were collected before 30 days post partum (Subgroup A, N = 49) and between postnatal days 34 and 108 (Subgroup B, N = 5). Samples were analyzed for eight different UV-filters (benzophenone-2, benzophenone-3, ethyl-hexyl cinnamate, homosalate, 3-(4-methyl-benzylidene) camphor, 3-benzylidene camphor, octocrylene and octyldimethyl PABA), sixteen different synthetic musks, seven PCBs and six PBDEs. UV filters were detected in 85.19% of breast milk samples with the rank order of frequency of detection corresponding to that of reported use of these filters. 15.1% of mothers reported use of homosalate exclusively in sunscreens with no additional use of other cosmetics. Homosalate was detected in 5.56% of total milk samples. No significant differences found between subgroups A and B in levels of chemicals. Comparison with a detailed questionnaire revealed that the presence of UV filters in human milk was closely linked with the use of cosmetics containing these chemicals, indicating that internal exposure resulted from repeated application of cosmetics rather than from general environmental exposure.

Ref.: 70

Barr et al. (2018) used high-performance liquid chromatography tandem mass spectrometry to measure concentrations of octylmethoxycinnamate, 4-methylbenzylidenecamphor, benzophenone and homosalate in human breast tissue from three serial locations across the breast from 40 women undergoing mastectomy for primary breast cancer. Homosalate was not detected in any sample.

Ref.: 8

Matta et al. (2020) conducted a randomized clinical trial in 48 healthy participants in order to assess the systemic absorption and pharmacokinetics of 6 active ingredients (avobenzone, oxybenzone, octocrylene, homosalate, octisalate, and octinoxate) in 4 sunscreen products under single- and maximal-use conditions. Participants were randomized to 1 of 4 sunscreen products, formulated as lotion (n = 12), aerosol spray (n = 12), nonaerosol spray (n = 12), and pump spray (n = 12). Sunscreen product was applied at 2 mg/cm^2 to 75% of body surface area at 0 hours on day 1 and 4 times on day 2 through day 4 at 2-hour intervals, and 34 blood samples were collected over 21 days from each participant. The maximum plasma concentrations of homosalate were 23.1 ng/mL (coefficient of variation [CV], 68.0%), 17.9 ng/mL (CV, 61.7%), and 13.9 ng/mL (CV, 70.2%) for aerosol spray, nonaerosol spray, and pump spray, respectively. The overall t_{max} ranged between 57 and 68.5 h. After single application, most participants had maximum plasma concentrations that reached or exceeded the 0.5-ng/mL threshold, with 86% of participants reaching that threshold within 3 hours for homosalate (n = 31/36). Observations at 23 hours remained above 0.5 ng/mL in all participants [n = 36/36] for homosalate. In addition, many participants had concentrations above 0.5 ng/mL until day 7 (55%; n = 17/31). Homosalate remained detectable through day 21 with concentrations of 0.2 ng/mL (CV, 273.0%, n=10, range: 0-1), 0.3 ng/mL (CV, 1654.1%, n=10, range: 0-2.9), and 0 ng/mL (CV, 1552.1%, n=10, range: 0-3.9) for aerosol spray, nonaerosol spray and pump spray, respectively. Terminal half-lives of 67.9 h (CV 55.5%), 78.4 h (CV 61.6%) and 46.9 h (CV 67.5%) were reported for aerosol spray, nonaerosol spray and pump spray.
Homosalate was detectable in skin following tape stripping, with greater amounts detectable at day 7 compared with day 14. The skin amounts for homosalate were 4517 ng/cm^2 (CV, 102.2%), 2814.9 ng/cm^2 (CV, 178.7%) and 2165.7 ng/cm^2 (CV, 164.1%) on day 7 and 190.2

ng/cm^2 (CV, 202.9%), 436.9 ng/cm^2 (CV, 253.6%) and 181.8 ng/cm^2 (CV, 157.1%) on day 14 for aerosol spray, nonaerosol spray and pump spray, respectively.
The continued presence of sunscreen active ingredients in skin at days 7 and 14, the long terminal half-life typically exceeding 48 hours and ingredients remaining detectable through day 21 suggest, according to the authors, that absorption through skin is the rate-limiting step.

Ref.: 55

Adamson et Shinkai (2020) commented on the study performed by FDA (Matta et al. 2020) that tape stripping evaluation on days 7 and 14 revealed persistence of sunscreen filters, raising the possibility that the skin could serve as a depot for ongoing absorption after daily sunscreen application is stopped. They mention that the study provides important additional information documenting systemic absorption of commonly available chemical sunscreen filters and strengthens the need for safety testing for certain chemical sunscreen ingredients to confirm they are generally recognized as safe and effective.

Ref.:1

***In vitro* metabolism of homosalate**

The *in vitro* metabolism of homosalate was investigated in commercially available (Celsis®-brand) rat and human liver microsomes. Homosalate at a final concentration of 10 mM was incubated with human or rat liver microsomes (1 mg/ml protein) containing 1 mM NADPH and 5 mM GSH for 20 min at 37°C and protected from light. Reaction was stopped by precipitation of proteins. Supernatants from centrifugation were analysed by LC/MS/MS. On one hand, homosalate was hydrolysed into salicylic acid and 3,3,5-trimethylcyclohexanol. On the other hand, conjugation and hydroxylation of intact homosalate was observed. Two peaks corresponded to ring hydroxylated and ring-GSH-conjugated metabolites (isomers) and a smaller peak corresponded to ring hydroxylated and ring GSH-conjugated metabolites with additional hydroxylation at the ester side chain.

Ref.: 38

SCCS comment

Studies demonstrate that homosalate becomes systemically bioavailable after dermal use of cosmetics containing homosalate. This could be demonstrated by the detection of homosalate in plasma of volunteers after topical application of sunscreen products containing homosalate (Ref. 50) but also by the detection of homosalate human milk samples from different cohorts (Ref. 64). However, homosalate could not be detected in either human breast samples (Ref. 7) or in urine after topical application in human volunteers (Ref. 9). *In vitro*, homosalate was hydrolysed into salicylic acid and 3,3,5-trimethylcyclohexanol. In addition, conjugation and hydroxylation of intact homosalate was observed (Ref. 34).
Maximum plasma concentrations of homosalate after topical application varied between 13.9 and 23.1 ng/ml and terminal half-lives varied between 46.9 and 78.4 h in an explorative study (Ref. 50).

Additional information received in 2021

Physiologically based pharmacokinetic (PBPK) modelling

A PBPK model has been developed (Najjar 2021) in order to determine the human internal dose that would correspond to the animal internal systemic dose at an oral external dose of 120 mg/kg bw/d, i.e. the dose level selected as NOAEL by the Applicant. Based on *in vivo* studies performed in rats (Kim et al., 2014, experimental phase was during 2013; the Applicant takes responsibility that the experimental *in vivo* phase of the study took place before animal testing ban (11 March 2013 for toxicokinetics)), an *in vivo* rat PBPK model has been calibrated for the intravenous (i.v.) route and C_{max}. Data from the i.v. route in rats was extrapolated to the oral route in order to calculate C_{max} and AUC that would result from an oral dose of 120 mg/kg bw/d. The rat PBPK model was then extrapolated to a human PBPK model which was refined by results from the study by Matta et al., 2019.

Ref.: 56

SCCS comments
The SCCS notes that the rat PBPK model for the i.v. route has been calibrated but not validated by independent data. The rat PBPK model for the oral route has neither been calibrated nor validated. Therefore, it is not possible to assess the reliability of this model to derive an internal PoD from an external oral dose. In consequence, the subsequent step (i.e. animal to human extrapolation) is not feasible.
According to the WHO guidance on PBPK (WHO 2010), the level of confidence of the rat PBPK model can be considered as low, especially regarding the reliability and performance.

Ref. 83

SCCS overall comment on Toxicokinetics
Several *in vitro* dermal penetration studies using rat and human skin have been performed. For MoS calculation, the SCCS selected a new skin penetration study using human skin from which a dermal absorption of 5.3% (mean + 1SD: 3.86±1.43) was derived. Systemic bioavailability of homosalate after dermal application was confirmed by the detection of homosalate in plasma of volunteers after topical application of sunscreen products containing homosalate but also by the detection of homosalate human milk samples. Maximum plasma concentrations of homosalate after topical application varied between 13.9 and 23.1 ng/ml and terminal half-lives varied between 46.9 and 78.4 h in an explorative study. *In vitro*, homosalate was hydrolysed into salicylic acid and 3,3,5-trimethylcyclohexanol. In addition, conjugation and hydroxylation of intact homosalate was observed.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

Taken from SCCP/1086/07
Homosalate is used as a broad-band UV filter in concentrations of up to 10% in the EU or 15% depending upon where the product is used (e.g. in the USA) in sunscreen products alone or in combination with other UV absorbers to protect the skin against harmful effects of the UV radiation.

Ref.: 74, 75

New information

A survey on the occurrence of organic UV filters in personal care products in Switzerland revealed that homosalate can be also found in such products.

Ref.: 54

In a survey performed by the Danish Environmental Protection Agency from October 2013 to August 2015, homosalate was found in 27 products out of 291, including 18 sunscreens. Products include face cream, body wash, cream; day cream, eau de toilette, foundation, hand cream, lip balm, makeup, perfume, sun oil and sunscreen.

Ref.: 81

3.3.2 Calculation of SED

The systemic exposure dose for homosalate used as a UV filter in cosmetic products is calculated using a dermal absorption value of 5.3% derived from an *in vitro* dermal

penetration study using viable human skin (Finlayson 2021) and a standard sunscreen formulation containing 10% homosalate.

Systemic exposure doses (SED) are also calculated for inhalation and oral exposure to product types containing 10% homosalate (Tables 5 and 6) separately and as aggregate exposure (Table 7)

Daily use of sunscreen lotion:

Amount of sunscreen applied	A	=	18 g/d
Concentration in the finished product		=	10%
Absorption through the skin	DA_p	=	5.3%
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)			
A x 1000 mg/kg x C/100 x DAp/100/60		=	1.59 mg/kg bw/d

Daily use of face cream containing UV-filter:

Amount of facecream applied	A	=	1.54 g/d
Concentration in the finished product		=	10%
Absorption through the skin	DA_p	=	5.3%
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)			
A x 1000 mg/kg x C/100 x DAp/100/60		=	0.136 mg/kg bw/d

Daily use of hand cream containing UV-filter:

Amount of facecream applied	A	=	2.16 g/d
Concentration in the finished product		=	10%
Absorption through the skin	DA_p	=	5.3%
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)			
A x 1000 mg/kg x C/100 x DAp/100/60		=	0.191 mg/kg bw/d

Inhalation exposure

The systemic exposure dose by the inhalation route was calculated using an adapted deterministic 2-box model as described in the Notes of Guidance 11th revision (SCCS/1628/21).

For the calculations (see Table 5) it was assumed that for both pump spray and propellant spray the same amount of sunscreen needs to reach the skin to ensure the necessary level of sun protection. For a propellant spray, this means that the additional amount of propellant gas needs to be added to the default value of 9 g/application, resulting in 15 g/application. By applying a factor of 0.6 for the proportion of non-propellant in the formulation based on information by the Applicant, this results in an amount of 9 g/application on the skin.

