Scientific Committee on Consumer Safety

SCCS

OPINION on

Benzyl Salicylate

(CAS No. 118-58-1, EC No. 204-262-9)

The SCCS adopted this document
during the plenary meeting on 26 October 2023

ACKNOWLEDGMENTS

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

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This Opinion has been subject to a commenting period of min eight weeks after its initial publication (from 13 June to 24 August 2023). Comments received during this period were considered by the SCCS. For this Opinion, main changes occurred in the following sections: 3.2.1.1 SCCS comment, 3.3.2.2. SCCS comment, SCCS comments under Table 10 and Table 11, SCCS conclusion on endocrine activity, 3.5 safety evaluation, as well as special investigation and safety evaluation under discussion section.

All Declarations of Working Group members are available on the following webpage:
Register of Commission expert groups and other similar entities (europa.eu)

1. ABSTRACT

The SCCS concludes the following:

(1) *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Benzyl Salicylate, does the SCCS consider Benzyl Salicylate safe when used up to the maximum concentrations provided in the dossier submission by the Benzyl Salicylate Consortium?*

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

(2) Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzyl Salicylate in cosmetic products?

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(3) Does the SCCS have any further scientific concerns with regard to the use of Benzyl Salicylate in cosmetic products?

The available data on Benzyl Salicylate provide some indications for an endocrine mode of action, but there is no evidence to suggest that this results in endocrine effects.

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

Keywords: SCCS, scientific opinion, Benzyl Salicylate, Regulation 1223/2009, CAS No. 118-58-1, EC No. 204-262-9

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Benzyl Salicylate (CAS No. 118-58-1, EC No. 204-262-9), preliminary version of 6-7 June 2023, final version of 26 October 2023, SCCS/1656/23

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems, which may pose an actual or potential threat.
These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

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

SCCS

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

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PDF ISSN 1831-4767 ISBN 978-92-68-19384-6 doi:10.2875/160899 EW-AQ-24-001-EN-N

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

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

Background on Benzyl Salicylate

Benzyl Salicylate (CAS No. 118-58-1, EC No. 204-262-9) with the chemical name '2-hydroxybenzoic acid phenylmethyl ester' is produced naturally in a variety of plants and plant extracts where it can be extracted. In addition, Benzyl Salicylate can be synthesised for use, typically as a fragrance ingredient, in a range of manufactured goods (cosmetics, household goods and medicines). In cosmetics, Benzyl Salicylate is used for its fragrance/perfuming function.

Benzyl Salicylate was assessed by the SCCNFP in 1999[4] and by SCCS in 2012[5] and it is considered as an established contact allergen in humans. It is currently regulated for labelling purposes as an allergen in entry 75 of Annex III to the Cosmetics Regulation. In particular, "*its presence must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products*".
During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Benzyl Salicylate as a fragrance ingredient in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Benzyl Salicylate in view of the information provided, taking into account the maximum concentration of Benzyl Salicylate in the different categories of cosmetic products listed in the table below:

[1] https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-739-F1-EN-MAIN-PART-1.PDF
[2] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic%20products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, Homosalate, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein
[4] https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out98_en.pdf
[5] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf

Table 1: Maximum use concentrations of Benzyl Salicylate in cosmetic products

Type of cosmetic product exposure	Maximum % concentration used
Hydroalcoholic-based fragrances (spray and non-spray)	4
Rinse-off skin & hair products (except rinse off body products)	0.5
Rinse off body products	1.3
Leave on skin & hair products (non-spray/non-aerosol)(except body lotion)	0.5
Leave on hair products (spray/aerosol)	0.5
Leave on body products (non-spray/spray/aerosol)	0.7
Face make-up products and make-up remover	0.2
Oral care	0.004
Deodorant products (spray/aerosol)	0.91

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Benzyl Salicylate, does the SCCS consider Benzyl Salicylate safe when used up to the maximum concentrations provided in the dossier submission by the Benzyl Salicylate Consortium?*

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

3. *Does the SCCS have any further scientific concerns with regard to the use of Benzyl 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

Benzyl Salicylate

3.1.1.2 Chemical names

IUPAC: benzyl 2-hydroxybenzoate

Synonyms: salicylic acid benzyl ester; 2-Hydroxybenzoic acid phenylmethyl ester; phenylmethyl 2-hydroxybenzoate; Benzyl o-hydroxybenzoate; Salicylic acid, benzylester;)

3.1.1.3 Trade names and abbreviations

Benzyl 2-hydroxybenzoate
Benzyl o-hydroxybenzoate
Benzyl Salicylate

Ref: ECHA: https://echa.europa.eu/el/substance-information/-/substanceinfo/100.003.876

3.1.1.4 CAS / EC number

CAS number: 118-58-1
EC number: 204-262-9

3.1.1.5 Structural formula

The chemical structure of Benzyl Salicylate is shown in Figure 1

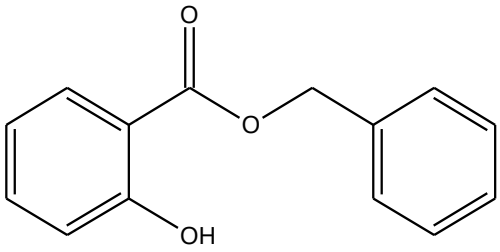

Figure 1. Chemical structure of Benzyl Salicylate

3.1.1.6 Empirical formula

Empirical formula: $C_4H_{12}O_3$

3.1.2 Physical form

Thick, colourless to pale yellow liquid.

Ref: 2021-11-18 SCCS Benzyl Salicylate Dossier_FINAL

3.1.3 Molecular weight

228.25 g/mol

3.1.4 Purity, composition and substance codes

> 99%

SCCS comment
A full report of the chemical characterisation of Benzyl Salicylate in terms of purity and identity in representative batches should be provided and the validity of the analytical methodologies used should be shown.

3.1.5 Impurities / accompanying contaminants

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SCCS comment
A full report on the impurity tests in representative batches of the test substance should be provided and the validity of the analytical methodologies used shown. Identity and concentration of any impurities that may be present should also be stated.

3.1.6 Solubility

Slightly soluble in water (8.8 mg/L at 20 °C). Soluble in organic solvents and oils.

3.1.7 Partition coefficient (Log P_{ow})

4.31 at 35 °C

3.1.8 Additional physical and chemical specifications

Where relevant:

- organoleptic properties: Benzyl Salicylate has a faint, sweet odour.
- melting point: 24 °C
- boiling point: 320 °C
- flash point: /
- vapour pressure: 7.8 x 10^{-5} mmHg at 25 °C; 0.0104 Pa at 25 °C (ECHA, 2020)
- density: /
- viscosity: /
- pKa: 9.82
- pH: 6.8-7.1
- refractive index: /
- UV/visible light absorption spectrum: /

3.1.9 Homogeneity and Stability

Stable under recommended storage conditions. Predicted to be stable in most organic solvents.

SCCS comment
Data on the stability of the test substance under the experimental conditions of the reported studies and under conditions of use, and information on any hydrolysis products, should be provided.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

3.2.1.1 *In vitro* human skin absorption studies

Two studies have been used to investigate absorption of Benzyl Salicylate *in vitro* using human skin: Jimbo (1983) and a new OECD Test Guideline 428 study (BASF, Oct 2021).

The skin absorption of Benzyl Salicylate through human epidermis was studied *in vitro* using a static cell glass chamber (Jimbo, 1983). The experiment was repeated six times. 0.2 mL of Benzyl Salicylate was applied neat to the skin. The upper surface of human epidermis was fixed to a glass tube which was then placed inside one arm of a U-shaped glass chamber. 0.5 mL saline was in contact with the underside of the epidermis. Parafilm was applied to occlude the skin. The chamber was kept at 21°C and 55% relative humidity for 72 h. Material that had penetrated into the receptor fluid was extracted in ether and analysed by gas chromatography. The amount of Benzyl Salicylate that penetrated human skin in this study was minimal; the percent penetration±S.E. through excised human skin was 0.031% ± 0.004%. This study did not meet the basic criteria for skin absorption in SCCS Notes of Guidance (2021) and therefore a new study was performed using human skin *in vitro*.

An OECD Test Guideline 428 skin absorption study was performed (BASF, Oct 2021). In preparation, prior to the performance of the main study, a stability study was performed that showed 5 mg/g (0.5% w/w) of Benzyl Salicylate was homogenously formulated and was stable in a representative blank (i.e without Benzyl Salicylate) body lotion formulation for at least 24 hours at 32°C. Benzyl Salicylate was also stable in receptor fluid (physiological saline with 5% bovine serum albumin (BSA)) for 24 hours at target concentrations of 0.09 mg/mL and 0.01 µg/mL at 32°C. The solubility of the test-substance in the receptor fluid was determined to be 0.91 mg/mL.

[^{14}C]-Benzyl Salicylate was applied to n=12 human skin samples (previously frozen and dermatomed, from 4 donors) at a target dose of 10 µg/cm^2 (actual 9.5 µg/cm^2) in application of representative body lotion formulation at 2 mg/cm^2 in Franz-type diffusion cells in flow-through mode (surface skin area approximately 1cm^2), using receptor fluid (physiological saline with 5% BSA and 0.01% (w/v) sodium azide (NaN_3)). The integrity of the skin barrier was checked via TEER (impedance value). A summary of the mean recoveries and kinetic parameters from this study is shown in Table 2.

Table 2: Mean recoveries and kinetic parameters for skin penetration of [^{14}C]-Benzyl Salicylate as applied to human skin *in vitro* (BASF, Oct 2021)

	recovery	
	mean amount of test substance [µg]	**mean % of applied dose**
dislodgeable dose		
membrane washing after 24 hours	7.64 ± 0.27	80.67 ± 2.80
donor chamber washing	0.42 ± 0.22	4.43 ± 2.27
sum	**8.06 ± 0.29**	**85.11 ± 3.10**
dose associated to tape strips		
tape strips 1+2	0.03 ± 0.03	0.27 ± 0.34
tape strips 3-5	0.02 ± 0.02	0.23 ± 0.22
tape strips 6-10	0.03 ± 0.02	0.28 ± 0.21
tape strips 11-15	0.02 ± 0.01	0.18 ± 0.09
tape strips 16-20	0.01 ± 0.01	0.15 ± 0.09
sum	**0.11 ± 0.08**	**1.11 ± 0.87**
dose associated to remaining skin		
epidermis excluding tape strips	0.06 ± 0.03	0.65 ± 0.29
dermis	0.09 ± 0.05	0.99 ± 0.57
sum	**0.16 ± 0.08**	**1.64 ± 0.79**
absorbed dose		
sum receptor samples 0 - 24 h	0.12 ± 0.03	1.28 ± 0.28
receptor fluid	0.03 ± 0.01	0.32 ± 0.13
receptor chamber washing	0.55 ± 0.11	5.76 ± 1.17
sum	**0.70 ± 0.14**	**7.36 ± 1.51**
sum of absorbed dose and dose associated to remaining skin	**0.85 ± 0.14**	**9.01 ± 1.46**
total recovery	**9.02 ± 0.19**	**95.23 ± 2.02**

	sample time [h]	**mean cumulative absorption [µg/cm²]**	**percentage absorption**
	0	0.00	0.00
	0.5	0.00	0.00
	2	0.00	0.01
	4	0.00	0.02
	8	0.01	0.11
	16	0.05	0.56
	24	0.12	1.28
Kp	**[×10^{-5} cm/h]**		0.18
absorption rate[1]	**[µg / (cm²*h)]**		0.01
lag time	**[h]**		9.78

[1]absorption rate was calculated to be 0.0085 µg/(cm²×h)

N.B. Mean values were calculated from cells 1-7 and 9-11. Cells 8 and 12 were excluded from statistics due to failed application (resulting in invalid recoveries that were calculated to be >120%).

The majority of the applied test substance was recovered as dislodgeable, calculated as the sum of skin wash and donor chamber washing (85.11 ± 3.10%). The amount associated to tape strips resulted in 1.11 ± 0.87% of the applied dose. Minor amounts of the test substance were associated with the skin after the exposure period. This amount accounted to 1.64 ± 0.79% of the applied dose. The recovery from the sum of absorbed dose and dose associated to remaining skin was 9.01 ± 1.46% of applied dose. The mean total recovery fulfilled the quality criteria put forward in the test guidelines, resulting in a mean total recovery of 95.23 ± 2.02 %. A value for skin absorption can be calculated from this study using the mean + 1SD i.e. 9 + 1.5 = 10.5%

SCCS comment
The results of the skin penetration study are based on radiolabelled compound ([^{14}C] on one of the benzyl rings) and as such it is not clear whether the amounts measured were for the intact compound or a breakdown product. This needs to be clarified. Apart from this, the study was well performed and follows the OECD 428 guidance and the SCCS basic criteria for skin penetration study (SCCS/1358/10). Assuming the amounts measured were for the worst case that included intact substance as well as breakdown products (salicylic acid), the value of mean+1SD i.e. 9±1.5 = 10.5% can be used for the MoS calculation.

3.2.1.2 *In vitro* animal skin absorption studies

Studies that have been used to investigate skin absorption of Benzyl Salicylate *in vitro* using animal skin are summarised in Table 3. There are two studies performed at Hoffmann LaRoche laboratories from 1983 and 1984, including data for rat and pig skin, respectively. These experiments used radiolabelled 14C-Benzyl Salicylate in a static cell system with physiological saline as receptor fluid. These studies show that as expected, the skin absorption through pig skin (which is most like human skin) is much less than is observed with naked rat skin (Figure 2). As outlined in the SCCS document 'Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients' (SCCS/1358/10), rat skin is not recommended for skin absorption studies given that rat skin is more permeable than human skin (mean difference about 10-fold) (van Ravenzwaay and Leibold, 2004), whereas pig skin is regarded as a suitable surrogate for human skin. Hence, the greatest weight in these available animal skin absorption studies should be given to the pig skin data.

Table 3: Summary of observations for Benzyl Salicylate from *in vitro* skin absorption studies using animal skin

Species/ number/sex	Exposure concentration	Application site details	Observations	Reference
Rat	1%, 3%, or 10% solution of ^{14}C-benzyl salicylate in ethanol for up to 24 h	Intact naked shaved rat skin. Test material applied to a 5cm^2 area at a dose of 12 µl/cm^2 Material was rinsed off at either 1, 6, 16 or 24h, and the % radioactivity calculated	As measured by radioactive scintillation counting, for each of 1%, 3% or 10% dose, respectively **after 24h exposure duration:** - 62.7, 58.8, 40.3% penetrated the skin into receptor fluid - 9.3, 12.0, 17.7% was present in the skin - 0.4, 1.6, 4.0% in the stratum corneum (horny layer) - 27.7, 27.5, 38.0% washed from the skin surface - Total recovery: 100.1, 99.9 and 100%. As measured by radioactive scintillation counting, for each of 1%, 3% or 10% dose, respectively **after 1h exposure duration:** - 0, 0, 0% penetrated the skin into receptor fluid - 21.9, 28.3, 17.2% was present in the skin - 7.9, 9.6, 5.3% in the stratum corneum (horny layer) - 70.3, 62.1, 77.5% washed from the skin surface - Total recovery:100.1, 100, 100%.	Hoffmann LaRoche Feb 1983 & Aug 1984 study reports (Givaudan)
Pig	1%, 3%, or 10% solution of ^{14}C-benzyl salicylate in ethanol for up to 16 h	Intact pig skin. Test material applied to a 5cm^2 area at a dose of 12 µl/cm^2 Material was rinsed off at either 1, 6, 16h and the % radioactivity calculated	As measured by radioactive scintillation counting, for each of 1%, 3% or 10% dose, respectively **after 16h exposure duration:** - 3.5, 1.7, 0.9% penetrated the skin into receptor fluid - 4.7, 4.1, 3.8% was present in the skin - 7.2, 5.5, 4.7% in the stratum corneum (horny layer)	Hoffmann LaRoche August 1984 study report (Givaudan)

			- 84.5, 88.7, 90.7% washed from the skin surface - Total recovery: 99.9, 100, 100.1%. As measured by radioactive scintillation counting, for each of 1%, 3% or 10% dose, respectively **after 1h exposure duration**: - 0, 0, 0% penetrated the skin into receptor fluid - 1.7, 1.5, 1.1% was present in stripped skin - 5.3, 6.3, 4.8% in the outer stratum corneum (horny layer) - 93.0, 92.1, 94.1% washed from the skin surface - Total recovery: 100, 99.9, 100	

The extent of skin penetration into receptor fluid was dependent upon the application dose, time of application and the species of skin used (Figure 2).

