Scientific Committee on Consumer Safety

SCCS

OPINION
on Methyl salicylate
(methyl 2-hydroxybenzoate)

The SCCS adopted this document
at its plenary meeting on 26-27 October 2021

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 9 July until 8 September 2021). Comments received during this time period are considered by the SCCS. For this Opinion, new calculations of the MoS were made by SCCS to take into account this revised concentration of methyl salicylate in mouth wash.

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the RAC Opinion on Methyl salicylate and the expected new classification as Toxic for Reproduction Category 2 of Methyl salicylate (to be introduced by an update of Annex VI Reg. 1272/2008), does the SCCS consider Methyl salicylate safe when used up to the maximum concentrations provided in the dossier submitted by the applicant?*

   The SCCS considers Methyl salicylate safe when used in cosmetic products up to the maximum concentrations provided in the dossier submitted by the applicant as detailed in Table 1.

2. *Does the SCCS have any further scientific concerns with regard to the use of Methyl salicylate in cosmetic products, also in relation to the RAC recommended classification of Methyl salicylate as 'Skin sensitizer Category 1B'?*

   Methyl salicylate should be considered as a weak skin sensitiser in humans and eye irritant.

   Moreover the SCCS would like also to express other concerns related to the use of methyl salicylate:

   - Methyl salicylate can be used in consumer products other than cosmetic products, such as household cleaning products, air care products, biocides (e.g. disinfectants, pest control products), polishes and waxes which may increase the systemic exposure dose with a possibility to exceed the safe level.

   - Methyl salicylate will be metabolised in the body to salicylic acid which is also classified as a reprotoxicant and used in cosmetic products (see opinion SCCS/1601/18). Therefore the combined exposure to cosmetic products containing various salicylates may increase the systemic exposure dose with a possibility to exceed the safe level.

   The SCCS also notices that wintergreen oil that is used in cosmetic products may contain up to 99% methyl salicylate. Therefore when calculating the content of methyl salicylate in a cosmetic product, any fraction coming from wintergreen oil should also be considered.

Keywords: SCCS, scientific opinion, methyl salicylate, methyl 2-hydroxybenzoate, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on methyl salicylate (methyl 2-hydroxybenzoate), preliminary version of 24-25 June, final version of 26-27 October 2021, SCCS/1633/21

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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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TABLE OF CONTENTS

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Methyl salicylate (CAS: 119-36-8; EC number: 204-317-7) is the INCI name of methyl 2-hydroxybenzoate an ingredient used in many fragrance mixtures and as flavouring and soothing agent in oral hygiene products. It is manufactured in and imported into the European Economic Area in quantities of 1000-10000 tonnes per year (ECHA website, 2018).

Methyl salicylate is not listed in the Annexes to the EU Cosmetic Products Regulation n. 1223/2009 on cosmetic products (Cosmetics Regulation) and its use is not otherwise restricted in cosmetic products.

The European Risk Assessment Committee (RAC) of ECHA issued in September 2019 an opinion recommending for Methyl salicylate a classification as 'Toxic for Reproduction Category 2' (i.e. suspected of damaging the unborn child) and 'Skin sensitizer Category 1B'. https://echa.europa.eu/documents/10162/ea33d742-d73f-a7a7-8bca-be3679b713e0. This opinion was based on a read across with data available on Salicylic acid.

Methyl salicylate is the ester of methyl alcohol and Salicylic acid. Different studies have shown that Salicylic acid is the main metabolic product of Methyl salicylate by hydrolysis. SCCS has issued an opinion on the safety of Salicylic acid in 2018 (Corrigendum 2019).

Following the RAC opinion, it is expected that the European Commission will propose a classification for Methyl salicylate as a 'Toxic for Reproduction Category 2' and 'Skin sensitizer Category 1B' (amending Annex VI to Regulation (EC) 1272/2008 on classification, labelling and packaging of chemical substances and mixtures, so called CLP Regulation).

Article 15 of the Cosmetics Regulation lays down that substances which have been classified as carcinogenic, mutagenic or toxic for the reproduction (CMR) under Annex VI to the CLP Regulation shall be prohibited for use in cosmetic products, unless an exemption has been granted.