Table 5: SED calculations after inhalation exposure

Description	Parameter	Propellant spray	Pump spray	Unit
Amount by application[1]	A	15 000	9 000	mg/application
Fraction of Homosalate in non-propellant	C	0.1	0.1	(w/w)
Proportion of non-propellant in formulation	P	0.6	1	-
Airborne fraction	AF	1	0.2	-

Potential amount to be inhaled	EA (A*C*P*AF)	900	180	mg
First step: Near-field, 1 m^3	V_1	1 000	1 000	L
Breathing rate	BR	13	13	L/min
2 min in near-field	t_1	2	2	min
Potential amount inhaled during t_1	IA_1 (EA/V_1*BR*t_1)	23.4	4.68	mg
Second step: Far-field 10 m^3	V_2	10 000	10 000	L
Breathing rate	BR	13	13	l/min
10 min in far-field	t_2	10	10	min
Potential amount inhaled during t_2	IA_2 (EA/V_2*BR*t_2)	11.7	2.34	mg
Substance availability fraction	G	0.75	0.75	-
Respirable fraction	RF	0.2	0.01	-
Frequency of application	F	2	2	d^{-1}
Default bodyweight	BW	60	60	kg
SED_{inhal}	$(IA_1*IA_2)*G*RF*F/BW$	**0.176**	**0.002**	**mg/kg bw/day**

[1]Adjusted for the proportion of propellant to achieve a final "on-body" amount of 9 000 mg

The airborne fraction AF was assumed according to the SCCS Notes of Guidance (SCCS/1628/21). The near-field zone of the two-compartment model was assumed to have a volume V_1 of 1 m^3 and the duration of staying in the near-field zone t_1 as 2 min. For the far-field a volume V_2 of 10 m^3 and a duration of 10 min (t_2) was assumed.
The factor for substance availability G is based on Guidance from the European Commission, 1996. The respirable fraction of 0.2 is based on Applicant information on the spray can types used for sunscreen sprays.

Ref.: 28

Oral exposure
The systemic exposure dose from lipstick was corrected for a 50% oral availability of homosalate due to lack of relevant bioavailability information (Table 6).

Table 6: SED calculation after oral exposure

Description	**Parameter**	**Lipstick**	**Unit**
Relative daily exposure	$E_{product}$	0.9	mg/kg bw/d
Concentration of Homosalate	C	10	%
Retention factor[1]	F_{ret}	100	%
Adjustment for oral bioavailability		50	%
SED_{oral}	$E_{product}*(C/100)*(F_{ret}/100)*(50/100)$	**0.045**	**mg/kg bw/day**

[1]Potential amount available for oral exposure

Aggregated exposure

Table 7: Calculation of total SED for aggregated exposures

SED_{dermal}	**SED_{inhal}**	**SED_{dermal}**	**SED_{dermal}**	**SED_{oral}**	**SED_{total}**
Sunscreen (lotion)		**Face cream**	**Hand cream**	**Lipstick**	
1.590	-	0.136	0.191	0.045	**1.962**

Sunscreen (propellant spray)					
1.590	0.176	0.136	0.191	0.045	**2.138**
Sunscreen (pump spray)					
1.590	0.002	0.136	0.191	0.045	**1.964**

3.4 TOXICOLOGICAL EVALUATION

3.4.1 Irritation and corrosivity

3.4.1.1 Skin irritation

Taken from SCCP/1086/07

The skin irritative property of homosalate was tested within a combined study according to a modified Harber et al. (1982, 1987) protocol in male and female guinea pigs as well as in a combined and optimized mice ear swelling study in female BALB/C mice. In these investigations it was shown that homosalate revealed no skin irritation potential in guinea pigs or mice.

Ref.: 34, 35

New information

Guideline:	EU Method B.46 *(In Vitro* Skin Irritation: Reconstructed Human Epidermis Model Test), OECD Guideline 439 *(In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method)
Species:	Seeded human epidermal keratinocytes supplied by SkinEthic Laboratories, Lyon, France
Test substance:	Homosalate (Neo Heliopan® HMS)
Batch:	1439
Purity:	99.8%
Amount applied:	10 µL of undiluted test item were applied to each of triplicate tissues.
Concentration:	Undiluted
Surface area	0.38 cm²
Exposure:	15 min at 37 ±1.5°C, 5 ±0.5% CO_2
Positive control:	Sodium lauryl sulphate (SLS), 5%
Negative control:	Deionised water
GLP:	in compliance
Study period:	May – July 2012

Skin irritation of homosalate 99.8% was tested using EPISKIN assay, a human epidermis skin model test. The negative and positive control and the test item were added into the insert atop the concerning EpiSkin™ triplicate tissues. The 12-well plates were placed into the incubator for 15 min at 37 ±1.5°C, 5 ±0.5% CO_2. Using a wash bottle, the tissues were gently rinsed with PBS to remove any residual test material. The inserts were placed in the plates with 2 mL maintenance medium. The tissues were incubated for approximately 42 hours at 37 ±1.5°C, 5 ±0.5% CO_2.

A 12-well plate was filled with 2 mL assay medium containing 0.3 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) per well.
After the treatment procedure was completed for all tissues of each time point, cell culture inserts were transferred from the holding plates to the MTT-plates. After a 3-hour incubation

period (37 ± 1.5°C, 5 ± 0.5% CO_2) MTT solution was aspirated from the wells and the wells were rinsed three times with PBS. Tissue samples were cut out of the inserts with a biopsy punch and transferred into plastic vials. The tissue samples were immersed into extractant solution by gently pipetting 0.5 mL extractant solution (isopropanol / 2 N HCl 49:1 (v/v)) into each vial.
Per each tissue sample 2 x 200 µL aliquots of the formazan blue solution were transferred into a 96-well flat bottom microtiter plate. OD was read in a microplate reader (Versamax ® Molecular Devices, 85737 Ismaning, Germany) with 570 ± 1 nm filter. Mean values were calculated from the 2 wells per tissue sample.
MTT reducing capability of the test item was tested (as described in section "9.5 Test for Direct MTT Reduction").

Treatment with the positive control induced a decrease in the relative absorbance as compared to the negative control to 24.2% thus ensuring the validity of the test system.

The relative mean absorbance of the test item treated tissues was 108.9% (relative to negative control) after a 15-minute exposure period. Homosalate had no effect on viability and is considered non-irritant.

Ref.: 39

SCCS comment
Under the experimental conditions reported, homosalate is considered not irritant to skin.

3.4.1.2 Mucous membrane irritation / eye irritation

Taken from SCCP/1086/07

The limited data in experimental animals in respect to the irritative potential of homosalate did not indicate an irritation potential to the skin or the mucous membranes. In addition, numerous clinical studies in human revealed no irritative potential, not even under enhanced conditions.

SCCS overall conclusion on skin and mucous membrane irritation
Homosalate is not considered as a skin irritant. Although the SCCS notes that there are self-classifications for Eye irrit. 2, the limited data available do not point to eye irritation of homosalate when used in concentrations up to 10% in sunscreen formulations.

3.4.2 Skin sensitisation

Taken from SCCP/1086/07

There is no study that conforms to guidelines available in respect to the skin sensitizing potential of homosalate in experimental animals.
However, the skin sensitizing property of homosalate was tested within a combined study according to a modified Harber et al. (1982, 1987) protocol in male and female guinea pigs as well as in a combined and optimized mice ear swelling study in female BALB/C mice. In these specific studies it was shown that homosalate revealed no specific or selective skin sensitizing potential in guinea pigs or mice.

Ref.: 34, 35

There exists a personal communication from Maibach in a publication on the quantitative structure-toxicity relationship (QSTR) that homosalate (homomenthyl salicylate, CAS 118-56-9) showed no sensitizing potential in the guinea pig maximization test. The model

calculations *per se* predicted homosalate as a substance with weak/moderate sensitizing potential according to the specific QSTR model of the authors.

Ref.: 26

Homosalate was tested for potential sensitization on human skin in a maximization test in 25 healthy volunteers. In the main phase of the study, the neat test substance was applied for five 48-hour periods under an occlusive dressing, each time at the same site.
The 25 volunteers revealed no signs of skin irritation or sensitization at any challenge readings.

Ref.: 50

There are several human studies (mainly human repeated insult patch test, HRIPT) which were not performed with homosalate *per se* but with representative products (mostly sunscreens) with a varying concentration of homosalate. In none of these studies was a clinically relevant potential for dermal irritation or sensitization observed.

Ref.: 13-14, 31-33, 40, 45, 46-48, 57, 60-61, 82

One publication describes two patients with follicular dermatitis after being in contact with a commercially available suntan lotion containing homosalate with contact sensitivity to homosalate confirmed with patch test.

Ref.: 63

Applicant's conclusion on sensitisation
Although only limited information on the skin sensitizing potential is available in experimental animals, the existing data obtained in guinea pigs and mice exhibited no sensitizing potential of homosalate. Furthermore, recent clinical studies in humans with different types of sunscreens and other cosmetic products containing homosalate up to 15% and performed under controlled and standardized conditions including GLP/GCP and under supervision or participation of a certified dermatologist revealed no skin sensitizing potential, not even under enhanced condition. Therefore, it is considered that homosalate is of no sensitization risk for the consumer from the usage in sunscreens at intended use conditions.

SCCS overall conclusion on skin sensitisation
The HRIPT data appear to be generated in the USA. The SCCS considers the HRIPT studies to be unethical.
The SCCS agrees with a Risk Management Option Analysis (RMOA) performed by the French REACH Competent Authority (ANSES 2018) that overall, homosalate does not present any concern for skin sensitisation considering the above data.

3.4.3 Acute toxicity

Taken from SCCP/1086/07

The acute oral and dermal toxicity of homosalate is very low. The respective LD50 values for the acute oral toxicity in rats and for the acute dermal toxicity in rabbits are far above >2000 mg/kg bw.

New information

The SCCP conclusion is supported by a more recent analysis based on ECHA disseminated information.

SCCS overall conclusion on acute toxicity
Homosalate is of low acute oral and dermal toxicity.

3.4.4 Repeated dose toxicity

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

Taken from SCCP/1086/07

Range-finding study

Guideline:	/
Species/strain:	Rat
Group size:	5 animals/sex/group
Test substance:	Homosalate
Batch:	/
Purity:	/
Doses:	0, 100, 300, 1000 mg/kg bw
Route of exposure:	Gavage
Observation:	2 weeks exposure period
GLP:	Not in compliance

Homosalate was investigated for its subacute toxicity in a 2-week range-finding study in male and female rats. Each of the 5 male and 5 female rats received the test substance at dose levels of 0, 100, 300 and 1000 mg/kg bw orally by gavage for 2-weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption, haematology and clinical chemistry including coagulation were performed. At termination of treatment, all animals were sacrificed and macroscopically examined.

Results
Wet fur and/or salivation were observed at 100, 300 and 1000 mg/kg bw (males: 2/5, 5/5, 5/5, females 0/5, 5/5, 5/5, respectively). However, this is not considered as a toxic effect but as an indication of the bad taste of the test substance preparation. With the exception of a slight retarded body weight gain in male animals and a corresponding reduction of food efficiency at 1000 mg/kg bw, there was no relevant effect on body weight, food consumption or food efficiency in the other groups. Haematology and gross pathology revealed no treatment-related findings at any dose level. Increases in APTT and/or PT were observed in males at >300 mg/kg bw and in females at 1000 mg/kg bw. Bilirubin was reduced at >100 mg/kg bw in males and at >300 mg/kg bw in females, while triglycerides were increased in both sexes at 1000 mg/kg bw. However, these effects were considered as not adverse (Bilirubin) or only potentially adverse (triglycerides) by the author (no data or further information supplied).

Conclusion
The author assumed a No Adverse Effect Level (NOAEL) of 100 mg/kg bw for repeated application in rats over a period of 14 days due to the effects on coagulation in males at >300 mg/kg bw and in females at 1000 mg/kg bw.