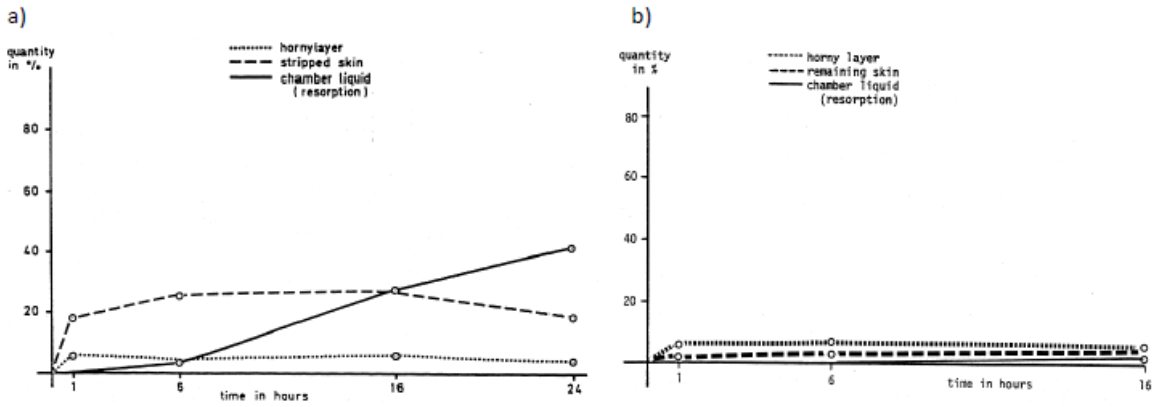

Figure 2. The skin absorption profile for a) naked shaved rat skin and b) intact pig skin over time, when a 10% dose of Benzyl Salicylate is applied for 1, 6, 16 (rat and pig) or 24h (rat only). (Hoffmann LaRoche study 1984)

Taking the pig skin data (Hoffmann LaRoche, 1984) as the most relevant for human exposure, more than 85% of the applied dose is removable from the surface of pig skin at all time points and all dose levels. The material removed and that in the stratum corneum are not bioavailable, and are not considered as 'absorbed'. The material measured within the skin and in receptor fluid is considered as absorbed and bioavailable. For the three doses tested at 16h, the % absorbed is 8.2, 5.8 and 4.7%, respectively.

A conservative skin absorption value of 10% is supportable from the *in vitro* pig skin study to account for the study duration only being 16h and not the usual 24h. No metabolism or speciation was investigated in these studies, these data are for total radioactive counts therefore there is no underestimation, although, it is not known what proportion of Benzyl Salicylate has been metabolised in the skin by esterases to benzyl alcohol and salicylic acid.

3.2.1.3 *In vivo* human skin absorption/kinetics studies

A study where 100 µL neat Benzyl Salicylate was applied to skin in humans, and measurements made in urine, is summarised in Table 4.

Table 4: Summary of observations in urine from *in vivo* skin absorption/kinetics studies in humans

Volunteers	Concentration & Vehicle	C_{max} (µg)	T_{max} (h)	Area Under the Curve (AUC) (µg) Total cumulative excretion	Reference
n = 3 male, n = 3 female participants	100µl (approx. 100 mg) of neat benzyl salicylate, pipetted onto one upturned forearm. (NB. Methyl salicylate was also administered in the same study on the other forearm).	557	21	773 - 1430	James *et al* (2020)

James *et al.* (2019) developed a new GC-MS based bioanalytical method for the detection of parent unmetabolized methyl salicylate and Benzyl Salicylate and applied it to analyse urine following human skin application (James *et al.,* 2020).

Six participants (n =3 female, n = 3 male; age range 22-44 years, median age 27.5 years) participated in the trial during April 2018. All participants were healthy and were instructed to avoid foodstuffs suspected to contain methyl salicylate and cosmetic products suspected to contain Benzyl Salicylate for 24 hours before and after the study during the urine collection period.

Participants provided a baseline urine sample ten minutes prior to the start of the study. Test material was pipetted (100µl of each simulant) onto the upturned forearm of each participant; Benzyl Salicylate was applied to the right forearm while the methyl salicylate was applied to the left forearm. The simulant was applied as a long droplet (~10cm). Participants were instructed to stay as still as possible to avoid simulant run-off until the simulant had dried.

At 60 minutes, the first of eight post-application urine samples were collected. Over the next 24 hours, urine samples were collected from each participant (at 2-hour, 4-hour, 6-hour, 8-hour, 12.5-hour, 21-hour, and 24-hour post-dose). Urine samples were returned shortly after 24 hours by all participants.

All 54 urine samples collected during the study contained detectable levels of methyl salicylate and Benzyl Salicylate above the assay limits of quantification (4.6 ng/mL). Recoveries are given as total Benzyl Salicylate excreted (µg) per urine sample provided. Baseline recoveries ranged between 8µg and 34µg (concentrations 257.5 ng/mL – 370.3 ng/ml non-normalized) with an average recovery of 21µg. The peak excretion of Benzyl Salicylate occurred in the 21-hour sample (Figure 3), with the highest value being 557 µg. Samples taken at 12.5 hours and 21 hours were both significantly higher than baseline levels ($P < 0.0001$). Cumulative excretion of Benzyl Salicylate over 24 hours ranged between 773 µg and 1430 µg.

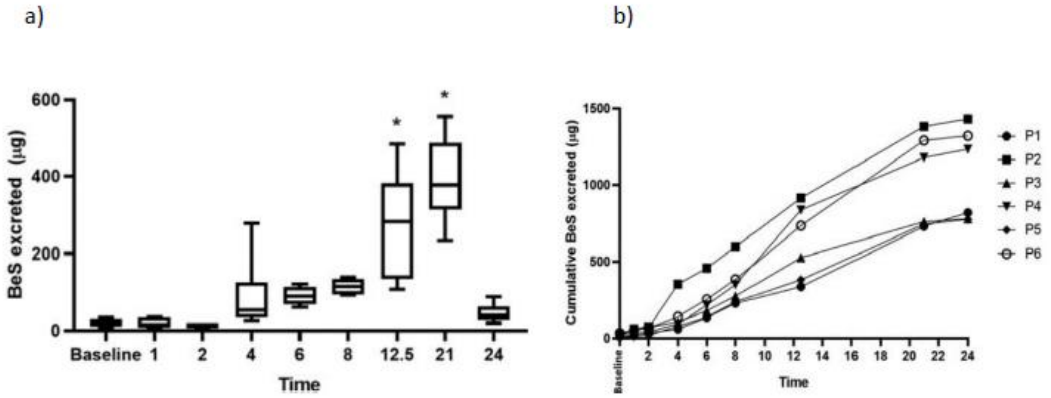

Figure 3. a) Total measured excretion and b) cumulative excretion over 24 h of Benzyl Salicylate in urine for all n=6 participants. Note: Values given have been normalized against total volume of each urination. 12.5-hour and 22-hour samples are the average values for urine collected before and after bed. Box and whisker plot shows the median and inter-quartile range, together with the maximum and minimum values. * = P <.0001 compared to baseline. Abbreviation: BeS, Benzyl Salicylate. (As appears in James *et al.,* 2020)

No significant increase in excreted Benzyl Salicylate levels was observed compared to baseline until four hours, when all participants' urinary Benzyl Salicylate levels increased (Figure 3). Levels of Benzyl Salicylate slowly increased between four hours and 12.5 hours, followed by a large increase overnight and into the post-sleep sample (21 hours) for all participants before dropping to near baseline levels at the 24-hour mark. Benzyl Salicylate was detectable in urine after skin exposure, with reproducible values across the 24 hours for all six participants and cleared effectively within 24 hours.

No metabolism was accounted for in this study, as the authors were looking for detectable Benzyl Salicylate as a qualitative marker substance only rather than considering total absorption, and it is likely that some absorption of benzyl alcohol and salicylic acid as the first pass primary metabolites via the skin would also have occurred and was not measured using this method.

SCCS comment
This study is of limited use as only the parent compound Benzyl Salicylate was measured, without measuring any metabolites, although it is well known that Benzyl Salicylate will be rapidly and extensively metabolised once absorbed by any route of exposure.

3.2.1.4 *In vivo* animal skin absorption studies

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3.2.1.5 Dermal Metabolism data

It is expected that Benzyl Salicylate, once absorbed by any route of exposure, will be rapidly and extensively metabolised by ubiquitous carboxylesterases *in vivo* (Williams 1985; Oesch *et al.,* 2018), thus, only salicylic acid and benzyl alcohol will be systemically available. Generally, skin esterase activity was shown to be predominantly localized in the epidermis and near hair follicles (using fluorescein- 5-isothiocyanate diacetate as substrate) (Sugibayashi *et al.*, 1999). As was seen above in the James *et al.,* 2020 study, a very low level of parent Benzyl Salicylate (<1.5%) is excreted in urine after a ~100 mg dermal dose. Benzyl Salicylate itself is not regarded as the main toxicant in animal toxicology studies, but salicylic acid as the chief hydrolysis product (Belsito *et al.*, 2007).

Conclusion from the Applicant on skin absorption:
Data from an OECD Test guideline 428 skin absorption study performed to GLP (BASF, Oct 2021) indicates that a value of 10.5% absorption can be used in the safety evaluation for Benzyl Salicylate. It is expected that the majority of systemic exposure is in the forms of salicylic acid and benzyl alcohol. Subsequent phase 2 conjugation of both leads to effective clearance.

SCCS overall comment on skin absorption
A value of 10.5% 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) (BASF, 2021) (see section 3.2.1.1). This value will be used for the calculation of the MoS.

3.2.2 Other studies on Toxicokinetics

3.2.2.1 Oral absorption, distribution, metabolism, excretion and kinetics

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 Benzyl Salicylate via the oral route in animals and humans, but oral absorption studies conducted on closely related hydroxy- and alkoxy-substituted benzyl derivatives 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 assumed (SCCS, 2018a). In addition, *in vitro* investigations demonstrate the rapid hydrolysis of Benzyl Salicylate to salicylic acid in rat and human tissues and plasma (see below).

- Oral ADME/kinetic data in animals or humans

No data

- Oral ADME/kinetic data *in vitro*

Ozaki *et al.* (2015) examined the hydrolysis of Benzyl Salicylate to salicylic acid (and benzyl alcohol) by various tissue microsomes and plasma of rats, and by human liver and small-intestinal microsomes. Benzyl Salicylate was readily hydrolysed by human and rat microsomes; this was most extensive in small intestine microsomes, followed by liver microsomes. It is evident that microsomal hydrolysis of Benzyl Salicylate *in vivo* leads to rapid production of salicylic acid in rats and humans.

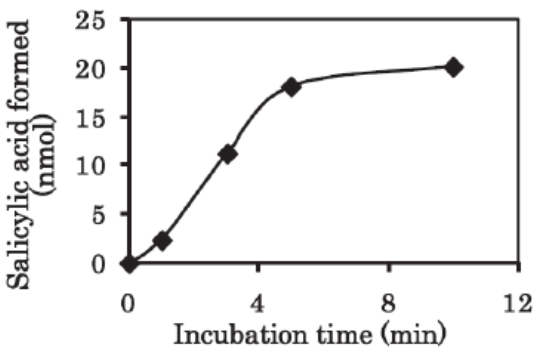

Figure 4. Rapid formation of salicylic acid from 100 nmol Benzyl Salicylate incubations in rat liver microsomes (Ozaki *et al.,* 2015).

A similar experiment was performed by Givaudan (Givaudan study, 2018), Benzyl Salicylate was incubated (100 µM) with rat and human hepatocytes. Rat hepatocytes were thawed, checked for viability by Trypan blue exclusion and adjusted to 106 cells per ml in WEM medium. Viability was at > 90%. Benzyl Salicylate was dissolved in methanol at 10 mM and a final concentration of 100 µM was added to the cells to start the experiment. Experiments were run in closed glass vials. To stop the reaction, samples were mixed with 1M HCl containing d4-deuterated salicylic acid as internal standard. Samples were then purified over SPE columns prior to injection into HR-LC-MS. With HR LC-MS the peak of salicylic acid and the internal reference d4-salicylic acid was determined, and calibration was performed with an external calibration row. Benzyl Salicylate was completely hydrolysed within 15 mins. The extent of hydrolysis was the same in both rat and human hepatocytes.

Table 5: Release of salicylic acid (given in % of added parent) from different salicylates in rat and human hepatocytes

Table 5. Release of salicylic acid (given in % of added parent) from different salicylates in rat and human hepatocytes

	Human 0.5 h	Human 2h	Rat 0.5h	Rat 2h
Benzyl-Sal.	105.6	105.0	103.7	98.5

Note: Experiments in human hepatocytes are from 1 repetition, while the rat data are average from 3 experiments.

- Salicylic acid metabolite - ADME

The oral ADME properties of salicylic acid were covered in the SCCS Opinion from 2018. The data will not be repeated here. Briefly, it was concluded that available evidence suggests that oral absorption can be regarded as 100% and that clearance of salicylic acid is rapid and complete within 24 hours in both rats and humans, after any route of exposure (Goodman & Gilman, 2006).

- Benzyl alcohol metabolite - ADME

A dossier has been prepared for benzyl alcohol by the Cosmetics Ingredient Review (CIR, 2017) and by OECD SIDS (2001). The OECD evaluation of benzyl alcohol toxicokinetics concludes: 'The studies clearly show that the compound is rapidly absorbed from the GI tract of rats and mice, and about 90% of the total dose is recovered as urinary metabolites after 24h. More than 90% of the radiolabel in the urine is present as hippuric acid, with minor amounts of benzyl alcohol and benzylmercapturic acid (up to 4%); no unchanged benzyl acetate was found. Only at very high doses, saturation of these pathways will occur. All supports very rapid ADME of these substances.

Conclusion on Oral ADME data:
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 benzyl alcohol following oral exposure in both rat and humans. With rapid hydrolysis in the gut and liver, systemic exposure is primarily to salicylic acid and benzyl alcohol, 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 Benzyl Salicylate, salicylic acid or benzyl alcohol can be regarded as a systemic point of departure (POD_{sys}).

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

3.2.2.2 Inhalation absorption, distribution, metabolism, excretion and kinetics

There are no data on the extent of Benzyl Salicylate absorption in the lung.

Ozaki *et al.* (2015) also used rat lung microsomes to investigate the hydrolysis of Benzyl Salicylate to salicylic acid and found lung microsomes to be equally as active as small intestinal microsomes and liver at hydrolysing Benzyl Salicylate.

SCCS comment
Due to the lack of data available data, the SCCS considers that an absorption value by inhalation of 100% can be used in the risk assessment.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

Benzyl Salicylate (CAS RN 118-58-1; EC No. 204-262-9) occurs naturally in a variety of plants and plant extracts such as ylang-ylang oil, carnation oil and tuberose absolute. It can be extracted or synthesised for use, typically as a fragrance ingredient, in a range of manufactured goods (cosmetics, household goods and medicines) and its use in Europe is greater than 1000 tonnes but less than 10000 tonnes per annum (ECHA, 2020).

- Cosmetic uses

Benzyl Salicylate has been used safely in cosmetic preparations for decades. It is used in the formulation of fragrances in cosmetics as it has a sweet odour. Benzyl Salicylate is used globally in a wide range of cosmetics [Lapczynski *et al.,* 2007; CIR 2019a]. The EU Cosmetic Regulation (Annex III-entry 75) does not restrict the use of Benzyl Salicylate in cosmetics and personal care products, but states that "its presence must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products". It can also be used as an UV absorber (CosIng database, consulted 04/05/23).

- Other non-cosmetic dermatological uses

Benzyl Salicylate can also be used in pediculicides for treatment of lice in humans and animals.

- Other non-cosmetic uses

Benzyl Salicylate is also used as a fragrance in household cleaners and it has been approved as a flavour by the US FDA in accordance with (21 CFR 172.515). Benzyl Salicylate has been granted Generally Recognized as Safe (GRAS) status as a flavouring ingredient by the Flavor and Extract Manufacturers Association (FEMA) (Adams *et al.,* 2005). In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the substance does not present a safety concern at current low levels of intake when used as a flavouring agent (JECFA, 2001).