In particular, Article 15.1 of the Cosmetics Regulation provides that a CMR classified in category 2 may be used in cosmetic products where the substance has been evaluated by the SCCS and found safe for use in cosmetic products.

Considering the abovementioned RAC opinion and the expected classification of Methyl salicylate as a 'Toxic for Reproduction Category 2' substance in Annex VI to the CLP Regulation, on 25th May 2020, Cosmetics Europe, or "the applicant", submitted a dossier to support the safety of Methyl salicylate and the granting of an exception under Article 15.1 of the Cosmetics Regulation. The Commission requests, therefore, the SCCS to carry out a safety assessment on Methyl salicylate in view of the information provided by the applicant, taking into account the maximum concentration of Methyl salicylate in the different categories of cosmetic products listed in the table below:

Type of cosmetic product exposure	Maximum % concentration
Hydroalcoholic-based fragrances	0.600%
Rinse-off skin & hair products (except hand wash products)	0.060%
Hand wash products	0.600%
Leave on skin & hair products (non aerosol)	0.060%
Face Make up products	0.050%
Lipsticks & lip balm	0.030%
Eye make up products & Make up remover	0.002%
Toothpaste	2.520%
Mouthwash	0.600%
Mouth spray	0.650%
Deo spray	0.003%
Hair products (spray/aerosol)	0.009%
Body Lotion Spray	0.040%

In the draft opinion, that was published for public consultation from 9 July until 8 September, SCCS concluded that Methyl salicylate is safe when used in cosmetic products up to the maximum concentrations provided in the dossier submitted by the applicant as detailed in Table 1, except for mouth washes used below 10 years old.

During the commenting period, the Applicant has proposed to reduce the concentration of methyl salicylate in mouthwash for children aged 10 or below from 0.6% to 0.1%. Therefore, new calculations of the MoS were made by SCCS to take into account this revised concentration of methyl salicylate in mouth wash. Only the updated concentrations were detailed in the final opinion.

Terms of reference

1. *In light of the data provided and taking under consideration the RAC Opinion on Methyl salicylate and the expected new classification as Toxic for Reproduction Category 2 of Methyl salicylate (to be introduced by an update of Annex VI Reg. 1272/2008), does the SCCS consider Methyl salicylate safe when used up to the maximum concentrations provided in the dossier submitted by the applicant?*

2. *Does the SCCS have any further scientific concerns with regard to the use of Methyl salicylate in cosmetic products, also in relation to the RAC recommended classification of Methyl salicylate as 'Skin sensitizer Category 1B'?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

Methyl salicylate (methyl 2-hydroxybenzoate; CAS 119-36-8 as 99% pure) is the methyl ester of salicylic acid and it is also the main component of the natural 'oil of wintergreen'. Methyl salicylate occurs naturally in wintergreen leaves or sweet birch bark [USP, 1995]. It can be obtained from such plants through distillation or derived synthetically through esterification of salicylic acid [USP (1995); Budavari, 1989]. Synthetic methyl salicylate (CAS 119-36-8), however, is the ingredient used in cosmetics products, as reviewed in this dossier. Although wintergreen oil is also considered a UVCB (unknown or variable composition, complex reaction products or of biological materials), spectroscopic analysis has demonstrated that the oil typically contains up to 99% methyl salicylate, a single chemical.

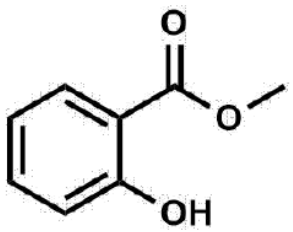


Figure 1 Chemical structure of methyl salicylate (CAS 119-36-8)

3.1.1.1 Primary name and/or INCI name

Methyl 2-hydroxybenzoate (INCI)

3.1.1.2 Chemical names

2-Hydroxybenzoic acid methyl ester
Benzoic acid, 2-hydroxy-, methyl ester
Methyl salicylate
Salicylic acid, methyl ester