Ref.: 42

New information

Guideline/method:	OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)
Species/strain:	Male and female RccHanTM: WIST(SPF) rats
Group size:	10/sex/dose
Batch:	1439
Purity:	99.8
Doses:	0 (control), 60, 120, 300 and 750 mg/kg bw/d
Frequency of administration:	Once daily
Vehicle:	Corn oil
Route of administration:	Oral (gavage)
Duration of treatment:	47 days (males); approximately 7 weeks (females)
GLP:	In compliance except for the occurrence of constant lighting during the conduct of the study

Homosalate was tested in a combined repeated dose and reproduction / developmental screening study in rats by gavage up to 750 mg/kg bw/day. For reproduction/development see section 3.4.5 (Reproductive Toxicity). One female animal of the highest dose was found dead on day 6 of the pre-pairing period and a body weight loss of 17% compared to start of treatment was noted. Another female animal of the high-dose group exhibited weakened condition (and also a 17% body weight loss) and was killed for ethical reasons on day 7 of the prepairing period. These deaths were considered test-item related by the Applicant. Decreased body weight and food consumption were reported in both males and females at 750 mg/kg bw/day from pre-pairing period.
Findings noted in functional observational battery: Mean total beam counts during the 30 minutes of measurement at the dose levels of 0, 60, 120, 300 and 750 mg/kg bw/day were respectively: 1417, 1374, 1441, 1295 and 1073 in males and 834, 1041, 796, 720 and 1074 in females giving birth. In females not giving birth, measured values were 833, 675 and 839 at the dose levels of 60, 120 and 750 mg/kg bw/day, respectively.
The following statistically significant changes in haematological parameters were not considered test-item related by the Applicant: In males: higher haemoglobin levels at 750 mg/kg bw/d, higher mean corpuscular haemoglobin concentration at 300 mg/kg bw/d, lower number of eosinophils at 60, 300 and 750 mg/kg bw/d and higher concentration of basophils at 60 mg/kg bw/d in males. Lower erythrocyte and haemoglobin concentrations as well as a higher number of neutrophils at the highest dose and lower number of basophils at 300 (but not at 750 mg/kg bw/d) in females. According to the Applicant, all haematological changes in males were minor, did not follow the dose dependency and the values remained in the range of the historical controls (number of eosinophils at the dose level of 750 mg/kg bw/day was slightly below the lower limit of the historical controls). All values in females remained in the range of the historical controls and number of basophils was not statistically significantly changed at the dose level of 750 mg/kg bw/day.
In males, statistically significantly increased concentration of albumin accompanied by lower globulin concentration was observed. Although remaining in the historical control range, at 300 mg/kg bw/d statistically significantly elevated albumin was observed. Therefore, changes in albumin were considered treatment-related. In females, all changes in biochemical parameters were reported to fall within the range of historical controls.
Several changes in organ weights were reported: statistically significantly increased absolute and relative liver weights in both sexes at the high dose. Significantly increased absolute and relative liver weights in females at 300 mg/kg bw/day. In males at 300 mg/kg bw/d the liver to body weight ration was statistically significantly increased. Statistically significantly increased kidney weights were reported in females from 300 mg/kg bw/day and increased absolute and relative kidney weights in males were reported from 60 mg/kg bw/d (without dose-dependency). Decreased thymus weight was observed in both sexes at 750 mg/kg bw/day and reduced prostate and seminal vesicles weights in males at 750 mg/kg bw/day.
Several target organs were identified with increasing doses of homosalate. Kidney was the

target organ at all doses (from 60 mg/kg bw/day), liver from 120 mg/kg bw/day and thyroid and spleen were the target organs from 300 mg/kg bw/day.
Histopathologically, a minimal to moderate increase in intra-epithelial hyaline droplets in the kidneys was found in all the male groups given homosalate. In a few of the affected animals the finding was associated with an increase in foci of basophilic (regenerating) tubules, single cell death and/or the presence of granular casts. The Applicant interpreted this as manifestations of hyaline droplet nephropathy without giving further evidence. Minimal or mild centrilobular hypertrophy of hepatocytes was reported in 1/5 males given 120 mg/kg bw/day, in all males and 4/5 females given 300 mg/kg bw/day and in all males and 6/7 females given 750 mg/kg bw/day. In the thyroid gland, there was a higher incidence and/or severity of diffuse hypertrophy of the follicular epithelium in males at 750 mg/kg bw/day and in females from 300 mg/kg bw/day. Finally, a greater incidence and severity of decreased cortical lymphocytes was noted in males from 300 mg/kg bw/day and in females at 750 mg/kg bw/day.

Reproductive data have been reported under the section 3.4.5.1 "Fertility and reproduction toxicity".

Ref.: 24

Re-Evaluation of Rat Organs from the Harlan Study D54938 by Immunohistochemical Analysis (alpha-2-microglobulin)

Paraffin blocks from the OECD TG422 study were used to stain for the presence of alpha-2-microglobulin. Tissue slides were prepared at 2-4 µm thickness for hematoxylin/eosin (H&E) staining and at 4 µm for immunohistochemical analysis against alpha-2-microglobulin. For immunohistochemistry, an antibody against alpha-2-microglobulin from R&D Systems was used. The immunohistochemistry was performed on a Bond III TM Autostainer combined with a manual staining step. Dehydration and coverslipping was performed on a Leica Autostainer XL combined with robotic coverslipping on a Leica CV5030.
One immunohistochemistry kidney section from all males in the study and one female from Group 1 (control) and 5 (dosed with 750 mg/kg bw/d) and positive and negative controls were imaged scanned by an Olympus VS200 slides canner using the 20x objective. Quantitative evaluation was made in Olympus imaging and image analysis software cellSens v1.18. The cortex of the kidney was set into a region of interest. The area of positive anti-alpha2u-globulin immunohistochemistry of 3,3'-Diaminobenzidine (DAB) staining was measured in the region of interest using consistent pixel thresholds by hue, saturation, and intensity. Arithmetic mean values of the percentage positive area of the cortex were used for further descriptive statistics. Statistical tests were performed using GraphPad Prism 8. The Shapiro-Wilk test for normality was performed. Since the data was following normal distribution, the comparisons were performed with the unpaired t-test or ordinary one-way ANOVA (p-values <0.05 were considered significant).

In recut H&E-stained sections, there was a minor increase of hyaline inclusions in epithelial tubular cells in all test item-treated groups but mainly at 120 to 750 mg/kg. In one single case, there were single cell necrosis of cells laden with hyaline inclusions.
There were no further lesions that could be attributed to treatment with the test item.
Other findings consisted of single cases of pelvic dilation, pelvic inflammation, mononuclear cell infiltration, tubular basophilia and one case of granular casts in a low dose animal.

The mean cortex area was similar in test item-treated groups compared to controls. At 120 mg/kg, the mean value of alpha-2-microglobulin was higher than in controls.
However, at 300 and 750 mg/kg, the differences of mean values were not higher compared to controls. The differences were not statistically significant between test item-treated groups and controls.

Ref.: 3

SCCS comments
The full study report of the OECD TG 422 study was made available to the SCCS during the commenting period.
Based on this study, the Applicant derived a NOAEL of 300 mg/kg bw/day for general toxicity based on mortality in high-dose females and decreased food consumption. However, it should be noted that at this dose, effects on kidneys, liver, thyroid and thymus had already occurred.
In male animals, histopathological kidney findings which occurred from the lowest dose level were stated to be attributed to hyaline droplet nephropathy but no scientific data was provided to underline this statement. It is of note that in males, higher kidney weights were also observed from the lowest dose (but without dose-dependency).
In the original study report and the reevaluation, the incidences of hyaline inclusions are almost identical (severity of changes slightly different but generally similar) pointing to reliability of the histopathological findings as obtained by H&E staining.

The dose-dependent increase in intra-epithelial hyaline droplets was, however, not reflected by immunohistochemical analysis. Moreover, staining for alpha-2-microglobulin was also observed in females (at 750 mg/kg comparable to males exposed the same way and higher when compared to male positive control), where much lower amounts of alpha-2-microglobulin would be expected.
Therefore, the results from immunohistochemical staining are not conclusive to show a clear picture. Based on the outcome of this study the SCCS cannot support the conclusion that observed kidney toxicity was dependent on alpha-2-microglobulin mediated mechanism, which is not relevant to humans.

No historical control data on haematological or biochemical parameters were available.
As effects were noted from the lowest dose of 60 mg/kg bw/d the SCCS considers this dose as LOAEL, in particular as human relevance of the kidney findings cannot be ruled out due to inconclusive results from immunohistochemical reanalysis of kidneys.
The SCCS further notes that the occurrence of a constant lighting during the conduct of the study significantly affects the reliability of this study, especially for developmental / reproductive effects.

It should be noted that, in the context of a compliance check process under REACH, the European Chemicals Agency adopted in March 2018 a decision requesting a sub-chronic toxicity study, a prenatal developmental toxicity study, an extended one-generation reproductive toxicity study, and the identification of degradation products (ECHA decision CCH-D-2114386909-26-01/F).

Ref: 19

An appeal was filed against this decision (Notice of appeal in Case No. A-009-2018) and a decision was adopted by the ECHA Board of Appeal on 18 August 2020. The Board of Appeal dismissed the appeal and decided that the information required by the Contested Decision must be provided by 25 February 2024. The Board of Appeal found that the REACH Regulation requires registrants to perform studies on vertebrate animals even if the substance is used exclusively as an ingredient in cosmetic products.
The information required by the Contested Decision is:
1. Sub-chronic toxicity study (90-day), oral route (test method: EU B.26.|OECD TG 408) in rats with homosalate;
2. Pre-natal developmental toxicity study (test method: EU 8.3I./OECD TG 414) in a first species (rat or rabbit), oral route with homosalate;
3. Extended one-generation reproductive toxicity study (test method: EU 8.56,/OECD TG 443) in rats, oral route with homosalate specified as follows:
   - Ten weeks premating exposure duration for the parental (PO) generation;
   - Dose level setting shall aim to induce some toxicity at the highest dose level;
   - Cohort 1A (Reproductive toxicity);

- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;
- Cohorts 2A and 28 (Developmental neurotoxicity); and
- Cohort 3 (Developmental immunotoxicity),

4. Identification of degradation products using an appropriate test method with homosalate.

Ref: 18

In view of the above-mentioned requests, new data may become available after finalisation of this SCCS Opinion and may trigger a new request for SCCS assessment.

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

No data submitted

3.4.4.3 Chronic (> 12 months) toxicity

No data submitted

3.4.5 Reproductive toxicity

New information

Fertility and developmental toxicity of homosalate were addressed in a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (see also section 3.4.4).

Males (10/group) were treated with 0, 60, 120, 300 or 750 mg/kg bw/day of homosalate in corn oil, by gavage once daily from 14 days pre-pairing and for a total of 47 days. Females (10/group) were treated with 0, 60, 120, 300 or 750 mg/kg bw/day homosalate in corn oil as vehicle, by gavage, once daily from 14 days pre-pairing and sacrificed on day 4 post-partum. Pups were sacrificed on day 4 post-partum.
A NOAEL for general toxicity was established at 300 mg/kg bw/day for both sexes based on maternal effects and developmental toxicity (adverse effects on food consumption and body weights in both sexes and mortality of females noted at higher dose).
No indication of any effect on reproduction was noted at the dose levels of 60 and 120 mg/kg bw/day (changes in sperm morphology and sperm motility correlating with reduced weights of prostate and seminal vesicles and increased post-implantation loss were noted at 750 and 300 mg/kg/day). However, because of low numbers of pregnant females, none of these dose levels could be conclusively confirmed as NOAEL.
The possible effects on fertility (increased infertility, sperm changes), development (higher post-implantation loss) and thyroid (hypertrophy of the follicular epithelium) noted in this study cannot be considered as conclusive and reliable due to a technical error that maintained the animals under a constant light.
Significant changes in sperm morphology (reduced number of normal complete sperm, increased number of sperm with normal head only and detached tail and of sperm with abnormal head and normal tail) and reduction in sperm motility were noted at 750 mg/kg bw/day. Increased infertility was reported without dose-response relationship at control, 60, 120, 300 and 750 mg/kg bw/day with 8, 4, 5, 7 and 3 pregnant females in each group.
At the highest dose, the three pregnant females presented a low number of corporea lutea and higher post-implantation loss. Only one female had living pup at first litter check (but missing on day 2 of lactation period). No birth was recorded in the 2 remaining pregnant

females. At 300 mg/kg bw/day, higher incidence of post-implantation loss was noted leading to a lower birth index but without any effect on litter size. There was no effect recorded on pups' body weight, sex ratio, post-natal loss and at macroscopical examination at all relevant doses up to 300 mg/kg bw/day. However, the low numbers of pregnancies per group questions the validity of data on development of offspring in this study.

