Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC OPINION

on Hexyl Salicylate

(CAS/EC No. 6259-76-3/228-408-6)

The SCCS adopted this document
at its plenary meeting on 26 October 2023

ACKNOWLEDGMENTS

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1. ABSTRACT

The SCCS concludes the following:

1. In light of the data provided and taking under consideration the CMR Cat.2 classification (to be introduced in Annex VI to Reg. 1272/2008), does the SCCS consider Hexyl Salicylate safe when used up to the maximum concentrations provided in the dossier?

   Based on the assessment of data provided and taking into consideration the concerns related to potential endocrine disrupting properties, the SCCS considers Hexyl Salicylate safe when used up to the maximum concentrations as provided in Table 1 of this Opinion.

2. Does the SCCS have any further scientific concerns with regard to the use of Hexyl Salicylate in cosmetic products?

   The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of Hexyl Salicylate for the environment.

Keywords: SCCS, scientific advice, Hexyl Salicylate, CAS/EC No. 6259-76-3/228-408-6, Regulation 1223/2009

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TABLE OF CONTENTS

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Hexyl Salicylate (CAS/EC No. 6259-76-3/228-408-6) is the INCI name of 'hexyl 2-hydroxybenzoate', an ingredient with sweet, floral, and fruity odour used in formulations of fragrances in multiple consumer goods including cosmetic, household cleaning products, detergents, and air care products.

Hexyl Salicylate is not listed in the Annexes to the Cosmetic Regulation (EC) No. 1223/2009 and its use is not otherwise restricted in cosmetic products.

The European Risk Assessment Committee (RAC) of ECHA issued in March 2022 an opinion[1] recommending a 'Toxic for Reproduction Category 2' (i.e., suspected of damaging the unborn child) and 'Skin sensitizer Category 1' classification for Hexyl Salicylate. These classifications were based on the results of an LLNA assay and on 'read across' from the structural analogue Methyl Salicylate and the metabolite Salicylic Acid, respectively.

Hexyl Salicylate is the ester of 1-hexanol and Salicylic Acid, with the latter being the main metabolite. Salicylic Acid has been subject to a safety evaluation by SCCNFP in 2002[2] and SCCS in 2018[3] and the SCCS is currently re-evaluating its safety in view of endocrine disrupting concerns. The scientific committee has concluded on the safety of Methyl Salicylate in 2021[4].

Following the RAC opinion, it is expected that the European Commission will propose a classification for Hexyl Salicylate as a 'Toxic for Reproduction Category 2' and 'Skin sensitizer Category 1' (CLP Regulation Annex VI).

In December 2022, stakeholders submitted a dossier to support the safe use of Hexyl Salicylate according to Art. 15(1) Reg. 1223/2009 with specific concentration limits for various product types (see Table 1). The Commission requests the SCCS to carry out a safety assessment on Hexyl Salicylate in view of the information provided.

Table 1: Maximum concentrations of Hexyl Salicylate in cosmetic products as reported in the dossier submission.

Product type, Body parts	**Maximum concentration**
Hydroalcoholic-based fragrances	2
All Rinse-off products	0.5
All Leave on products	0.3
Oral care (toothpaste and mouthwash)	0.001

[1] https://echa.europa.eu/documents/10162/88845f59-c1f3-1302-2701-e684a9193ef7
[2] https://ec.europa.eu/health/ph_risk/committees/sccp/documents/out170_en.pdf
[3] https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_223.pdf
[4] https://health.ec.europa.eu/publications/methyl-salicylate-methyl-2-hydroxybenzoate_en

Terms of reference

1. In light of the data provided and taking under consideration the CMR Cat.2 classification (to be introduced in Annex VI to Reg. 1272/2008), does the SCCS consider Hexyl Salicylate safe when used up to the maximum concentrations provided in the dossier?

2. Does the SCCS have any further scientific concerns with regard to the use of Hexyl Salicylate in cosmetic products?

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Hexyl Salicylate

3.1.1.2 Chemical names

IUPAC, EC name: Hexyl Salicylate

Synonyms: hexyl 2-hydroxybenzoate; salicylic acid hexyl ester; benzoic acid, 2-hydroxy-,n-hexyl ester

3.1.1.3 Trade names and abbreviations

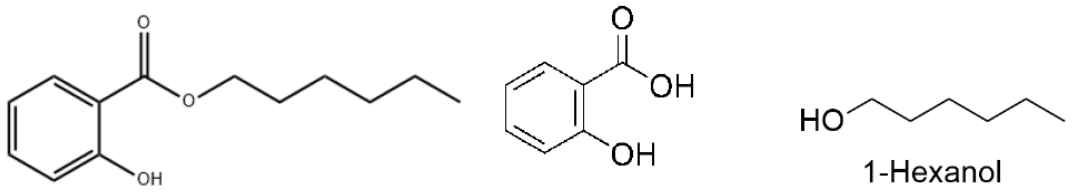
Benzoic acid, 2-hydroxy-, hexyl ester Hexyl Salicylate, Hexyl o-hydroxybenzoate, n-Hexyl Salicylate

3.1.1.4 CAS / EC number

CAS No: 6259-76-3
EC No: 228-408-6

3.1.1.5 Structural formula

The chemical structure of Hexyl Salicylate is shown in Figure 1a. Hexyl Salicylate is an ester of salicylic acid (Figure 1b) and 1-hexanol (Figure 1c).



a) b) c)

Figure 1 Chemical structure of a) hexyl salicylate (CAS 6259-76-3; EC number 228-408-6), b) salicylic acid (CAS 69-72-7; EC number 200-712-3) and c) 1-hexanol (CAS 111-27-3; EC number 203-852-3).

3.1.1.6 Empirical formula

$C_{13}H_{18}O_3$

3.1.2 Physical form

At 20°C colourless liquid.

3.1.3 Molecular weight

222.28 g/mol

3.1.4 Purity, composition and substance codes

> 99%

3.1.5 Impurities / accompanying contaminants

/

3.1.6 Solubility

2 mg/L in water at 23°C and pH 7
Registration Dossier - ECHA (europa.eu) consulted 6 September 2023

3.1.7 Partition coefficient (Log P_{ow})

5.5 at 30°C and pH 7 (OECD Test Guideline 117)

3.1.8 Additional physical and chemical specifications

Density: 1.038 at 20°C g/cm^3
Melting point: -4.15°C (equivalent to 269 ± 0.5K at 101.325 kPa (OECD Test Guideline 102)
Boiling point: 297.84°C (equivalent to 571 ± 0.5K at 100.62 kPa (OECD Test Guideline 103)
Vapour Pressure: 7.7 x 10^{-5} kPa at 23°C
Flash point: 151°C (EU Method A.9) at 101.1 kPa
pKa: 8.17±0.30 (Predicted)
https://www.chemicalbook.com/ProductChemicalPropertiesCB4430407_EN.htm
Consulted 6 September 2023

3.1.9 Homogeneity and Stability

According to the Applicant, compound is stable under recommended storage conditions.

SCCS comment
According to the Applicant, the information was taken from ECHA website (https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14766/3/1/6) and the SCCS has corrected some points accordingly.

Information on the analytical methods used for the determination of purity and impurities of the test substance should be provided in accordance with the SCCS Notes of Guidance. Relevant data should be provided.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

Hexyl Salicylate (CAS 6259-76-3; EC No. 228-408-6) can be synthesised for use in a range of manufactured goods including cosmetic products, household cleaning products, detergents and air care products (Lapczynski *et al.* 2007).

3.2.1.1 Cosmetic uses

Hexyl Salicylate is used in the formulation of fragrances in cosmetics as it has a sweet, floral and fruity odour. Hexyl Salicylate is used globally in a wide range of cosmetics (Lapczynski *et al.*, 2007; CIR 2019).

The EU Cosmetics Regulation (Annex III) does not list Hexyl Salicylate as a restricted substance in cosmetics and personal care products.

EU companies in the Hexyl Salicylate Consortium surveyed the typical use concentrations of Hexyl Salicylate in cosmetic products: the maximum use in fine fragrance products was up to 2%, with up to 0.5% in rinse off products and 0.3% in leave on products. A value of 0.001% is nominally applied for prospective use in oral care products, though no current use was reported by Consortium members. These % w/w use levels will be applied in the aggregate exposure assessment in this cosmetics safety evaluation.

3.2.1.2 Other uses

Hexyl Salicylate is also used as a fragrance ingredient in household cleaning products, detergents and air care products. A range of non-cosmetic consumer uses are included in the EU REACH dossier (https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14766/3/1/6).

3.2.2 Dermal / percutaneous absorption

3.2.2.1 *In vitro* animal skin absorption studies

No data available

3.2.2.2 *In vivo* animal skin absorption studies

No data available

3.2.2.3 *In vitro* human skin absorption studies

An OECD Test Guideline 428 study performed to GLP has been used to investigate absorption of Hexyl Salicylate *in vitro* using human skin as summarised in Table 2.

Table 2: Summary of method details from an OECD Guideline 428 *in vitro* human skin absorption study for Hexyl Salicylate

Exposure concentration/vehicle	Application site details	Observations	Reference
0.1, 20 or 100% ^{14}C-hexyl salicylate (99.8% purity) in dipropylene glycol. The corresponding amounts as applied in 6.4 µL were: 10.2 ± 0.1; 2130 ± 15; 10745 ± 57 µg/cm^2, respectively.	Split-thickness (0.2-0.4mm) previously frozen human abdominal or breast skin membrane (n=8) from 4 female donors. Hexyl salicylate applied for 8 hours duration using automated flow-through cells maintained at 32°C. The experiment was terminated at 24 hours by washing the skin with 3% soap solution, the skin was tape stripped. Receptor fluid (physiological saline with 6% PEG 20) measurements were taken.	Overall recovery of hexyl salicylate in human skin was 93.5% ± 2.0%, 97.6% ± 0.9%, and 98.5% ± 1.9% for the 0.1%, 20%, and 100% solutions, respectively.	Maas *et al* 2016 (Triskelion report)

An *in vitro* human skin absorption study for Hexyl Salicylate (CAS 6259-76-3) was conducted following OECD TG 428 guidelines for 24 hours and according to the guidelines expected by the SCCS (2021), except that the dose was in contact with the skin surface for 8 hours (as might mimic worker exposures) and not 24 hours as requested for the general population. Skin membrane integrity was assured by performing a tritiated water test. The results from the OECD TG 428 study by Maas (2016) are summarised below in Table 3.

Table 3: Skin absorption results for Hexyl Salicylate as applied to human skin *in vitro*: Group A = 100% neat liquid; Group B = 20% and Group C = 0.1% in solvent dipropylene glycol. RF = receptor fluid.

Group	A	B	C
Number of replicates	8	8	8
75 % absorbed in RF in first half of study	No	No	No
Maximal flux (µg.cm^{-2}.h^{-1})	0.84 ± 0.12	0.83 ± 0.21	0.007 ± 0.001
	Recovery (% of dose, mean ± SD)		
Amount in RF	0.15 ± 0.02	0.64 ± 0.15	1.00 ± 0.16
Amount in receptor compartment wash	0.009 ± 0.001	0.072 ± 0.017	0.037 ± 0.018
Amount in (stripped) skin	0.38 ± 0.14	2.33 ± 1.32	1.30 ± 0.62
Amount in tape strips 1+2	0.12 ± 0.09	2.62 ± 1.76	0.12 ± 0.08
Amount in tape strips 3-last	0.12 ± 0.08	2.16 ± 1.11	0.24 ± 0.15
Amount in skin wash	97.6 ± 1.8	87.9 ± 4.3	90.0 ± 3.1
Total recovery	98.5 ± 1.9	97.6 ± 0.9	93.5 ± 2.0
Absorbed dose [1]	0.53 ± 0.14	3.04 ± 1.43	2.34 ± 0.69
Potentially absorbed dose [2]	0.65 ± 0.19	5.20 ± 2.41	2.58 ± 0.77

[1] The absorbed dose is defined as the amount in the receptor fluid, the receptor compartment wash and skin membrane, excluding tape strips.
[2] The potentially absorbed dose is defined as the amount in the receptor fluid, the receptor compartment wash, the skin and stratum corneum (except for the first 2 tape strips)

Illustrative data from the tape strips from Group B in this experiment are shown in Figure 2.

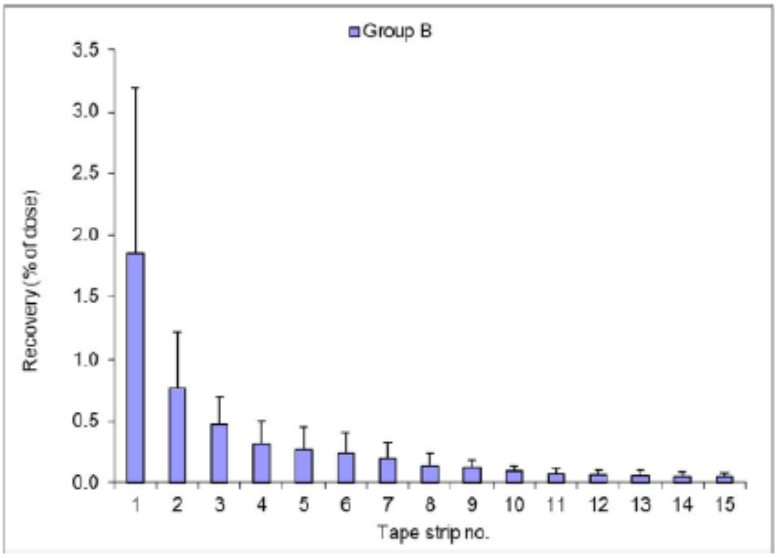


Figure 2: Distribution of [^{14}C]-Hexyl Salicylate in tape strips at 24 hours (Group B – 20% dose).

As can be seen in Table 3, the majority of the test material is rinsed from the skin surface and removed in the first few tape strips at 24 hours. Very low amounts penetrated into receptor fluid with the majority of the low-level absorbed dose remaining in the skin. The degree of total skin absorption is low for Hexyl Salicylate which is supported by its lipophilic nature.

The mean absorbed dose at 24 hours, as per SCCS Notes of Guidance (2021), which is measured as the compound-related radioactivity present in the receptor fluid + receptor compartment + epidermis + dermis, was 0.53 ± 0.14% (100% solution), 3.04 ± 1.43% (20 % solution) and 2.34 ± 0.69% (0.1% solution) of the applied dose, respectively.