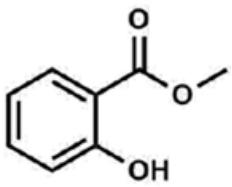
3.1.1.3 Trade names and abbreviations

2-HYDROXYBENZOATE DE METHYLE
2-Hydroxybenzoic acid methyl ester
Benzoic acid, 2-hydroxy-, methyl ester
HYDROXY-2 BENZOATE DE METHYLE
Methyl 2-hydroxybenzoate
Methyl salicylate
SALICYLATE DE METHYLE
Salicylic acid, methyl ester
Wintergreen oil

3.1.1.4 CAS / EC number

CAS No: 119-36-8
EC No: 204-317-7

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Empirical formula: $C_8H_8O_3$

3.1.2 Physical form

Colourless to slightly yellow liquid

Ref: Budavari 1989; REACH dossier

3.1.3 Molecular weight

Molecular Weight (g/mol): 152.15

3.1.4 Purity, composition and substance codes

Purity: ≥ 99% (trace impurities: methanol, anisole, methylbenzoate esters) (European Pharmacopoeia)

SCCS comment
A full report of the chemical characterization of methyl salicylate in terms of purity and identity in representative batches should be provided and the validity of the analytical methodologies used must be shown.

3.1.5 Impurities / accompanying contaminants

Information on impurities has not been provided.

SCCS comment
According to US Pharmacopoeia, salicylic acid and dimethyl 4-hydroxyisophthalate are organic impurities in methyl salicylate. A full report in terms of impurity tests in representative batches of the test substance should be provided and the validity of the analytical

methodologies used must be shown. Identity and concentration of any impurities that may be present must also be stated.

3.1.6 Solubility

Slightly soluble in water, 625 mg/L at 30°C. Soluble in organic solvent, including ethanol, chloroform, ether, glacial acetic acid and acetone.

Ref: Budavari 1989; REACH dossier

SCCS comments
Methyl salicylate should be considered as very slightly soluble according to the table in NoG.

3.1.7 Partition coefficient (Log P_{ow})

Log Pow: 2.55

Ref: REACH dossier

3.1.8 Additional physical and chemical specifications

- organoleptic properties : characteristic odour and taste of wintergreen
- melting point: −8.6 °C at 101.3 kPa
- boiling point: 221 °C at 101.3 kPa
- flash point: 95.5 °C at 101.3 kPa
- vapour pressure: 13 Pa at 20 °C density: 1.184 at 25 °C
- relative density: 1.1782 at 25 °C
- viscosity: 1.535 mPa.s at 25.15°C
- pKa: 10.13 (Calculated value); 9.8 and 9.87 (experimental value) refractive
- index (n 20/D) 1.534-1.538
- UV/ light absorption spectrum in methanol λmax: 305 nm

Ref: Budavari 1989; REACH dossier

3.1.9 Homogeneity and Stability

Stable. No oxidizing properties. Can react with oxidizing materials.

Ref: Sax, 1979

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, have to be provided.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

Use of methyl salicylate in cosmetic products

The use of methyl salicylate in cosmetics is not currently regulated under the Cosmetic Products Regulation EC 1223/2009 of the European Union. It is used as a flavouring agent and smoothing agent in oral hygiene products such as toothpastes, mouthwashes and breath fresheners (Norwegian FSA 2012; CIR, 2003, 2019). It is also used in perfumery, bathing products such as soaps, detergents and oils, body and hand preparations and mud packs, skin care preparations, foot powders and hair products such as shampoo and conditioners (CIR 2003, 2019; Norwegian FSA 2012).

Table 1: Maximum concentration of methyl salicylate in cosmetic products

Type of cosmetic product exposure	**Maximum % concentration used**
Hydroalcoholic-based fragrances	0.600%
Rinse-off skin & hair products (except hand wash products)	0.060%
Hand wash products	0.600%
Leave on skin & hair products (except spray and aerosol products)	0.060%
Face Make up products	0.050%
Lipsticks & lip balm	0.030%
Eye make up products & Make up remover	0.002%
Toothpaste	2.520%
Mouthwash for age 6-10 years	0.100%
Mouthwash for age above 10 years	0.600%
Mouth spray	0.650%
Deo spray/aerosol	0.003%
Leave on hair products spray/aerosol	0.009%

Body Lotion spray/aerosol	0.040%

Use of methyl salicylate in pharmaceuticals

Methyl salicylate is also used at high concentrations in some medical creams (see Table 2).