Ref.: 24

SCCS comment

The full study report was made available the SCCS during the commenting period. The study protocol used was the 1996 version of OECD test guidelines 422, which covers less parameters compared to the 2015 version, which in particular addresses endocrine related mode of action. The SCCS notes that the occurrence of a constant lighting during the conduct of the study significantly affects the reliability of this study, especially for developmental/reproductive effects.
In addition, the low numbers of pregnancies per group questions the validity of data on development of offspring in this study.
The SCCS is aware that new data could become available (see section 3.4.4.1 above).

3.4.6 Mutagenicity / genotoxicity

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

Taken from SCCP/1086/07

No genotoxic/mutagenic potential was noted in three bacterial gene mutation assays in Salmonella typhimurium strains in the presence or absence of metabolic activation. In mammalian cells systems, homosalate showed no clastogenic potential with or without metabolic activation.

New information

Mammalian cell gene mutation assay

Guideline/method:	OECD Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test)
Test system:	Chinese hamster lung fibroblasts (V79)
Test substance:	Homosalate
Purity:	99.8%
Concentrations:	Experiment I 4 hours without metabolic activation: 0.08, 0.15, 0.3, 0.6, 1.2 µg/ml 4 hours with metabolic activation: 20.0, 40.0, 80.0, 160.0, 640.0 µg/ml Experiment II 24 hours without metabolic activation: 1.3, 2.5, 5.0, 10.0, 20.0 µg/ml 4 hours with metabolic activation: 20.0, 40.0, 320.0, 640.0 µg/ml
Solvent:	Ethanol
Positive Controls:	Without metabolic activation: ethylmethanesulphonate, 150 µg/ml With metabolic activation: 7,12-dimethylbenzanthracene, 1.1 µg/mL With S9 mix: Cyclophosphamide, 1.4 µg/ml
GLP:	In compliance

The study was performed to investigate the potential of homosalate to induce gene mutations at the HPRT locus in V79 cells of the Chinese hamster. The study is comprised of a pre-experiment and two independent main experiments. In the pre-experiment the cell cultures

were treated with homosalate for 4 hours with metabolic activation and for 4 and 24 hours without metabolic activation. In the first experiment, the treatment period was 4 hours with and without metabolic activation. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.
The maximum concentration of the pre-experiment (2700 µg/mL) was equal to a molar concentration of about 10 mM. The concentration range of the main experiments was limited by cytotoxic effects and phase separation. Ethanol was used as solvent.
No substantial and reproducible dose dependent increase of the mutation frequency was observed in either of the main experiments.

Authors conclusion on mutagenicity in vitro:
Under the experimental conditions reported, homosalate did not induce gene mutations at the HPRT locus in V79 cells. Therefore, it is considered to be non-mutagenic in this HPRT assay.

Ref.: 25

Single cell gel electrophoresis (SCGE-comet) assay
The potential genotoxic effect of homosalate has been evaluated using the single cell gel electrophoresis (SCGE-comet) assay in isolated human peripheral lymphocytes. Human peripheral blood lymphocytes were exposed to different concentrations (10, 50, 100 and 200 µg/mL) of homosalate for 30 and 120 min. After the treatment, cell viability was determined by using the trypan blue exclusion method and it was found to be above 90% for all samples. The data indicated that all concentrations of homosalate did not produce statistically significant differences in cell viability when compared to control ($p>0.05$). According to the data obtained from three separate experiments, DNA damage was significantly increased at 200 µg/mL, compared with those of untreated cells. The increase was dose dependent. The toxicity of homosalate at 10, 50 and 100 µg/mL concentration was not statistically significant ($p>0.05$).

Authors' conclusion:
Our results revealed that homosalate increased the DNA migration both in the time and concentration manner. DNA damage was higher in cells that had been incubated with the greatest concentration of 200 µg/mL ($p<0.05$). Results obtained by the present study indicate that homosalate would be genotoxic at higher concentration and incubation time.

Ref.: 85

SCCS comment
The study is of limited value for the assessment of the genotoxicity of homosalate. The study has methodological deficiencies mainly due to low viability of lymphocytes observed after the exposure and unclear description of statistical analysis performed.

Micronucleus test with human breast cell line MCF-7
In a recent study, the cytotoxic and genotoxic effects of homosalate in MCF-7 cells were evaluated. Cell viability was examined by the MTT assay, cell membrane integrity by the lactate dehydrogenase release assay (LDH) and genotoxicity by using the micronucleus (MN) test at 250, 500, 750, 1000, 1500, and 2000 µM.
Results showed that homosalate affected the cell viability dose-dependently at concentrations above 1000 µM. Micronucleus formation was significantly induced at 750 and 1000 µM within 24 hours due to an increase in cytostatic effect. Viability of cells exposed to homosalate decreased to 57% at a concentration of 2000 µM and a sufficient number of binucleated cells could not be obtained to count. Homosalate was also clastogenic when the cells were incubated at cytotoxic concentrations.
Under the conditions of the study described, the authors concluded that homosalate had cytotoxic and genotoxic effects in MCF-7 cells.

Ref.: 86

SCCS comment
The study is of limited value for the assessment of mutagenicity of homosalate.
- The results of cell viability after 24 and 72 h of exposure to the same concentrations of homosalate (up to 2000 µM) are unexpectedly almost at comparable level (even higher after 72 h exposure to 1500 µM). Normally, cell viability after 72 h would be expected to be lower.
- MCF-7 is not among the cell lines recommended by OECD TG 487.
- Only MN test without metabolic activation (-S9) was used.
- As MCF-7 cells are breast cancer cells and most probably show quite high spontaneous MN frequency, having no historical control values, it is difficult to assess if the increase reported in this paper, i.e. 1.13% (control) vs. 9 (1500 µM) is biologically relevant.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

No data submitted.

SCCS overall comment on mutagenicity based on the information from SCCP/1086/07 and the new *in vitro* studies

Homosalate was investigated in valid GLP genotoxicity tests for the three types of genotoxic endpoints: gene mutations, structural and numerical chromosome aberrations.
Homosalate did not induce gene mutations in bacteria and it did not induce gene mutations at the HPRT locus in V79 Chinese hamster cells. Homosalate did not induce chromosomal aberrations in CHO cells.
Two recent studies (Yazar et al., 2018; Yazar et al., 2019) suffer from methodological deficiencies, therefore the results were not included in the overall WoE.
Overall, the SCCS is of the opinion that homosalate can be considered to have no genotoxic potential.

3.4.7 Carcinogenicity

No data submitted.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

Taken from SCCP/1086/07

In vitro homosalate was proven to be not phototoxic in the NRU assay using murine BALB/c fibroblasts. *In vivo* there exists also no indication for a phototoxic potential in experimental animals.

Photosensitisation *in vivo*

No photosensitisation was found after topical treatment in male and female guinea pigs and female mice.

3.4.8.2 Photomutagenicity / photoclastogenicity

Taken from SCCP/1086/07

No photo-genotoxic/mutagenic potential was noted in the bacterial gene mutation assays in *Salmonella typhimurium* strains and no photo-clastogenic potential was recorded in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation.

3.4.9 Human data

Taken from SCCP/1086/07

Whenever studies with different sunscreens and cosmetic products were performed under controlled and standardized conditions including GLP/GCP and under supervision or participation of a certified dermatologist, homosalate was shown to have no irritative or sensitization potential and was proven to be not photoirritant and posesses no photo-allergic potential even under enhanced conditions. Although homosalate is widely used and has a long history of usage, only very isolated cases of allergic/photoallergic reactions are available in the open literature. This is considered as further indication that homosalate has a negligible potential to induce adverse skin reactions in the human population.

3.4.10 Special investigations

3.4.10.1. Effects on human cell lines

Proliferative acitivity in a human trophoblast cell line

The proliferative activity of homosalate was recently investigated by Yang et al. (2018) using human trophoblast cells, HTR8/SVneo cells, treated with 0, 50, 100, 200, and 400 μM homosalate for 48 h. It was found that homosalate inhibited proliferation of HTR8/SVneo cells in a dose-dependent manner. As proliferation reduced 66% in response to 100 μM homosalate, this concentration was the maximum concentration used for further cellular experiments. The expression of PCNA, which is a DNA clamp protein crucial for cell proliferation, decreased at 100 μM homosalate. PCNA expression in the nucleus of HTR8/SVneo cells treated with homosalate was reduced by 58% compared with that in the non-treated cells. Based on these results, the authors concluded that homosalate reduces the proliferation of human trophoblast cells.

HTR8/SVneo cells were treated with homosalate in a dose-dependent manner (0, 20, 50, and 100 μM) for 48 h, and annexin V and propidium iodide staining was performed for estimating the distribution of apoptotic cells. The number of annexin V-positive apoptotic cells increased following homosalate treatment. Furthermore, homosalate (100 μM)-treated HTR8/SVneo cells showed a 1.7-fold increase in apoptotic cell ratio compared with that in non-treated cells. Authors concluded that homosalate stimulates apoptosis of human trophoblast cells.

Homosalate induced abundant expression of Alexa 488, indicating lipid peroxidation and increased their intensity approximately 4.9-fold compared with non-treated cells. These results suggest that homosalate can alter characteristics of HTR8/SV neo cells by changing the intracellular oxidative condition.

Homosalate treatment promoted depolarization of mitochondrial membranes in HTR8/SVneo cells by 6.4-fold at the maximum concentration. These results demonstrated that homosalate induced intracellular oxidative stress to control the proliferation of human trophoblast cells.

The effects of homosalate on invasion of HTR8/SVneo cells indicate that homosalate suppressed the invasive process of human trophoblast cells, which was followed by poor placentation and unsuccessful implantation.

The authors also investigated whether homosalate regulates signaling kinase proteins involved in PI3K/AKT and MAPK pathways related to cell survival and invasiveness of human trophoblast cells.
In summary, Yang et al. (2018) concluded that homosalate promoted the death of HTR8/SVneo cells with elevated lipid peroxidation and intracellular Ca^{2+} concentration. It also induced endoplasmic reticulum stress and mitochondrial morphological disturbances associated with the differentiation of human trophoblast cells. However, when the intracellular Ca^{2+} or reactive oxygen species were removed using BAPTA-AM, a Ca^{2+} chelator, or N-acetyl-L-cysteine (NAC), the cell proliferation suppressed by homosalate was restored. Homosalate also significantly inhibited the invasion of HTR8/SVneo cells. Furthermore, it modulated phosphoinositide 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK) signaling pathways, which were involved in the cross-talk between both signaling pathways in HTR8/SVneo cells. It was concluded that homosalate adversely affects the survival, proliferation and invasiveness of human trophoblast cells.

Ref.: 84

Effects on migration and invasion of human breast cancer cell lines
The effects of exposure to six UV filters (Benzophenone-1, Benzophenone-2, Benzophenone-3, Octyl methoxycinnamate, 3-(4-Methylbenzilidene) camphor and homosalate) were studied by Alamer and Darbre (2017) using MCF-7 and MDA-MB-231 human breast cancer cell lines. Increased motility of oestrogen-responsive MCF-7 human breast cancer cells was observed after long-term exposure (>20 weeks) to each of the six compounds at concentrations $\geq 10^{-7}$ M using three independent assay systems (scratch assay, live cell imaging, xCELLigence technology) and increased invasive activity was observed through matrigel using the xCELLigence system. Increased motility of oestrogen-unresponsive MDA-MB-231 human breast cancer cells was observed after 15 weeks of exposure to each of the six compounds by live cell imaging and xCELLigence technology, implying that the increased migratory activity was not confined to oestrogen-responsive cells. Molecular mechanisms varied between compounds and cell lines. Using MCF-7 cells, reduction in E-cadherin was observed following 24 weeks' exposure to 10^{-5} M homosalate. According to the authors these results demonstrate that exposure to any of these six compounds (including homosalate) can increase migration and invasion of human breast cancer cells.