To be conservative, taking the highest of these mean values for Group B, 3.04% plus 1 standard deviation of 1.43%, this leads to a total measured skin absorption value in this study of 4.47%.

Given that the dose was only on the skin for an 8-hour duration (to mimic worker exposure conditions), a correction factor of 3 has been applied to yield a value of 13.4% skin absorption to mimic the 24-hour use in a consumer safety assessment. This amount is based on measured radioactivity and it was hypothesised that this goes on to be absorbed mainly as salicylic acid following first pass skin metabolism by esterases; this was tested in the same study as described below.

3.2.2.4 *In vivo* human skin absorption

No data available.

3.2.3 Other studies on toxicokinetics

3.2.3.1 Dermal Metabolism data

From previous reviews on salicylates (CIR, 2019), it is known that n-alkyl salicylates are converted by skin esterases and systemically (liver) to salicylic acid as the main metabolite with the corresponding alcohol, in this case 1-hexanol. Various salicylates may be converted to salicylic acid at different kinetic rates and overall extent by the skin and systemically. The amount of systemic salicylic acid produced in the body is a function of both the dermal absorption of the salicylate across the *stratum corneum* and the extent of metabolic conversion of the absorbed substance by esterases. Not all salicylates may be substrates for the active site of the esterase enzymes. However, this can be tested *in vitro*.

In the same study by Maas (2016), dermal metabolism of Hexyl Salicylate was assessed using fresh human skin *in vitro*. Human breast skin was provided by female Donor 1 (n=3) and human abdomen skin by female Donor 2 (n=3). Upon arrival at the laboratory, subcutaneous fat was removed from the skin. Skin membranes were cut to a target thickness of 0.3-0.4 mm using a Dermatome (25 mm, Nouvag GmbH, Germany). The thickness of all skin membranes was measured with a digimatic micrometer (Mitutoyo Corporation, Japan). For logistic reasons, the skin discs were stored overnight, epidermal side up, on a gauze tissue slightly wetted with phosphate buffered saline (PBS) at 2-10°C until washing and start of exposure the following day. A two-compartment static diffusion cell system was used. 14C-Hexyl Salicylate was applied for 8 hours at 0.1% in dipropylene glycol. Approximately 15 µL of dose solution was applied to each 1.5 cm^2 skin sample, approximately 10 µl/cm^2. 24 hours later receptor fluid was collected as a single sample and skin samples were also stored at -18°C until processing and analysis. Radio high performance liquid chromatography (HPLC) was used to characterise and quantify Hexyl Salicylate and salicylic acid.

Analysis of the low level of radioactive substance that had penetrated into receptor fluid showed almost no parent compound (<1%). Salicylic acid was present at >94% in donor 1 and at >92% in donor 2. Analysis of the skin extracts showed some parent compound present, ranging from 5.73% to 10.70% in three replicates obtained from donor 1 and from 19.95% to 37.36% in three replicates isolated from donor 2. Salicylic acid was present at >86% in donor 1 and at >59.5% in donor 2. Hydrolysis of Hexyl Salicylate to salicylic acid was almost complete when applying a dose of 0.1% at 10 µl/cm^2 skin, therefore it will be assumed that at the low doses applied to the skin, 100% of the Hexyl Salicylate applied to human skin could be converted to salicylic acid *via* skin esterases within a period of 24 hours.

Dermal Systemic Availability - Conclusions from the Applicant:

The following generic statements regarding dermal absorption and metabolism are supported by the current body of data:

• There is good evidence that absorption of parent Hexyl Salicylate is low across human skin.

• A conservative skin absorption value of 13.4%, using a mean of (3.04% +1SD) x a correction factor of 3 to convert 8-hour worker exposure to 24-hour consumer exposure, will be taken forward from the OECD guideline 428 study (Maas 2016) for this safety evaluation. The plausibility of this skin absorption rate is supported by the good comparability with the skin absorption value for benzyl salicylate of 10.5% derived from an OECD Test Guideline 428 study, both compounds sharing similar LogP and MW.

• It is expected, also based on the evidence from methyl- and benzyl salicylate, that the majority of the substance measured by total radioactivity is in the form of salicylic acid.

• It is expected that subsequent phase 2 conjugation leads to effective clearance of salicylic acid (SCCS Opinion 2018).

SCCS comment
A value of 13.4% (Mean + 1SD corrected for 24 hours) was calculated following a recent *in vitro* study using human skin that meets the basic criteria for skin absorption in SCCS Notes of Guidance (2021). This value will be used for the calculation of the MoS.

3.2.3.2 Oral ADME/kinetic data in animals or humans for salicylates

Salicylates are known to be well absorbed across the gut (Goodman & Gilman, 2006). There are no specific quantitative *in vivo* studies available on the ADME properties and kinetics of Hexyl Salicylate *via* the oral route in animals and humans, but oral absorption studies conducted on a similar analogue methyl salicylate indicate a rapid and nearly complete absorption following ingestion. As a result, for the assessment of potential effects of oral exposures to the salicylates from their use as cosmetic ingredients, an oral bioavailability of 100% is assumed (Belsito *et al.*, 2007). This is supported by the 2018 SCCS opinion on salicylic acid, where a 100% oral absorption was previously used (SCCS, 2018).

- Oral bioavailability

Davison *et al.* (1961) performed two studies in both rats and dogs for a similar substance, methyl salicylate.

Rat – 300 mg/kg bw (body weight) methyl salicylate in 2 % methylcellulose was administered by oral gavage as a single dose, to groups of 10 male Wistar rats (200-350g). Blood samples were taken at 20 and 60 min after administration. Plasma and brain tissues were analysed for the presence of methyl salicylate and free salicylate. Methyl salicylate was completely hydrolysed within 20 min of a single oral dose. After 20 min, 217 and 8 mg/L free salicylate were found in the plasma and brain, respectively. After 60 min, these values were 278 and 42 mg/L, respectively. Methyl salicylate values were negligible.

Dogs – 300 mg/kg bw methyl salicylate was administered as a capsule in fasting male dogs weighing 12-15 kg. Blood was taken from the cephalic vein at 1 h and 4 h intervals, and the plasma was analysed. Hydrolysis was ~95% complete at both time points.

- Distribution

After absorption, salicylates and salicylic acid are distributed throughout most body tissues and most transcellular fluids, primarily by pH dependent passive processes. Salicylates are actively transported by a low-capacity, saturable system out of the cerebrospinal fluid (CSF) across the choroid plexus. Salicylates readily crosses the placental barrier. The plasma half-life for methyl salicylate is 2 to 3 h in low doses but may be as long as 15 to 30 h at high therapeutic doses or when there is intoxication (Gilman *et al.*, 1990) *e.g.* at high doses in animal toxicity studies. The *in vitro* protein binding of methyl salicylate is assumed to be around 86% based on a study in rats where the unbound fraction was found to be 14% (Dancik *et al.*, 2011). The plasma protein binding may be a major and important factor determining the extent of placental transfer and possibly also the teratogenicity of highly protein-bound drugs (Nau, 1986). The protein binding of acetyl salicylic acid is species dependent. In rats the protein binding is lower (30%) as compared to monkeys (~70%) and hence the placental transfer of free unbound salicylic acid is more from mother to fetus in rats as compared to monkeys.

- Excretion

Salicylates are excreted in the urine as free salicylic acid (10%), salicyluric acid (75%), salicylic phenolic (10%) and acyl (5%) glucuronides, and gentisic acid (less than 1%). However, excretion of free salicylate is extremely variable and depends upon both the dose and the urinary pH (Gilman, 1990).

3.2.3.3 Oral bioavailability of 1-hexanol metabolite

Upon hydrolysis of Hexyl Salicylate, salicylic acid and 1-hexanol are formed *in situ*. It is expected that the 1-hexanol will be absorbed rapidly across the gut and further metabolised in the liver by aldehyde and alcohol dehydrogenase enzymes to the corresponding hexanal and hexanoic acid metabolites. Endogenous Phase 2 metabolism in the body *via* the fatty acid pathway and tricarboxylic acid cycle will rapidly degrade 1-hexanol to carbon dioxide and its metabolites; excretion will be complete within 24 hours.

Conclusion on Oral ADME data from the Applicant:

Hexyl Salicylate, like methyl salicylate and benzyl salicylate, is expected to be rapidly and completely absorbed and metabolised, in both gut and liver tissue by first pass metabolism, to salicylic acid and 1-hexanol following oral exposure in both rat and humans. With rapid hydrolysis in the gut and liver, systemic exposure is primarily to salicylic acid and 1-hexanol, which do not accumulate in the body, and are rapidly excreted. This means that any point of departure from an oral toxicology study on either Hexyl Salicylate, salicylic acid or 1-hexanol can be regarded as a systemic point of departure (POD_{sys}).

SCCS comment
Based on the available data indicating rapid and complete absorption, the SCCS considers that an absorption value by oral route of 100% can be used in the risk assessment.

3.2.3.4 Inhalation and absorption through the lung

There are no data on the extent of Hexyl Salicylate absorption in the lung. Based on the high log Po/w and low water solubility it is expected that Hexyl Salicylate will be poorly absorbed by the inhalation route. An assumption of 100% absorption is therefore very conservative.

3.2.4 Calculation of SED/LED

Exposure assessment is, by necessity, an iterative process that begins as simple as possible and moves to more complexity, bringing in more data as and when available to refine the assessment (Meek *et al.*, 2011). Deterministic additive methods for calculating aggregate exposure assume that everybody in the population uses all the products each day, and that all of the products contain the chemical of interest at a fixed maximum concentration, which is not a realistic scenario but is a simple place to start. This technique is the basis of the current SCCS Notes of Guidance (2021) approach to aggregate exposure assessment. However, as this approach grossly exaggerates realistic aggregate exposure, a more realistic and refined risk assessment should be used for aggregate exposures where data allow and there is a need for further refinement. With good data on habits and practices of cosmetic product use and distributions of concentration use data in products, a probabilistic approach to estimating exposure can be performed and so where data exist, further refinements of the risk assessment can be performed. A consistent approach to describing a tiered exposure assessment for cosmetics safety evaluation was proposed in Alexander-White *et al.* 2022 (Figure 3).

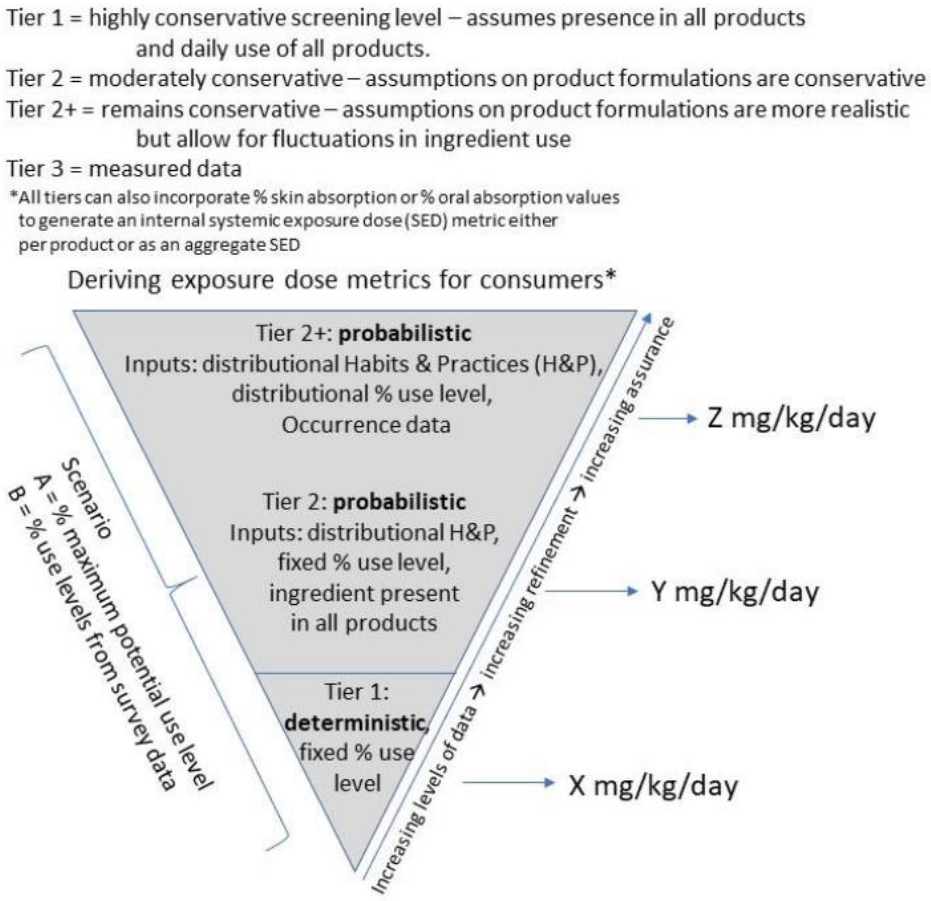


Figure 3: A tiered approach to exposure evaluation (Tier 1 deterministic; Tier 2 probabilistic) for scenarios as indicated relating to the safety assessment of cosmetic ingredients (reproduced from Alexander-White *et al.* 2022). The values generated as output from the different tiers can be used in the safety evaluation. Values can also be taken into PBK modelling approaches for further exposure refinement if useful.

For Hexyl Salicylate, separate exposure assessments are performed in this section for the dermal and oral, and inhalation routes. As can be seen, a Tier 1 deterministic approach with maximum % use levels yields a favourable outcome and in this case of assessing the aggregate exposure to Hexyl Salicylate (and the resulting systemic exposure to salicylic acid) further Tier 2 modelling was not necessary to refine exposure. Refinement may be undertaken if considering the use of Hexyl Salicylate in the context of combined exposure to salicylic acid from multiple sources, but such evaluations are the subject of a separate exercise.

3.2.4.1 Calculation of Systemic Exposure Dose (SED) - dermal route

- **In adults**

In the SCCS Notes of Guidance 11th revision (Table 5 in the SCCS document of October 2021), values are provided for the amount of product exposure an individual consumer could experience in g product per day, for 17 different cosmetic products, and as calculated in mg/kg bw/day (see Table 4).

To estimate a systemic exposure dose (SED) following dermal exposure, the standard worst case deterministic aggregate Tier 1 exposure assessment approach, as per the SCCS 11th

revision of the Notes of Guidance, has been performed and is presented in Table 5, with the addition of an 18th product type – hydroalcoholic fragrances. In this approach the exposures for individual product types are calculated, but also aggregated in a worst-case assumption. This exposure scenario assumes 100% occurrence of Hexyl Salicylate at the maximum use concentrations in cosmetic products used simultaneously by an individual in a day, which is highly unrealistic.