Table 2: Topical over-the-counter medical products and the % of methyl salicylate (Muniandy *et al.*, 2012)

Products	% Methyl-salicylate
Oil of wintergreen	98
Icy Hot Extra Strength Cream	30
Bayer Muscle Joint Strength Cream	30
Muscle Ryb Extra Strength Cream	30
Ben-Gay Extra Strength Cream	30
Tiger Balm Liniment	28
Ben-Gay Original Cream	18.3
Pain Bust-R ii Ointment	17
Thera-Gesic Cream	15
Banalg Hospital Strength Lotion	4.9
Red Flower Oil	67
White Flower Medicine Oil	40
Tiger Oil	38
Kwan Loong Medicated Oil	15

Wintergreen oil

Wintergreen oil is used in cosmetics such as bath products, cleansers, fragrance products, massage products, and moisturizers, among others. The concentration of wintergreen oil in these products ranged from less than 0.1% to 100%. The substance is also reported as a non-medicinal ingredient in topical, oral, and nasal natural health products (Health Canada, 2020). Concentrations of wintergreen in dermally applied natural health products ranged from less than 1% to 20% and the types of products were analgesic creams. Orally administered natural health products that contain wintergreen oil as a non-medicinal ingredient include antacids and an herbal tea with therapeutic effects.

Exposure assessment

Values for the maximum % level of methyl salicylate that are used in Europe, in each of the standard 17 product types, have been provided by the members of the Cosmetics Europe Methyl Salicylate consortium and are used to calculate the total dermal exposure to methyl salicylate (in mg/kg/day) from each product for adults.

3.2.2 Dermal / percutaneous absorption

Several studies have measured skin penetration of MS either *in vitro* or *in vivo* in animals. However, most of these studies are quite old and/or have serious shortcomings.

Many studies on human skin or in humans have been performed. A summary table of data for dermal absorption has been provided in the applicant dossier to facilitate selection of an appropriate dermal absorption value for risk assessment. Values for total absorption (methyl salicylate plus salicylic acid and all related salicylate metabolites) range from 3% to 93%

depending upon the species, the concentration applied, condition of application (open/occlusive) and vehicle (Table 3 for the *in vitro* studies).

It is also observed that methyl salicylate readily hydrolyses to salicylic acid and methanol on and in the skin and in the body (see Figure 2). The total absorbed values refer to all salicylate derivatives of methyl salicylate in the experiment, i.e. total absorption could include parent methyl salicylate, salicylic acid and phase 2 metabolites of both.

Table 3: Summary of observations from *in vitro* skin penetration studies using human skin

Exposure concentration/vehicle	Application site details	Observations	Reference
Topically, commercial formulation containing 20% MS	Full thickness breast skin or epidermal membrane; 15 mm pieces mounted stratum corneum uppermost in Franz-type glass diffusion cells; surface area 1.3 cm^2; 1 g of topically commercial formulation (200mg MS) applied to stratum corneum side of the skin; exposed for 24h.	Permeability as flux was 11.2 ± 0.7 µg/cm^2/h for full-thickness skin and 32.8 ± 2.0 µg/cm^2/h for epidermal membrane. The amount remaining in skin was 86.7 ± 28.7 µg/100 mg for full-thickness skin and 41.1 ± 44.1 µg/100 mg for epidermal membrane.	Cross et al 1998
2, 20 and 200 mM concentrations in acetone.	Fresh dermatomed (0.3 to 0.4mm) female breast skin. Bronaugh flow-through cells. 14C radiolabelled MeSal. 30 minutes exposure at 29°C temp. 6.5 h receptor fluid collection into Hank's HEPES buffered saline with 4% bovine serum albumin [BSA], pH 7.4	32% absorption into receptor solution at 2 mM, 17% at 20 mM and 11% at 200 mM dose. Including the skin depot absorption was 40 (2mM), 26%, (20mM), and 13% (200mM).	Moody et al 2007
1.0% (w/w) [phenyl-U-^{14}C] MS in cream (light soft body lotion)	**OECD 428 study; GLP** Split-thickness human skin samples were mounted into flow-through diffusion cells (1 cm^2). Application volume was 10 µL. 7 donors; n=2 samples. 24 hours exposure with carbon filter cap occlusion.	**Total absorbed dose = 20.17%** Dislodgeable dose = 64.70% Mass balance = 85.06%	Charles River Study No. 20144966 (2018)