3.3.2 Calculation of SED/LED

Separate exposure assessments were performed by the Applicant for the dermal and inhalation routes. The use in oral care products is low and leads to negligible exposure. As

the deterministic approach yields a favourable outcome, further exposure modelling was not necessary to refine exposure.

3.3.2.1 Calculation of Systemic Exposure Dose (SED) following dermal exposure

To estimate an 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 by the Applicant and is presented in Table 6, with the addition of an 18th product type – hydroalcoholic fragrances.

Scenario A uses the maximal concentrations of Benzyl Salicylate in products in weight %, and Scenario B uses the P90 of concentrations used in products. Maximal and P90 use levels are taken from an RIFM survey performed in 2018. In this approach the exposures for individual product types are calculated, but also aggregated in a worst-case assumption. Exposure Scenarios A and B assume 100% occurrence of Benzyl Salicylate at the maximum potential % use concentration in all cosmetics products used simultaneously by an individual in a day.

A measured value of 10.5% (see section 3.2) is used for skin absorption of the dermally applied substance from all products.

- Deterministic worst case aggregated exposure assessment for the dermal route

The starting point is a Tier 1 Scenario A deterministic exposure assessment based on maximum concentrations of Benzyl 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 'Benzyl Salicylate consortium' and are used to calculate the total systemic exposure to Benzyl Salicylate (in mg/kg/day) from each product for adults (see Table 6).

Benzyl Salicylate is also used as a fragrance ingredient in hydroalcoholic fragrances (e.g. aftershave, eau de toilette, cologne) and, therefore, this is added in this case into the calculation as an 18th product type in Table 6. The exposure to product (Eproduct) value normalised by weight is calculated by including the respective retention factor for each product type (SCCS, 2021).

A measured value for skin penetration of Benzyl Salicylate of 10.5% (see section 3.2) has been used for all products in these calculations where dermal absorption needs to be factored in to calculate a systemic exposure dose (SED). For lipstick and oral care products a worst-case value of 100% absorption is used to account for passage across the oral mucosa and/or 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 6).

Table 6: Tier 1 Scenario A Deterministic worst case systemic exposure dose calculation for the dermal route using maximum % levels of Benzyl Salicylate. (NB. exposure to spray/aerosol products are taken into account in section 3.3.2.3 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) per product	Total dermal exposure (mg/kg bw/day) Per product	% absorbed dermally	Calculated SED[2] (mg/kg bw/day) Per product
Hydroalcoholic-based fragrances (non-spray)		4	4.67*	0.1868	10.5	0.0196
Rinse-off skin & hair products	Shower gel	1.3	2.79	0.03627	10.5	0.0038
	Hair conditioner	0.5	0.67	0.00335		0.0004
	Shampoo	0.5	1.51	0.00755		0.0008
	Hand wash soap	0.5	3.33	0.01665		0.0017
Leave on skin & hair products	Body lotion	0.7	123.20	0.8624	10.5	0.0906
	Face cream	0.5	24.14	0.12070		0.0127
	Hand cream	0.5	32.70	0.16350		0.0172
	Deodorant non-spray	0.5	22.08	0.11040		0.0116
	Hair Styling	0.5	5.74	0.02870		0.0030
Face Make-up products	Liquid foundation	0.2	7.90	0.01580	10.5	0.0017
	Lipstick, lip salve[3]	0.2	0.90	0.00180	100	0.0018
	Make-up remover for face	0.2	8.33	0.01666	10.5	0.0017
	Eye make-up	0.2	0.33	0.00066		0.0001
	Mascara	0.2	0.42	0.00084		0.0001
	Eyeliner	0.2	0.08	0.00016		0.00002
Oral care products	Toothpaste[3]	0.004	2.16	0.00009	100	0.0001
	Mouthwash[3]	0.004	32.54	0.00130	100	0.0013
	Aggregate					**0.179**

1.According to values in Table 3 of the SCCS 2021 notes of guidance; 2. Total external dermal exposure x 10.5% skin absorption;
3. 100% oral/mucosal absorption is applied here as a worst case assumption; retention factors have already been factored into the $E_{product}$ calculation in Table 3 of the SCCS 2021 notes of guidance, and it is assumed all of the retained benzyl salicylate can enter the systemic circulation via dermal and oral routes.
* Data from Ficheux & Roudot 2017.

Table 7 shows the outcomes following a similar method but using P90 % use levels for the same set of product types as determined in a RIFM survey from 2018. This provides reassurance that current use levels are in reality lower than the maximal values.

Table 7: Tier 1 Scenario B Deterministic aggregate exposure calculations for the dermal route using P90 use levels as defined by RIFM in 2018. Values of SED are calculated according to the SCCS 11th notes of guidance approach to calculating aggregate exposure on the basis of deterministic additive methods. Benzyl Salicylate is assumed to undergo 10.5% skin penetration for all product types (except oral care and lipstick where 100% is assumed) in this assessment.

	Examples of Product	P90 concentration (w/w%) in the finished product	$E_{product}$ normalised by body weight [1] (mg/kg bw/day) per product	Total dermal exposure (mg/kg bw/day) Per product	% absorbed dermally	Calculated SED[2] (mg/kg bw/day) Per product
Hydroalcoholic-based fragrances (non-spray)		1.2	4.67*	0.05604	10.5	0.0059
Rinse-off skin & hair products	Shower gel	0.09	2.79	0.00251	10.5	0.00026
	Hair conditioner	0.05	0.67	0.00034		0.00004
	Shampoo	0.05	1.51	0.00076		0.00008
	Hand wash soap	0.09	3.33	0.00299		0.00031
Leave on skin & hair products	Body lotion	0.22	123.20	0.27104	10.5	0.02846
	Face cream	0.025	24.14	0.00604		0.00063
	Hand cream	0.033	32.70	0.01079		0.00113
	Deodorant non-spray	0.11	22.08	0.02429		0.00255
	Hair Styling	0.03	5.74	0.00172		0.00018
Face Make-up products	Liquid foundation	0.02	7.90	0.00158	10.5	0.00017
	Lipstick, lip salve[3]	0.01	0.90	0.00009	100	0.00009
	Make-up remover for face	0.03	8.33	0.00250	10.5	0.00026
	Eye make-up	0.011	0.33	0.00004		0.000004
	Mascara	0.011	0.42	0.00005		0.000005
	Eyeliner	0.011	0.08	0.000009		0.0000009
Oral care products	Toothpaste[3]	0.0002	2.16	0.000004	100	0.000004
	Mouthwash[3]	0.000003	32.54	0.000001	100	0.000001
	Aggregate					**0.04**

1.According to values in Table 3 of the SCCS 2021 notes of guidance; 2. Total external dermal exposure x 10.5% skin absorption;
3. 100% oral/mucosal absorption is applied here as a worst case assumption; retention factors have already been factored into the $E_{product}$ calculation in Table 3 of the SCCS 2021 notes of guidance, and it is assumed all of the retained benzyl salicylate can enter the systemic circulation via dermal and oral routes.
* Data from Ficheux & Roudot 2017.

SCCS comment
The SCCS considers Scenario A "Deterministic worst case systemic exposure dose calculation for the dermal route using maximum % levels of Benzyl Salicylate" as the more appropriate as it also covers some highly concentrated products. Therefore, the SCCS will use Scenario A for the MoS calculation.

3.3.2.2 Calculation of Systemic Exposure Dose (SED) – inhalation route

Based on the vapor pressure of Benzyl Salicylate, inhalation exposure through volatilisation is not expected.

Therefore, the systemic exposure through inhalation will be performed only for the sprayed fraction of finished products.

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 on page 27.

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

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;)**

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

Calculation of SED_{inh} for Benzyl Salicylate in four main spray products (hydroalcoholic fragrance spray, deodorant spray, hair spray and body lotion spray) are presented in Table 8, 9, 10and 11, respectively.

Table 8: Deterministic systemic exposure dose after inhalation exposure (SED_{inh}) to 4% Benzyl Salicylate in a **hydroalcoholic fragrance spray formulation** (e.g. eau de toilette, perfume, cologne etc)

Description	Parameter	Pump spray	Unit
Amount by application*	A	280*	mg/application
Fraction of benzyl salicylate in non-propellant	C	4	(% 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	2.24	mg
First step: near-field, 1m³	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)	**0.058**	mg
Second step: far-field 10m³	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)	**0.058**	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	**0.015**	**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. Also, the frequency of application refers to a mean value, according to Ficheux & Roudot, 2017. Therefore, the SCCS has recalculated the presented exposure with the P95 of 618 mg/application. This results in an exposure of 0.032 µg/kg/d to hydroalcoholic fragrance spray.

Table 9: Deterministic systemic exposure dose after inhalation exposure (SED_{inh}) to 0.91% Benzyl Salicylate in **a deodorant spray formulation**

Description	Parameter	Propellant spray	Unit
Amount by application*	A	3050*	mg/application
Fraction of benzyl salicylate in non-propellant	C	0.91	(% 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	14.75	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)	**0.384**	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)	**0.384**	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}	$(IA_1+IA_2)*G*RF*F/BW$	**3.84**	**µg/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). α20% for aerosolised deodorants (Delmaar & Bremmer, 2009).

SCCS comment

The value of 0.886 for the airborne fraction has been derived based on a 2012 study by Steiling *et al.* that reports a worst case for dermal exposure to deodorant (i.e. 11.4% deposited). Since the worst-case inhalation exposure cannot be derived from subtracting a worst-case dermal exposure from 100%, and since no data were provided, the SCCS uses 100% availability as a worst case. The resulting exposure is 4.64 µg/kg/day for inhalation exposure to deodorant.

Table 10: Deterministic systemic exposure dose after inhalation exposure (SED_{inh}) to 0.5% Benzyl Salicylate **in hair spray formulations**

Description	Parameter	Propellant spray	Pump spray	Unit
Amount by application	A	5965*	3158**	mg/application
Fraction of benzyl salicylate in non-propellant	C	0.5	0.5	(% 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	17.9	3.2	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)	**0.465**	**0.083**	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)	**0.465**	**0.083**	mg
Substance availability fraction	G	0.75	0.75	
Respirable fraction α	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	**2.6**	**0.024**	**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). α 1% for pump hairspray and 20% for aerosol (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, for propellant spray SCCS uses the P95 (9890 mg/application) of Loretz *et al.*, 2006 for daily use combined with a frequency of 1. This results in an exposure value of 3.86 µg/kg/d. For pump spray, the amount is a P50 derived from Loretz *et al.*, 2006. The SCCS therefore uses the P95 (15620 mg/application) from Loretz *et al.*, 2006 for daily use in combination with a frequency of 1. This results in an exposure value of 0.102 µg/kg/d for pump spray.

Table 11: Systemic exposure dose (SED) after inhalation exposure to 0.7% Benzyl Salicylate in a **body lotion spray formulation**

Description	Parameter	Propellant spray	Pump spray	Unit
Amount by application	A	5720*	3430*	mg/application
Fraction of benzyl salicylate in non-propellant	C	0.7	0.7	(% 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	24.0	4.8	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)	**0.624**	**0.124**	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)	**0.624**	**0.124**	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	**7.11**	**0.07**	**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. [α]Assumed similar to sunscreen lotion products (SCCS 2021). [$]Table 4 of the 11th SCCS NoG (2021)

SCCS comment

The SCCS agrees with the Applicant's calculation of systemic exposure dose from the use of Benzyl 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 Benzyl Salicylate in cosmetic products is 179 µg/kg/day. This value will be taken forward into the safety evaluation.

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 7 will be used, not both simultaneously. Typically, the non-spray version of a product leads to the higher SED, and inhalation exposure (in the range here from 0.015 to 7.11 µg/kg/day) is often much lower than dermal exposure.

SCCS comment

SCCS agrees that for most of the products, the non-spray products lead to a much higher systemic exposure when compared to the spray products. The exceptions are the hair styling products: systemic exposure *via* non sprayed hair styling products is 3 µg/kg/d whereas it is 3.86 µg/kg/d when using hair styling spray products. Therefore, for the MoS calculation, for all products except hair styling products, only non-spray products will be considered in a worst-case scenario.

3.4 TOXICOLOGICAL EVALUATION

For the hazard assessment of Benzyl Salicylate, the Applicant mainly relied on several literature reviews such as Belsito *et al.,* 2007; Lapczynski *et al.* (2007); Cosmetics Ingredient Review (2019a) and on the EU REACH substance dossier available at https://echa.europa.eu/registration-dossier/-/registered-dossier/16100

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

- Animal data

The available irritation studies in animals are listed in Table 12.

Table 12: Skin irritation studies in animals

Method	Dose	Species	Results	References
Irritation evaluated as a part of LD_{50} study	100%	Rabbit	No irritation	RIFM (1970b)
24-h closed patch test	10% in alcohol SDA39C	Rabbit	No irritation	RIFM (1975d)
OECD guideline 404 GLP compliant	0.5ml neat applied for 4h; observations at 1,24,48,72 and 168h after patch removal.	Rabbit	No irritation	RIFM (1984) & RIFM (1985)
Irritation evaluated during pre-test and induction phase of OET	0.1%	Guinea pig	Minimal irritating concentration	Klecak *et al.* (1977)
Irritation evaluated during phototoxocity test	5% and 10% in ethanol	Guinea pig	No irritation	RIFM (1983a)
Irritation evaluated during phototoxicity test	5%, 10% and 30% in acetone	Guinea pig	No irritation observed at 5%; irritation observed at 10% and 30%	RIFM (1997b)
Irritation evaluated as a part of Draize test	2%	Guinea pig	Irritation observed (ACC)	Sharp (1978)

In a study according to OECD guideline 404 (RIFM 1985), approximately 0.5mL of neat Benzyl Salicylate was applied to the shorn flanks of three rabbits, wrapped in semi-occlusive dressing for a period of 4 hours. One hour after the dosing period, well defined erythema of the treated skin was apparent in one rabbit and very slight erythema was observed in the remaining three rabbits of the group. Slight oedema was noted in one rabbit and very slight oedematous reaction was observed in the treated skin of the second rabbit at this time. Very slight erythema remained in three rabbits at 24-hour observation, very slight oedema also being observed in two rabbits. This response declined, very slight erythema remaining in two animals 72 hours after dosing and no irritation remaining visible 7 days after dosing.

The averages calculated from the numerical values given to the irritation observed at the 24, 48 and 72 -hour observations were 0.6 for erythema and 0.2 for oedema.

The effects seen do not trigger classification according to Regulation (EC) No. 1272/2008 (CLP).

- Human data

A range of skin irritation tests in humans are presented in Table 13.

Table 13: Skin irritation studies with Benzyl Salicylate in humans

Method	Dose (%)	Vehicle	Results		References
			Reactions	Incidence (%)	
Induction (HRIPT)	5	Dimethyl phthalate	0/8	0	RIFM (1968)
Induction (HRIPT)	10	Alcohol SDA39C	0/35	0	RIFM (1975c)
Induction (HRIPT)	15	3:1 DEP:EtOH	0/101	0	RIFM (2004a)
Maximisation (pre-test)	30	Petrolatum	0/5	0	RIFM (1975a)
Maximisation (pre-test)	30	Petrolatum	2/22	9.09	RIFM (1975b)
48 h closed patch test	20	Petrolatum or Unguentum cream	0/5	0	Fujii *et al.* (1972)
24-72 h closed patch test	2	Unguentum cream	0/30	0	Fujii *et al.* (1972)
24-48 h closed patch test	0.2	Ethanol	5/313	1.6	Fujii *et al.* (1972)
24 h closed patch test	5	Petrolatum	0/25	0	RIFM (1997a)
4 h closed patch test	100	N/A	0/30	0	Basketter *et al.* (2004)

Human repeat insult patch test (HRIPT) studies:

In agreement with earlier studies (RIFM 1968, 1975c), RIFM (2004a) observed no irritation in the induction phase of a HRIPT (n = 29 males and n = 72 females). 0.3 ml of 15% Benzyl Salicylate in 3:1 DEP:EtOH vehicle was applied to an adhesive patch (25 mm Hilltop® Chamber System) and then applied to the back of each subject for 24h. Nine induction patches were completed based on Monday, Tuesday, Wednesday and Friday schedule over a period of approximately three weeks. Reactions were scored at patch removal. **No irritation was observed.**

Human maximisation tests:

No irritation was observed following 48-h closed patch with 30% Benzyl Salicylate in petrolatum applied to normal sites on the volar forearms of five volunteers (RIFM, 1975a). In a second study, two irritant reactions were observed from 22 male volunteers conducted with 30% Benzyl Salicylate in petrolatum in a 48h patch under occlusion (RIFM, 1975b).