Ref.: 2

SCCS comment
Due to limited data available, no conclusion on the effect of homosalate to human breast cancer cells can be drawn from this study.

3.4.10.2 Endocrine activity

3.4.10.2.1 Non-test information, in silico, read across, in chemico.

According to Danish QSAR database, homosalate is predicted to activate the oestrogen receptor (according to Leadscope and SciQSAR models) and to act as an antagonist of androgen receptor (AR) (according to CASE Ultra and Leadscope models).

Ref.: 4

3.4.10.2.2 *In vitro* and other assays

Oestrogenic potential *in vitro*

Taken from SCCS/1086/07

Guideline/method:	Mechanistic study on oestrogen receptor binding properties according to a modified protocol of Bosel and Shain (1974)

Test system:	Human recombinant oestrogen receptor (ER), α-subtype (PanVera, Madison, WI; USA)
Replicates:	Two separate experiments with triplicate concentration levels
Test substance:	Homosalate
Batch:	50446454
Purity:	99.6%
Concentrations:	100, 1000, 10000, 100000 nM
Solvent:	DMSO
Positive Controls:	Genistein: 0.03 -100 nM Estradiol: 10 - 10000 nM
GLP:	Not in compliance

The potential interaction of homosalate with the oestrogen receptor (ER) was examined in a receptor binding assay with human recombinant ER of the α-subtype as receptor and radiolabelled oestradiol as ligand. Oestradiol (0.03 -100 nM) with strong ER affinity and genistein (10 -10000 nM) with weak ER affinity were used as positive control substances. A modified method according to Boesel and Shain (1974) was applied. 100 µl assay buffer containing BSA and 2% DMSO (± test compounds) and 50µl of 4.8 nM solution of radiolabelled oestradiol in assay buffer were mixed in microtiter plate wells. Following incubation at 4°C overnight under continuous shaking, charcoal suspension in assay buffer was added. After mixing the samples, charcoal was sedimented by centrifugation and 50 µl aliquots of the clear supernatant were analyzed by liquid scintillation counting (^{3}H activity). Aliquots (50 µL containing the ER-ligand complex were mixed with 200 µl of the scintillation cocktail and radioactivity was counted for 10 min in the reader with 1 hour delay allowing samples to equilibrate. Receptor binding was corrected for unspecific binding. Binding of radiolabelled oestradiol in the presence of test compound was related to binding in its absence. IC50 values were calculated, if possible. Two separate experiments were conducted each with triplicate concentrations and nine fold incubations of vehicle (DMSO) alone.
No affinity of homosalate to the oestrogen receptor (ER) at the maximum applicable concentration of 100000 nM was observed. The quantity of radiolabelled ligand oestradiol binding in the presence of the test compound was comparable to that of the control. The sensitivity of the test system was shown as the positive controls oestradiol and genistein displaced the radiolabelled oestradiol from the ER with IC50 values of 1.7 nM and 1.85 nM and 165 nM and 145 nM, respectively.

The authors concluded that homosalate showed no affinity to the human recombinant oestrogen receptor up to the highest concentration technically applicable of 100000 nM.

Ref.: 7

Schlumpf et al. (2001a, 2001b) investigated the oestrogenic potential of homosalate among other sunscreens *in vitro* using MCF-7 human breast cancer cells The MCF-7 cells were exposed to concentrations in the range between $1x10^{-7}$ -$5x10^{-5}$ M. Induction of proliferation in the MCF-7 cells was noted with an EC50 value of 1.56 µM and this was interpreted by the authors as a positive result.
Further evidence for oestrogenic activity was the induction of pS2 protein in MCF-7 cells.

Ref.: 68, 69

New information

The interaction of seven UV filters: benzophenone-3, octyl-methoxycinnamate, 4-methylbenzylidene camphor, butyl-methoxydibenzoylmethane, homosalate (purity not given), octyl-dimethyl-p-aminobenzoic acid, 3-benzylidene camphor and five polycyclic musk fragrances (Tonalide, Galaxolide, Celestolide, Versalide, Phantolide) with the oestrogen receptor (ER), androgen receptor (AR), and progesterone (PR) receptor, was assessed using 293HEK cells (human embryonal kidney cells), which lack significant endogenous levels of ER. The cell line was stably transfected with a reporter construct, consisting of 3 oestrogen

response elements upstream from a TATA box in front of luciferase cDNA and a hERα or hERβ expression plasmid. To measure anti-oestrogenicity, cells were incubated with both the chemical to be tested and an E2 concentration of 3 and 100pM for hERa and hERb, respectively. 17beta-estradiol was used as positive control. The authors report oestrogenic effects, because homosalate activated transcription of ERα (EC_{50} of 1.6 µM compared to 2.1 pM for oestradiol) and ERβ to a limited extent (dose response reached a plateau level at 32% of oestradiol for which EC_{50} = 83 pM). Repression of hERα and hERβ was also tested, but no clear dose-dependent antagonistic effects were observed for either receptor.

Ref.: 71, 72

A classical receptor binding assay using human recombinant oestrogen receptor (ER) of α-subtype as receptor source and radiolabeled oestradiol as ligand was used in order to investigate the potential affinity of homosalate (purity 99.6%) for the ER. The physiological oestrogen oestradiol and the phytoestrogen genistein served as reference compounds with strong and weak affinity for the ER, respectively. The affinity of the test substance for the ER was investigated by evaluating the replacement potential of test substance for "radiolabeled oestradiol". The authors report no affinity of homosalate for ER at the maximum nominal concentration of 100 µM. The quantity of radiolabeled ligand oestradiol bound in the presence of the test compound was not significantly different than that of the control. The reference substances oestradiol (E2) and genistein (GEN) reproducibly displaced radiolabeled oestradiol from the ER with IC50 - values of 1.7 nM and 1.85 nM for E2 and 165 nM and 145 nM for GEN, respectively, in two parallel tests.

Ref.: 23

Analysis of the oestrogenic and antiestrogenic activity of homosalate (> 99% pure) together with 17 other UV filters and one metabolite was performed *in vitro* with recombinant yeast systems carrying either a human oestrogen (hERα) or androgen receptor (hAR).The yeast (*Saccharomyces cerevisiae*) genome carries a stably integrated DNA sequence of the human oestrogen receptor (hERα).Yeast cells also contain expression plasmids carrying androgen responsive elements regulating the expression of the reporter gene lacZ, (encoding the enzyme β-galactosidase). Thus, when an active ligand (i.e., E2 or an oestrogenic UV filter) binds to the receptor, β-galactosidase is synthesised and secreted into the medium, where it hydrolyzes chromogenic substrate chlorophenol red β-d-galactopyranoside leading to a colour change from yellow to red.
Potential cytotoxicity caused by the test compounds was checked, by measuring yeast growth (620 nm) besides β-galactosidase activity (540 nm). The authors report that homosalate was not oestrogenic up to 10^{-2} M. Anti-oestrogenic responses were detected for the highest concentration of homosalate (10^{-2} M and 10^{-3} M) obtaining an IC_{50} of 2.06 mM compared to 0.5 µM for 4-hydroxytamoxifen. However, the cytotoxicity was not evaluated with these concentrations. It completely inhibited the activity of E2 (EC65) at the highest concentrations tested and produced full dose–response curves. This indicates a much higher frequency of antiestrogenic than oestrogenic activity.

Ref.: 52

The oestrogenic activity of 23 UV filters, including homosalate (> 99% pure), and one UV filter metabolite was determined by employing a recombinant yeast carrying the oestrogen receptor of rainbow trout (rtERa). Comparisons were made with yeast carrying the human hERa for receptor specificity. The authors reported that no oestrogenic activity was detected with both receptors and that the system with the hERα assay was 62 times more sensitive than rtERα toward E2.

Ref.: 51

The interaction of homosalate (purity not mentioned) with the human oestrogen receptor alpha (hERα) was investigated in MCF-7 cells, using E-screen bioassay. In this cell line, the full ER agonist E2 induces significant proliferation in a clear dose-dependent manner. Oestradiol was used as positive control for MCF-7 proliferative test. Homosalate showed oestrogenic activity (EC_{50} = 5.53 µM) increasing the number of viable cells by 3.5 fold,

compared with control-treated cells (hormone-free medium). Homosalate failed to antagonize oestradiol-induced proliferation in MCF-7 cells up to the concentration of 10 µM. The latter finding is in contrast with Kunz and Fent (2006b), who reported a strong anti-oestrogenic activity of homosalate at high concentrations. The authors attributed these contradictory results to the different concentration ranges used in the studies, i.e., maximum concentration of 10 µM in their study versus substantially higher concentrations (up to 1mM) in Kunz and Fent (2006b).

Ref.: 44

The oestrogenic activity of homosalate (purity not mentioned) was tested with the use of 3 reporter cell lines: HELN (ER negative), HELN hERα (expressing human ERα) and HELN hERβ (expressing human ERβ). HeLa cell lines expressing luciferase constitutively were transfected with the appropriate plasmid (ERE-(βGlob-Luc-SVNeo) to stably express hERα or hERβ. Oestradiol was used as positive control. Luciferase assays were performed at concentrations between 10^{-7} *M* and 10^{-5} *M*. Homosalate was first tested in HELN cell line and a non-specific response was observed (activation of luciferase expression in the absence of ER). Then, homosalate was assayed in HELN ERα and HELN ERβ cell lines. At 1 µM, the substance activated ERα while it had no non-specific response on HELN. A weak oestrogenic activity towards ERβ was observed but this response could be due to non-specific induction. According to the authors, these results indicate that homosalate is a clear agonist of ERα but show a much less activation of ERβ, if any.

Ref.: 36

Androgenic potential *in vitro*

Taken from SCCP/1086/07

Guideline/method:	Mechanistic study on androgen receptor binding properties according to a modified protocol of Bosel and Shain (1974)
Test system:	Rat recombinant fusion protein to thioredoxin containing the hinge region and ligand binding domain of the androgen receptor (AR, PanVera, Madison, WI; USA)
Replicates:	Two separate experiments with triplicate concentration levels
Test substance:	Homosalate
Batch:	50446454
Purity:	99.6%
Concentrations:	100, 1000, 10000, 100000 nM
Solvent:	DMSO
Positive Controls.	Dihydrotestosterone: 0.1 - 300 nM Androstendione: 30 -100000 nM
GLP:	Not in compliance
Published:	No

Homosalate was tested for its potential to interact with the androgen receptor (AR) in a receptor binding assay with rat recombinant fusion protein containing the hinge region and ligand binding domain of the androgen receptor as receptor source and radiolabelled methyltrienolone (R 1881) as ligand. Dihydrotestosterone (0.1 - 300 nM) with strong AR affinity and androstendione (30 - 100000 nM) with weak AR affinity were used as positive control substances. A modified method according to Boesel and Shain (1974) was applied. 100 µl assay buffer containing γ-globulin and 2% DMSO (± test compounds), 50µl of 8 nM solution of radiolabelled R1881 in assay buffer were mixed in microtiter plate wells. Following incubation at 4°C overnight under continuous shaking, charcoal suspension in assay buffer was added. After mixing the samples charcoal was sedimented by centrifugation and 50 µl aliquots of the clear supernatant were analyzed by liquid scintillation counting (^{3}H activity). Aliquots (50 µl containing the AR-ligand complex were mixed with 200 µl the scintillation cocktail and radioactivity was counted for 10 min in the reader with one hour delay allowing

samples to equilibrate. Receptor binding was corrected for unspecific binding. Binding of radiolabelled R1881 in the presence of the test compound was related to binding in the absence. IC50 values were calculated, if possible. Two separate experiments were conducted, each with triplicate concentrations and six fold incubations of vehicle (DMSO) alone.