A conservative value of 13.4% (see section 3.2.2) is used for skin absorption of the dermally applied substance from all products, which is likely to be an overestimate.

Table 4: Calculation of 90th percentile individual and aggregate product exposure for 17 different types of cosmetic product (reproduced from Table 5 of the SCCS Notes of Guidance 11th revision, October 2021).

Type of cosmetic product exposure	***Product category***	***Exposure product ($E_{product}$)*** *(g/d)*	***$E_{product}$ normalized by body weight[1]*** *(mg/kg bw/d)*
Rinse-off skin& hair cleansing products	Shower gel	0.19	2.79
	Hand wash soap	0.20	3.33
	Shampoo	0.11	1.51
	Hair conditioner	0.04	0.67
Leave on skin& hair cleansing products	Body lotion	7.82	123.20
	Face cream	1.54	24.14
	Hand cream	2.16	32.70
	Deodorant non-spray	1.50	22.08
	Hair styling	0.40	5.74
Make-up products	Liquid foundation	0.51	7.90
	Make-up remover	0.50	8.33
	Lipstick	0.06	0.90
	Eye make-up	0.02	0.33
	Mascara	0.025	0.42
	Eyeliner	0.005	0.08
Oral care Products[2]	Toothpaste	0.14	2.16
	Mouthwash	2.16	32.54
TOTAL		**17.4**	**269**

1. The specific body weight of the persons involved in the study is used and not the default value of 60kg
2. Oral care product categories are not corrected and are presumed here to only represent dermal exposure (mucosa)

– Deterministic worst-case aggregated exposure assessment for the dermal and oral route

Values for the maximum % level of Hexyl Salicylate that are used in Europe, in each of the standard 17 product types plus hydroalcoholic fragrances (non-spray), have been provided in a recent analysis of use by the members of the 'Hexyl Salicylate Consortium', and are used to calculate the total systemic exposure to Hexyl Salicylate (in mg/kg/day) from each product (see Table 5).

Hexyl Salicylate is also used as a fragrance ingredient in hydroalcoholic fragrances and therefore, this is added into the calculation as an 18th product type in Table 5. The exposure to product (Eproduct) value normalised by weight is calculated by including the respective retention factor for each product type (SCCS, 2021).

A generic maximal (and conservative) value for skin penetration of Hexyl Salicylate of 13.4% (see section 3.2.2) has been used for all products in these calculations where dermal absorption needs to be factored in to calculate a SED. For lipstick and oral care products, a worst-case value of 100% absorption is used for passage across the oral mucosa/incidental ingestion. A SED via the dermal route was calculated for each product in mg/kg/day and also an aggregate systemic exposure dose for the 18 products (see Table 5).

As the metabolism data suggests rapid and extensive metabolism to salicylic acid, 100% metabolic conversion has been assumed, and after correction for molecular weight, an aggregate SED as a salicylic acid equivalent has also been derived (Table 5).

Table 5: Deterministic worst case systemic exposure dose (SED) calculation for the dermal and oral route using maximum % levels of Hexyl Salicylate and the derivation of salicylic acid equivalent SED. (NB. dermal exposure to spray/aerosol products are taken into account in section on inhalation below)

	Examples of Product	Maximum use (w/w%) in the finished product	$E_{product}$ normalised by body weight [1] (mg/kg bw/day)	Total dermal exposure (mg/kg bw/day)	% Abs	Calculated SED[2] HexSal (mg/kg bw/day)	Calculated SED Sal Acid equiv[4] (mg/kg bw/day
Hydroalcoholic-based fragrances (non-spray)		2	4.67*	0.0934	13.4	0.0125	0.0078
Rinse-off skin & hair products	Shower gel	0.5	2.79	0.0140	13.4	0.0019	0.0012
	Hair conditioner	0.5	0.67	0.0034	13.4	0.0005	0.0003
	Shampoo	0.5	1.51	0.0076	13.4	0.0010	0.0006
	Hand wash soap	0.5	3.33	0.0167	13.4	0.0022	0.0014
Leave on skin & hair products	Body lotion	0.3	123.20	0.3696	13.4	0.0495	0.0308
	Face cream	0.3	24.14	0.0724	13.4	0.0097	0.0060
	Hand cream	0.3	32.70	0.0981	13.4	0.0132	0.0082
	Deodorant (non-spray)	0.3	22.08	0.0662	13.4	0.0089	0.0055
	Hair Styling (non-spray)	0.3	5.74	0.0172	13.4	0.0023	0.0014
Face Make-up products	Liquid foundation	0.3	7.90	0.0237	13.4	0.0032	0.0020
	Lipstick, lip salve[3]	0.3	0.90	0.0027	100	0.0027	0.0017
	Make-up remover for face	0.3	8.33	0.0250	13.4	0.0034	0.0021
	Eye make-up	0.3	0.33	0.0010	13.4	0.00013	0.0001
	Mascara	0.3	0.42	0.0013	13.4	0.00017	0.0001
	Eyeliner	0.3	0.08	0.0002	13.4	0.00003	0.00002
Oral care products	Toothpaste[3]	0.001	2.16	0.00002	100	0.00002	0.00001
	Mouthwash[3]	0.001	32.54	0.00033	100	0.00033	0.00020
	Aggregate					**0.112**	**0.069**

1.According to values in Table 5 of the SCCS 2021 Notes of Guidance;
2. Total external dermal exposure x 13.4% skin absorption to calculate an SED for Hexyl Salicylate parent substance;
3. 100% oral/mucosal absorption is applied here as a worst case assumption; retention factors have already been

factored into the Eproduct calculation in Table 5 of the SCCS 2021 Notes of Guidance, and it is assumed all of the retained Hexyl Salicylate can enter the systemic circulation via dermal and oral routes.
4. 1 mole of Hexyl Salicylate (MW = 222 g/mole) is assumed to be 100% metabolised to 1 mole of salicylic acid (MW = 138 g/mole), therefore a conversion factor can be applied to account for this, using % metabolised and using relative molecular weight conversion this leads to the calculation of salicylic acid equivalents in mg/kg/day. (SEDParent x 100% x 138(MW metabolite)/222(MW parent) = SED Metabolite equivalent).
* Data from Ficheux & Roudot, 2017.

3.2.4.2. Calculation of Systemic Exposure Dose (SED) – inhalation route

Based on the vapour pressure of Hexyl Salicylate, inhalation exposure through excessive volatilisation is not expected. Therefore, the systemic exposure through inhalation will be performed for the sprayed fraction of finished products as below.

$$SED_{inh} = (IA1 + IA2) \times G \times RF \times DA/BW$$

Where:

SED_{inh} = Systemic Exposure Dose from the inhalation route (mg/kg/day)

IA1 = the potential amount inhaled during the first 2 min (in mg)

$$IA1\ (EA/V1*BR*t1)$$

IA2 = the potential amount inhaled during the subsequent 10-20 min (in mg)

$$IA2\ (EA/V2*BR*t2)$$

EA = potential amount to be inhaled

$$EA = (A*C*P*AF)/100$$

A = Amount of product by application (mg/application) (user defined or default SCCS, 2021)*
C = Percentage concentration of ingredient in product (%) (user defined)*
P = Proportion of non-propellant in formulation (no units) (user defined)* or default 60% (propellant, 100% pump spray) (Bremmer/RIVM, 2006)
AF = airborne fraction (no units); 1 (propellant spray); 0.2 (pump spray) (Bremmer/RIVM 2006)
V1 = First step: near-field, $1m^3$ (SCCS, 2021)
V2 = Second step: far-field, $10m^3$ (SCCS, 2021)
BR = breathing rate, 13 L/min (SCCS, 2021; US EPA, 2011)**
t1 = 2 minutes in near field (SCCS, 2021; Rothe *et al.,* 2011)
t2 = 10-20 minutes in far field (SCCS, 2021; Rothe *et al*., 2011)
G = default factor substance lung retention 0.75 (25% is exhaled) (Rothe *et al.*, 2011; SCCS, 2021)
RF = respirable fraction: propellant & pump spray specific (user defined experimental value*)
DA = Daily frequency of application (user defined* or SCCS, 2021)
BW = body weight = adult 60 kg (SCCS, 2021)
*Product-dependent parameter value
** highest median among several adult age categories

A systemic exposure dose from the potential for inhalation (SED_{inh}) from spray products can be calculated by assuming instant release of the ingredient in a defined box (1-Box model) or by applying a 2-Box model based on principles in Rothe *et al.* (2011). In Section 3-3.5.4.1 and Appendix 11 of the 11th Notes of Guidance (2021), a deterministic approach for 2-box modelling was presented for propellant and pump spray products according to the generic equations and associated parameters in the box above.

Calculation of SED_{inh} for Hexyl Salicylate in four main spray products (hydroalcoholic fragrance spray, deodorant/antiperspirant spray, hair spray and body lotion spray) are presented in Table 6, 7, 8 and 9, respectively.

Table 6: Deterministic systemic exposure dose after inhalation exposure (SED_{inh}) to 2% Hexyl Salicylate **in a hydroalcoholic fragrance spray formulation**

Description	Parameter	Pump spray	Unit
Amount by application*	A	280*	mg/application
Fraction of hexyl salicylate in non-propellant	C	2	(% w/w)
Proportion of non-propellant in formulation	P	1	-
Airborne fraction	AF	0.2#	-
Potential amount to be inhaled	EA = (A*C*P*AF)/100	1.12	mg
First step: near-field, $1m^3$	V_1	1000	L
Breathing rate	BR	13	L/min
2 min in near field	t_1	2	min
Potential amount inhaled during t_1	**IA_1** = (EA/V_1*BR*t_1)	**0.029**	mg
Second step: far-field $10m^3$	V_2	10000	L
Breathing rate	BR	13	L/min
20 min in far-field	t_2	20	min
Potential amount inhaled during t_2	**IA_2** (EA/V_2*BR*t_2)	**0.029**	mg
Substance availability fraction	G	0.75	-
Respirable fraction	RF	0.01**	-
Frequency of application$	F	1	d^{-1}
Default body weight	BW	60	kg
SED_{inh}	(IA_1+IA_2)*G*RF*F/BW	**0.007**	**µg/kg/day**

*Based on the daily amount used reported by Ficheux & Roudot (2017) and corrected by the frequency of use. #Bremmer/RIVM 2006. $Table 4 of the 11th SCCS NoG (2021).
** Delmaar and Bremmer, 2009 (1% for hydroalcoholic fragrances)

SCCS comment

The amount of 280 mg/application refers to the arithmetic mean for the use amounts. Therefore, the SCCS has recalculated the presented exposure with the P95 of 618 mg/application This results in an exposure of **0.015 µg/kg/d** to hydroalcoholic fragrance spray.

Table 7: Deterministic systemic exposure dose after inhalation exposure (SEDinh) to 0.3% Hexyl Salicylate **in a deodorant/antiperspirant spray formulation**

Description	Parameter	Propellant spray	Unit
Amount by application*	A	3050*	mg/application
Fraction of hexyl salicylate in non-propellant	C	0.3	(% w/w)
Proportion of non-propellant in formulation	P	0.6#	-
Airborne fraction	AF	0.886**	-
Potential amount to be inhaled	EA(A*C*P*AF)/100	4.86	mg
First step: near-field, $1m^3$	V_1	1000	L
Breathing rate	BR	13	L/min
2 min in near field	t_1	2	min
Potential amount inhaled during t_1	**IA_1** (EA/V_1*BR*t_1)	**0.126**	mg
Second step: far-field $10m^3$	V_2	10000	L
Breathing rate	BR	13	L/min
20 min in far-field	t_2	20	min
Potential amount inhaled during t_2	**IA_2** (EA/V_2*BR*t_2)	**0.126**	mg
Substance availability fraction	G	0.75	-
Respirable fraction	RF	0.2 [α]	-
Frequency of application[$]	F	2	d^{-1}
Default body weight	BW	60	kg
SED_{inh}	$(IA_1+IA_2)*G*RF*F/BW$	**0.001**	**mg/kg/day**

*Based on daily amount used reported by Hall *et al.* (2007) and corrected by the frequency of use. #Bremmer/RIVM 2006.
**From Table 2 in Steiling *et al.* 2012 based on 11.4% deposited. Table 4 of the 11th SCCS NoG (2021). 20% for aerosolised deodorants (Delmaar & Bremmer, 2009).

SCCS comment

To be consistent with the other Tables, it would be better to express the SED_{inh} in µg/kg/day.

The value of 0.886 for the airborne fraction is based on an experiment (Steiling *et al.*, 2014) that derives a worst case of 11.4% product available for dermal exposure. This is therefore not a worst case for inhalation exposure, and in the absence of other data, 100% of the applied amount should be assumed to be available for inhalation. With this consideration, the resulting exposure is 6.89 instead of 1.13 µg/kg/d.

Table 8: Deterministic systemic exposure dose after inhalation exposure (SEDinh) to 0.3% Hexyl Salicylate **in hair spray formulations**

Description	Parameter	Propellant spray	Pump spray	Unit
Amount by application	A	5965*	3158**	mg/application
Fraction of hexyl salicylate in non-propellant	C	0.3	0.3	(% 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)/100	10.7	1.89	mg
First step: near-field, $1m^3$	V_1	1000	1000	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)	**0.278**	**0.049**	mg
Second step: far-field $10m^3$	V_2	10000	10000	L
Breathing rate	BR	13	13	L/min
20 min in far-field	t_2	20	20	min
Potential amount inhaled during t_2	**IA_2** (EA/V_2*BR*t_2)	**0.278**	**0.049**	mg
Substance availability fraction	G	0.75	0.75	-
Respirable fraction#	RF	0.2	0.01	-
Frequency of application$	F	1.14	1.14	day^{-1}
Default body weight	BW	60	60	kg
SED_{Inh}	$(IA_1+IA_2)*G*RF*F/BW$	**1.58**	**0.014**	**µg/kg/day**

* Based on daily amount used reported by Steiling *et al.* (2014) and corrected by the frequency of use;
**Loretz *et al.* 2006. #Bremmer/RIVM 2006. $Table 4 of the 11th SCCS NoG (2021). 1% for pump hairspray and 10% for aerosol hairspray (Delmaar & Bremmer, 2009).