Closed patch tests:

Benzyl Salicylate at 20% in vaseline or unguentum cream applied to the back of each subject (n = 5 males; n = 5 females) produced no irritation (Fujii *et al.*, 1972). Irritation was not observed when a 24–72h closed patch with 2% Benzyl Salicylate in an unguentum cream was applied to the upper inside of arm of 30 male and female volunteers (Fujii *et al.*, 1972). **Five (5/313) positive reactions were observed** when 0.2% Benzyl Salicylate in 99% ethanol was applied under occlusion for 24–48h to the upper inside of arm of 313 volunteers (Fujii *et al.*, 1972).

Benzyl Salicylate at 5% in petrolatum was applied to adhesive patches for 1 or 24h which were then applied to the upper arms of each subject (n = 12 males; n = 13 females). **No irritation was observed** (RIFM, 1997a).

The potential of Benzyl Salicylate to produce irritation was evaluated in a patch test which was conducted on 30 volunteers. A 0.2 ml aliquot of neat Benzyl Salicylate was applied using 25 mm Hilltop® Chambers which were then applied to the skin of the upper outer arm for up to 4h. Benzyl Salicylate was applied progressively for 15 and 30 min through 1, 2, 3 and 4h, each, to a new skin site. Reactions were scored at 24, 48 and 7 h after patch removal. **No irritation was observed** (Basketter *et al.*, 2004).

Skin Irritation & Corrosivity Applicant Conclusion:

Overall, there is no evidence of a skin irritation potential of Benzyl Salicylate in humans at a range of high concentrations, including neat. Consequently, there is no risk of skin irritation at the maximum concentrations used in cosmetic products.

3.4.1.2 Mucous membrane irritation / eye irritation

- *In vitro* data

A GLP-compliant OECD Guideline 437 (Bovine Corneal Opacity and Permeability Test for Identifying Ocular Corrosives and Severe Irritants) is available (Givaudan report, 2012).

Method:

0.75 mL of the neat Benzyl Salicylate was applied to triplicate corneas. The undiluted test material was applied for 10 minutes followed by an incubation period of 120 minutes. At the end of the exposure period the test material was removed from the anterior chamber and each cornea was rinsed three times with fresh complete MEM containing phenol red before a final rinse with complete MEM. Results from the two test method endpoints, opacity and permeability, were combined in an empirically derived formula to generate an *In Vitro* Irritancy Score. Opacity Measurement- The change in opacity for each cornea (including the negative control) was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value of each treatment group was then calculated by averaging the corrected opacity values of each cornea for that treatment group.

Permeability Measurement - The corrected OD492 was calculated by subtracting the mean OD492 of the negative control corneas from the OD492 value of each treated cornea. The OD492 value of each treatment group was calculated by averaging the corrected OD492 values of the treated corneas for the treatment group. The following formula was used to determine the *in vitro* score:

In Vitro Irritancy Score = mean opacity value + (15 x mean OD492 value)

A test material that induces an *in vitro* irritancy score > than or equal to 55.1 is defined as an ocular corrosive or severe irritant. Additionally, the opacity and permeability values were evaluated independently to determine whether the test material induced a response through only one of the two endpoints. The condition of the cornea was visually assessed immediately after rinsing and at the final opacity measurement.

The test results are summarised in Table 14.

Table 14: Summary of results from the OECD Guideline 437 study on Benzyl Salicylate

Treatment	*In Vitro* Irritancy Score
Benzyl Salicylate	0.0
Negative Control	3.8
Positive Control	31.2

- *In vivo* data

A Draize rabbit eye study is available, that was performed pre-GLP but as the study was similar to OECD guideline 405 and conducted in accordance with good scientific principles, the data reported was sufficient to **classify the substance as a mild to moderate eye irritant**, and in the interest of animal welfare it was considered unecessary to repeat the study (ECHA, 2020).

Approximately 0.5mL of neat test substance was instilled into the eyes of three albino rabbits. 1, 2, 3, 4, 7 and 10 days after exposure reactions were recorded. The effects on the conjunctivae (redness) were sufficient in 2 out of 3 animals tested to trigger classification as category 2 eye irritation in accordance with Regulation (EC) 1272/2008 (CLP).

All adverse reactions were reversed between 3-7 days.

Eye Irritation Applicant Conclusion:
Benzyl Salicylate was not an eye irritant *in vitro*/ex vivo, but did show irritant properties in a pre-GLP *in vivo* Draize rabbit eye test in undiluted form. However, there is no risk of eye irritation at the maximum concentrations used in cosmetic products.

SCCS comment
SCCS considers that Benzyl Salicylate is non irritating to the skin but may cause eye irritation.

3.4.2 Skin sensitisation

- Animal data

All of the available skin sensitisation studies in guinea-pig are listed in Table 15 below. And two murine LLNA studies in Table 16.

Table 15: Available skin sensitisation studies for Benzyl Salicylate in guinea-pigs

Method	Concentration	Results	References
GPMT	5% and 25% in white petrolatum	Not sensitising	Klecak *et al.* (1977)
GPMT	1% in ethanol for induction; 100% for challenge	Not sensitising	Tsuchiya *et al.* (1982)
GPMT	10% for induction and challenge	Sensitiser	Ishihara *et al.* (1986)
GPMT	10% in liquid paraffin for intradermal induction; 30% in ethanol for topical induction; 0.003%, 0.01% and 0.03% in liquid paraffin for challenge	Sensitiser	Kashima *et al.* (1993)
GPMT	10% in liquid paraffin for intradermal induction; 50% in white petrolatum for topical induction; 5%, 10% and 20% in white petrolatum for challenge	No sensitisation at 5% and 10%; Sensitiser at 20%	Kozuka *et al.* (1996)
GPMT	10% in FCA for induction; 5%, 10% and 20% in acetone for challenge	Sensitiser	RIFM (1997c)
OET	0.03% and 30%	0.03% – minimum eliciting concentration; 30% – minimum sensitisation concentration	Klecak *et al.* (1977)
OET	10% for induction and challenge	Not sensitising	Klecak (1979)
OET	30% for induction and challenge	Not sensitising	Klecak (1985)
CCET	10%, 30% and 100% for induction application; 50% for challenge	No sensitisation at 10%; Sensitiser at 30 and 100%	Tsuchiya *et al.* (1982)
CET	30% – induction; 1% – challenge	Sensitiser (3/20)	Ishihara *et al.* (1986)
CCET	30% in ethanol for induction; 1%, 3% and 10% in ethanol for challenge	Sensitiser	Kashima *et al.* (1993)
CCET	100% for induction application; 50% in ethanol for challenge application	Sensitiser	Imokawa and Kawai (1987)
DCHA	30% for induction application: 1%, 3% and 10% for challenge	Sensitiser	Kashima *et al.* (1993)
FCAT	50% in FCA for induction application; 0.1% for challenge application	Not sensitising	Klecak *et al.* (1977)
Modified FCAT	10% in FCA for intradermal induction; 10% in acetone for challenge	Sensitiser	Hausen and Wollenweber (1988)
DRAIZE (Modified)	0.1% in isotonic saline for intradermal induction; 0.1% in isotonic saline for challenge	Not sensitising	Klecak *et al.* (1977)
DRAIZE (Modified)	0.5% for intradermal induction; 0.5% for intradermal challenge and 2% for dermal challenge	Not sensitising	Sharp (1978)

Abbreviations: Guinea-pig maximisation test (GPMT); Open Epicutaneous Test (OET); Closed Epicutaneous Test (CET); cumulative contact enhancement test (CCET); delayed contact hypersensitivity assay (DCHA); Freund's complete adjuvant test (FCAT); DRAIZE test (Draize, 1959).

Table 16: Murine local lymph node assays (LLNA) for Benzyl Salicylate

EC3 value (%)	EC3 value (µg/cm²)	Vehicle	References
2.9	725	DEP : EtOH	RIFM (2005)
1.5	375	Acetone:olive oil	Yoshida *et al.* (2000)

A GLP compliant OECD Guideline 429 murine LLNA is available (RIFM, 2005) and acts as the pivotal animal sensitisation assay for Benzyl Salicylate.

Five groups of n=4 animals received Benzyl Salicylate at 2.5, 5, 10, 25 or 50% in 1:3 ethanol/diethylphthalate and a negative control group of n=4 animals received vehicle. α-hexylcinnamaldehyde (HCA) was used successfully as a positive control to validate the study. 25 µL of benzyl benzoate or vehicle was applied to each ear for three consecutive days. Following two rest days, each animal received a single intravenous injection of 250 µl of phosphate buffered saline containing 20 µci of 3H-TdR. Approximately 5 hours later, auricular lymph nodes were excised, and lymphocyte proliferation was quantified by beta scintillation counting. The Stimulation Index (SI) was obtained by mean dpm per node of treated divided by mean dpm per node of vehicle control group. The SI of 2.6, 5.5, 6.0, 18.9 and 26.2 were observed for 2.5, 5, 10, 25 or 50% w/v Benzyl Salicylate, respectively.

Under conditions of this study, Benzyl Salicylate induced contact sensitization and an EC3 value was calculated to be 2.9%, or 725 µg/cm².

- Human data

There have been a few case reports indicating Benzyl Salicylate exposure may lead to contact allergy (Fernández-Canga *et al.,* 2017; Tous-Romero *et al.,* 2018). However, this is a rare observation; Benzyl Salicylate is not a common allergen in the population (Schnuch, 2007). This is supported by the results of human repeated insult patch tests (HRIPT) and maximization (HMT) tests for Benzyl Salicylate indicating that Benzyl Salicylate may be only a weak sensitiser in humans, if any (Table 17).

Table 17: Confirmative human *in vivo* study data for skin sensitisation

Test method	Test concentration	Results		References
		Reactions	Incidence (%)	
HRIPT	5% in dimethyl phthalate	0/52	0	RIFM (1968)
HRIPT	10% in alcohol SD39	0/35	0	RIFM (1975c)
HRIPT	15% in DEP:EtOH	0/101	0	RIFM (2004a)
HMT	30% in petrolatum	0/25	0	RIFM (1970c)
HMT	30% in petrolatum	0/25	0	RIFM (1975a)
HMT	30% in petrolatum	0/22	0	RIFM (1975b)
HMT	20% in petrolatum	1/25	4	RIFM (1979)
HMT	20% in petrolatum	2/25	8	RIFM (1980)

Human repeat insult patch test (HRIPT); human maximization test (HMT)

Diagnostic patch testing in human volunteers has also been performed for Benzyl Salicylate

Table 18: Summary of human diagnostic patch test studies

Concentration tested	Results		References
	Reactions	Incidence (%)	
0.1% in petrolatum	1/65	1.54	Kozuka *et al.* (1996)
0.2% in perfumed base cream	3/313	0.96	RIFM (1974)
0.2%, 1%, or 10% in ethanol	0/10538	N/A	Kohrman *et al.* (1983)
0.05–0.5% in a base cream or 99% ethanol	5/313	1.6	Takenaka *et al.* (1986)
1% in petrolatum	5/180	2.78	Ishihara *et al.* (1979)
1% in petrolatum	6/394	1.52	Ueda (1979)
1%, 2%, 5% in petrolatum	1/394	0.25	Ueda (1979, 1994)

Concentration tested	Results		References
	Reactions	Incidence (%)	
1% in petrolatum	6/394	1.5	MJDRG (1984)
1% in petrolatum	0/100	N/A	Frosch *et al.* (1995b)
1% in petrolatum	3/201	1.49	Kozuka *et al.* (1996)
1% in petrolatum	3/201	1.49	Kozuka *et al.* (1996)
1% in petrolatum	3/747	0.4	Wohrl *et al.* (2001)
2% in an unspecified vehicle	4/183	2.1	Rudner (1977, 1978)
2% in petrolatum	9/180	5.0	Ishihara *et al.* (1979)
2% in petrolatum	9/394	2.28	Ueda (1979)
2% in paraffin	1/457	0.22	Addo *et al.* (1982)
2% in petrolatum	77/1255	6.1	Sugai (1982)
2% in petrolatum	9/394	2.3	MJDRG (1984)
2% in an unspecified vehicle	13/200	6.5	Asoh *et al.* (1985a)
2% in petrolatum	5/157	3.18	Hayakawa (1986)
2% in petrolatum	38/788	4.8	Sugai (1986)
2% in petrolatum	1/89	1.12	Nethercott *et al.* (1989)
2% in petrolatum	8/436	1.83	Nagareda *et al.* (1992)
2% in petrolatum	7/706	0.99	Katoh *et al.* (1995)
2% in petrolatum	5/167	3	Larsen *et al.* (1996)
2% in petrolatum	4/482	0.83	Nagareda *et al.* (1996)
2% in petrolatum	1/386	0.26	Sugai (1996)
2% in an unspecified vehicle	2/103	1.94	Fujimoto *et al.* (1997)
2% in petrolatum	10/1825	0.5	deGroot *et al.* (2000)
2% in petrolatum	3/102	2.94	Hausen (2001)
5% in petrolatum	16/254	6.29	Ishihara *et al.* (1979)
5% in petrolatum	23/394	5.84	Ueda (1979)
5% in petrolatum	20/362	5.52	Ishihara *et al.* (1981)
5% in an unspecified vehicle	12/155	7.74	Itoh (1982)

Concentration tested	Results		References
	Reactions	Incidence (%)	
5% in petrolatum	14/176	7.95	Shoji (1982)
5% in petrolatum	12/212	5.66	Hada (1983)
5% in petrolatum	25/181	13.8	Hayakawa *et al.* (1983)
5% in petrolatum	23/394	5.8	MJDRG (1984)
5% in an unspecified vehicle	24/522	4.6	Nishimura *et al.* (1984)
5% in an unspecified vehicle	27/680	3.97	Itoh *et al.* (1986)
5% in an unspecified vehicle	30/756	4	Itoh *et al.* (1988)
5% in petrolatum	1/64	1.6	Haba *et al.* (1993)
5% in petrolatum	1/100	1	Frosch *et al.* (1995b)
5% in petrolatum	8/167	4.8	Larsen *et al.* (1996)
5% in petrolatum	0/315	N/A	Heydorn *et al.* (2002)
5% in petrolatum	2/658	0.3	Heydorn *et al.* (2003)

- *In silico* and *in vitro* data

The chemical structure of Benzyl Salicylate indicates that it theoretically has intrinsic reactive properties, and it would be expected to react with skin proteins directly (Roberts, 2007; OECD toolbox v 4.3), but it would have to penetrate the skin to act as a skin sensitiser.

Benzyl Salicylate was found to be negative in the *in vitro* direct peptide reactivity assay (DPRA); 1% and 2.65% mean depletion of cysteine and lysine peptides was observed, respectively, demonstrating minimal reactivity of Benzyl Salicylate to skin proteins (RIFM, 2014; Urbisch, 2015; Avonto, 2019).

In two KeratinoSens assays, Benzyl Salicylate induced ARE dependent gene activity with an EC1.5 of 9.63 µM and 8.42 µM, respectively (RIFM, 2015; Emter, 2010) and in the U-Sens assay, induced CD86 expression with an EC150 of 63 µg/mL (276.03 µM) (Piroird, 2015). In an *in vitro* assay looking at the upregulation of IL-18, Benzyl Salicylate showed no response (Galbiati *et al.,* 2017). These assays represent key events in the adverse outcome pathway (AOP) for skin sensitisation as developed by the OECD (2012).

Sensitisation Applicant Conclusion:

The skin sensitisation potential of Benzyl Salicylate has been extensively investigated. Overall, from the weight of evidence, the available data indicate that Benzyl Salicylate is only a weak sensitiser in humans. This is supported by the available data from *in vitro* or *in chemico* new approach methodologies (NAMs) predicting the lack of a relevant sensitisation hazard as well as the absence of a skin sensitisation potential of salicylic acid. In addition, in both animal tests and studies in humans, a relevant sensitisation response was only induced at concentrations higher than the maximum concentrations used in cosmetic products.