Results
Homosalate showed a weak affinity to the androgen receptor (AR) but the concentration-response relationships were flat and even the highest technically achievable concentration of 100000 nM revealed a displacement of 32% or 41% in the two experiments. Therefore, no IC50 value could be calculated. The sensitivity of the test system was shown as the positive controls dihydrotestosterone and androstendione displaced the radiolabelled methyltrienolone from the androgen receptor. In contrast to homosalate, the concentration-response curves were steep and parallel. The IC50 values were 5.2 nM or 4.4 nM for dihydrotestosterone and 2.5 µM or 1.8 µM for androstendione, respectively.

Conclusion from the authors
Homosalate showed only a weak affinity to the rat androgen receptor with a flat concentration-response relationship up to the highest concentration technically applicable of 100000 nM in contrast to the reference androgens. This result is not considered as an indication for a specific interaction with the androgen binding domain of the androgen receptor.

Ref.: 6

The human breast carcinoma cell line MDA-kb2 cell was used to screen several UV filters including homosalate *in vitro* for its potential to influence the androgen receptor. In this specific *in vitro* assay, homosalate was found to antagonize dihydrotestosterone induced androgen activation in concentrations below cytotoxicity as an indication for anti-androgenic activity *in vitro.* No agonistic activity was observed. However, these preliminary results of a screening assay were considered as of no relevance for the *in vivo* situation.

Ref.: 53

Kunz & Fent (2006b) commented that the absence of androgenic activity of homosalate in MDA-kb2 cells could be due to low endogenous occurrence of hAR in this cell line.

Ref.: 52

New information

(Anti)androgenic activity of homosalate (> 99% pure) was investigated using recombinant yeast systems carrying human androgen receptor (hAR). From the dose-response curves in this study, the authors concluded that homosalate demonstrated both androgenic [EC_{50} (effective concentration 50%) = 170 µM, compared to 2.07 nM for dihydrotestosterone] and antiandrogenic (IC_{50} = 107 µM, compared to 4.3 µM for flutamide) effects. However, it should be noted that androgenic activity was only observed at very high concentration (10^{-3} M).

Ref.: 52

The AR CALUX® bioassay was used to measure the agonistic and antagonistic effects of homosalate (purity not mentioned) on the androgen receptor. This assay is based on the generation of stable human AR transfectants of U2-OS cells (human osteosarcoma cell line) and contains a 3 x ARE-TATA-Luc-reporter construct in combination with a pSG5-neo-hAR expression plasmid. The natural AR ligand dihydrotestosterone was used as a positive control for AR agonism, while flutamide and vinclozolin were used as controls for AR antagonism. A 5a-dihydrotestosterone (DHT) concentration of 0.1nM (EC_{50}) was used for the measurement of anti-androgenicity. From this study, the authors concluded that homosalate acts as an AR antagonist although no AR transactivation was observed. The IC_{50} value was 1.7 µM compared to 0.5 and 0.1 µM for flutamide and vinclozolin, respectively. The antagonistic effect of all

compounds was reversed by coincubation with excess ORG2058 (100 times the EC_{50} value), showing the specificity of the response.

Ref.: 71

The potential androgenic and anti-androgenic activity of homosalate (purity not mentioned) via hAR was investigated, using *in vitro* bioassay based on transfected bioluminescent PALM cells (from human prostate carcinoma). The synthetic androgen R1881 at 0.2 nM was used as AR agonist. The authors concluded that homosalate did not show androgenic activity in the concentration range used (0.01–10 µM) in PALM cells, whereas the substance was found to be a potent hAR antagonist at 10 µM (IC_{50} = 2.66 µM) strongly inhibiting the luciferase activity induced by R1881.

Ref.: 44

Progesteronic potential *in vitro*

A PR CALUX® bioassay was used to measure the agonistic and antagonistic effects of homosalate among other substances at the progesterone receptor (PR). This bioassay is based on the generation of stable human PR transfectants of U2-OS cells (human osteosarcoma cell line) and contain a pSG5-neo-hPR expression vector in combination with a 3x ARE-TATA-Luc-reporter construct. ORG2058 was used as a positive control for agonism and RU486 as control for antagonism. According to the authors, homosalate (purity not mentioned) exerted a slightly PR antagonist effect (with an IC_{50} of 3.0 µM, compared to 4.9 pM for RU486). The effect was reversed by coincubation with excess ORG2058 (100 times the EC_{50} value), showing the specificity of the response. The EC_{50} for hERα was 1.6 µM. No PR transactivation was observed.

Ref.: 71

In mammalian sperm, CatSper (cationic channel of sperm), represents the principal Ca^{2+} channel, controlling intracellular Ca^{2+} concentration and motility. Mutations in CatSper genes have been correlated with male infertility. In human sperm, progesterone and prostaglandins directly activate CatSper. The action of homosalate on human sperm was studied using the CatSper inhibitor MDL12330A (MDL) at 100µM to examine whether EDC-induced Ca^{2+} signals involve CatSper. The potency and lowest effective dose for homosalate were quantified. Analysis of Ca^{2+} signals evoked by homosalate yielded an EC_{50} of 5.70 µM while from dose-response relationship a value of 232.1 nM was obtained for EC_{02}. Ca^{2+}signals evoked by homosalate were suppressed by 77% by MDL. The authors concluded that homosalate primarily acts via activation of CatSper.

Ref.: 67

Homosalate was investigated *in vitro* for its ability to induce Ca^{2+} signals in human sperm cells by applying measurements of the intracellular free Ca^{2+} concentration. It was tested at 10µM along with a positive control (progesterone, 5µM) and negative control (HTF^{+} with vehicle) and found to induce a significant Ca^{2+}signal. The Ca^{2+} signals induced by homosalate at 50 µM were compared with progesterone at 5 µM in the presence or absence of 20 µM CatSper inhibitor MDL 12330A, and found similar to progesterone, highly inhibited with MDL 12330A. It was suggested that homosalate induces Ca^{2+} signals primarily by activating the CatSper (cationic channel of sperm) channel, either by acting agonistically on the binding pockets of progesterone or prostaglandin or by affecting CatSper through another unknown mechanism independent of changes in pH. Homosalate produced saturating dose-response curves, with a mean EC_{50} 1.40 µM and a lowest effective dose value (EC_{02}) 62.9 nM. Because Ca^{2+} signaling controls important sperm functions, including chemotaxis, motility, capacitation, and acrosome reaction, all of which are essential for fertilization, the authors concluded that homosalate might interfere with the normal human fertilization process and thus impair fertility.

Ref.: 62

3.4.10.2.3 Animal data

***In vivo* studies**
Oestrogenic potential *in vivo*

Taken from SCCP/1086/07

Guideline/method:	Uterotrophic assay in immature rats according to OECD Validation Work and OECD Protocol (Draft, 2000)
Species/strain:	Rat/Wistar ((HsdCpb:WU)
Group size:	6 animals/sex/group
Test substance:	Homosalate
Batch:	507 57115
Purity:	89.64%
Dose levels:	0, 200, 1000 mg/kg bw
Vehicle:	Corn oil
Positive Control:	Ethinylestradiol (EE); 0.3 and 1.0 µg/kg bw
Route:	Subcutaneous (sc.)
Exposure period:	3 consecutive days
GLP:	In compliance

Homosalate was investigated for its oestrogenic potential in the uterotrophic assay in immature rats. Each 6 juvenile female Wistar rats received the test substance dissolved in corn oil at dose levels of 200 and 1000 mg/kg bw by subcutaneous injections, once a day on three consecutive days. Treatment started after an acclimatization period of three days when the juvenile rats were 19 days old. For control purposes, each of the 6 rats remained untreated and one additional group of 6 rats received the carrier (corn oil). Ethinylestradiol was selected as positive control substance and each 6 rats were subcutaneously injected with 0.3 and 1.0 µg/kg bw according to the same schedule. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed. At termination of treatment, all animals were sacrificed, macroscopically examined and the uterus weight (wet and blotted) was determined.

There was no mortality and no effect on the general state of health. Body weights and food consumption was comparable to the control groups. No effects on the uterus weights after sub-cutaneous treatment with homosalate at 200 and 1000 mg/kg bw was observed. The sensitivity of the juvenile female rats was demonstrated as the positive control caused an enlargement of the uterus accompanied by an increase in uterus weight.

The authors concluded that the repeated subcutaneous injection of homosalate at dose levels up to 1000 mg/kg bw to juvenile female Wistar rats on three consecutive days revealed no oestrogenic potential in the uterotrophic assay.

Ref.: 5

A further uterotrophic assay was performed in Long Evans immature rats receiving homosalate (purity not mentioned) in the diet at 491 or 892 mg/kg bw/day for 4 days from post-natal day 21. The phytoestrogen levels in the diet were not reported in the publication. Ethynilestradiol was used as positive control. No oestrogenic effect of homosalate was noted *in vivo* by the authors.

Ref.: 69

Zebrafish, in which an oestrogen responsive luciferase reporter gene has been stably introduced, were used for *in vivo* testing of six UV filters, including homosalate. Exposure studies were carried out with heterozygous F4 juvenile fish of 4–5 weeks of age. Fish (n=5–6) were exposed for 96 h in 200 ml acclimatized tap water (26–27°C). Homosalate (dissolved in ethanol) was added to the water in a 1:10,000 dilution. Fish were fed once daily with live

brine shrimp (*Artemia salinas*) and were exposed to a single concentration of homosalate (10µM). Luciferase activity was measured in a scintillation counter. In this transgenic zebrafish assay homosalate showed no oestrogenic activity at the concentration tested. The authors stated that one should be aware of over-interpretation when predicting *in vivo* effects from weak *in vitro* data.

Ref.: 72

In the risk management option analysis of homosalate performed by ANSES, France, it is concluded that no final conclusion can be drawn from this study due to the low sensitivity of the test and the fact that only one concentration was tested.

Ref.: 4

Dermal study

In a study using female and male Wistar Hanover rats, the effects of dermal exposure to homosalate (purity unknown) during the prenatal, lactation and early infancy periods on pubertal development and thyroid function were investigated. A 10% homosalate paraffin solution was topically applied to shaved areas on the rats' back at doses of 2 mg/cm^2. The back of each rat was shaved once per week to expose an area of 9 cm^2 during the pregnancy and lactation periods or an area of 4 cm^2 during infancy. To determine the effects of homosalate during the prenatal period, the solution was topically applied to 5 pregnant Wistar Hannover rats from gestation day 1 until delivery. The expected day of delivery, that is, GD 22, was designed as postnatal day (PND) 0 for the pups. The pups of all mothers that had received applications were included in the study. To determine the effects of homosalate during the lactation period, the solution was applied at the same dosage to 5 maternal rats between post-natal day 2 to 21. To determine the effects of homosalate after exposure during infancy period, the solution was applied for 6 consecutive days between post-natal day 21-26 to 10 pups. The pups from the different groups of exposure were examined daily from post-natal day 26 for signs of puberty. Vaginal opening, vaginal smear and preputial separation were examined as sign of puberty onset. Vaginal patency is oestrogen dependent and generally indicative of the occurrence of the first ovulation and the onset of oestrous cyclicity in the rat. Thyroid gland, testes, prostate, seminal vesicles, uterus, bilateral oviduct and ovaries were weighted. Serum thyroid-stimulating hormone (TSH), thyroxine (T4), follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and oestradiol levels were measured. Uteri, ovaries and testes were histopathologically examined.
After prenatal exposure and compared to control groups, the average thyroid gland weight, and TSH levels were reduced in treated females, while T4, LH and oestradiol levels were higher. In treated males, there was an increase of FSH and LH and a decrease in testosterone levels. No significant differences were noted between the control and experimental groups in the histopathological examinations of the ovaries, uteri and testes. After lactation exposure a decrease of oestradiol level was noted in treated females compared to control groups. The number of Graaffian follicles significantly increased, while no significant histopathological differences were observed in the uteri. In treated males, TSH levels decreased whereas T4 levels increased. The weight of testis, prostate and seminal vesicle increased. No significant effect was reported in the structure of seminiferous tubules. After exposure during infancy period and compared to control groups, treated females exhibited increased thyroid gland weight and decreased oestradiol levels. Treated males exhibited higher TSH, T4, LH and FSH levels. No effect was observed histopathologically in the ovaries, uterus and testis.