SCCS comment

The use amount derived from Steiling *et al.*, 2014 goes back to Bremmer *et al.,* 2006, and is a P75. Therefore, SCCS has used the P95 of Loretz *et al.,* 2006 for daily use combined with a frequency of 1. This results in an exposure value of **2.31 µg/kg/d for propellant spray**. The amount for pump spray is a P50 derived from Loretz *et al.*, 2006. The SCCS has therefore used the P95 from Loretz *et al.*, 2006 for daily use in combination with a frequency of 1. This results in an exposure value **of 0.07 µg/kg/d for pump spray.**

Table 9: Systemic exposure dose (SED) after inhalation exposure to 0.3% Hexyl Salicylate in **a body lotion spray formulation**

Description	Parameter	Propellant spray	Pump spray	Unit
Amount by application	A	5720*	3430*	mg/application
Fraction of hexyl salicylate in non-propellant	C	0.3	0.3	(% 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)/100	10.3	2.1	mg
First step: near-field, $1m^3$	V_1	1000	1000	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)	**0.268**	**0.055**	mg
Second step: far-field $10m^3$	V_2	10000	10000	L
Breathing rate	BR	13	13	L/min
20 min in far-field	t_2	20	20	min
Potential amount inhaled during t_2	**IA_2** (EA/V_2*BR*t_2)	**0.268**	**0.055**	mg
Substance availability fraction	G	0.75	0.75	-
Respirable fraction[α]	RF	0.2	0.01	-
Frequency of application[$]	F	2.28	2.28	d^{-1}
Default body weight	BW	60	60	kg
SED_{inh}	$(IA_1+IA_2)*G*RF*F/BW$	**3.06**	**0.03**	**µg/kg/day**

*Based on daily amount of SCCS (Notes of Guidance 11th, 2021) and corrected by the frequency of use. Propellant spray adjusted to yield the same 'on body' amount of 3430 mg/application.
#Bremmer/RIVM 2006. αAssumed similar to sunscreen lotion products (SCCS 2021). Table 4 of the 11th SCCS NoG (SCCS/1628/2021).

SCCS comment

The SCCS agrees with the Applicant's calculation of systemic exposure dose from the use of Hexyl Salicylate in sprayable body lotion.

Overall Exposure Assessment Conclusion from the Applicant:

A deterministic worst-case systemic exposure dose (SED) estimate from aggregated dermal exposure modelling of Hexyl Salicylate in cosmetic products is 0.112 mg/kg/day (see table 5). Assuming this is converted 100% to salicylic acid (SA), 1 mole of Hexyl Salicylate (MW = 222 g/mole) is assumed to be metabolised to 1 mole of salicylic acid (MW = 138 g/mole), therefore a conversion factor can be applied to account for this using relative molecular weight conversion, leading to the calculation of salicylic acid equivalents in mg/kg/day : SED Hexyl Salicylate x 100% metabolism x 138 (MW salicylic acid)/222(MW Hexyl Salicylate) = SED salicylic acid equivalent). The SA equivalent systemic exposure dose is 0.069 mg SA/kg/day. This value will be taken forward into the safety evaluation and compared with the POD for salicylic acid.

It is not necessary to add the dermal aggregate outcome for Total SED to the SED values from inhaled spray products, as on any one day, only one (spray or non-spray versions) of the type of products in Table 5 will be used, not both simultaneously. Typically, the non-spray version of a product leads to the higher dermal SED and secondary incidental inhalation exposure is often much lower than dermal exposure.

SCCS comment

SCCS agrees that, for most of the products, the non-spray products lead to an equivalent or much higher systemic exposure when compared to the spray products. Therefore, for all dermally applied products, the non-spray products will be considered in the MoS calculation.

For hairspray, no exposure experiments are available. Therefore, considering that in contrast to deodorant, hairspray is not applied on naked skin, the SCCS uses a fraction of 10% that is considered a worst case for availability for skin exposure. Dermal exposure to hair spray (using the same application amount and frequency as for the inhalation exposure calculation) is then 6.6 µg/kg bw/d and aggregate (inhalation + dermal) is 8.94 µg/kg bw/d.

3.2.4.3 Exposure of children <3 years to Hexyl Salicylate in cosmetics products

Taken from the Applicant:

Due to the restriction of salicylic acid-containing cosmetic products for children below the age of 3 years a specific risk assessment is presented here for the use of 0.1% use of Hexyl Salicylate in cosmetic products for infants and children at the age of 0-3 years.

The exposure assessment is derived from data in 0-3 years old European infants and children from the recent Cosmetics Europe study in European Infants & Children (0–3 years old), Creme Report, 14^{t} October 2022. Based on this, the following Margin of Safety for the aggregate exposure to all products (including toothpaste) for the age group 0-3 years is calculated:

Table 10: Calculated **SED for the aggregate exposure** to all products (including toothpaste) for the age group 0-3 years:

Cosmetic product	Estimated exposure to product (P95) (mg/kg bw/day)*	Maximum use concentration (%)	Calculated HexSal SED (mg/kg/day) 13.4 % dermal penetration	Calculated Sal Acid equiv SED (mg/kg/day), 13.4% dermal penetration
All products	1200	0.1	0.16	0.1

* Total product exposure from the Cosmetics Europe study in European Infants & Children (0–3 years old), Creme Report, 14th October 2022

SCCS comment

The SCCS assumes that the Applicant will use the same concentration (0.001%) in toothpaste both for adults and children.

The SCCS agrees that the amount of toothpaste used by children aged 6 years and under is generally 0.25 grams. Since children up to 6 years of age can be expected to ingest more toothpaste than adults, a retention factor of 0.4 for children aged 3-6 years, and 0.1 for children aged 6-10 years, is appropriate for SED calculations.

The Applicant did not provide any specific scenarios for children applying cosmetic products on their skin (dermal exposure), also taking into consideration the differences between age categories in some exposure parameters (body weight, amount of the products applied, body's surfaces, etc). As the concern for this Opinion is on ED, which may lead to some specific effects in vulnerable populations, such as children, specific exposure calculations for children (between 3 to 10 years old) are needed. Table A.7.2 in the SCCS Note of Guidance provides examples of the different cosmetic product categories that are generally used for children of different ages.

3.3 TOXICOLOGICAL EVALUATION

3.3.1. Irritation and corrosivity

3.3.1.1 Skin irritation

- Animal data

A summary of the available skin irritation data in animal models is provided in Table 10 below.

Table 10: Skin irritation studies in animals for Hexyl Salicylate

Method	Dose (%)	Species	Results	References
Preliminary intradermal irritation screen (for modified Draize test)	0.1% (ICC)	Guinea pigs	Slight but perceptible irritation ICC = 0.1% ACC = 5%	Sharp (1978)
Preliminary topical irritation screen (for modified Draize test)	5% (ACC)	Guinea pigs	No irritation was observed	Sharp (1978)
Preliminary topical irritation screen (for maximization test)	10%, 20%, 50% 25% 50%	Guinea pigs	No irritation at 10% Slight erythema at 25% and 50%	RIFM (1981)
Preliminary intradermal irritation screen (for maximization test)	0.1%, 0.25%, 0.5%, 1.0%, 2.0% in DOBS/saline	Guinea pigs	Very slight erythema observed at 0.1% Slight erythema and edema observed at 0.25-2%	RIFM (1981)
Irritation evaluated as part of a photoallergy study	1-50% in 3:1 DEP/EtOH 100%	Guinea pigs	No irritation	RIFM (2003)
Irritation evaluated as part of phototoxicity study	100%	Miniature swine	No irritation	RIFM (1975b)
Primary irritation test	10%, 15%, 50% in DEP, 100%	Rabbits	Irritation observed at 50% and 100%	RIFM (1984); RIFM (1985); RIFM (1986a); RIFM (1986b)
Irritation evaluated as part of acute toxicity study	100%	Rabbits	Irritation observed	RIFM (1975a)
Irritation evaluated as part of phototoxicity study	100%	Mice	No irritation	RIFM (1975b)

Moderate skin irritation was reported in an OECD Guideline 404 study available in the ECHA registration dossier (RIFM, 1986). In this study, female rabbits were exposed to 50% and 100% Hexyl Salicylate in DEP for 4 hours under semi-occlusive conditions. At 50% Hexyl Salicylate, the mean erythema and oedema scores were respectively 2.0 and 1.4. The observed effects were fully reversible within 7 days. For the undiluted substance, the mean scores for erythema and oedema over the 24-72 hour period were respectively 2.0 and 2.16. In this case, it was reported that one rabbit showed remaining erythema and oedema after 7 days. Nevertheless, these effects concerned only one animal and no information was available for 14 days, which is the normal observation period recommended by OECD Guideline 404. Overall, the results of the study could not trigger a classification for skin irritation according to the CLP criteria.

Pivotal animal irritation study: Mild to moderate irritation can occur, but Hexyl Salicylate is not a classifiable irritant.

- Human data

A range of skin irritation tests in humans are presented in Table 11.

Table 11: Skin irritation studies with Hexyl Salicylate in humans

Method	Dose (%)	Vehicle	Results	References
HRIPT (induction phase)	30	3:1 DEP:EtOH	3/103	RIFM (2004a)
Maximization pre-test	3	Petrolatum	No irritation	RIFM (1975b)
Primary irritation	100	N/A	No irritation	Basketter *et al* (2004)
Irritation evaluated as a part of phototoxicity study	0.3, 3, 30	3:1 DEP:EtOH	No irritation	RIFM (2004b)

In a 24-h patch test involving 56 subjects, Hexyl Salicylate was evaluated for skin irritation potential at concentrations of 0.3%, 3%, or 30% in 3:1 diethyl phthalate:ethanol. Results indicated Hexyl Salicylate was not an irritant of concern.

Skin Irritation & Corrosivity Conclusion from the Applicant:

Overall, Hexyl Salicylate shows some irritant reactions in animal models at concentrations of 25% and above and very low skin reactions in one human study at 30%, but it is not considered to be an irritant of concern at the concentrations used in cosmetic products.

3.3.1.2 Mucous membrane irritation / eye irritation

- *In vitro* data

No data

- *In vivo* data

An *in vivo* rabbit eye irritation study is reported in the ECHA REACH dossier (Schreiter U, 2000; https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14766/7/4/3), which is an OECD Guideline 405/EU Method B.5 GLP compliant study. Hexyl Salicylate was applied undiluted to the eyes of 4 female SPF albino rabbits in a volume of 0.1ml. Slight to well-defined signs of irritation were observed in the treated eyes at the 1, 24 and 48 hour examination time-points. However, all effects were fully reversible and no signs of irritation were observed after 72 hours. Under the conditions of this study, Hexyl Salicylate does not induce irritation of the eyes following its application. Based on these results, it does not need to be classified according to Regulation EC No. 1272/2008.

Eye Irritation Conclusion from the Applicant:

Undiluted Hexyl Salicylate is not an eye irritant *in vivo* and there is no risk of eye damage at the maximum concentrations of Hexyl Salicylate used in cosmetic products.

3.3.2 Skin sensitisation

From the Applicant:
Data for assessing the skin sensitisation endpoint are available for Hexyl Salicylate from new approach method (NAM) assays, animal models (LLNA and GPMT assays) and human studies.

For the purposes of cosmetics safety evaluation, in this dossier we present the body of evidence for evaluation in the context of risk assessment (see Table 12).

Table 12: A summary of skin sensitisation data available for Hexyl Salicylate: negative = non-sensitising.

Assay type	Methods & Observations	Outcome	Reference
OECD Toolbox v4.2 In silico predictions	No alerts found for hexyl salicylate (parent, autoxidation/metabolism)	Negative	Personal communications with RIFM
In chemico Direct Peptide Reactivity Assay (DPRA)	Hexyl salicylate analysed in triplicate, following a protocol equivalent to OECD TG 442C: 1.96%, 2.83%, and 0% depletion.	Negative	RIFM (2014)
In chemico Direct Peptide Reactivity Assay (DPRA)	Hexyl salicylate analysed in duplicate, following a protocol equivalent to OECD TG 442C: 3.9% and 1.1% depletion.	Negative – key event 1	Urbisch *et al* (2015)
In vitro KeratinoSens cell-based assay	Hexyl salicylate analysed in triplicate, following a protocol equivalent to OECD TG 442D. ARE-dependent luciferase gene activity >1.67-fold compared to the solvent control (cf. cell viability was <70%) A mean Imax (maximal induction factor of luciferase activity compared to solvent control) value of 2.64 was reported, while the EC 1.5 and mean IC50 were 28.67 µM and 58.29 µM, respectively	Negative – key event 2	RIFM (2015)
In vitro KeratinoSens cell-based assay	Hexyl salicylate analysed in triplicate, following a protocol equivalent to OECD TG 442D.	Negative – key event 2	Urbisch *et al*. (2015)

	Nrf2 gene expression was not induced when tested up to 4 mM		
In vitro h-CLAT cell-based assay	Hexyl salicylate stimulated CD54 2-fold with an EC200 of 52.73 µg/mL but did not result in stimulation of CD86 1.5-fold at the highest tested concentration of 177.20 µg/mL. Cell viability not reported.	Low level response – key event 3 Equivocal	Urbisch *et al*. 2015
In vitro U-SENS cell-based assay	Hexyl salicylate was found to induce CD86 expression 1.5-fold at 27 µg/mL.	Low level response - key event 3 Equivocal	Piroird *et al*. 2015
Murine local lymph node assay (LLNA)	5 treated groups of 4 animals received hexyl salicylate at 1%, 2.5%, 5%, 10%, or 25% w/v in 1:3 ethanol:diethyl phthalate. Negative control group of n=4 animals vehicle only. 25% α-hexylcinnamald-ehyde (HCA), a sensitiser, in 4:1 acetone:olive oil. SIs of 1.9, 3.6, 5.6, 10.8, and 10.8 were observed with 0.05%, 0.25%, 0.5%, 1%, and 2.5% w/v hexyl salicylate in 1:3 ethanol:diethyl phthalate, respectively. The EC3 value was calculated to be 0.18% (45 µg/cm$_2$).	Positive	Betts, 2006
Guinea-pig maximisation test (GPMT)	10 test and 8 control albino guinea pigs (Dunkin Hartley strain). Dose range finding: 0.1%, 0.25%, 0.5%, 1%, and 2% hexyl salicylate in 0.01% Dobs/saline (intradermal injections). 10%, 25%, and 50% hexyl salicylate in acetone was used for topical application. The concentration suitable for intradermal injection was found to be 1% in 0.01% Dobs/saline, while 40% and 10% hexyl salicylate in acetone were chosen for topical induction and challenge.	Negative	RIFM (Quest) 1981
Guinea-pig modified Draize test	n=10 inbred Hartley albino guinea pigs. Four intradermal injections were administered with 0.1 ml of hexyl salicylate at 2.5 times the ICC (Injection Challenge Concentration = 0.1%) at four sites overlying the two auxillary and the two inguinal lymph nodes. 14 days challenge with intradermal injection in one flank and a topical application in the other flank using 0.1 ml hexyl salicylate at 0.1% (ICC) and 5% (ACC), respectively. A second challenge was conducted 7 days later. Sensitisation reactions were observed after the second challenge.	Positive	Sharp 1978