Nevertheless, the SCCS opinion on fragrance materials (SCCS, 2011) lists Benzyl Salicylate as a contact allergen in humans that is less frequently reported compared to other allergens. Consequently, it is one of the 26 fragrance allergens currently requiring individual labelling in the EU. Risk management of the use of Benzyl Salicylate as a fragrance material is very effective through the use of quantitative risk assessment (QRA) within the IFRA Standards and its use is not considered to present a significant problem in the consumer population. Such QRA method has been further refined within the International Dialogue for the Evaluation of Allergens IDEA (www.ideaproject.info).

SCCS comment
Benzyl Salicylate is classified as a Category 1B skin sensitiser in CLP.
Benzyl Salicylate is listed as a contact allergen in humans (SCCS, 2011). Consequently, it is one of the fragrance allergens that currently require individual labelling in the EU.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

There is one study in animals covering the acute oral toxicity of Benzyl Salicylate as summarised in Table 19.

Table 19: Acute oral toxicity studies for Benzyl Salicylate

Reference	Species	Dosing (g/kg)	Oral LD_{50} (g/kg)	Observed effects
RIFM 1970a	Rat	1.25, 2.5, or 5.0	2.23 (range 1.93–2.58)	1.25 g/kg, 0/6 deaths 2.5 g/kg 4/6 deaths 5.0 g/kg (6/6) deaths

Three groups of rats (6/dose) weighing approximately 100–200 g were dosed orally (gavage) at levels of 1.25, 2.5, or 5.0 g/kg. The calculated LD_{50} was 2.23 g/kg (1.93–2.58 g/kg). Observations for mortality and systemic effects were made over a 7-day period. At 1.25 g/kg, no deaths (0/6) were observed; 4/6 deaths were observed at 2.5 g/kg and all (6/6) animals died at 5.0 g/kg. The principal toxic effect observed before death was depression (RIFM, 1970a).

3.4.3.2 Acute dermal toxicity

The acute dermal data is summarised in Table 20.

Table 20: Acute dermal toxicity study for Benzyl Salicylate

Reference	Species	Dosing	Dermal LD_{50} (g/kg bw)	Observed effects
RIFM 1970b	Rabbit (albino) n = 3 per dose	5, 10, or 20 g/kg applied neat to clipped area of skin, 24h occluded	14.15 g/kg (95% CI 4.56–43.86 g/kg)	5 g/kg 0/3 deaths 10 g/kg 1/3 deaths 20 g/kg 2/3 deaths Depression, slow respiration, loss of righting reflex and coma.

The animals in this study were observed daily for a period of seven days for any signs of systemic toxicity. On day 5, blood was drawn from the marginal ear vein for haematology and clinical chemistry evaluation. No effects were observed at 5.0 g/kg. Animals that received the 10 and 20 g/kg dosage were depressed and showed slow respiration. On day 5, one of three rabbits (1/3) at the 10 g/kg level and two (2/3) at the 20 g/kg level died. Among animals dying, intoxication persisted, and gradually deepened to severe depression, loss of the righting reflex, coma and death. Survivors appeared normal on day 5. No significant gross pathology was noted in animals that died during the study. Haematological and clinical chemistry values from the survivors were within normal limits when compared to control

animals, except for a low hemoglobin value that was noted for the only survivor animal treated at the 20 g/kg level (RIFM, 1970b).

3.4.3.3 Acute inhalation toxicity

There are no acute inhalation toxicity data available in animals for Benzyl Salicylate. The use of Benzyl Salicylate in consumer products for decades has revealed no adverse effects in the lung. Benzyl 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.

Given hydrolysis of Benzyl Salicylate has been shown to occur via lung microsomes *in vitro* (Ozaki *et al.*, 2015), it may be relevant to consider the benzyl alcohol data that is available, showing the very low toxicity of benzyl alcohol in the lung. Also, there are data on acute inhalation for methyl salicylate to provide supporting evidence that salicylates in general are not acute lung toxicants.

Conclusion from the Applicant on acute Toxicity

Benzyl Salicylate is only acutely toxic at very high doses.
The LD50 via the oral route in rabbits was 2.23 g/kg.
The LD50 via the dermal route was g/kg was in the range 4.6 to 43.9 g/kg.
Benzyl Salicylate is not expected to be irritating or toxic to respiratory tract and lung.

SCCS comment
Benzyl Salicylate is acutely toxic at high doses.

3.4.4 Repeated dose toxicity

No repeat dose toxicity data were available on Benzyl Salicylate, as performed before the March 2013 animal testing ban. For the purposes of performing a cosmetics safety assessment and given that Benzyl Salicylate itself is not regarded as the main toxicant, but salicylic acid as the chief hydrolysis product, this section also discusses the available repeat dose toxicity data and conclusions for the primary Benzyl Salicylate metabolites salicylic acid and benzyl alcohol (Belsito *et al.*, 2007).

All data for salicylic acid were recently reviewed by the SCCS in its recent opinion (SCCS/1646/22) and therefore are not reported in this opinion.

Concerning benzyl alcohol, a summary of the repeat dose oral studies cited in the ECHA REACH registration dossier for benzyl alcohol (https://echa.europa.eu/registration-dossier/-/registered-dossier/14748/7/6/2) are presented in Table 21.

Table 21: Oral (gavage) repeat dose studies in animals for benzyl alcohol

Study duration	Species	Doses mg/kg/day	Observations	Reference
13-week dose-finding study	Rat (male and female)	0, 50, 100, 200, 400, 800	NOAEL 400 mg/kg/day, based on mortality (partially related to the gavage procedure), signs of neurotoxicity and reduced body weight gain at 800 mg/kg/day	NTP (1989)
13-week dose-finding study	Mice (male and female)	0, 50, 100, 200, 400, 800	NOAEL 400 mg/kg/day, based on temporary clinical signs and slightly reduced body weight gain at 800 mg/kg/day.	NTP (1989), US EPA (2009)

The NOAEL from these studies was regarded as 400 mg/kg/day which was subsequently confirmed in an OECD 451 carcinogenicity study. EFSA (2019) also used this NOAEL to determine an ADI for benzyl alcohol in foods of 4 mg/kg/day.

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

14 days range finding study mentioned in the reach dossier.

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

- Oral route

Following ECHA decision (CCH-D-2114379324-45-01/F) on Benzyl Salicylate it was requested to conduct additional toxicological studies:
Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance.

The objective of the following study was to evaluate the potential toxicity of the Benzyl Salicylate when administered via the diet to Sprague Dawley rats for at least 90 consecutive days.

Guideline:	OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents)
Species/strain:	female and male Sprague-Dawley rats
Group size:	4 groups of 10 male and 10 female rats
Test substance:	Benzyl Salicylate
Physical form:	/
Batch:	/
Purity:	/
Vehicle:	not need (dietary study)
Dose levels:	1500, 3000, and 6000 ppm equivalent to 86, 177 and 357 mg/kg for males and 106, 204 and 429 for females
Route:	oral feed
Administration:	continuously
GLP:	Yes
Observation period:	90 days
Study period:	IN-LIFE DATES: From:02 Jan 2019 To: 04 Apr 2019

Taken from the REACH dossier

Animals were administered the test substance continuously in the diet for at least 90 consecutive days. The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, ophthalmology, clinical pathology parameters (haematology, coagulation, serum chemistry, and urinalysis), thyroid hormone concentration (T3, T4, and TSH), gross necropsy findings, organ weights, and histopathologic examinations. Average compound consumption for the 1500, 3000, and 6000 ppm groups was 86, 177, and 357 mg/kg/day for males and 106, 204, and 429 mg/kg/day for females, respectively. All animals survived to the scheduled necropsy.

There were no direct test substance-related clinical, ophthalmic, macroscopic, or microscopic observations or effects on serum chemistry, haematology, coagulation, urinalysis, thyroid hormone concentration, and organ weights. Adverse test substance-related lower body weights and body weight gains were noted in the 6000 ppm group males and females generally throughout the dosing period. In the 6000 ppm group males, lower food consumption was noted during the first week of dosing and lower food efficiency was noted throughout the first month of the dosing period. Lower mean food efficiency was also noted for females in the 6000 ppm group during 7 of the 13 weekly intervals during the study.

Effects on mean food efficiency were considered adverse as they were a contributing factor to the adverse effects on body weights in males and females at 6000 ppm. Based on the results of this study, dietary administration of Benzyl Salicylate to Crl:CD(SD) rats at concentrations of 1500, 3000, and 6000 ppm for a minimum of 90 days resulted in adverse lower body weights, lower body weight gains, and decreased food efficiency for males and females at 6000 ppm.

Therefore, the no-observed-effect level (NOEL) was considered to be 3000 ppm (equivalent to 177 and 204 mg/kg/day for males and females, respectively).

Ref: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16100/7/6/1

- Inhalation route

There are no specific repeat dose toxicity data by the inhalation route for Benzyl Salicylate.

Given hydrolysis has been shown to occur via lung microsomes (Ozaki *et al.*, 2015), it may be relevant to consider the benzyl alcohol data that is available. Also, there are data on acute inhalation for methyl salicylate to provide supporting evidence that salicylates in general are not acutely toxic to respiratory tract and lung.

- Dermal route

No study available

3.4.4.3 Chronic (> 12 months) toxicity

3.4.5 Reproductive toxicity

No reproductive toxicity data were available on Benzyl Salicylate, as performed before the March 2013 animal testing ban.

Taken from the REACH dossier on Benzyl Salicylate

- Following ECHA decision (CCH-D-2114379324-45-01/F) on Benzyl Salicylate it was requested to conduct additional toxicological studies: a Screening study for reproductive/developmental toxicity (Annex VIII, Sections 8.6.1 and 8.7.1.; test method: OECD TG 421) in rats, oral route
- a Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD 414) in a first species (rat or rabbit), oral route.

3.4.5.1 Fertility and reproduction toxicity

The objective of the following study was to provide preliminary information on the potential adverse effects of the test substance on male and female reproduction within the scope of a screening study. This encompassed gonadal function, mating behavior, conception, parturition, and lactation of the parental generation and the development of offspring from conception through day 13 of postnatal life.

Guideline:	OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)
Species/strain:	female and male Sprague-Dawley rats
Group size:	4 groups of 10 male and 10 female rats
Test substance:	Benzyl Salicylate
Physical form:	/
Batch:	/
Purity:	/
Vehicle:	not need (dietary study)
Dose levels:	500, 750, and 2500 ppm equivalent to 32, 48 and 158 mg/kg for males and 34, 49 and 166 for females
Route:	oral feed
Administration:	continuously
Exposure period:	FO males for 14 days prior to mating and continuing through the day of euthanasia (minimum 28 days of dose administration); F0 females were dosed for 14 days prior to mating and continuing through Lactation Day 13. The F1 animals were not directly exposed to the test substance at any time during the study; the offspring of the F0 parental generation were potentially exposed to the test substance in utero and while nursing.
GLP:	Yes
Study period:	IN-LIFE DATES: from 30 May 2019 to 27 Aug 2019

Taken from the REACH dossier

Animals were administered the test substance continuously in the diet. F0 males were dosed for 14 days prior to mating and continuing through the day of euthanasia. F0 females were dosed for 14 days prior to mating and continuing through Lactation Day 13. The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, oestrous cycles, reproductive performance, parturition, litter viability and survival, anogenital distance, areolae/nipple anlagen, thyroid hormones, gross necropsy findings, organ weights, and histopathologic examinations.

Mean compound consumption was 34, 49, and 166 mg/kg/day in the 500, 750, and 2500 ppm group F0 males during the premating treatment period (Study Days 0–14). Mean compound consumption was 32, 48, and 158 mg/kg/day during the premating period (Study Days 0–14), 33, 51, and 170 mg/kg/day during gestation (Gestation Days 0–20), and 67, 101, and 324 mg/kg/day during lactation (Lactation Days 1–13) in the 500, 750, and 2500

ppm group females, respectively. All F0 males and females in the control, 500, 750, and 2500 ppm groups survived to the scheduled necropsy. There were no test substance-related clinical observations noted at the daily examinations at any exposure level.

No test substance-related effects on F0 body weight or food consumption parameters were noted for males and throughout the study at any exposure concentration.

F0 male and female mating and fertility, male copulation and female conception indices, oestrous cycle lengths, mean number of days between pairing and coitus, gestation lengths, and the process of parturition were unaffected by test substance administration at all dietary concentrations.

No test substance-related effects on mean T4 levels were noted in the 500, 750, and 2500 ppm group F0 males on Study Day 28.

There were no test substance-related effects on gross observations, organ weights, or histologic changes observed in the F0 generation.

There were no test substance-related effects on the mean number of former implantation sites or unaccounted-for sites at any exposure level.

Mean absolute F1 birth weights (PND 1) in the 2500 ppm group males and females were 4.9% and 5.2% lower, respectively, than the control group. Lower mean body weight gains in these pups during PND 4–13 resulted in mean absolute male and female body weights that were up to 7.8% and 10.4% lower, respectively, than the control group. These differences were not statistically significantly different compared to the control group except for female F1 pups in the 2500 ppm group on PND 13. Also, the lower mean pups body weight in the 2500 ppm group was mainly due to lower body weights in a single litter (No. 4892) and the body weight mean in other litters in the 2500 ppm group were all within Charles River historical control data range; therefore, the effects on mean body weights and body weight gains at 2500 ppm were considered test substance-related and no adverse.

Mean pup body weights and body weight gains in the 500 and 750 ppm groups were unaffected by test substance administration.

There were no test substance-related effects on mean number of pups born, pup survival, liver litter size, mean sex ratio, anogenital distance, areolae/nipple anlagen (males only), thyroid hormone levels (total T4) on PND 13, and thyroid weights. There were no clinical observations or necropsy findings that could be attributed to F0 maternal administration of the test substance at any exposure concentration.

Under the conditions of this screening study, no test substance-related effects were noted on reproductive performance in F0 males and females at any exposure concentration. Based on the lack of F0 parental toxicity at any exposure concentration, an exposure concentration of 2500 ppm (the highest dose level tested) was considered to be the no-observed-adverse-effect level (NOAEL) for F0 systemic and reproductive toxicity of Benzyl Salicylate when administered in the diet to Crl:CD(SD) male and female rats. Based on the lack of test substance-related effects at any dose level, an exposure concentration of 2500 ppm (the highest dose level tested) was considered to be the no-observed-adverse-effect level (NOAEL) for F1 neonatal toxicity. The 2500 ppm dose level corresponded to actual consumption of 166 mg/kg/day for males during the pre-mating period and 158, 170, and 324 mg/kg/day for females during pre-mating, gestation, and lactation, respectively.

Ref: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16100/7/9/2

SCCS comment
The SCCS agrees that the NOAEL derived from this study should be 166 mg/kg/day for male rats and 158 mg/kg/day for female rats.

3.4.5.2 Developmental Toxicity

The objectives of the following study were to determine the potential of the test substance to induce developmental toxicity after maternal exposure from implantation to expected parturition, to characterize maternal toxicity at the exposure levels tested and to determine a no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity.

Guideline:	OECD Guideline 414 (Prenatal Developmental Toxicity Study)
Species/strain:	female Sprague-Dawley rats
Group size:	4 groups of 25 female rats
Test substance:	Benzyl Salicylate
Physical form:	/
Batch:	/
Purity:	/
Vehicle:	not need (dietary study)
Dose levels:	1000, 3000, and 4000 ppm equivalent to 72, 214 and 289 mg/kg
Route:	oral feed
Administration:	continuously
Exposure period:	From gestation Day 6 to through 21.
GLP:	Yes
Study period:	IN-LIFE DATES: From: 04 March 2019 To: 29 March 2019

All females survived to the scheduled necropsy on Gestation Day 21; there were no test substance-related clinical observations during the study.

Mean maternal body weight loss in the 4000 ppm group and lower mean body weight gain in the 3000 ppm group were noted following administration of the first dose (Gestation Days 6–7), and lower mean food consumption was noted in these groups during Gestation Days 6–9 (both groups) and 9–12 (3000 ppm only).

Mean absolute body weights in the 4000 ppm group were up to 6.4% lower than the control group during Gestation Days 7–21. Some of the aforementioned differences in mean body weight changes and food consumption and all of the differences in mean absolute body weights were statistically significant compared to the control group. The effects on mean body weights, body weight gains, and food consumption at 4000 ppm were considered test substance-related and adverse due to the magnitude of the changes.