Ref.: 27

SCCS comment
This is a study reported in the open literature. In agreement with the RMOA, the SCCS considers this study not sufficiently robust to properly assess the endocrine-disrupting effects of homosalate. There are some inconsistencies in the interpretation of results in regard to the reported effects. In addition, as only one dose was tested, dose-response relationship cannot be established. The tested material was not the pure substance but a 10% solution.

Ref.: 4

An overview on ED-related *in vitro* and *in vivo* studies available for homosalate is given in Table 8.

Table 8: Summary of endocrine effects (Levels 2, 3 and 4) reported for homosalate (slightly modified from RMOA)

Endocrine activity	**Results reported by the authors**		**Method**	**Reference**
	In vitro (level 2)	***In vivo***		
Oestrogenic	No affinity for the oestrogen receptor was detected up to the highest concentration tested of 0.1 mM (Concentrations used 0.1-100 µM)		hER a recombinant binding assay	23, 66
		No oestrogenic effect at doses 200 and 1000 mg/kg bw subcutaneously injected	Uterotrophic assay (level 3)	23, 66
	Agonism toward hERa (EC_{50} = 1.6µM compared to 2.1µM of oestradiol) and hERβ to a limited extent. Dose-response curve of HMS on hERβ reached its plateau level at 32%. Concentration range tested (0.1 - 100 µM)		*In vitro*: gene expression (ER a) assay in HEK293 reporter cell lines	72, 71
		No oestrogenic activity at a single concentration of 10µM	*In vivo*: transgenic zebrafish assay (level 3)	72
	Dose-dependently increase in cell proliferation EC50 = 1.56 µM Concentrations used: 0.1-100 µM		*In vitro*: E-SCREEN assay in MCF-7 cells	69, 70
		Inactive at the doses tested 491 and 892 mg/Kg/bw (oral administration)	*In vivo*: Uterotrophic assay (level 3)	
	Activation of ERα at 1µM. ERβ: only weak response. Concentrations used 0.1-10 µM		Gene expression assay (ERα and ER) in HELN cell lines	36
	No oestrogenic activity up to 10 mM		Yeast hERα transactivation assay	52
	Inactive up to 25mM		Yeast rtERα transactivation assay	51
	Oestrogenic activity (EC50 = 5.53 µM). At HMS concentration of 10µM the number of viable cells increased by 3.5 fold, compared with control-treated cells. Range of concentrations tested 0.01–10 µM.		E-SCREEN in MCF-7 cells (ERα)	44
		No-conclusion can be drawn 10% paraffin solution	Repeated-dose toxicity study in rats exposed dermally *in utero* or during lactation or during infancy (level 4)	27
Anti-oestrogenic	No anti-oestrogenic effects. Concentration range used (0.1 - 100 µM).		Gene expression (ERα) assay in	72, 71

			HEK293 reporter cell lines	
	Anti-oestrogenic activity at the highest concentrations (10mM and 1 mM) tested. IC50 = 2.06 mM compared to 0.5 µM for 4-hydroxytamoxifen. Complete inhibition of E2 activity with full dose–response curves. (concentrations used 6µM – 10 mM)		Yeast hERα transactivation assay	52
	No anti-oestrogenic activity up to the concentration of 10 µM (range of concentrations tested 0.01–10 µM)		E-SCREEN in MCF-7 cells (ERα)	44
		No conclusion can be drawn 10% paraffin solution	Repeated-dose toxicity study in rats exposed dermally *in utero* or during lactation or during infancy (level 4)	27
Androgen	HMS weakly displaced the radiolabeled ligand methyltrienolone (< 50% at max conc. of 100 µM) in a concentration-dependent manner (Concentrations used 0.1-100µM)		Rat recombinant AR binding assay	23, 66
	No agonistic activity (concentrations used 0.1-100µM)		MDA-kb2 cell transactivation-activation assay	53
	No AR transactivation		AR CALUX assay	71
	Androgenic effect at very high concentration (1mM) producing full dose-response curve. EC_{50} = 170 µM, compared to 2.07 nM for dihydrotestosterone. (concentrations used 6 µM – 10 mM)		Yeast hAR transactivation assay	52
	No androgenic activity at the concentrations tested (0.01–10 µM)		Gene expression bioassay in PALM cells	44
Anti-androgenic	Positive. Reduction of DHT-induced AR activation in MDA-KB2 cells in a concentration-dependent manner (IC50 = 5.57 µM in 0.1 nM DHT and 13.1 µM in 0.5 nM DHT, HMS concentrations used 0.01-100 µM)		MDA-kb2 cell transactivation-activation assay	53
	AR antagonist (IC50=1.7 µM compared to 0.5 and 0.1 µM for flutamide and vinclozolin, respectively). The antagonistic effect was reversed by coincubation with excess ORG2058. (Concentrations used 0.1-10µM)		AR CALUX Yeast hAR transactivation assay	71
	Antiandrogenic effects (IC50 = 107 µM, compared to 4.3 µM for flutamide), producing full dose-response curve with complete inhibition of DHT activity. (Concentrations used 0.4 – 500 µM)		Yeast hAR transactivation assay	52
	Potent hAR antagonist at 10 µM (IC_{50} = 2.66 µM, HMS concentrations used 0.01-10µM)		Gene expression bioassay in PALM cells	44

	No-conclusion can be drawn 10% paraffin solution		Repeated-dose toxicity study in rats exposed dermally *in utero* or during lactation or during infancy.	27
Progesterone	No PR transactivation was observed		Calux assay	71
	HMS at 50 µM was suggested to induce Ca^{2+} signals primarily by activating the CatSper channel		*In vitro* study in human sperm cells	62
Anti-progesterone	Slightly PR antagonistic effect (IC50 3.0 µM, compared to 4.9 pM for RU486), which was reversed by coincubation with excess ORG2058 (concentrations used 1pM-10µM)		Calux assay	71
Glucocorticoid	No glucocorticoid effects. Concentrations used 0.01-100 µM		MDA-kb2 cell transactivation-activation assay	53
Thyroid-related activity		No-conclusion can be drawn 10% paraffin solution	Repeated-dose toxicity study in rats exposed dermally *in utero* or during lactation or during infancy (level 4)	27

3.4.10.2.4 Human data

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SCCS overall conclusions on endocrine disruption properties of homosalate

The overall conclusion is based on an analysis of the most appropriate risk management option (RMOA) https://echa.europa.eu/documents/10162/ccaa4d83-f34c-ebe5-1939-b9a4e4536738 which was performed by ANSES in the framework on the French National Strategy on Endocrine Disruptors in 2017 and further supported by with the assessment of ED-mediated properties performed by other bodies (see Annex I).

Level 1: non-testing methods:
QSAR gives some indications that homosalate can activate the oestrogen receptor α and act as an antagonist of androgen receptor.

Level 2: *in vitro* assays:
Anti-androgenic and oestrogenic activities are reported in *in vitro* studies (see Table 8). Furthermore, there were some contradictory interactions with the progesterone signalling pathway of unknown relevance. Doses used in *in vitro* studies are also deemed outside any biological plausibility reasoning and therefore do not consitute a convincing evidence for extrapolation to hormonal activity *in vivo.*

Level 3: *in vivo* assays with data regarding MoA:
Negative results have been reported from uterotrophic assays performed up to 1000 mg/kg bw/d. However, it is notable that this type of assay is only based on an assessment of uterus weight and such a test cannot allow a firm conclusion on all possible oestrogenic modes of action. In addition, although this assay has a good sensitivity for strong oestrogenic compounds, the sensitivity is lower for weaker oestrogenic compounds. Negative results have

also been reported in transgenic zebrafish but this study is not appropriate for drawing a firm conclusion on the mode of action of homosalate.

Level 4: *in vivo* assays with data regarding adverse effects:
Two *in vivo* studies are available. A 14 day range-finding study (see section 3.4.4) is considered inadequate to assess ED-related properties as it investigated limited parameters. The other study (OECD TG 422, described in sections 3.4.4 and 3.4.5) is considered to be of limited value to conclude on ED-related properties because the animals were under constant light conditions, which might have affected the reliability of the reported effects. In addition, possible effects on fertility (increased infertility, sperm changes), development (higher post-implantation) and thyroid (hypertrophy of the follicular epithelium) were identified.
A further study (Ref. 27, described in section 3.4.10.2.3), considered by the SCCS of insufficient robustness, has reported some findings that could be linked to an endocrine mediated mode of action: e.g. some variations in hormone levels when rats were exposed during different sensitive periods. However, no clear trend could be identified in the hormonal fluctuations and there was no effect reported from histopathological examination of the reproductive organs.

Overall, the RMOA has indicated that the available data from level 1 and 2 information and the inadequate *in vivo* studies provides indications for an ED potential of homosalate, whereas the available level 3 studies are of limited relevance and do not indicate the potential for ED concern. Despite the poor quality of the *in vivo* studies, findings that could be linked to an endocrine disruption were identified, in particular fluctuations of hormones, sperm changes and effects on the thyroid. These effects raised some concerns regarding ED properties of homosalate.

The SCCS agrees with the conclusions drawn in the French RMOA document that from the currently available dataset, no conclusion can be drawn on the endocrine potential of homosalate. The available data on homosalate provide some indications for potential endocrine effects. However, the current level of evidence is not sufficient to conclusively regard it as an endocrine disrupting substance, or to derive a toxicological point of departure based on endocrine disrupting properties for use in human health risk assessment. It was brought up during the commenting period that homosalate is metabolized to e.g. salicylic acid which is considered as endocrine disruptor by the Danish EPA. However, salicylic acid is not the only metabolite formed from homosalate (see chapter on toxicokinetics above). Furthermore, in its Opinion on Salicylic acid (SCCS/1601/18), the SCCS derived an NOAEL of 75 mg/kg bw/d as point of departure for risk assessment, which is higher than the LOAEL currently used by the SCCS for the safety assessment of homosalate (60 mg/kg bw/d).

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MoS)

The calculation of the systemic exposure dose (SED) was carried out as laid down in the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 11th revision, adopted during the plenary meeting of 30-31 March 2021 (SCCS/1628/21).

CALCULATION OF THE SYSTEMIC EXPOSURE DOSE

As point of departure for risk assessment, a LOAEL of 60 mg/kg bw/d, based on a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (OECD Guideline 422) was used (see section 3.4.4.1). Since the point of departure is based on a LOAEL, an assessment factor of 3 was added to account for LOAEL-NOAEL extrapolation. Furthermore, due to lack of information or oral bioavailability, 50% of the administered dose was used as the default oral absorption value, resulting in an adjusted NOAEL of 10 mg/kg bw/day.

For dermal absorption of test substance, the value of 5.3% (*mean + 1SD: 3.86±1.43)* was used [derived from dermatomed human skin, *in vitro,* Finlayson (2021)].

Considering only dermal exposure the MoS for homosalate when used daily in sunscreen lotion is 6.3.

For use as UV-filter in sunscreens			
Amount of sunscreen applied	A (g/d)	=	18
Concentration in the finished product	C (%)	=	10%
Dermal Absorption	Dap (%)	=	5.3%
Typical bodyweight of human		=	60kg
Systemic exposure dose (SED)	A x 1000 mg/kg x C/100 x Dap/100/60 =		
		=	1.59 mg/kg bw
Lowest observed adverse effect level (OECD TG 422 study, oral, rat)	LOAEL	=	60 mg/kg bw/d
NOAEL/LOAEL adjustment			20 mg/kg bw/d
Bioavailability 50%		=	10 mg/kg bw/d
Margin of Safety	**adjusted NOAEL/SED**	**=**	**6.3**

In order to derive at a MoS of 100, the SED should be maximally 0.1 mg/kg bw meaning that

A x 1000 mg/kg x C/100 x Dap/100/60 = 0.1.