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Guinea-pig photoallergy test	Groups of n=5 Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs. Topical induction: 0.3 ml dose of 100% hexyl salicylate applied on days 3,5,8,10,12 of induction phase. Day 22, topical challeng: 50% hexyl salicylate in 3:1 diethyl phthalate (DEP):ethanol and 100% hexyl salicylate. Test sites were observed at 1, 4 hours, and days 1,2 and 3. No sensitisation was observed.	Negative	RIFM (2003)
Human Maximisation Test	Patch sites pre-treated with 5% SLS in water. 3% (2070 µg/cm2) hexyl salicylate applied in petrolatum, under occlusion on the volar forearm n=22 subjects, 5 alternate day 48 hour periods. 10-14 days rest period. Challenge with 3% (2070 µg/cm2) hexyl salicylate.	Negative	RIFM (Epstein) 1975
Human Diagnostic patch test multicentre study	5% Hexyl Salicylate in petrolatum. No reactions were observed in 218 fragrance sensitive patients with proven contact dermatitis.	Negative	Larsen *et al* 2002
Human Repeat Insult Patch Test (HRIPT)	n = 103 males and females. 25-mm Hill Top Chamber System® was used occlusively. 0.3 mL of 30% or 35,433 µg/cm² hexyl salicylate in 3:1 diethyl phthalate:ethanol was applied to each patch on the left side of the back for 24 hours; Mon, Wed, Fri schedule. 9 patches over 3 weeks in total. 2 week rest then challenge with 0.3 mL of 30% hexyl salicylate. No reactions were observed.	Negative A No Expected Sensitisation Induction Level (NESIL) was derived as 35,400 µg/cm²	RIFM (Harrison) 2004

***In silico*, mechanistic and *in vitro* skin sensitisation (new approach method, NAM) data**

From the chemical structure of Hexyl Salicylate (see Figure 1a), it does not contain any electrophilic protein reactive groups and mechanistically, from its parent structure, as predicted in the OECD Toolbox v4.2, it is not expected to be a skin sensitiser.

As per the skin sensitisation adverse outcome pathway (OECD, 2014), the *in vitro* direct peptide reactivity assay (DPRA), Keratinosens and the h-CLAT/U-Sens tests represent key events 1, 2 and 3, respectively. The results were evaluated following the OECD Guideline No. 497: Defined Approaches on Skin Sensitisation (OECD, 2021). Based on the two out of three 'Defined Approach', Hexyl Salicylate is predicted *in vitro* to be a non-sensitiser (see Table 12).

Animal skin sensitisation study data

Local Lymph Node Assay: An OECD Guideline 429 murine (n=4 female CBA mice) LLNA was performed on Hexyl Salicylate (using the vehicle 1:3 ethanol: diethylphthalate) to GLP (Betts, 2006). A very low EC3 (0.18%; EC3 = effective concentration that induces a 3-fold increase in local lymph node proliferative activity) was reported, indicating Hexyl Salicylate to be a classifiable sensitiser in this assay. This is a surprising result for Hexyl Salicylate that requires mechanistic interrogation. It is unlikely that an impurity has caused this strong reaction, as Hexyl Salicylate had an analytical purity of 98.5%. The detailed results of this

LLNA show that the increase in stimulation index is not always dose-dependent which may indicate that an additional mechanism may be involved. It is known (Maas 2016) that a metabolite (1-hexanol) can be formed *in situ* in the skin *via* the action of esterases, and the alcohol could go on to be oxidised further to 1-hexanal quite rapidly by skin alcohol dehydrogenase enzymes. If the test substance was applied to the mouse ear and 1-hexanal was formed but not effectively cleared, 1-hexanal could be the sensitising metabolite in this assay. Another explanation proposed was that irritation, being a confounding factor in the LLNA, may have led to false positive responses (Kolle *et al.*, 2019). However, overall, there is no apparent chemical or biological reason for the positive response.

Guinea-pig Maximisation Test: A Magnusson-Kligman Guinea-Pig Maximisation Test (GPMT) according to OECD Guideline 406 was performed using a group of 10 albino Dunkin Hartley guinea pigs weighing 440-554g. Induction consisted of intradermal injection followed one week later by a 48h occluded patch. The six intradermal injections were made to a 2.4 cm clipped, shaved area in the dorsal shoulder region. There were two 0.1 ml injections of 1% Hexyl Salicylate in 0.01% DOBS/saline, two 0.1 ml injections of 1% Hexyl Salicylate in 50% Complete Freund's Adjuvant, and two 0.1 ml injections of 50% Complete Freund's Adjuvant. Seven days later, the site was clipped and shaved, and induction was supplemented topically with a 48h occluded patch with 40% Hexyl Salicylate in acetone over the shoulder injection sites. Thirteen to fourteen days after application of the shoulder patch, the guinea pigs were challenged on the clipped and shaved flank using an 8 mm diameter filter paper patch saturated with 10% Hexyl Salicylate in acetone which was applied for 24 h under occlusion. Reactions were assessed at 24 and 48 h after patch removal. Three additional challenge applications with 10% Hexyl Salicylate in acetone were made at weekly intervals on the contralateral flanks. No sensitisation reactions were observed under the conditions of this study (RIFM, 1981).

Guinea-pig modified Draize test: Hexyl Salicylate was tested in guinea pigs using a modified Draize sensitisation study procedure in n=10 inbred Hartley albino guinea pigs initially weighing approximately 350 g each. Four intradermal injections were administered with 0.1 ml of Hexyl Salicylate at 2.5 times the ICC (Injection Challenge Concentration = 0.1%) at four sites overlying the two axillary and the two inguinal lymph nodes. Fourteen days later the animals were challenged with an intradermal injection in one flank and a topical application in the other flank using 0.1 ml Hexyl Salicylate at 0.1% (ICC) and 5% (ACC), respectively. A second challenge was conducted 7 days later. Sensitisation reactions were observed after the second challenge (Sharp, 1978).

Sensitisation was also assessed during a photoallergy test using groups of five Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs. Topical induction was performed using a 0.3 ml dose of 100% Hexyl Salicylate. Doses were applied on days 3, 5, 8, 10 and 12 of the induction phase. On day 22, topical challenge was performed with 50% Hexyl Salicylate in 3:1 diethyl phthalate (DEP):ethanol and 100% Hexyl Salicylate. Test sites were observed at 1 and 4 hours, and days 1, 2 and 3. No sensitisation was observed (RIFM, 2003).

Human data

Diagnostic patch test studies: In a multicenter study, 218 fragrance sensitive patients with proven contact dermatitis were patch tested with various fragrance materials according to internationally accepted criteria. No reactions were observed with 5% Hexyl Salicylate in petrolatum (Larsen *et al.*, 2002).

Human Maximisation Test (HMT): A human maximisation test according to the method of Magnusson and Kligman (1969) was carried out with 3% Hexyl Salicylate in petrolatum on 22 adult volunteers. Application was under occlusion to the same site on the volar forearms or backs for five alternate-day 48-h periods. Patch test sites were pre-treated for 24 h with 5% aqueous sodium lauryl sulphate under occlusion. Following a 10-day rest period, a

challenge patch was applied to a fresh site for 48 h under occlusion. The challenge sites were pre-treated for 30 min with 2% aqueous SLS under occlusion on the left side of the back whereas Hexyl Salicylate was applied without SLS on the right side. Reactions to challenge were read at patch removal and 24 h after patch removal. No reactions were produced (RIFM (Epstein), 1975).
Human Repeat Insult Patch Test (HRIPT): A repeated insult patch test was conducted in 103 subjects (29 males and 74 females) to provide confirmatory evidence that Hexyl Salicylate is not a skin sensitiser in humans. During the induction phase a 0.3 ml aliquot of 30% Hexyl Salicylate in 3:1 DEP:EtOH was applied to Webril/adhesive patches (25 mm Hilltop_ Chamber System) on the left side of the back of each subject. This represented a dose of 35,433 µg/cm2. Patches remained in place and were kept dry for approximately 24 h and then removed. A series of nine induction applications were completed over a period of three weeks. A rest period of approximately 2 weeks followed the last induction. At the challenge phase, patches were applied as in the induction phase and kept in place for 24 h, after which time they were removed and the challenge sites were scored. The test sites were also scored at 48, 72 and 96 h post-patching. No sensitisation reactions were observed (RIFM (Harrison), 2004). **The HRIPT NOEL was therefore 35,433 µg/cm^2**.

Hexyl Salicylate has been classified **as a Category 4 substance** (infrequent cause of contact allergy in relation to level of exposure) with regard to its human skin sensitisation potential (Basketter *et al.*, 2014). This classification by authors of the study is based on an analysis of human data adapted from a number of published references. Substances in Category 4 are rarely important clinical allergens, because they require considerable/prolonged exposure to higher dose levels to produce sensitisation, which even then is unlikely to exceed 0.01% of the exposed population.

Conclusion from the Applicant:

The skin sensitisation potential of Hexyl Salicylate has been investigated extensively. All studies in humans, which carry the greatest weight, have been consistently negative indicating that Hexyl Salicylate is a rare sensitiser in humans, if any. NAM *in silico* and *in vitro* data also support this conclusion. Two rodent assays show anomalous results for Hexyl Salicylate, indicating that rodent ADME or mechanisms of action may be different to humans.

<u>Taken from the RAC opinion</u>

Conclusion:

With EC3 values =< 2% in the LLNA, Hexyl Salicylate fulfils criteria for classification Skin Sens. 1A according to the CLP guidance. Regarding human data, the HRIPT cannot be used for the purpose of classification due to its low reliability. Nevertheless, the maximisation assay and both diagnostic studies were negative and were considered reliable.

There are several possible reasons for the absence of sensitising reactions in these studies:

- The patch test for Hexyl Salicylate is not marketed. In fact, 46 fragrances are marketed by Chemotechnique for patch testing, but Hexyl Salicylate is not part of the list. Hexyl Salicylate was therefore only tested for prospecting purposes. This could explain why only 2 diagnostic studies with different concentrations of this substance have been published.
- Hexyl Salicylate is not included in the list of 26 sensitising fragrances for humans that require labelling. Therefore, it would be difficult to determine whether Hexyl Salicylate is responsible for contact dermatitis following exposure to a fragrance.
- Although this substance is widely used in perfumes, the concentrations used are low. In leave-on products for face and body, the concentrations are between 0.02 and 0.03 % and between 0.08 and 0.12 %, respectively. The highest concentrations are used in rinse-

off products, reaching 0.52 % in soaps and cleansers (Cosmetic Ingredient Review on salicylic acid and salicylates (2018)). These concentrations are below the concentration limits recommended by the International Fragrance Association (IFRA).

Therefore, the absence of sensitising reactions observed in humans could be due to primary prevention related to these concentration limits, more than the absence of sensitising properties.
Due to the significant discrepancies between positive animal data and negative human studies, sub-categorisation does not seem appropriate according to the CLP-guidance.
With the positive results of the LLNA of good quality, Category 1A would be justified. However, since data are not sufficient for sub-categorisation, RAC agrees with the DS that Hexyl Salicylate should be classified Skin Sens. 1 – H317.

SCCS comment
The SCCS agrees with the Applicant and the RAC opinion that the available human, animal and NAM data are contradictory. In a well-conducted LLNA, Hexyl Salicylate was positive at a relatively low concentration, whereas the GPMT was negative. *In silico* data showed no protein binding alerts, which was confirmed in the DPRA. In addition, the Keratinosens was negative, whereas the h-CLAT and U-SENS were positive. If for example the 2o3 DA (two out of three defined approach) was performed, according to OECD Guideline 497, Hexyl Salicylate would have been considered as a non-sensitiser. Human evidence is limited, but the data available is all negative. This was observed already in an earlier SCCS Opinion on fragrance allergens (SCCS/1459/11) and no new evidence indicating that Hexyl Salicylate is a relevant skin sensitiser in humans has emerged in literature.

Taking all the evidence together, the SCCS concludes that although Hexyl Salicylate is classified as a skin sensitiser, based on clinical evidence, the risk of skin sensitisation in humans from the use in cosmetic products can be considered negligible.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

There is one study in animals (RIFM 1975; Lapczynski *et al.,* 2007) covering the acute oral toxicity of Hexyl Salicylate as summarised in Table 13.

Table 13: Acute oral toxicity studies for Hexyl Salicylate

Reference	Species	Dosing (g/kg)	Oral LD_{50} (mg/kg)	Observed effects
RIFM 1975	Rats	Single oral dose N=10 animals per group	>5000	1/10 deaths at the top dose on Day 4. Urinary incontinence observed at 24h.

The animals in this study were observed daily for a period of 14 days for any signs of systemic toxicity.

3.3.3.2 Acute dermal toxicity

There is one study in animals (RIFM 1975; Lapczynski *et al.,* 2007) covering the acute dermal toxicity of Hexyl Salicylate as summarised in Table 14.

Table 14: Acute dermal toxicity study for Hexyl Salicylate

Reference	Species	Dosing	Dermal LD_{50} (mg/kg bw)	Observed effects
RIFM 1975	Rabbit	Single dermal dose. Neat hexyl salicylate N=10 animals per group	>5000	0/10 deaths. No clinical signs of toxicity.

The animals in this study were observed daily for a period of 14 days for any signs of systemic toxicity.

3.3.3.3 Acute inhalation toxicity

There are no acute inhalation toxicity data available in animals for Hexyl Salicylate. The use of Hexyl Salicylate in consumer products for decades has revealed no adverse effects in the lung. Hexyl Salicylate is not irritating to the skin and eye at the concentrations used in cosmetic products and therefore, is not expected to be irritating or toxic to respiratory tract and lung at the concentrations used in cosmetic products.

3.3.3.4 Acute subcutaneous toxicity

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3.3.3.5 Acute intraperitoneal toxicity

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Acute Toxicity Conclusion from the Applicant:

Hexyl Salicylate is not acutely toxic *via* any route of exposure.