<u>Conclusions from the applicant</u>

- Methyl salicylate is readily absorbed through the skin but the extent of absorption is highly dependent on the vehicle, being the most from acetone and the least from petrolatum based creams and ointments.
- Total absorption following dermal exposure to methyl salicylate was observed up to 93% in one study in humans (Yano *et al.* 1986) where acetone was used as a vehicle under highly occluded conditions, i.e. where taped foil was used to occlude the site. However, it would be inappropriate to use this value for a cosmetics risk assessment, given the high degree of occlusion and a vehicle that does not reflect real cosmetics product formulations.

- In the majority of the *in vitro* and *in vivo* dermal absorption studies reviewed above using commercial product bases, values were in the range of 12 to 37% (11 to 32% in Valussi, 2015).
- In ethanolic vehicles, methyl salicylate has been seen to vaporise as much as 82% from the skin surface, reducing the value for penetration significantly in the case of ethanol based products.
- A conservative estimate of dermal absorption for use in the risk assessment in the dossier therefore selected as 50% based on the weight of evidence from human *in vivo* studies and in relation to product base formulae.

SCCS comment
As the methyl salicylate absorption observed in *in vitro* studies was highly variable, SCCS agrees that a default value of 50% skin absorption, based on the data reported in human *in vivo* studies and on the physico-chemical properties of methyl salicylate, can be used to estimate systemic exposure following skin application.

3.2.3 Other studies on toxicokinetics

As toxicological effects of methyl salicylate depend on the absorption route, it is appropriate to consider route-specific data on systemic exposure for performing route-specific safety evaluations.

- Dermal distribution, metabolism and excretion

From the studies on absorption, hydrolysis has been observed for methyl salicylate in viable (metabolically active) skin, and to a lesser extent in non-viable skin. Hydrolysis of methyl salicylate is catalysed by esterase enzymes in the skin (Williams 1985). Under certain conditions, chemical hydrolysis of esters can occur, usually at more alkaline pH >7. Given that the skin is typically acidic to neutral, it is expected that hydrolysis is mediated by esterases in skin. Irrespective of the route of exposure (dermal, oral or *via* the lung), or the specific esterase that catalyses the reaction, the resulting products of first step esterase hydrolysis are salicylic acid and methanol (see Figure 2).

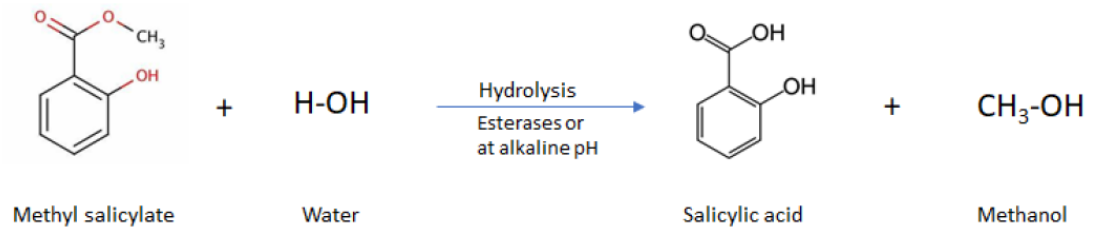


Figure 2: Hydrolysis of methyl salicylate to salicylic acid and methanol

The rate of production of these primary metabolites *in situ* in the body, as methyl salicylate enters the body *via* the different routes, may give rise to different systemic concentrations of each metabolite and the parent methyl salicylate. Esterases are in gut and liver tissue, and there are also plasma esterases, but there is no specific data on howmethyl salicylate ishydrolysed once inside the body *via* the dermal route.

The primary metabolites are in turn metabolised further. Salicylic acid (SA) is detoxified to form salicyluric acid (SUA) and glucuronide conjugates to facilitate excretion. Methanol is rapidly metabolised to form formaldehyde, but both methanol and formaldehyde are generated in the body in normal metabolic processes and the body has evolved highly efficient clearance mechanisms to deal with these two toxic metabolic by-products. Whether methanol

or formaldehyde is toxic in the body is dependent upon the levels that are formed and upon where and whether in-built metabolism can cope in detoxifying these levels.