Mean body weights in the 3000 ppm group were slightly lower than the control group (sporadically statistically significant) during Gestation Days 7–15; however, the values were ≤ 4.3% lower than the control group. The effects on mean body weights, body weight gains, and food consumption at 3000 ppm were considered test substance-related but not adverse due to the low magnitude of the changes and the transient nature. There were not test substance-related effects on maternal body weight and food consumption parameters in the 1000 ppm group.

There were no test substance-related macroscopic findings noted at the scheduled necropsy.

Mean foetal body weights (male, female, and combines sexes) were 7.1% and 10.7% lower in the 4000 ppm group compared to the concurrent control group and resulted in a lower mean gravid uterine weight in the 4000 ppm group; the differences in mean foetal weights were statistically significant, and the values were below the minimum mean values in the Charles River Ashland historical control data, therefore were considered test substance-

related and adverse. Intrauterine growth in the 1000 ppm group and intrauterine survival in the 1000, 3000, and 4000 ppm groups were unaffected by test substance administration.

There were no test substance-related foetal malformations noted in any group. A higher mean litter proportion of 14th rudimentary rib(s) was noted in the 4000 ppm group (statistically significant) and higher mean litter proportions of bent rib(s) were noted in the 3000 and 4000 ppm groups compared to the concurrent control group; the values were also above the maximum mean values in the Charles River Ashland historical control data.

These findings corresponded to the test substance-related lower mean foetal body weights observed at 3000 and 4000 ppm but were not considered adverse because both have been noted to resolve postnatally and should not be used in risk assessment to set standards for exposure to chemical agents (Kimmel, *et al.*, 2014).

Ref: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16100/7/9/3/?documentUUID=59df934a-3aff-4c34-b016-61e23a9c1936

SCCS comment
Based on adverse mean body weight loss, lower mean body weights and food consumption at 4000 ppm, a dosage level of 3000 ppm, equivalent to **214 mg/kg/day**, was considered to be the **NOAEL for maternal toxicity.**

Based on lower mean foetal body weights in the 4000 ppm group, a dosage level of 3000 ppm, equivalent to **214 mg/kg/day**, was considered to be the **NOAEL for embryo/foetal development** when Benzyl Salicylate was administered orally (via the diet) to time-mated Crl:CD(SD) rats.

3.4.6 Mutagenicity / genotoxicity

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

Bacterial gene mutation tests

Three Ames tests in Salmonella typhimurium were performed for Benzyl Salicylate in the presence and absence of metabolic activation, using the following strains: TA98, TA100, TA1535, and TA1537, and/or TA97 (Zeiger *et al.,* 1987; NTP (2018a,b)). Doses of 3.3 to 333 µg Benzyl Salicylate/plate in dimethyl sulfoxide (DMSO) vehicle did not produce any mutagenic effects with or without metabolic activation.

Table 22: *In vitro* bacterial assays for Benzyl Salicylate

Method	Test concentration	Method details	Results	Reference
Ames test (as per OECD 471)	3.3 to 333 µg/plate in dimethyl sulfoxide (DMSO) with and without S9	*Salmonella typhimurium* strains: TA98, TA100, TA1535, and TA1537, and/or TA97	Not genotoxic with or without metabolic activation	Zeiger *et al* 1987
OECD Guideline 471 Ames Test	1, 3.3, 10, 33.3, 100, 333 µg/plate in dimethyl sulfoxide (DMSO) with and without rat or hamster S9	*Salmonella typhimurium* strains: TA100, TA1535, TA1537, TA98	Not genotoxic with or without metabolic activation	NTP (2018a) study number 023477
OECD Guideline 471 Ames Test	1, 3.3, 10, 33.3, 100, 333, 666 µg/plate in dimethyl sulfoxide (DMSO) with and without rat or hamster S9	*Salmonella typhimurium* strains: TA100, TA1535, TA1537, TA98	Not genotoxic with or without metabolic activation	NTP (2018b) study number 521477

Mammalian gene mutation test

The following *in vitro* assays were available using mammalian cells (summary in Table 23 and abstracts of the studies below the Table).

Table 23: *In vitro* mammalian clastogenicity and gene mutation

Methods	Test Article	Method details	Results	Reference
OECD Guideline 473 (In Vitro Mammalian Chromosomal Aberration Test)	0 - 170 µg/mL in DMSO with and without S9	Chinese hamster lung fibroblastic cells (CHL)	No chromosome aberrations	Bozo Research Centre Inc. Japan (2017)
OECD Guideline 476 In Vitro Mammalian Cell Gene Mutation (an HPRT) assay	125, 250, 500, 1000 and 2000 µg/mL in DMSO with and without rat S9	Chinese hamster ovary (CHO) cells	Not mutagenic	Bioreliance study no. AF45GY.783.BTL (2019)
OECD Guideline 487 In vitro	Due to cytotoxicity, doses selected	Human peripheral blood lymphocytes	Not mutagenic	Bioreliance, (2021): also updated on EU REACH record 2021

mammalian cell micronucleus test	for evaluation of micronuclei were 19.4, 38.8, and 56.4 µg/mL for the non-activated 24-hour exposure group; 22.5, 65.6, and 81 µg/mL for the non-activated 4-hour exposure group; and 90, 162, and 180 µg/mL for the S9-activated 4-hour exposure group			

***In Vitro* Mammalian Cell Gene Mutation Test** (according to OECD Guideline 476) (2019)

The test substance, Benzyl Salicylate (CAS# 118-58-1), was evaluated for its ability to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (hprt) of Chinese hamster ovary (CHO) cells, in the presence and absence of an exogenous metabolic activation system, as assayed by colony growth in the presence of 6-thioguanine (TG resistance, TG^r). Dimethyl sulfoxide (DMSO) was used as the vehicle.

In the preliminary toxicity assay, the concentrations tested were 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/mL. The maximum concentration evaluated approximated the limit dose for this assay. Visible precipitate was observed at concentrations ≥31.3 µg/mL at the beginning of treatment and at concentration 2000 µg/mL by the end of treatment with S9. Adjusted relative survival was 44.74 and 64.94% at a concentration of 2000 µg/mL with and without S9, respectively. Based upon these results, the concentrations chosen for the definitive mutagenicity assay were 125, 250, 500, 1000 and 2000 µg/mL with and without S9.

In the definitive mutagenicity assay, visible precipitate was observed at all concentrations at the beginning of treatment and at concentrations ≥1000 µg/mL by the end of treatment with S9. The average adjusted relative survival was 21.80 and 78.14% at a concentration of 2000 µg/mL with and without S9, respectively. Cultures treated at all concentrations with and without S9 were chosen for mutant selection. No statistically significant increases in mutant frequency, as compared to the concurrent vehicle controls, were observed at any concentration evaluated with or without S9 ($p > 0.01$). The positive controls induced significant increases in mutant frequency ($p < 0.01$).

These results indicate Benzyl Salicylate (CAS# 118-58-1) was negative for the ability to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (*Hprt*) of Chinese hamster ovary (CHO) cells, in the presence and absence of an exogenous metabolic activation system.

Conclusion:

Under the conditions of the assay, Benzyl Salicylate (CAS# 118-58-1) was concluded to be negative for the induction of forward mutations at the hypoxanthineguanine phosphoribosyl transferase (HPRT) locus (*Hprt*) of Chinese hamster ovary (CHO) cells, in the presence and

absence of an exogenous metabolic activation system, in the *in vitro* mammalian cell forward gene mutation (CHO/HPRT) assay.

Ref: BioReliance Corporation, BioReliance Study Number AF45GY.783.BTL (2019)

SCCS comment
In the main (definitive) experiment with S9-mix, the concentrations 250 and 500 µg/mL gave high cytotoxicity (9.1 and 10.9% of viability, respectively), which is not in accordance with the OECD Guideline. Historical positive control B(a)P has a high range of mutant frequency from 6-323 mutants per million of cells, while the negative control shows a much lower range from 0-15 mutants per million of cells. The SCCS therefore considers this study as not valid.

New study submitted by Applicant in March 2023

***In vitro* Cell Gene Mutation Test (according to OECD Guideline 476)**

An *in vitro* mammalian cell gene mutation assay was performed in CHO K1 Chinese hamster ovary cells at the Hprt locus to evaluate the potential of Benzyl Salicylate (batch number VE00790747) to cause gene mutation. Treatments were carried out for 5 hours with and without metabolic activation (±S9-mix – rat metabolic activation induced by phenobarbital) and for 24 hours without metabolic activation (-S9-mix). The study was performed in compliance with the OECD Principles of Good Laboratory Practice.

Positive controls without metabolic activation - Ethyl methanesulfonate (EMS, 0.4 µL/mL) was used. For metabolic activation 7,12 dimethylbenz[a]anthracene (DMBA, 15 µg/mL) was used. Dimethyl sulfoxide (DMSO, 1%) was used as the vehicle (solvent) of the test item in this study. Stock solution 200mg/mL was prepared. Treatment concentrations for the mutation assays of the main tests were selected based on the results of a preliminary toxicity test as follows:

Assay 1
5-hour treatment in the presence of S9-mix: 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 µg/mL.
5-hour treatment in the absence of S9-mix: 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 µg/mL.

Assay 2
5-hour treatment in the presence of S9-mix: 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 µg/mL.
24-hour treatment in the absence of S9-mix: 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 µg/mL.

In the main assays, a measurement of the survival (colony-forming ability at the end of the treatment period) and viability (colony-forming ability at the end of the 7 day expression period following the treatment) and mutagenicity (colony-forming ability at the end of the 7 day expression period following the treatment, in the presence of 6-thioguanine as a selective agent) was determined.

In Assay 1, insolubility (precipitate) was detected in the final treatment medium at the end of the treatment in the experiments with and without metabolic activation at the concentration range of 2000 - 125 and/or 62.5 µg/mL.

In Assay 2, insolubility (precipitate) was detected in the final treatment medium at the end of the treatment in the experiment with and without metabolic activation at the concentration range of 2000 - 500 and/or 250 µg/mL. There were no large changes in pH and osmolality after treatment in any cases.

In Assay 1, in the presence of S9-mix (5-hour treatment), marked cytotoxicity of the test item was observed at only 500 µg/mL concentrations (with a relative survival of 44%). In the absence of S9-mix (5-hour treatment), cytotoxicity was observed in 2000, 1000, 500 and 250 µg/mL, with a relative survival of 38%, 33%, 37% and 49%, respectively.

In Assay 2, in the presence of S9-mix (5-hour treatment), cytotoxicity of the test item was observed in 2000, 1000, 500 and 250 µg/mL, with a relative survival of 79%, 51%, 30% and 59%, respectively. In the absence of S9-mix (24-hour treatment), cytotoxicity was observed in 2000, 1000, 500, 250 and 125 µg/mL, with a relative survival of 25%. 28%, 23%, 35% and 58%, respectively. Therefore, the evaluation was made using data of all ten concentrations (up to the recommended maximum concentration).

No statistically significant increases in the mutant frequency were observed at any examined concentrations when compared to the negative (vehicle) control data and there was no dose response to the treatment (a trend Test Facility analysis showed no effect of treatment (R2 = 0.447)). **This experiment is considered to be negative**.

In Assay 1, without metabolic activation, statistically significant increase in the mutation frequencies was observed in this experiment at a single value (at the concentration of 31.25 µg/mL, at $p< 0.05$ level), but the observed increase was within the general historical control range. Furthermore, the observed mutant frequency (8.4×10^{-6}) was within the expected range of the negative control samples according to the relevant OECD guideline (expected range: $5\text{-}20 \times 10^{-6}$). No dose response to the treatment was observed (a trend analysis showed no effect of treatment (R2 = 0.021)). **This experiment is considered to be negative.**

In Assay 2, with metabolic activation statistically significant increase in the mutation frequencies (at $p < 0.01$ level) was observed in this experiment at a single value (at the concentration of 62.5 µg/mL), but the observed increase was within the general historical control range. Furthermore, the observed mutant frequency (10.7×10^{-6}) was within the expected range of the negative control samples according to the relevant OECD guideline (expected range: $5\text{-}20 \times 10^{-6}$). No dose response to the treatment was observed (a trend analysis showed no effect of treatment (R2 = 0.067)). **This experiment is considered to be negative.** Moreover, it confirmed the result of the Assay 1.

In Assay 2, in the absence of S9-mix (24-hour treatment) no statistically significant increases in the mutation frequency were observed at any examined concentrations when compared to the negative (vehicle) control data and there was no dose response to the treatment (a trend analysis showed no effect of treatment (R2 = 0.003)). This experiment is considered to be negative.

The spontaneous mutant frequency of the negative (vehicle) control was in accordance with the general historical control range in all assays. The positive controls gave the anticipated increases in mutation frequency over the controls in line with the historical data in all assays. Ten evaluated concentrations were presented in all assays. The cloning efficiencies for the negative controls at the beginning and end of the expression period were within the target range. The evaluated concentration ranges were considered to be adequate (concentrations were tested up to the maximum achievable cytotoxicity, which plateaued well below the target 10-20% survival as concentrations increased. The overall study was considered to be valid.

In conclusion, no mutagenic effect of Benzyl Salicylate was observed either in the presence or absence of a metabolic activation system under the conditions of this HPRT assay. The study was considered valid based on the negative and positive control values.

Ref: Charles River Laboratories Hungary Kft. Final report.
Study Code: 22/215-015C, (2023)

SCCS comment
According to OECD TG 476, the top concentration should be considered based on cytotoxicity and precipitation. If no precipitate or limiting cytotoxicity is observed, the highest test concentration should correspond to 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest. As stated in the Guideline, it is advisable to test only one concentration producing turbidity or with a visible precipitate because artifacts may result from the precipitate. However, in all preliminary as well as the main experiments (5h treatment with and without S9-mix) and 24h treatment without S9-mix, the precipitates appeared already in lower/middle concentrations (65.5 -125-250ug/mL) before and after the treatment. Nevertheless, the study was considered valid by the SCCS.

***In Vitro* Mammalian Chromosomal Aberration Test in Chinese hamster cells (according to OECD Guideline 473) (2018)**

In order to evaluate the clastogenic potential of Benzyl Salicylate, a chromosomal aberration test using cultured Chinese hamster (CHL/IU) cells was conducted (Study period: December 14, 2016 to March 27, 2017).

As a preliminary study to select dose levels for the chromosomal aberration test, a cell-growth inhibition test was conducted setting the highest dose level at 2000 µg/mL, and this dose diluted using a common ratio of 2 to prepare a total of 8 concentrations. In the results, cell growth inhibition effects of more than 50% were recorded at the dose levels of 125 µg/mL and above for the short-term treatment method without metabolic activation and for the continuous treatment method and at the dose levels of 250 µg/mL and above for the short-term treatment method with metabolic activation, and thus the 50% cell growth inhibition concentration (approximate value) was calculated to be 92 µg/mL for the short-term treatment method without metabolic activation, 178 µg/mL for the short-term treatment method with metabolic activation, and 97 µg/mL for the continuous treatment method.

Based on these results, chromosome aberration study was conducted setting the maximum dose concentration at 120 µg/mL and using 5 dose concentrations with common difference of 20 µg/mL for the short-term treatment method without metabolic activation and for the continuous treatment method, and setting the maximum dose concentration at 200 µg/mL and using 5 dose concentrations with common difference of 30 µg/mL for the short-term treatment method with metabolic activation.

In the results of the chromosome aberration study, the incidence of the occurrence of cells with chromosomal aberrations not containing gaps, an index for the chromosome structural aberration (TA value), and the incidence of the occurrence of cells with polyploidy (poly value) were not higher than those in the negative control group with statistical significance for any treatment method, and the values for the negative control group within the range of 95% probability distribution of the negative control values in the historical background data of the test facility. Therefore, the test article was judged to be negative for chromosome aberration effects.

For all the treatment methods, the incidence of the occurrence of cells with chromosome structural aberrations and the incidence of the occurrence of polyploidy in the negative control group were within the range of 95% probability distribution of the historical background data of the test facility. In the positive control group, a statistically significant increase in the incidence of cells with chromosome structural aberration was recorded in comparison with that of the negative control group. Therefore, it was judged that the study was conducted appropriately.

Conclusion: It was concluded that Benzyl Salicylate had neither chromosome structural aberration inducibility nor chromosome numerical aberration inducibility under the conditions of this study.