3.3.4 Repeated dose toxicity

No repeat dose toxicity data are available on Hexyl Salicylate. Given that Hexyl Salicylate itself is not regarded as the main toxicant, but rather salicylic acid as the chief hydrolysis product, for the purposes of performing a cosmetics safety assessment, this section discusses the available repeat dose toxicity data and conclusions for the primary Hexyl Salicylate metabolites salicylic acid and 1-hexanol (Belsito *et al.*, 2007). All data for salicylic acid were recently reviewed by the SCCS in its recent opinion (SCCS, 2018).

SCCS comment
As the SCCS recently published a new Opinion on salicylic acid, data on this metabolite are not reported in this Opinion. Only data provided by the Applicant on 1-Hexanol are reported in this Opinion.

3.3.4.1 Repeated dose sub-acute and sub-chronic oral / dermal / inhalation toxicity

1-Hexanol

- Oral route

As summarised in the ECHA REACH dossier for 1-hexanol, a 13-week dietary study in rats using hexan-1-ol reported a No Observed Adverse Effect Level (NOAEL) of 1127 mg/kg (study reported as by Scientific Associates Inc., 1966). No adverse effects were noted at any of the dose levels administered during the study. The results of this key study are supported by the reliable (Klimisch score 2) 3-week feeding study in rats which reported a NOAEL of approximately 1000 mg/kg bw/day (Moody and Reddy, 1978, 1982). In addition, a 13-week study in dogs reported a NOAEL for 370 mg/kg bw/day for male dogs and 435 mg/kg bw/day for female dogs (study reported as by Scientific Associates, 1966). Although this study had some methodology discrepancies, it is still considered to be reliable (Klimisch score 2).

- Dermal route

No data reported on 1-hexanol

- Inhalation

There are no data for Hexyl Salicylate that were generated before March 2013. Reference to the similar ingredient methyl salicylate is provided below.

Methyl salicylate

Gage (1970) reported a study on methyl salicylate in rats (n=4; average weight 200g) where 20 x 7 h exposures were administered at 120ppm in a saturated atmosphere of 700 mg/m^3. The atmospheres were dynamic and passed through the exposure chamber. Haematological parameters were measured and the following organs were taken for microscopical examination after fixation in formol-corrosive: lungs, liver, kidneys, spleen, and adrenals; and occasionally heart, jejunum, ileum, and thymus. No toxicity was reported and all organs appeared normal at necropsy. This provides evidence for a NOEC for methyl salicylate of 700 mg/m^3.

3.3.4.3 Chronic (> 12 months) toxicity

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Conclusions from the Applicant on repeat dose toxicity studies:

As there are no pre-2013 repeat dose data on Hexyl Salicylate, evidence that can be used to perform a cosmetics safety assessment has long been provided from data on its main metabolites, salicylic acid and 1-hexanol, to which the body may be systemically exposed.

No significant concerns are presented for salicylic acid in terms of repeat dose endpoints as summarised by the SCCS conclusion below. Similarly, there are no safety concerns regarding the generation of a 1-hexanol metabolite.

SCCS comment

SCCS noticed there are no repeated dose toxicity studies available on Hexyl Salicylate and will therefore rely on its main metabolite salicylic acid for the safety assessment.

3.3.5 Reproductive toxicity

No reproductive/developmental toxicity data are available on Hexyl Salicylate. The proposal for classification is based upon read across to salicylic acid and methyl salicylate data, and the assumption that Hexyl Salicylate is metabolised to the same common metabolite, salicylic acid.

Given that Hexyl Salicylate itself is not regarded as the main toxicant, but rather salicylic acid as the chief hydrolysis product, for the purposes of performing a cosmetics safety assessment, this section also discusses the available reproductive/developmental toxicity data and conclusions for the primary metabolite salicylic acid and 1-hexanol to which the body may be principally exposed.

SCCS comment
As the SCCS recently published a new Opinion on salicylic acid, data on this metabolite are not reported in this Opinion. Only data provided by the Applicant on other analogues are reported in this Opinion.

3.3.5.1 Reproductive and Developmental – oral

As the main premise for the safety evaluation (given there are no repeat dose toxicology data for Hexyl Salicylate) is a confident analogue read across using data on the metabolite salicylic acid, it is not necessary to rely on safety data for other structural analogues in this dossier.

However, for awareness and completeness, there is evidence on other simple alkyl salicylates that can add to the confidence of the outcome from the safety evaluation performed in this dossier.

Cyclohexyl Salicylate

An OECD Guideline 415 one-generation reproduction toxicity study was performed to GLP in Wistar rats treated orally with Hexyl Salicylate (Schmidt, 1995). Dose levels were 60, 180 and 540 mg/kg bw/day in corn oil. Some general toxicity effects were seen in the F0 generation at the top dose, and a NOAEL could be determined at 180 mg/kg bw/day; this dose had no effects on reproduction. In males a NOAEL was defined as 540 mg/kg bw/day. With the F1-generation, a NOAEL of 180 mg/kg bw/day as effects on litter responses, survival, growth and behaviour were seen at the top dose.

An embryotoxicity study (including teratogenicity) was performed to GLP (Pitterman, 1996). Dose levels were 0, 40, 120 and 360 mg/kg bw/day, dosed daily in arachidis oil from day 6 to 15 of gestation. A standard dose volume of 5ml/kg bw was used. Each group was n=24 female rats. There were no effects of treatment seen in dams and there were no embryotoxic or teratogenic effects seen up to 360 mg/kg/day.

SCCS comments
Full reports of these 2 studies on cyclohexyl salicylate have not been provided to the SCCS and only limited information is available on the ECHA website.

Methyl salicylate

An SCCS Opinion was published for methyl salicylate in 2021 (SCCS, 2021). The pivotal study that can be used to derive a point of departure (POD) for reproductive and development toxicity is that from a 3-generation study by Collins *et al.* (1971), where the derived POD is 75 mg/kg bw/day. This provides further assurance that the POD derived from salicylic acid data is relevant to use in this safety evaluation, also for Hexyl Salicylate.

3.3.5.2 Reproductive and Developmental – dermal

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Conclusions from the Applicant on reproduction and developmental toxicology studies:

Salicylates do not affect fertility or reproduction. As there are no specific reproductive and developmental data on Hexyl Salicylate from before March 2013, evidence is provided for cosmetics safety assessment from data on its main metabolites, salicylic acid and 1-hexanol, to which the body may be principally exposed. Since 1-hexanol is not classified for reproductive toxicity, the focus is on the more relevant metabolite salicylic acid data. The Point of Departure for salicylic acid, as summarised by the SCCS conclusion in their 2018 Opinion, is the following:

SCCS agrees with RAC that salicylic acid is a developmental toxicant. Harmonised classification of salicylic acid was recently published in Regulation 2018/1480 and is classified as Repr. 2 (H361d Suspected of damaging the unborn child). For MoS calculation, SCCS uses the developmental NOAEL of 0.1% (75 mg/kg bw/day) derived from Tanaka *et al.* (1973a). The developmental effects observed in this study are the most sensitive effects after repeated exposure to salicylic acid. This is also in agreement with the previous SCCNFP Opinion (2002) and is also supported by Tanaka *et al.* (1973b).'

In addition, due to the evidence for high (100%) oral bioavailability in humans, the oral NOAEL of 75 mg/kg bw/day is defined as systemic NOAEL ($NOAEL_{sys}$) by SCCS for salicylic acid.

This POD for salicylic acid can act as a conservative surrogate POD for Hexyl Salicylate. On a molar basis, 1 mole of Hexyl Salicylate is converted to 1 mole of salicylic acid. An assumption is made that 100% of Hexyl Salicylate is metabolised to salicylic acid and 1-hexanol, and salicylic acid is the driver of any observed Hexyl Salicylate toxicity. The salicylic acid $NOAEL_{sys}$ = 75 mg/kg bw/day. A salicylic acid equivalent SED can be calculated and compared with this POD as per Table 16.

3.3.6 Endocrine disrupting effects

Hexyl Salicylate has not been identified by the European Commission as a suspected endocrine disruptor. However, its major metabolite is salicylic acid, which is on the Commission List of suspected endocrine disruptors. There is no convincing evidence that reproductive and developmental effects are caused by an endocrine mechanism of action: the rationale for reaching this conclusion is provided below. SCCS has recently adopted an Opinion on Salicylic acid (SCCS/1646/22, June 2023).

In December 2017, the Danish Centre on Endocrine Disrupters published a report in which it was stated that salicylic acid meets the European Commission's criteria to be identified as an endocrine disruptor (ED) with an anti-androgenic mode of action. Although the toxicology evidence suggests salicylic acid is a developmental toxicant (observations are on skeletal abnormalities), there is no definitive evidence that salicylic acid causes adverse health effects in an intact organism directly as a result of an endocrine mechanism. Nor is an anti-androgenic mode of action hypothetically linked to skeletal abnormalities. This connection between mode/mechanism of action and observed adverse effect is a basic requirement for substances falling within the Commission's ED criteria and the ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 and based upon the WHO IPCS 2002 Definition.

Level 1 and 2 evidence is available for evaluating salicylic acid within the OECD Endocrine Disruption Framework. Salicylic acid has been tested in the US EPA Endocrine Screening Program in a range of *in vitro* assays (Level 2) and there was no evidence of an endocrine activity in the assays tested.

There are no *in vivo* assays with salicylic acid that explicitly investigated a potential endocrine mode of action. However, as mentioned above, there is no evidence from the available data of an adverse effect of salicylic acid as a result of an endocrine mechanism, *e.g.*, there is no adverse effect on fertility.

The evidence given the greatest weight in the Danish review for salicylic acid in drawing their conclusions is from Level 4 data on read-across analogue aspirin (acetylsalicylic acid, ASA) and are of questionable quality with limitations in the data as discussed below. There are no level 5 studies.

Level 1 data: Existing Data and Non-Test Information

Chemical structure and physicochemical properties *e.g*. as per Table 1.

Level 2 data: *In vitro* assays providing data about selected endocrine

Mechanism(s)/pathways(s) (Mammalian and non-mammalian methods)
Salicylic acid has been tested (purity >90%) in the Endocrine Disruptor Screening Program (EDSP) within the US EPA Tox21 programme.
https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID7026368#invitrodb-bioassays-toxcast-data
There was no activity seen in 18 oestrogen receptor assays, there was no activity in 9 thyroid receptor assays, and it was not steroidogenic in 2 assays. Of the 15 androgen receptor assays, only one was registered as positive above a cut-off value but this was a marginal and inconclusive observation. There was no evidence that salicylic acid was endocrine active in these systems.
Similarly, no *in vitro* assays were positive in the EDSP for the structurally related substance acetylsalicylic acid (aspirin).

Level 3 data: *In vivo* assays providing data about selected endocrine

mechanism(s)/pathway(s)
No data

Level 4 studies (on a read-across analogue aspirin):

Study 1

In a developmental toxicity study (Gupta *et al.*, 2003), 7 pregnant rats were given ASA by oral (gavage) administration between days 6 to 17 after mating. At the highest dose (250 mg/kg bw/day) 2 fetuses from 2 litters were observed to have hypoplastic testes. There were no hypoplastic testes in the control group. There were no other findings in that study suggestive of an anti-androgenic mode of action (*e.g.* cryptorchidism or hypospadias). The testes findings were accompanied by severe maternal and fetal toxicity at the same dose, which strongly suggests an absence of a specific endocrine mode of action but rather an adverse secondary impact by general toxicity.

Study 2

In another *in vivo* rat study (Kristensen *et al.*, 2011) females were treated with ASA at doses of 150, 200 or 250 mg/kg bw/day between gestation days 13-21. The Danish report states

that reduced anogenital distance in male rats (a marker of impaired androgen signalling) was seen. However, when corrected for bodyweight, no effect on anogenital distance was apparent. This is significant because proper evaluation of changes in this parameter requires correction for bodyweight. That study also claimed to show reduced *in vivo* production of testicular testosterone, however, evaluation of the data clearly shows that there is no dose-response for this effect and it is unlikely to be related to treatment. It is interesting to note that these data are not presented in the main article but embedded in the supplementary materials. In that same study, questionnaires were given to Danish and Finnish women to report on their analgesic use during pregnancy. The authors discovered that in the Danish cohort, an association was found between cryptorchidism and use of analgesics. The association was not found in the Finnish cohort. The numbers of pregnancies evaluated were small, and several factors were not taken into account, such as the familial occurrence of undescended testes and the association of this finding with multiple gestation. In general, human data from observational epidemiological studies based on questionnaires are susceptible to recall bias since self-reporting information may be incomplete or inaccurate (as the authors mentioned themselves). The critical factor is the lack of robust exposure data which is needed to strengthen the assumed association between cryptorchidism and use of analgesics. Another confounding factor with respect to this study may be that humans are generally exposed to measurable levels of salicylic acid *via* dietary sources such as fruits and vegetables; one study even found that vegetarians not taking aspirin had urinary levels of salicylic acid higher than the non-vegetarians (Lawrence *et al.*, 2003).

Study 3

The suggested link between ASA and effects on spermatogenesis comes from a study published in 1980, in which groups of 6 young (21-24 days of age) or adult (age not specified) rats received 50 mg ASA/kg bw/day for 30 days (Didolkar *et al.*, 1980). After treatment, testes were weighed and subjected to analysis of the activity of certain enzymes and histopathology. These assessments suggested a significant decrease in testicular weight in the younger (but not in the older) animals, and reduced activity of testicular sorbitol dehydrogenase and hyaluronidase. Fewer spermatids were also observed in the treated groups. The results of this study need to be viewed with caution, since it was a low-powered study (6 animals/group) and not thoroughly documented. If these effects were real, they do not necessarily indicate an endocrine mode of action. Given the many other *in vivo* studies available on ASA and methyl salicylate as summarised in the draft SCCS opinion on SA, none shows any clear adverse effects relevant to an anti-androgenic mode of action (neither in terms of development nor fertility), so it seems unlikely that SA is anti-androgenic *in vivo*.

The overall conclusion is therefore that there is insufficient data to show that SA causes adverse effects arising from an ED mode of action. The Danish Report fails to sufficiently link the proposed mode of action (anti-androgenism) with the adverse outcome. Specifically, no molecular initiating event (MIE) is proposed, and no alternative explanations for the observed findings are discussed.