With the above values, C is 0.63, meaning the safe level is maximally 0.63% homosalate in final product (sunscreen).

Although homosalate is mainly reported to be used as a UV filter in sunscreen product, use in other cosmetic products has been reported (Manova, 2012; Danish Environmental Protection Agency, 2015) to occur occasionally. Therefore, the SCCS has also calculated safety of homosalate from combined use in sunscreen products, hand cream and face cream. According to the SCCS NoG 11th revision (SCCS/1628/21), the daily applied amount of face cream is 1.54 g/d and the daily applied amount of hand cream is 2.16 g/d. Considering concomitant use of sunscreen product, face cream and hand cream containing homosalate, the total applied amount (A) of cosmetic product is 21.7 g/d.

With the above values, C is 0.5, meaning the safe level is maximally **0.5%** homosalate when used in sunscreen, hand cream and face cream.

MoS calculations for separate product types and aggregated exposures are shown in Table 9.

Table 9: MoS calculations for the different product types and aggregated exposures

Products	**Conc (%)**	**Surface**	**Systemic Exposure Dose (SED) (mg/kg bw/day)**				**Adjusted NOAEL (mg/kg bw/day)**	**MoS**
			Dermal	**Inhalation**	**Oral**	**Total**		
Lipstick	10	Lips	0	0	0.045	0.045	10	**222**
Face cream	10	Face	0.136	0	0	0.136	10	**74**
Hand cream	10	Hand	0.191	0	0	0.191	10	**52**

Sunscreen cream/lotion	10	Whole body	1.590	0	0	1.590	10	**6**
Sunscreen propellant spray	10	Whole body	1.590	0.176	0	1.766	10	**6**
Sunscreen pump spray	10	Whole body	1.590	0.002	0	1.592	10	**6**
Aggregated exposure (face cream, hand cream, sunscreen cream/lotion)			1.917	0	0	1.917	10	**5**
Aggregated exposure (face cream, hand cream, sunscreen as propellant spray)			1.917	0.176	0	2.093	10	**5**
Aggregated exposure (face cream, hand cream, sunscreen as pump spray)			1.917	0.002	0	1.919	10	**5**
Aggregated exposure (face cream, hand cream)			0.327	0	0	0.327	10	**31**
Aggregated exposure (lipstick, face cream, hand cream, sunscreen cream/lotion)			1.917	0	0.045	1.962	10	**5**
Aggregated exposure (lipstick, face cream, hand cream, sunscreen as propellant spray)			1.917	0.176	0.045	2.138	10	**5**
Aggregated exposure (lipstick, face cream, hand cream, sunscreen as pump spray)			1.917	0.002	0.045	1.964	10	**5**
Aggregated exposure (lipstick, face cream, hand cream)			0.327	0	0.045	0.372	10	**27**

3.6 DISCUSSION

Physicochemical properties

The stability of the test substance in the marketed product (and in the test solutions) was not reported.

A full report of the chemical characterization of homosalate in terms of purity, identity and impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown. Identity and concentration of any impurities that may be present must also be stated.

Toxicokinetics

Several *in vitro* dermal penetration studies using rat and human skin have been performed. For MoS calculation, the SCCS selected a new skin penetration study using human skin from which a dermal absorption of 5.3% (mean + 1SD: 3.86±1.43) was derived. Systemic bioavailability of homosalate after dermal application was confirmed by the detection of homosalate in plasma of volunteers after topical application of sunscreen products containing homosalate but also by the detection of homosalate human milk samples. Maximum plasma concentrations of homosalate after topical application varied between 13.9 and 23.1 ng/ml and terminal half-lives varied between 46.9 and 78.4 h in an explorative study. *In vitro*, homosalate was hydrolysed into salicylic acid and 3,3,5-trimethylcyclohexanol. In addition, conjugation and hydroxylation of intact homosalate was observed.

Exposure
The systemic exposure dose for homosalate used as a UV filter in cosmetic products is calculated using a dermal absorption value of 5.3% derived from an *in vitro* dermal penetration study using viable human skin and a standard sunscreen formulation containing 10% homosalate.

Toxicological Evaluation

General toxicity

Irritation and corrosivity
Homosalate is not considered as a skin irritant. Although the SCCS notes that there are self-classifications for Eye irrit. 2, the limited data available do not point to eye irritation of homosalate when used in concentrations up to 10% in sunscreen formulations.

Skin sensitisation
The SCCS agrees with the French RMOA (ANSES 2018) that overall, homosalate does not present any concern for skin sensitisation considering the above data.

Acute toxicity
Homosalate is of low acute oral and dermal toxicity.

Repeated dose toxicity
The full study report of an OECD TG 422 study (1996 version) was made available to the SCCS during the commenting period.
Based on this study, the authors derived a NOAEL of 300 mg/kg bw/day for general toxicity based on mortality in high dose females and decreased food consumption. However, it should be noted that at this dose, effects on kidneys, liver, thyroid and thymus had already occurred.
In male animals, histopathological kidney findings which occurred from the lowest dose level were stated to be attributed to hyaline droplet nephropathy but no scientific data was provided to underline this statement. It is of note that in males, higher kidney weights were also observed from the lowest dose (but without dose-dependency).
In the original study report and the histopathological reevaluation (completed in 2021), the incidences of hyaline inclusions are almost identical (severity of changes slightly different but generally similar) pointing to reliability of the histopathological findings as obtained by H&E staining.
The dose-dependent increase in intra-epithelial hyaline droplets was, however, not reflected by immunohistochemical analysis. Moreover, staining for alpha-2-microglobulin was also observed in females (at 750 mg/kg comparable to males exposed the same way and higher when compared to male positive control), where much lower amounts of alpha-2-microglobulin would be expected.
Therefore, the results from immunohistochemical staining are not conclusive to show a clear picture. Based on the outcome of this study the SCCS cannot support the conclusion that observed kidney toxicity was dependent on alpha-2-microglobulin mediated mechanism, which is not relevant to humans.
No historical control data on haematological or biochemical parameters were available.
As effects were noted from the lowest dose of 60 mg/kg bw/d, the SCCS considers this dose as LOAEL, in particular as human relevance of the kidney findings cannot be ruled out due to inconclusive results from immunohistochemical reanalysis of kidneys.
The SCCS further notes that the occurrence of a constant lighting during the conduct of the study significantly affects the reliability of this study, especially for developmental/reproductive effects.
It is also important to note that, in the context of a compliance check process under REACH, the European Chemicals Agency adopted in March 2018 a decision requesting a sub-chronic toxicity study, a pre-natal developmental toxicity study, an extended one-generation reproductive toxicity study, and the identification of degradation products.

An appeal was filed against this decision (Notice of appeal in Case No. A-009-2018) and a decision was adopted by the ECHA Board of Appeal on 18 August 2020. The Board of Appeal dismissed the appeal and decided that the information required by the Contested Decision must be provided by 25 February 2024. The Board of Appeal found that the REACH Regulation requires registrants to perform studies on vertebrate animals even if the substance is used exclusively as an ingredient in cosmetic products.
Based on these requests, new data may become available after finalisation of this SCCS Opinion and may necessitate a revision of this Opinion.

Reproductive toxicity
The full study report of the Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test was made available during the commenting period. The study protocol used was the 1996 version of OECD test guidelines 422, which covers less parameters compared to the 2015 version, which in particular addresses endocrine related mode of action.
The SCCS notes that the occurrence of a constant lighting during the conduct of the study significantly affected the reliability of this study, especially for developmental/reproductive effects. In addition, the low numbers of pregnancies per group casts doubt on the validity of data on development of offspring in this study.

Mutagenicity / genotoxicity
Homosalate was investigated in valid GLP genotoxicity tests for the three types of genotoxic endpoints: gene mutations, structural and numerical chromosome aberrations.
Homosalate did not induce gene mutations in bacteria and it did not induce gene mutations at the HPRT locus in V79 Chinese hamster cells. Homosalate did not induce chromosomal aberrations in CHO cells.
Two recent studies (Yazar et al., 2018; Yazar et al., 2019) suffer from methodological deficiencies, therefore the results were not included in the overall WoE.
Overall, the SCCS is of the opinion that homosalate can be considered to have no genotoxic potential.

Carcinogenicity
Due to limited data available, no conclusions can be drawn.

Photo-induced toxicity
No photo-genotoxic/mutagenic potential was noted in the bacterial gene mutation assays in Salmonella typhimurium strains and no photo-clastogenic potential was recorded in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation.

Endocrine activity
The available data on homosalate provide some indications for potential endocrine effects. However, the current level of evidence is not sufficient to regard it as an endocrine disrupting substance, or to derive a toxicological point of departure based on endocrine disrupting properties for use in human health risk assessment. It was brought up during the commenting period that homosalate is metabolized to e.g. salicylic acid which is considered as endocrine disruptor by the Danish EPA. However, salicylic acid is not the only metabolite formed from homosalate (see chapter on toxicokinetics above). Furthermore, in its Opinion on Salicylic acid (SCCS/1601/18), the SCCS derived an NOAEL of 75 mg/kg bw/d as point of departure for risk assessment, which is higher than the LOAEL currently used by the SCCS for the assessment of homosalate (60 mg/kg bw/d).

The SCCS assessment did not cover the safety of homosalate for the environment.

4. CONCLUSION

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

On the basis of safety assessment of homosalate, and considering the concerns related to potential endocrine disrupting properties, the SCCS has concluded that homosalate is not safe when used as a UV-filter in cosmetic products at concentrations of up to 10%.

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

In the SCCS's opinion, the use of homosalate as a UV filter in cosmetic products is safe for the consumer up to a maximum concentration of 0.5% homosalate in the final product.

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

It needs to be noted that the SCCS has regarded the currently available evidence for endocrine disrupting properties of homosalate as inconclusive, and at best equivocal. This applies to all of the available data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, when considered individually or taken together. The SCCS considers that, whilst there are indications from some studies to suggest that homosalate may have endocrine effects, the evidence is not conclusive enough at present to enable deriving a specific endocrine-related toxicological point of departure for use in safety assessment.

Exposure to homosalate from other products than those in this Opinion has not been considered.

Combined exposure to salicylic acid either formed by metabolic transformation from homosalate, other salicylates (e.g. methylsalicylate) or directly from salicylic acid itself has not been considered in this opinion.

The use of Homosalate at the lower concentrations may have a bearing on efficacy as UV-filter, however this is outside the SCCS remit to assess the efficacy of cosmetic ingredients.

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.

RMOA	Risk Management Option Analysis
MED	Minimal Erythemal Dose
PCNA	Proliferating Cell Nuclear Antigen

ANNEX I

Assessment of ED-mediated properties performed by other bodies

Homosalate is reported as an endocrine disrupting chemical (EDC) in the TEDX List of Potential Endocrine Disruptors and in BCPP Red list of chemicals of concern.

Ref.: 80, 9

In the context of the Endocrine Disruptor Screening Program (EDSP), the US-EPA screened bioactivity of various substances on oestrogen receptor based on ToxCastTM "ER model" (June 2015). The ER bioactivity of homosalate was estimated at 0.0217.
According to Danish QSAR database, homosalate is predicted to activate the oestrogen receptor α (based in positive predictions in Battery, Leadscope and SciQSAR models) and to act as an antagonist of androgen receptor (AR) (based in positive experimental results and predictions in Battery, CASE Ultra and Leadscope models).

Further assessments identified the need for further (*in vivo*) investigations to clarify the concern of an ED-potential of homosalate:
A report was prepared by the Danish Centre on Endocrine Disrupters (CeHoS) with the overall scope of providing a science based consolidated list of EDCs and suspected EDCs. Homosalate was one of the 12 substances selected among the top prioritized substances, in the first literature screening phase. It was then excluded from further assessment due to limited data (only *in vitro* data, environmental relevant literature, and two negative *in vivo* studies).

Ref.: 17