Ref: Tokyo Laboratory, BoZo Research Center Inc., Study Number: T-G239 (2018)

SCCS comment
The SCCS agrees that Benzyl Salicylate was negative in the chromosome aberration test.

***In Vitro* Mammalian Cell Micronucleus Assay in human peripheral blood lymphocytes (according to OECD Guideline 487)**

The test substance, Benzyl Salicylate, was tested to evaluate the potential to induce micronuclei in human peripheral blood lymphocytes (HPBL) in both the absence and presence of an exogenous metabolic activation system. HPBL were treated for 4 hours in the absence and presence of S9 mix, and for 24 hours in the absence of S9 mix. Dimethyl sulfoxide (DMSO) was used as the vehicle.

In the preliminary toxicity assay (A1), the doses tested ranged from 0.2 to 2000 µg/mL, which was the limit dose for this assay. Cytotoxicity [>= 55± 5%cytokinesis-blocked proliferation index (CBPI) relative to the vehicle control] was observed at doses >=200 µg/mL in the non-activated and S9-activated 4-hour exposure groups, and at doses >= 60 µg/mL in the non-activated 24-hour exposure group. At the conclusion of the treatment period, visible precipitate was observed at doses >= 600 µg/mL in all three exposure groups. Based upon these results, the doses chosen for the micronucleus assay ranged from 38.8 to 156 µg/mL for the non-activated 4-hour exposure group, from 38.8 to 280 µg/mL for the S9-activated 4-hour exposure group, and from 9.70 to 69.6 µg/mL for the non-activated 24-hour exposure group.

In the initial micronucleus assay (B1), cytotoxicity (55± 5% CBPI relative to the vehicle control) was observed at doses >= 50.7 µg/mL in the non-activated 24-hour exposure group. At the conclusion of the treatment period, visible precipitate was not observed at any dose in any of the exposure groups. In the non-activated and S9-activated 4-hour exposure group, due to lack of requisite cytotoxicity, the micronucleus assay was repeated at doses ranging from 22.5 to 100 µg/mL for the non-activated 4-hour exposure group and from 45 to 250 µg/mL for the S9-activated 4-hour exposure group.

In the repeat assay (B2), cytotoxicity (55± 5% CBPI relative to the vehicle control) was observed at doses >= 81 µg/mL in the non-activated 4-hour exposure group and at doses >= 180 µg/mL in the S9-activated 4-hour exposure group. At the conclusion of the treatment period, visible precipitate was not observed at any dose in both exposure groups.

The doses selected for evaluation of micronuclei were 19.4, 38.8, and 56.4 µg/mL for the non-activated 24-hour exposure group; 22.5, 65.6, and 81 µg/mL for the non-activated 4-hour exposure group; and 90, 162, and 180 µg/mL for the S9-activated 4-hour exposure group.

Neither statistically significant nor dose-dependent increases in micronuclei induction were observed at any dose in treatment groups wth or without S9 ($p > 0.05$; Fisher's Exact and Cochran-Armitage tests). The results were within the 95% control limit of the historical negative control data.

These results indicate Benzyl Salicylate was negative for the induction of micronuclei in the presence and absence of the exogenous metabolic activation system.

Conclusion: Under the conditions of the assay described in this report, Benzyl Salicylate was concluded to be negative for the induction of micronuclei in the non-activated and S9-activated test systems in the *in vitro* mammalian micronucleus test using human peripheral blood lymphocytes.

Ref: BioReliance Corporation. BioReliance Study Number AF45GY.348REACH.BTL (2021)

SCCS comment
The SCCS agrees that Benzyl Salicylate was negative in the *in vitro* mammalian micronucleus test.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

There are no *in vivo* data for Benzyl Salicylate.

The Applicant's conclusion on mutagenicity and genotoxicity of Benzyl Salicylate

Benzyl Salicylate is not genotoxic or mutagenic in any of the OECD guideline *in vitro* assays performed. The mutagenicity and genotoxicity data for salicylic acid were recently reviewed by the SCCS (SCCS, 2018) and benzyl alcohol is also not genotoxic or mutagenic (CIR, 2017). There are no concerns from these endpoints for Benzyl Salicylate.

The SCCS overall comment on mutagenicity and genotoxicity of Benzyl Salicylate
Benzyl Salicylate was tested in 3 bacterial gene mutation tests with negative results. However, the SCCS noted that in the studies 1 strain combination recommended by the OECD TG 471 was not represented (E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102).

Benzyl Salicylate was tested in 2 mammalian gene mutation tests in *Hprt* locus on Chinese hamster ovary (CHO) cells with negative results. However, the first study was not considered valid due to several shortcomings highlighted above. The second *in vitro* mammalian gene mutation study was considered valid even if precipitation appeared already in middle concentrations.

Benzyl Salicylate was tested in 1 valid *in vitro* mammalian chromosomal aberration test on Chinese hamster cells and 1 valid *in vitro* mammalian cell micronucleus assay on human lymphocytes, both with negative results.

After analysis of the available data, the SCCS considers that Benzyl Salicylate does not pose a genotoxic/ mutagenic potential.

3.4.7 Carcinogenicity

There are no carcinogenicity studies for Benzyl Salicylate. However, given 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 benzyl alcohol may act as carcinogens, then there are no known concerns to address for Benzyl Salicylate.

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

SCCS comment
After analysis of the available *in vitro* mutagenicity studies, the SCCS considers that Benzyl Salicylate is not likely to be a human carcinogen.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

Based on the available *in vivo* and human data Benzyl Salicylate does not present a concern for phototoxicity or photoallergenicity.

UV Spectra Analysis: UV/Vis absorption spectra (OECD test guideline 101) were generated for Benzyl Salicylate. Benzyl Salicylate absorbs in the range of 290 to 700 nm. Furthermore, the molar absorption coefficient for λ max within this range is above 1000 $L \cdot mol^{-1} \cdot cm^{-1}$, the benchmark of concern for phototoxic effects (Henry, 2009).

Studies in mouse

Forbes (1977); In a phototoxicity study, 12 Skh:hairless mice (6/group) received 1 application of 20 µl/5 cm^2 of 25% and 100% Benzyl Salicylate in methanol. Thirty minutes later the animals were irradiated. One group was irradiated at a distance of 0.65 meters or less by fluorescent blacklight (a bank of 6 Sylvania F40T12BL PUVA lamps with a broad band output of 350 nm) for 1 hour to provide a measure dose of 200 RB units. The second group was irradiated at a distance of 1 meter from a simulated sunlight (Atlas Xenon light source, 6.5 KW long-arc xenon high pressure burner with power supply, igniter and water-cooling system) for 1 hour providing a dose of 200 RB units. The treated areas were examined for presence or absence of erythema, scaling, edema, or fissuring at 4, 24, 48, 72, and 96 hours after exposure. **No effects were observed**.

Studies in guinea-pig

RIFM/Givaudan (1982); Two open applications of 0.025 mL/2 cm^2 of 1 and 3% Benzyl Salicylate in ethanol with 2% DMSO were made to a 2 cm^2 area of the skin of 10 shaved outbred Himalayan white spotted guinea pigs (300-450 grams) per dose. Thirty minutes after application, one site was irradiated with UV light (Westinghouse FS 40 "Black Lamp" with energy of 1x10(4) Ergs/cm^2/sec and spectrum of 320-400 nm). The radiation dose was 20 J/cm^2. Reactions were graded at 4, 24, and 48 hours after application. No reactions were observed at 1%. At 3% a slight reaction was seen in all 10 guinea pigs at the 4 and 24 hour readings. Eight (8/10) guinea pigs scores returned to normal at the 48 hour reading.

RIFM/Rhodia (1983); Phototoxicity and photoallergy were evaluated in twenty (10/sex) adult albino Dunkin Hartley guinea pigs weighing 300-400 grams. The animals received a single application of 0.5 mL of 10% Benzyl Salicylate in absolute ethanol under an occlusive patch for 1 hour 30 minutes on the anterior part of the back. Irradiation was carried out using a system of fluorescent lamps with continuous spectrum emission: 4000-3100, Mazadaflour black light fluorescent map: 3500-2850 Westinghouse sun fluorescent lamp. Radiation emitted by these lamps was principally in the UVA range (wavelength from 4000-3150 A) and in the UVB range (wavelength from 3150-2900 A). The 2 lamps used were placed 10 cm from the back of each animal and irradiated for 5 minutes. The total energy was 12.5 J/cm^2 and the rage of UVS was 1%. Evaluation of the test sites was made at 6 and 24 hours after irradiation. **No effects were observed**.

RIFM/Takasago International (1997); In a phototoxicity study, open applications of 5%, 10% and 30% Benzyl Salicylate in acetone were made to clipped skin sites on 5 female albino Hartley-Dunkin guinea pigs. The application sites were irradiated with UV light (array of 5 National FL 20 S.BLB tubes (UV-A black light 300-400 nm, max 360nm)) at 10 cm for 60 minutes. All irradiation energy was about 13 J/cm^2. Reactions were graded according to Draize method at 24 & 48 hours after application. **No effects were observed.**

Study in humans

RIFM/Givaudan (1983); A phototoxicity test was conducted on 6 volunteers. Patches were prepared with 0.025 mL/cm^2 of 3% and 10% Benzyl Salicylate in 1:1 ethanol/acetone was applied to the left and right side on the back of each subject. The right test side was covered and served as an irritancy control site. Thirty minutes after application the test sites were exposed to non-erythrogenic UVA irradiation at 1, 2.5, 5, 10 and 20 J/cm^2. The light source was a bank of 4 "blacklight" fluorescent tubes with an emission spectrum of 320-400 mm

housed in a reflector unit. Following irradiation, each test site was examined 4, 24, 48, and 72 hours after application. **No phototoxic responses were observed.**

3.4.8.2 Photomutagenicity / photoclastogenicity

No data were provided

3.4.9 Special investigations / Endocrine Activity

Benzyl Salicylate has been identified by the European Commission (and *via* the German regulatory authorities in the CoRAP process) as a substance in cosmetic products that merits further review as a suspected endocrine disrupting chemical (EDC), based on some observations reporting endocrine activity (Miller *et al.,* 2001; Charles & Darbre 2009; Zhang *et al.,* 2012), and one in relation to fish (Kunz & Fent, 2006).

The available data as published for Benzyl Salicylate looking at the potential for endocrine activity *in vitro* and in an uterotrophic assay, as relevant to mammalian systems, are reviewed below. In addition, new *in vitro* evidence has been provided by the Applicant (Natsch *et al.,* 2021).

Endocrine activity *in vitro* – OECD level 2 studies

Benzyl Salicylate has been included in the US EPA's Endocrine Disruptor Screening Program for the 21st Century (EDSP21) and returned positive results in 9 Estrogen Receptor (ER) assays (out of 33), in 0 Androgen Receptor (AR) assays (tested in 17), 0 out of 9 Thyroid Receptor (TR) assays and 3 out of 26 steroidogenesis assays. The relative potency was significantly lower for Benzyl Salicylate compared to endogenous endocrine modulators. Based on the US EPA's EDSP ToxCast Estrogen Receptor (ER)/Androgenic receptor (AR) Model, Benzyl Salicylate was considered inconclusive for estrogenic potential and negative for androgen activity.

In the study from **Charles and Darbre (2009),** Benzyl Salicylate was reported to have estrogenic activity in assays using the estrogen-responsive MCF7 human breast cancer cell line. At 3 000 000-fold molar excess, they were able to partially displace [^{3}H]-estradiol from recombinant human estrogen receptors Erα and Erβ, and from cytosolic ER of MCF7 cells. At concentrations in the range of 5×10^{-5} to 5×10^{-4} M, Benzyl Salicylate was able to increase the expression of a stably integrated estrogen-responsive reporter gene (ERE-CAT) and of the endogenous estrogen-responsive pS2 gene in MCF7 cells, albeit to a lesser extent than with 10^{-8} M 17β-estradiol.

In the study from **Miller *et al.* (2001),** the authors have used a recombinant yeast estrogen assay to assess the activity of 73 phenolic additives that were used as sunscreens, preservatives, disinfectants, antioxidants, flavorings, or for perfumery. Benzyl Salicylate showed a very weak response (600,000 times weaker than 17β-estradiol), as shown in the table underneath.

Table 24: Estrogenic potency values (10% response level) for compounds displaying submaximal responses

Compound	CAS registry no.	Estrogenic potency
Benzophenone-3	131-57-7	1/100,000
Benzophenone-6	131-54-4	1/20,000,000
Benzophenone-7	85-19-8	1/300,000
Benzyl salicylate	118-58-1	1/600,000
Menthyl salicylate	89-46-3	1/200,000
Ethylhexyl salicylate	118-60-5	1/2,000,000
Nordihydroguaiaretic acid	500-38-9	1/600,000
Butylated hydroxytoluene	128-37-0	1/8,000,000
2,6, Di-*t*-butylphenol	128-39-2	1/20,000,000
Butylated hydroxyanisole	25013-16-5	1/2,000,000

Zhang *et al.* (2012) have evaluated the estrogenic potentials of phenyl salicylate (PhS), Benzyl Salicylate (BzS), phenethyl salicylate (PES), ethyl salicylate (ES) and methyl salicylate (MS) using an *in vitro* human estrogen receptor α (hERα)-coactivator recruiting assay. They found that PhS, BzS and PES showed obvious *in vitro* hERα agonistic activities. BzS has the highest activity among the tested Ses. The REC10 value of BzS is 1.58×10^{-8} M, which is approximately 257- and 0.06-fold that of E2 and BPA, respectively.

In their study, **Jimenez-Diaz *et al.* (2013)** have applied a new liquid chromatography–tandem mass spectrometry (LC–MS/MS) method to assess the presence of six UV-filters in current use (Benzyl Salicylate, phenyl salicylate, octyl salicylate, homosalate, 3-(4-methylbenzylidene) camphor, and 3-benzylidene camphor) in human placental tissue. Moreover, the interactions of these compounds with the human estrogen receptor alpha (hERα) and androgen receptor (hAR), using two *in vitro* bioassays based on reporter gene expression and cell proliferation assessment, were also investigated. All tested compounds, except Benzyl Salicylate and octyl salicylate, showed estrogenic activity in the E-Screen bioassay whereas only homosalate and 3-(4- methylbenzylidene) camphor were potent hAR antagonists.

SCCS comment
Benzyl Salicylate is not listed in the Annex VI of the EU Cosmetic Regulation (allowed UV-filters) but is listed in the Cosing database as a UV-Absorber.

Natsch *et al.* (2021) reviewed the *in vitro* evidence for Benzyl Salicylate. Given the limited data, two new *in vitro* assays were performed to assess the relative potency of Benzyl Salicylate *vs.* Estradiol (E2) at binding the estrogen receptor (ER). The 2 tests are i) a T47D-Kbluc cell-based assay with luciferase reporter gene under control of the estrogen response element (ERE) and ii) 6 days MCF-7 cell proliferation assay, cell yield by PrestoBlue®. In both assays, very similar results for BS were found. In the T47D-Kbluc cell assay Benzyl Salicylate was a partial agonist with a weak potency of 21,000,000- fold below E2; in the MCF-7 cell assay Benzyl Salicylate was a partial agonist with potency for equal proliferation ca. 36,000,000- fold below E2. The authors concluded that this potency is significantly below the agonistic activity of known chemicals which cause estrogenic effects in *in vitro* assays.

Applicant conclusion:

In vitro assays confirm that Benzyl Salicylate is an extremely weak partial agonist of the ER, seven orders of magnitude lower than the natural substrate E2. It is highly unlikely given such a weak potency, that any effects are exerted *in vivo* by an ER mechanism. That potency makes the difference in relation to endocrine activity was highlighted in the review by Borgert *et al.* (2013). Chemicals with low potency such as Benzyl Salicylate are not able to exert

detectable endocrine activity against the background of highly potent endogenous hormones already occupying receptors, especially not at relatively low use concentrations.

Endocrine activity *in vivo* – mouse uterotrophic assay (OECD Level 3 studies)

Uterotrophic assay :

Zhang *et al.* (2012) have evaluated the estrogenic potentials of phenyl salicylate (PhS), Benzyl Salicylate (BzS), phenethyl salicylate (PES), ethyl salicylate (ES) and methyl salicylate (MS) using an *in vivo* immature rodent uterotrophic bioassays. They found that PhS, BzS and PES showed obvious *in vitro* hERα agonistic activities; BzS in particular exhibited a higher estrogenic activity compared to bisphenol A (BPA). The uterine weights were significantly increased in mice treated with 11.1, 33.3, 100 and 300 mg/kg/day BzS and 33.3mg/kg/day PES and rats treated with 3.7, 11.1, 33.3 and 100mg/kg/day BzS for 3 days (P<0.05).