The appendices of the Danish report also discuss the ability of SA to displace thyroid hormones from plasma proteins. However, the report concludes that 'no studies investigated endpoints relevant for evaluation of adverse effects related to thyroid disruption were found'. It should however be noted that the SCCS Opinion quotes historical carcinogenicity studies on ASA performed in rats and mice, both of which were negative.

Conclusion from the Applicant:

On the basis of evidence available to date, there is no definitive data to show that salicylic acid (and hence Hexyl Salicylate) causes adverse effects in an intact organism arising from an endocrine mode of action.

SCCS comment
For the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, and based upon the WHO IPCS 2002 Definition, a connection between mode/mechanism of action and observed adverse effect is a basic requirement for substances falling within the Commission's ED criteria and the ECHA/EFSA Guidance. There is no evidence from the available *in vivo* data of an adverse effect of Hexyl Salicylate and salicylic acid resulting from an endocrine mechanism, *e.g.,* there is no adverse effect on fertility. The available evidence is therefore not strong enough to conclude on an endocrine effect linking a mode of action with an adverse outcome. *In vitro* data did not show any estrogenic, nor any androgenic, thyroid or steroidogenic properties of Hexyl Salicylate and salicylic acid.

3.3.6 Mutagenicity / genotoxicity

The *in vitro* mutagenicity and genotoxicity studies that have been performed for Hexyl Salicylate are summarised below.

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

Bacterial gene mutation assay (Ames test)

Guideline:	OECD 471, EU B.13/14
Species/strain:	*S. typhimurium*, TA98, TA100, TA102, TA1535, TA1537;
Replicates:	Triplicates in two independent tests
Test substance:	Hexyl Salicylate
Batch:	50554361
Purity:	data not provided
Solvent:	dimethyl sulfoxide (DMSO; 50 µl/plate)
Positive controls:	Sodium azide, 2-nitrofluorene, 9-aminoacridine, mytomycin C, and 2-aminoanthracene
Metabolic activation:	S9 liver fraction from Aroclor 1254 pretreated male rats
Concentrations:	experiment I: 50, 150, 500, 1500, 5000 µg/plate without S9-mix 50, 150, 500, 1500, 5000 µg/plate with S9-mix experiment II: 15 (for TA102 and TA 1537), 50, 150, 500, 1500, 5000 µg/plate without S9-mix 5 and 15 (for TA102 and TA 1537), 50, 150, 500, 1500, 5000 µg/plate with S9-mix
Treatment:	experiment I: direct plate incorporation method with 48-72 h incubation without and with S9-mix experiment II: direct plate incorporation method with 48 – 72 h incubation without S9-mix
GLP:	in compliance
Study period:	30.5.2000

The mutagenicity of the substance Hexyl Salicylate was studied with five mutant strains of Salmonella typhimurium (TA1535, TA1537, TA98, TA100, and TA102). The investigations were carried using the standard plate incorporation assay with and without liver homogenate (S9) from Aroclor 1254 pre-treated male rats as metabolic activation system.

Hexyl Salicylate was dissolved in DMSO and tested in concentrations of 5 to 5000 µg per plate in the presence and 15 to 5000 µg per plate in the absence of S9. In the presence of S9-mix Hexyl Salicylate was bacteriotoxic towards the strain TA1537 at 500µg/plate, towards the strains TA100 and TA102 at 1504 µg/plate and towards the strains TA98 and TA1535 at 5000

µg/plate. In the absence of S9-mix Hexyl Salicylate was bacteriotoxic towards the strain TA1537 at 500 µg/plate and towards the strains TA98, TA100, and TA102 at 500 µg/plate. Precipitation of the test compound at the plates was observed at 1500 and 5000 µg/plate. Sodium azide, 2-nitrofluorene, 9-aminoacridine, mytomycin C, and 2-aminoanthracene served as positive controls to confirm the reversion properties and the specificity of the bacterial strains as well as the efficacy of the metabolising system.
In the concentration range investigated, Hexyl Salicylate did not induce a significant increase in the mutation frequency of the tester strains in the presence or absence of a metabolic activation system.

In conclusion, these results indicate that Hexyl Salicylate under the experimental conditions described, was not mutagenic to Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and TA102 in the presence and absence of a metabolizing system.

King MT, 28 February 2000 Study (AM02000N)

SCCS comment
Data on the purity of test item have not been provided. Hexyl Salicylate was dissolved in DMSO, but no description of the test solution preparation was given. Precipitation of the test substance on the plates was observed at 1500 and 5000 µg/plate. The SCCS noted that Hexyl Salicylate was bacteriotoxic in the presence and absence of S9-mix towards several strains at concentrations 500 or 1500 µg/plate and above.

***In vitro* micronucleus assay**:

Guideline:	OECD 487 (2016)
Test system:	Isolated human lymphocytes
Replicates:	duplicates
Test substance:	Hexyl Salicylate
Batch (Purity):	80854 (99.6%)
Solvent:	dimethyl sulphoxide (DMSO)
Metabolic activation:	Phenobarbital-5,6 Benzoflavone-induced rat liver (S9 mix),
Concentrations and treatment:	
	3h without S9: 43.90, 65.84, 98.77 µg/mL
	3h + S9-mix: 98.77, 148.1, 222.2 µg/mL
	24h without S9-mix: 29.26, 43.90, 65.84 µg/mL
After treatment exposure:	Cytochalasin B (cytoB) 6 µM (after 3h exposure cyoB 21h, in 24h exposure added simultaneously with test item)
Positive controls:	-S9: Mitomycin C (MMC): 50 ng/mL (3 h), 30 ng/mL (24 h)
	Colchicine: 7.5 ng/mL (24 h)
	+S9: Cyclophosphamide (CP) 4 /mL (3h)
Negative control:	Vehicle
Statistics:	Pair-wise statistical analysis employing a one-sided Fisher's Exact test, Cochran-Armitage trend
GLP:	in compliance
Study period:	November 3, 2022

Hexyl Salicylate was tested for its potential to induce micronucleus formation in the *in vitro* micronucleus test. Lymphocytes isolated from fresh whole human blood were used in the study. In all treatments, the solvent used for the test item was dimethyl sulphoxide (DMSO). Based on findings in the preliminary assessment of solubility, the test item was prepared in DMSO at 50.00 mg/mL. The test item was then prepared in a dilution series, typically with a 1.5-fold dilution factor. Cells were cytokinesis blocked using cytochalasin B (cytoB). To enable calculation of the Cytokinesis Block Proliferation Index (CBPI), in order to determine cytotoxicity, at least 500 cells from appropriate cultures were scored. For the selected

concentrations, the micronucleus frequency was determined in 2000 binucleated cells per concentration. The final concentration ranges analysed for micronuclei were: 3h +S9 treatment schedule 98.77 to 222.2 µg/mL 3h -S9 treatment schedule 43.90 to 98.77 µg/mL continuous treatment schedule (24h -S9) 29.26 to 65.84 µg/mL

The recommended maximum level of cytotoxicity (55 ± 5%) was achieved in all treatment schedules. At the highest concentration selected for analysis, the following levels of cytotoxicity were observed: 3h +S9 treatment schedule 57.65% at 222.2 µg/mL 3h -S9 treatment schedule 51.43% at 98.77 µg/mL continuous treatment schedule (24h -S9) 54.08% at 65.84 µg/mL. Upon addition of test item to the culture medium, a precipitate of the test item was observed at a concentration of 222.2 µg/mL and above in all treatment schedules. At the end of the treatment period, precipitate was observed at a concentration of 333.3 µg/mL and above in the 3h -S9 and continuous treatment schedules, and at a concentration of 500.0 µg/mL in the 3h +S9 treatment schedule. The observations of precipitate did not affect the selection of dose levels for evaluation of micronucleus frequency. For all treatment schedules, data for background micronucleus induction in the solvent controls were consistent with the test facility's historical control databases (based on 95% Poisson confidence limits) for human lymphocyte cells.

The number of micronuclei analysed from 2000 binucleated cells for each selected test item dose was compared with that from the concurrent solvent control.

The concurrent positive controls produced statistically significant increases in micronuclei compared with the concurrent negative controls. No statistically significant increases in micronucleus formation in the test item-treated cultures were observed as a result of any of the treatment schedules.

There were no concentration-related increases when evaluated with Cochran-Armitage trend tests. All criteria for a negative result were met after expert evaluation of the data.

It was concluded that Hexyl Salicylate did not induce the formation of micronuclei (MN) in human lymphocytes in the presence or absence of S9, under the test conditions used. The criteria for a negative response, as defined in OECD 487 and in the Study Plan, were met.

SCCS comment

A precipitate was noted at final concentrations of 250.0, 500.0, 1000 and 2000 µg/mL. The SCCS agrees with conclusion of the study that Hexyl Salicylate did not induce the formation of micronuclei in human lymphocytes in the presence or absence of S9, under the test conditions used.

3.3.6.2 Mutagenicity / genotoxicity *in vivo*

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Mutagenicity and Genotoxicity overall conclusion from the Applicant:

Hexyl Salicylate is not genotoxic or mutagenic in the OECD guideline *in vitro* assays performed. The mutagenicity and genotoxicity data for salicylic acid were recently reviewed by the SCCS (SCCS, 2018) and 1-hexanol is also not genotoxic or mutagenic (CIR, 2017). There are no concerns in relation to these endpoints for Hexyl Salicylate.

Overall SCCS comment

SCCS agrees that Hexyl Salicylate was not genotoxic or mutagenic in the *in vitro* assays performed.

3.3.7 Carcinogenicity

There are no carcinogenicity studies for Hexyl Salicylate. However, given that there are no genotoxicity/ mutagenicity concerns from *in vitro* assays with or without S9, and it is also highly unlikely that the main metabolites salicylic acid and 1-hexanol may act as carcinogens, then there are no known concerns to address for Hexyl Salicylate.

Conclusion from the Applicant:
Hexyl Salicylate is not expected to be a carcinogen in animals.

SCCS comment
After analysis of the available data, the SCCS considers that Hexyl Salicylate is not likely to be a carcinogen.

3.3.8 Photo-induced toxicity

The photoirritation/photoallergenicity endpoints were evaluated based on data and ultraviolet/visible (UV/Vis) spectra, *in vitro* data and *in vivo* data as described below.

UV Spectra Analysis (RIFM (Sears), 2014):

The available UV/Vis spectra (OECD TG 101) for Hexyl Salicylate indicate significant absorbance between 290–700 nm, with peak absorbance at 305 nm and returning to baseline by 330 nm. The molar absorption coefficient for wavelengths between 290–700 nm is above the benchmark of concern (1000 L · mol-1 · cm-1) for photoirritating effects.

***In vitro* 3T3 cells (RIFM (Harbell), 2002):**

Hexyl Salicylate was tested in the 3T3 Neutral Red Uptake (NRU) Photoirritation Assay. Duplicate 96-well monolayers of 3T3 fibroblast were exposed to dilutions of test material (up to 100 µg/mL); 1 plate was exposed to 5 J/cm2 UVA irradiation (photoirritation), and the other was not exposed to UV irradiation (cytotoxicity). The treatment medium was then replaced by a culture medium, and, at approximately 24 hours after treatment, the number of viable cells was determined by NRU. The number of viable cells present for each concentration of test material was compared to that of untreated controls, and the percent inhibition of growth was calculated. The IC50 concentration (i.e., the concentration producing 50% inhibition of growth) was calculated and expressed as µg/mL for both the photoirritation and cytotoxicity plates. A photoirritancy factor was then calculated by comparing the IC50 value obtained with and without UVA exposure. The results indicate that Hexyl Salicylate does not have a photoirritating potential. No photoirritating responses were observed.

Mouse study (RIFM (Urbach), 1975):

Undiluted Hexyl Salicylate (20 µl) was applied to a 2cm2 area of the back in n=6 Skh:hairless-1 mutant mice exposed to light from a long arc xenon lamp and fluorescent blacklight lamps. Six mice were used as a positive control group using 8-methoxypsoralen (8-MOP) in methanol (0.01% w/v). Treated animals were exposed to a 6-kW long arc xenon lamp (distances = 1 meter, intensity = 0.1667 W/m2) for 40 minutes and 4 fluorescent F40BL-type blacklight lamps with exposure for 1 hour with an intensity of 3 W/m2. The irradiation area was defined by a 1 cm diameter hole punched in an aluminium foil adhesive tape, and the tape masked the skin surrounding the exposure area. Reactions were assessed at 4, 24, 48, 72, and 96

hours. Phototoxic reactions were observed at the irradiated positive control sites. No reactions were observed at either the irradiated or non-irradiated test material treated sites. Hexyl Salicylate was not phototoxic.

Miniature pig study (RIFM (Urbach), 1975):

Phototoxicity was also not observed in two miniature swine tested with undiluted Hexyl Salicylate (20 µl), according to the same procedure as for mouse test described above.

Guinea-pig (RIFM (Learn), 2003):

Photoirritation of Hexyl Salicylate was evaluated in two groups of n=5 Crl:IAF (HA)-hrBR outbred albino hairless guinea pigs. A 0.3-mL aliquot of Hexyl Salicylate at 0%, 5%, 10%, 50%, and 100% in 3:1 DEP:EtOH was topically administered using Hilltop chamber patches (25-mm diameter) to the dorsal skin along the midline of each guinea pig and occluded with a dental dam. Two hours later, the patches were removed, and the application sites were gently wiped with disposable paper towels moistened with deionized water. The animals were exposed to UV radiation using a 6.5-kW long arc xenon water-cooled lamp with a filter used to attenuate mid-range ultraviolet radiation (UVB). A dose of about 2.25 instrumental MED was delivered for each exposure session (approximately 2.25 hours). MED refers to a UVR dose adequate to elicit a barely perceptible response in human skin. Clinical observations were made immediately, 1 and 4 hours, and 1, 2, and 3 days after test material administration and UV exposure. Hexyl Salicylate did not cause skin changes indicative of photoirritation.