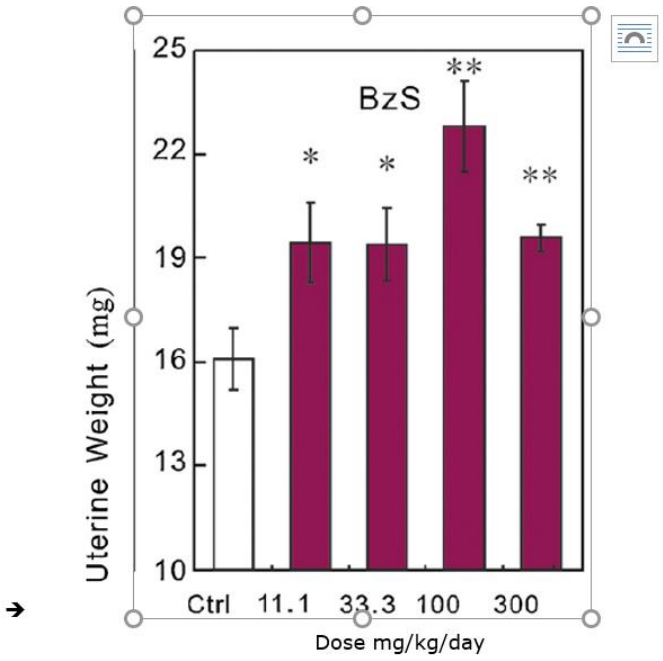

Figure 5. uterine weight of mice administered SE chemical for 3 days beginning on PND21. Values shown are the mean +/- SD. *Significantly different from to the corresponding control (vehicle control) at P < 0.05. ** *Significantly different from to the corresponding control) at P < 0.01

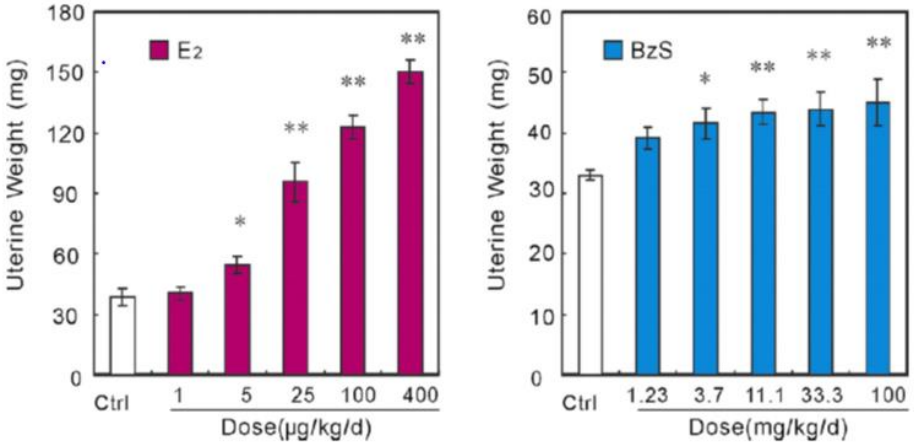

Figure 6. uterine weight of rats administered E2 or BzS for 3 days beginning on PND21. Values shown are the mean +/- standard error. *Significantly different from to the corresponding control (vehicle control) at $P < 0.05$. ** *Significantly different from to the corresponding control (vehicle control) at $P < 0.01$.

Finally, the authors have transformed the daily intakes and the dermal exposures of Ses in the "real world" into estradiol equivalent concentrations (EEQs). They found that the EEQ of BzS daily intake in consumers in the U.S. and the EEQs of dermal BzS and PES exposure among high-volume users worldwide were higher than the maximum secure daily estradiol intake recommended by the U.S. Food and Drug Administration (FDA). In particular, the EEQ for dermal BzS exposure was up to 162 ng EEQ/kg, which is 3.3 times higher than the maximal acceptable daily E2 intake recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Applicant observation

It has to be noted that a positive response in an uterotrophic assay only indicates some biological activity, but it does not indicate an adverse effect. Furthermore, the variability of the measured estrogen-dependent parameters is a major potential confounding factor for the scientific evaluation of this assay (Ashby, 2003).

SCCS conclusion on endocrine activity
In vitro results on Benzyl Salicylate did not show androgenic activity, and only a weak estrogenic activity. The estrogenic activity is confirmed by the *in vivo* studies in mouse. The uterotrophic assay is the gold standard to demonstrate an estrogenic effect *in vivo*, and the *in vivo* studies used in the Opinion clearly showed a weak estrogenic potential.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

Selection of the Key study and the POD

There are no repeat dose toxicity data for Benzyl Salicylate from before 11 March 2013 to use in a cosmetic safety assessment. However, as Benzyl Salicylate itself is not regarded as the main toxicant, but salicylic acid as the main hydrolysis product via all routes of exposure.

Salicylic acid was reviewed recently by the SCCS in its opinion from SCCS/1646/22. 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. Using molecular weight correction (Salicylic acid MW = 138 g/mol ; Benzyl Salicylate MW = 228 g/mol) the adjusted POD (that would yield the same amount of salicylic acid systemically) for Benzyl Salicylate is 123 mg/kg/day (= 75 mg/kg/day x (228/138)).

Based on the exposure information in section 3.3.2, the Margins of Safety for consumer exposures to Benzyl Salicylate in cosmetic products are shown for Scenario A maximum % use levels:

Table 24: Margins of Safety for consumer exposures to Benzyl Salicylate in cosmetic products based on Scenario A and maximum % use levels

Product families	Product categories	Maximum conc BzS	Dermal absorption	Eproduct normalized	Tier 1, dermal/oral SED	NOAEL	MOS
		(w/w %)	(%)	(mg/kg bw /d)	(mg/kg bw /d)	(mg/kg bw /d)	
	Hydroalcoholic-based fragrances	4	10.5	4.67	0.019614	123	6271
Rinse-off skin & hair cleansing products (except hand wash)	Shower gel	1.3	10.5	2.79	0.00380835	123	32297
	Hair conditioner	0.5	10.5	0.67	0.00035175	123	349680
	Shampoo	0.5	10.5	1.51	0.00079275	123	155156
Hand wash soap	Hand wash soap	0.5	10.5	3.33	0.00174825	123	70356
Leave on skin and hair products	Body lotion	0.7	10.5	123.2	0.090552	123	1358
	Face cream	0.5	10.5	24.14	0.0126735	123	9705
	Hand cream	0.5	10.5	32.7	0.0171675	123	7165
	Deodorant non-spray	0.5	10.5	22.08	0.011592	123	10611
	Hair styling*	0.5	10.5	5.74	0.00386	123	31865
Face make-up products	Liquid foundation	0.2	10.5	7.9	0.001659	123	74141
	Make-up remover	0.2	10.5	8.33	0.0017493	123	70314
Eye make up	Eye make-up	0.2	10.5	0.33	0.0000693	123	1774892
	Mascara	0.2	10.5	0.42	0.0000882	123	1394558
	Eyeliner	0.2	10.5	0.08	0.0000168	123	7321429
oral care products	Toothpaste	0.004	100	2.16	0.0000864	123	1423611
	Mouthwash	0.004	100	32.54	0.0013016	123	94499
Lip products	Lipstick, lip salve	0.2	100	0.9	0.0018	123	68333
	Mouthspray	0.004	100	26.7	0.001066667	123	115313
	aggregate dermal/oral			**273.490**	**0.170**	123	724

* values taken from the exposure via spray see section 3.3

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

As an estrogenic effect of Benzyl Salicylate at relatively high doses of exposure has been observed (see section 3.4.9 above), following the Note of Guidance 12th recommendations, specific calculations for children of different categories of ages have been considered by the SCCS. However, in view of the high values of the MoS for all product categories, even if specific scenario of exposure in children may lead to higher exposure (due to the difference of ratio in body surface and body weight depending of the ages) the MoS will still be above 100 and therefore these specific calculations are not included in the opinion.

3.6 DISCUSSION

Physicochemical properties

Benzyl Salicylate (CAS No. 118-58-1, EC No. 204-262-9) with the chemical name '2-hydroxybenzoic acid phenylmethyl ester' is produced naturally in a variety of plants and plant extracts where it can be extracted. In addition, Benzyl Salicylate can be synthesised.

A full report of the chemical characterization of Benzyl Salicylate in terms of purity and identity in representative batches is missing and therefore should be provided and the validity of the analytical methodologies used shown.
A full report in terms of impurity tests in representative batches of the test substance is missing and therefore should be provided and the validity of the analytical methodologies used must be shown. Identity and concentration of any impurities that may be present should also be stated.
Moreover, data on the stability of the test substance under the experimental conditions of the reported studies and under conditions of use, and information on any hydrolysis are missing and therefore, should be provided.

Toxicokinetics

Skin absorption

Data from an OECD Test guideline *in vitro* skin absorption study performed following the GLP (BASF, Oct 2021) indicates a value of 10.5% absorption, which is used in this opinion for the safety evaluation of Benzyl Salicylate. It is expected that the majority of systemic exposure is in the forms of salicylic acid and benzyl alcohol.

Oral exposure

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 benzyl alcohol following oral exposure in both rat and humans. With rapid hydrolysis in the gut and liver, systemic exposure is primarily to salicylic acid and benzyl alcohol, which do not accumulate in the body, and are rapidly excreted. Therefore, 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

Due to the lack of data available data, the SCCS considers that an absorption value by inhalation of 100% can be used in the risk assessment.

Exposure

Benzyl Salicylate can be used typically as a fragrance ingredient, in a range of manufactured goods (cosmetics, household goods and medicines). In cosmetics, Benzyl Salicylate is used for its fragrance/perfuming function.

Separate exposure assessments were performed for the dermal, oral and inhalation routes. As the deterministic approach - based on maximal concentrations used (scenario A) - yields a favourable outcome, further exposure modelling was not necessary to refine exposure. Scenario A is based on maximum concentrations of Benzyl 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 'Benzyl 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 17th cosmetic products usually considered, an 18th product type – hydroalcoholic fragrances was included in the scenario.

A measured value for skin penetration of Benzyl Salicylate of 10.5% (see section 3.2) has been used for all products in these calculations where dermal absorption needs to be factored in to calculate a systemic exposure dose (SED). For lipstick and oral care products a worst-case value of 100% absorption is used to account for passage across the oral mucosa and/or ingestion. A SED via the dermal route was calculated for each product in mg/kg/day and an aggregate systemic exposure dose for the 18 products (see Table 7).

Exposure via inhalation for products used in spray or pump forms was also calculated. 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 7 will be used, not both simultaneously. For most of the products the non-spray products lead to a much higher systemic exposure when compared to the spray products. The exception are the hair styling products: systemic exposure *via* non sprayed hair styling products is 3 µg/kg/d whereas it is 3.86 µg/kg/d when using hair styling spray products. Therefore, for the MoS calculation, for all products except hair styling products only non-spray products will be considered in a worst-case scenario.

A deterministic worst case systemic exposure dose (SED) estimate from aggregated dermal exposure modelling of Benzyl Salicylate in cosmetic products is 179 µg/kg/day. This value will be taken forward into the safety evaluation.

Toxicological Evaluation

For the toxicological evaluation of Benzyl Salicylate, SCCS mainly relied on the dossier from the Applicant and on the EU REACH substance dossier available at https://echa.europa.eu/registration-dossier/-/registered-dossier/16100

Irritation and corrosivity

SCCS considers that Benzyl Salicylate is non irritating to the skin but it may cause eye irritation.

Skin sensitisation

Benzyl Salicylate is currently regulated for labelling purposes as an allergen in entry 75 of Annex III to the Cosmetics Regulation. In particular, "*its presence must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products*".

Acute toxicity

Benzyl Salicylate is acutely toxic at high doses.

Repeated dose toxicity

No repeat dose toxicity data were available on Benzyl Salicylate, performed before the March 2013 animal testing ban. For the purposes of cosmetics safety assessment and given that Benzyl Salicylate itself is not regarded as the main toxicant, but salicylic acid as the main hydrolysis product, this section also discusses the available repeat dose toxicity data and conclusions for the primary Benzyl Salicylate metabolites salicylic acid and benzyl alcohol.

All data for salicylic acid were recently reviewed by the SCCS in its recent opinion (SCCS, 2023) and therefore are not reported in this opinion.

Concerning benzyl alcohol, a summary of the repeat dose oral studies cited in the ECHA REACH registration dossier for benzyl alcohol (https://echa.europa.eu/registration-dossier/-/registered-dossier/14748/7/6/2) has been provided to SCCS.
A no-observed-effect level (NOEL) was of 3000 ppm (equivalent to 177 and 204 mg/kg/day for males and females, respectively). This was derived from an oral 90 days study in rats performed after 2013 following a request from ECHA to comply with the Reach regulation.

Reproductive toxicity

Following ECHA decision (CCH-D-2114379324-45-01/F) on Benzyl Salicylate, additional toxicological studies were performed in 2019:

- a Screening study for reproductive/developmental toxicity (Annex VIII, Sections 8.6.1 and 8.7.1.; test method: OECD TG 421) in rats, oral route leading to a NOAEL of 166 mg/kg/day for males and 158 mg/kg/day for females

- a Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD 414) in a first species (rat or rabbit), oral route leading to a NOAEL of 214 mg/kg/day for maternal toxicity and for embryo/fetal development, when Benzyl Salicylate was administered orally (via the diet) to time-mated Crl:CD(SD) rats.

Mutagenicity / genotoxicity

Benzyl Salicylate was tested in 3 bacterial gene mutation tests with negative results. However, the SCCS noted that in the studies 1 strain combination recommended by the OECD TG 471 has not been represented (E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102).
Benzyl Salicylate was tested in 2 mammalian gene mutation tests in *Hprt* locus on Chinese hamster ovary (CHO) cells with negative results. However, first study was not considered valid due to several shortcomings highlighted above. The second *in vitro* mammalian gene mutation study was considered valid even if precipitation appeared already in middle concentrations.
Benzyl Salicylate was tested in 1 valid *in vitro* mammalian chromosomal aberration test on Chinese hamster cells and 1 valid *in vitro* mammalian cell micronucleus assay on human lymphocytes, both with negative results.

The SCCS, after analysis of the available data, considers that Benzyl Salicylate does not pose a genotoxic/ mutagenic potential.

Carcinogenicity

The SCCS, after analysis of the available *in vitro* mutagenicity studies, considers that Benzyl Salicylate is not likely to be a human carcinogen.

Photo-induced toxicity

Based on the available *in vivo* and human data Benzyl Salicylate does not present a concern for phototoxicity or photoallergenicity.

Special investigation

In vitro results on Benzyl Salicylate did not show androgenic activity but a weak estrogenic activity. The estrogenic activity is confirmed by the *in vivo* studies in mouse. The uterotrophic assay is the gold standard *in vivo* to demonstrate an estrogenic effect and the *in vivo* studies used in the opinion showed a weak estrogenic potential.

Safety evaluation

Considering all cosmetic products applied either directly on the skin or by spray, at the maximum concentrations of Benzyl Salicylate reported in Table 1 above, taken individually and also the aggregated exposure, the margin of safety is above 100. As an estrogenic effect of Benzyl Salicylate has been observed at relatively high doses of exposure, specific calculations for children of different categories of ages have been considered by the SCCS (see section 3.4.9 above), following the 12th revision of the SCCS Notes of Guidance (SCCS/1647/22). However, in view of the high values of the MoS for all product categories, even if specific scenario of exposure in children could lead to higher exposure (due to the difference of ratio in body surface and body weight depending on the ages), the MoS will still be far above 100, and therefore these specific calculations have not been considered in the Opinion.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Benzyl Salicylate, does the SCCS consider Benzyl Salicylate safe when used up to the maximum concentrations provided in the dossier submission by the Benzyl Salicylate Consortium?*

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

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

   /

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

   The available data on Benzyl Salicylate provide some indications for an endocrine mode of action, but there is no evidence to suggest that this results in endocrine effects.

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

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

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

8. LIST OF ABBREVIATIONS

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