Guinea-pig (RIFM (Learn) 2003):

Photoallergy was not observed in two groups of n=5 Crl:IAF (HA)-hrBR outbred albino hairless guinea pigs exposed to Hexyl Salicylate (50% and 100%). A nuchal area of skin, approximately 2.5 cm2, was defined by intradermal injections (0.1 mL/corner) with a formulation of sterile water and Freund's complete adjuvant (1:1 v/v) in each animal. This skin area was then tape stripped 5 times. A 0.3-mL aliquot of Hexyl Salicylate in 3:1 DEP:EtOH was applied to Hilltop® chamber patches (25-mm diameter) and then applied to the nuchal area and occluded with a dental dam. After 2 hours, the patches were removed, and the application sites were gently wiped with disposable paper towels moistened with reverse osmosis membrane processed deionized water. The nuchal area of animals was exposed to UVR for approximately 2.25 hours. The UVR source was a 6.5kw long arc xenon water-cooled lamp with a filter used to attenuate mid-range ultraviolet radiation (UVB). Exposures were monitored by a customised detector that records both intensity and UVR dose. A dose of about 2.25 instrumental MED was delivered for each exposure session. Procedures were repeated once daily on days 3, 5, 8, 10, and 12 of the induction phase. On day 22, using the induction procedure, Hexyl Salicylate at 50% and 100% was topically applied to each animal. Animals were exposed to UVR for 2.25 hours, 2 hours after patch application. The sites were scored 1 and 4 hours after dosage administration and/or UVR exposure. Minimal flaking was observed. Based on the results from the study, Hexyl Salicylate was not considered a photoallergen.

3.3.9 Human data

Human studies (RIFM (Potrebka), 2004):

Photoirritation potential was studied in 56 subjects (41 females and 15 males) who were patch tested with Hexyl Salicylate (0.3%, 3%, and 30% in 3:1 DEP:ethanol), followed by irradiation of sites with UVA and UVB. The test materials or controls were applied to 25-mm Hilltop® Chambers, which were applied to the back of each subject. Each subject received duplicate patches of the 3 concentrations of test material, and of 3 controls (vehicle alone, saline, and a blank (no test material patch)) which were placed on both sides of the spine. Patches remained in place for 24 hours. After 24 hours, the patches on the left paraspinal region were

removed, and the skin sites were irradiated with 16 Joules/cm2 of UVA irradiation for 10 minutes. Then the sites were irradiated with 0.75 Minimal Erythema Doses (MED) UVB. A 150-Watt Berger Solar Ultraviolet Simulator was used as the ultraviolet radiation source in the study. Patches were removed from the non-irradiated test sites on the right paraspinal region after the UVA/UVB dosing was complete. The non-irradiated sites were used as controls to assess the irritation potential of the test material. Reactions were assessed at 1, 24, 48, and 72 hours following UVA and UVB irradiation. No reactions were observed.

Conclusion from the Applicant on Photo-induced toxicity:

Based on the available *in vitro*, *in vivo* and human data, Hexyl Salicylate is not phototoxic or photoallergenic.

3.3.10 Special investigations

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3.4 SAFETY EVALUATION (including calculation of the MoS)

There are no repeat dose toxicity data for Hexyl Salicylate to use in a cosmetic safety assessment. However, given that Hexyl Salicylate itself is not regarded as the main toxicant, but rather salicylic acid as the chief hydrolysis product via all routes of exposure, it is possible to perform a cosmetic safety evaluation for Hexyl Salicylate using the study by Tanaka *et al.* 1973 (from the reproductive/developmental data shown in section 3.4.5) for its principal primary metabolite, salicylic acid.

Salicylic acid was reviewed recently by the SCCS in its Opinion from December 2023. The POD for salicylic acid was selected as a no observed adverse effect level (NOAEL) of 75 mg/kg/day based upon the most sensitive observations in orally dosed rats, of teratogenic effects, in the Tanaka *et al.* 1973 study.

A value for skin penetration of 13.4% (mean (3.04 +1SD) x a correction factor of 3) has been used in all calculations of systemic exposure dose for dermally applied products (see section 3.2.2).

- **MoS for Adults**

Based on the exposure information in section 3.2, the Margins of Safety for consumer exposures to Hexyl Salicylate in 18 cosmetic products are shown in Table 15 below.

Table 15: Deterministic worst-case margin of safety calculations for the dermal and oral route using maximum % use levels as defined by the European Cosmetics Industry. Values of SED are calculated according to the SCCS 11th Notes of Guidance approach for calculating a worst-case aggregate exposure on the basis of deterministic additive methods. Hexyl Salicylate is assumed to undergo 13.4% skin penetration for all product types (except oral care and lipstick where 100% is assumed) in this assessment. 100% metabolic conversion to salicylic acid equivalent has been assumed, as per Table 5.

Categories	Products	Max [C] in the fisnished product (%)	Calculated SED SA eq (mg/kg bw/day)	MOS using a POD of 75 mg/kg bw/day
Hydroalcoholic-based Fragrances		2	0.0078	9615
Rinse-off skin and Hair Products	Shower gel	0.5	0.0012	62500
	Hair Conditioner	0.5	0.0003	250000
	Shampoo	0.5	0.0006	125000
	Hand and Wash soap	0.5	0.0014	53571
Leave-on skin and Hair Products	Body Lotion	0.3	0.0308	2435
	Face Cream	0.3	0.006	12500
	Hand Cream	0.3	0.0082	9146
	Deodorant	0.3	0.0055	13636
	Hair Styling	0.3	0.0014	53571
Face-Make-up Products	Liquid Foundation	0.3	0.002	37500
	Lipsticks, Lip salve	0.3	0.0017	44118
	Make-up remover for face	0.3	0.0021	35714
	Eye make-up	0.3	0.0001	750000
	Mascara	0.3	0.0001	750000
	Eyeliner	0.3	0.0002	375000
Oral Care products	Tootpaste	0.001	0.00001	7500000
	Mouthwash	0.001	0.0002	375000
Aggregate			0.06961	1077
difference with the applicant figures				

For systemic effects, considering all applied cosmetic products either directly on the skin or by spray, at the maximum concentrations of Hexyl Salicylate reported in Table 1 above, taken individually and also the aggregated exposure, the margin of safety is above 100.

3.5 DISCUSSION

Hexyl Salicylate (CAS/EC No. 6259-76-3/228-408-6) is the INCI name of 'hexyl 2-hydroxybenzoate', an ingredient with sweet, floral, and fruity odour used in formulations of fragrances in multiple consumer goods including cosmetic, household cleaning products, detergents, and air care products.

Hexyl Salicylate is not listed in the Annexes to the Cosmetic Regulation (EC) No. 1223/2009 and its use is not otherwise restricted in cosmetic products.

Physicochemical properties

Information on the analytical methods used for the determination of purity and impurities of the test substance and their results should be provided in accordance with the SCCS Notes of Guidance.

Exposure

Dermal/percutaneous absorption

A value of 13.4 % (Mean + 1SD corrected for 24 hours) was calculated following a recent *in vitro* study using human skin that meets the basic criteria for skin absorption in SCCS Notes of Guidance (2021). This value will be used for the calculation of the MoS.

Toxicokinetics

Hexyl Salicylate, like methyl salicylate and benzyl salicylate, is expected to be rapidly and completely absorbed and metabolised, in both gut and liver tissue by first-pass metabolism, to salicylic acid and 1-hexanol following oral exposure in both rat and humans. With rapid hydrolysis in the gut and liver, systemic exposure is primarily to salicylic acid and 1-hexanol, which do not accumulate in the body, and are rapidly excreted.

Exposure by oral route

Based on the available data, the SCCS considers that an absorption value by oral route of 100% can be used in the risk assessment.

Exposure by inhalation

There are no data on the extent of Hexyl Salicylate absorption in the lung. Based on the high log Po/w and low water solubility, it is expected that Hexyl Salicylate will be poorly absorbed by the inhalation route. An assumption of 100% absorption can be used in the risk assessment, which is therefore conservative.

Systemic Exposure

Separate exposure assessments were performed for the dermal, oral and inhalation routes. As the deterministic approach - based on maximal concentrations used - yields a favourable outcome, further exposure modelling was not necessary to refine exposure. This Tier 1 scenario is based on maximum concentrations of Hexyl Salicylate that are used in products in Europe, in each of the standard 17 product types as included in an aggregate exposure assessment (according to the SCCS Notes of Guidance 2021). These levels have been provided in a recent use survey by the members of the 'Hexyl Salicylate consortium', and are used to calculate the total systemic exposure to benzyl salicylate (in mg/kg/day) from each product for adults. In addition to the 17 cosmetic products usually considered, an 18th product type – hydroalcoholic fragrances - was included in the scenario.

Toxicological Evaluation

Irritation and corrosivity

Hexyl Salicylate shows some irritant reactions in animal models at concentrations of 25% and above and very low skin reactions in one human study at 30%, but it is not considered to be an irritant of concern at the concentrations used in cosmetic products.

Undiluted Hexyl Salicylate is not an eye irritant *in vivo* and there is no risk of eye irritation at the maximum concentrations of Hexyl Salicylate used in cosmetic products.

Skin sensitisation

Available human, animal and NAM data on Hexyl Salicylate are contradictory. In a well-conducted LLNA, Hexyl Salicylate was positive at a relatively low concentration, whereas the

GPMT was negative. *In silico* data showed no protein binding alerts, which was confirmed in the DPRA. In addition, the Keratinosens was negative, whereas the h-CLAT and U-SENS were positive. If, for example, the 2o3 DA (two out of three defined approach) was performed according to OECD Guideline 497, Hexyl Salicylate would have been considered as a non-sensitiser. Human evidence is limited, but the data available is all negative. This was already observed in an earlier SCCS Opinion on fragrance allergens (SCCS/1459/11) and no new evidence indicating that Hexyl Salicylate is a relevant skin sensitiser in humans has emerged in literature.

Taking all the evidence together, the SCCS concludes that although Hexyl Salicylate is classified as a skin sensitiser, based on clinical evidence, the risk of skin sensitisation in humans from the use in cosmetic products can be considered negligible.

Acute toxicity

Hexyl Salicylate is not acutely toxic *via* any route of exposure.

Repeated dose toxicity

No repeat dose toxicity data are available on Hexyl Salicylate. Given that Hexyl Salicylate itself is not regarded as the main toxicant, but rather its chief hydrolysis product, salicylic acid, the available repeat dose toxicity data and conclusions for the primary Hexyl Salicylate metabolites salicylic acid and 1-hexanol were reviewed for the purposes of performing a cosmetics safety assessment. All data for salicylic acid were also recently reviewed by the SCCS in its recent Opinion (SCCS, 2018).

Reproductive toxicity

No reproductive/developmental toxicity data are available on Hexyl Salicylate. The proposal for classification is based upon read across to salicylic acid and methyl salicylate data, and the assumption that Hexyl Salicylate is metabolised to the same common metabolite, salicylic acid.

Given that Hexyl Salicylate itself is not regarded as the main toxicant, but rather salicylic acid as the chief hydrolysis product, available reproductive/developmental toxicity data and conclusions for the primary metabolite salicylic acid and 1-hexanol to which the body may be principally exposed were reviewed for the purposes of performing a cosmetics safety assessment.

SCCS considers that salicylic acid is a developmental toxicant. Harmonised classification of salicylic acid was recently published in Regulation 2018/1480 and is classified as Repr. 2 (H361d Suspected of damaging the unborn child). For MoS calculation, SCCS uses the developmental NOAEL of 0.1% (75 mg/kg bw/day) derived from Tanaka *et al.* (1973a).

In addition, due to the evidence for high (100%) oral bioavailability in humans, the oral NOAEL of 75 mg/kg bw/day is defined as systemic NOAEL (NOAELsys) by SCCS for salicylic acid.

This POD for salicylic acid can act as a conservative surrogate POD for Hexyl Salicylate. On a molar basis, 1 mole of Hexyl Salicylate is converted to 1 mole of salicylic acid. An assumption is made that 100% of Hexyl Salicylate is metabolised to salicylic acid and 1-hexanol, and salicylic acid is the driver of any observed Hexyl Salicylate toxicity. The salicylic acid NOAELsys = 75 mg/kg bw/day. A salicylic acid equivalent SED can be calculated and compared with this POD.

Mutagenicity / genotoxicity

SCCS concludes that Hexyl Salicylate was not genotoxic or mutagenic *in the vitro* assays performed.

Carcinogenicity

After analysis of the available data, the SCCS considers that Hexyl Salicylate is not likely to be a carcinogen.

Photo-induced toxicity

Based on the available *in vitro*, *in vivo* and human data, Hexyl Salicylate is not phototoxic or photoallergenic.

Special investigation: endocrine disrupting effects

For the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 and based upon the WHO IPCS 2002 Definition, a connection between mode/mechanism of action and observed adverse effect must be demonstrated for substances falling within the Commission's ED criteria and the ECHA/EFSA Guidance. There is no evidence from the available *in vivo* data of an adverse effect of Hexyl Salicylate and salicylic acid as a result of an endocrine mechanism, *e.g.,* there is no adverse effect on fertility. The available evidence is not strong enough to conclude on an endocrine effect linking a mode of action with an adverse outcome. *In vitro* data did not show any estrogenic effects, nor any androgenic, thyroid or steroidogenic properties of Hexyl Salicylate and salicylic acid.

4. CONCLUSION

1. In light of the data provided and taking under consideration the CMR Cat.2 classification (to be introduced in Annex VI to Reg. 1272/2008), does the SCCS consider Hexyl Salicylate safe when used up to the maximum concentrations provided in the dossier?

   Based on the assessment of data provided and taking into consideration the concerns related to potential endocrine disrupting properties, the SCCS considers Hexyl Salicylate safe when used up to the maximum concentrations as provided in Table 1 of this Opinion.

2. Does the SCCS have any further scientific concerns with regard to the use of Hexyl Salicylate in cosmetic products?

   The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of Hexyl Salicylate for the environment.

5. MINORITY OPINION

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Appendix 1 Literature & Data Search for toxicology evidence on Hexyl Salicylate

It is assumed in the preparation of this dossier that, in addition to available industry studies, the following authoritative public reviews on Hexyl Salicylate can be drawn upon for necessary safety information for the dossier.

- Cosmetics Ingredient Review (CIR) 2018-2019 (Final June 2019 report) https://www.cir-safety.org/sites/default/files/salicy042019FAR.pdf
- EU REACH substance dossier for Hexyl Salicylate https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14766/7/1
- European Chemicals Agency RAC CLH Opinion (CLP)(ECHA) 2022 https://echa.europa.eu/fr/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e18471782f
- RIFM Fragrance Material Review (Lapczynski *et al.* 2007; Belsito *et al.* 2007)
- SCCNFP/0017/98 Final report 1999 Fragrance Allergy https://ec.europa.eu/health/ph_risk/committees/sccp/documents/out98_en.pdf
- SCCS 2011 report Fragrance allergens opinion SCCS/1459/11 https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf

7. GLOSSARY OF TERMS

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

8. LIST OF ABBREVIATIONS

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