Metabolism *via* the dermal route is rapid, with maximal absorption between 1-4 h, and mostly as salicylic acid and its secondary metabolites. Some studies indicate that methyl salicylate conversion to salicylic acid systemically could be assumed to be 50% as it passes through the skin, but then any parent material that enters the blood is hydrolysed rapidly in the blood and by the liver such that within only a few hours, no parent substance can be detected, only free salicylate/salicylic acid.

Studies by Morra *et al.* 1996 and Cross *et al.* 1998 indicated that metabolism was effectively complete and no unchanged methyl salicylate was measured in human urine.

Table 4 shows kinetic data for methyl salicylate in human volunteers.

Table 4. Dermal kinetics data in humans for methyl salicylate

Volunteers	Concentration & Vehicle	C_{max} (mg/L)	T_{max} (h)	Terminal Half-Life (h)	AUC (mg h/L)	Reference
10 humans took a 20 minute bath	30mg/L MS together with Siberian spruce-needle oil, thyme oil and camphor	Plasma conc: 0.4526	1	2.4 to 4	1 to 3.9	Pratzel et al 1990
Twelve subjects (6 male; 6 female).	Ointment containing 12.5% methyl salicylate	1.7±0.7 (Day 1) 3.9±1.2* (Day4)	6.0±2.0 4.4±1.3	NR	15.3±6.6 (0-12h)	Morra et al 1996

Conclusions from the applicant:

- Methyl salicylate is rapidly and extensively metabolised to salicylic acid and methanol *via* hydrolysis, on and in the skin by carboxylesterases, and in the body by ubiquitous esterase enzymes, as it enters the systemic circulation.

- In addition, a reasonable assumption could be made that 50% (if not all) parent methyl salicylate applied to the skin is hydrolysed by esterases by first pass metabolism in the skin to salicylic acid and further metabolism occurs in the blood, liver and other tissues, such that the body is actually exposed systemically to the primary metabolite salicylic acid, as generated from ubiquitous esterase hydrolysis.

- Oral absorption, distribution, metabolism, excretion and kinetics

Limited studies are available on the ADME properties and kinetics of methyl salicylate *via* the oral route in animals and humans. However, available data provide evidence that methyl salicylate is rapidly and extensively absorbed across the gut and is completely hydrolysed to its primary metabolites salicylic acid and methanol. An oral absorption value of 100% can be used in risk assessment, as proposed in Belsito *et al.* (2007), but it should be noted that this would most likely be in the form of free salicylate and a corresponding concentration of methanol.

- Inhalation absorption, distribution, metabolism, excretion and kinetics data

In a study by Buchbauer *et al.* (1993), groups of four female outbred Swiss mice (body weight 28.5g) were housed in cages where air was passed through a glass tube containing 1.5 ml methyl salicylate. The total methyl salicylate volume was 20-50mg. Blood samples were taken

at 0, 30, 60, and 90 mins. Plasma was analysed and in all samples taken after 1h, only traces of salicylate were found in blood samples. This limited evidence suggest that the kinetics of inhalation are different from the oral and dermal routes.
For the calculation of the internal dose following exposure by inhalation, the applicant has considered that 100% of inhaled Methyl salicylate is bioavailable.

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

3.2.4 Calculation of SED/LED

Dermal and oral exposure

Adults

Values for the maximum % level of methyl salicylate that are used in Europe, in each of the standard 17 product types, have been provided by the members of the Cosmetics Europe Methyl Salicylate consortium and are used to calculate the total dermal exposure to methyl salicylate (in mg/kg/day) from each product for adults.

Methyl salicylate is also used as a fragrance ingredient in hydroalcoholic fragrances (e.g. aftershave, eau de toilette, cologne), and therefore this has been added into the calculation as an 18th product type in Table 5. The exposure to product ($E_{product}$) value normalised by weight is calculated by including the respective retention factor for each product type (SCCS, 2021).

A generic maximal value for skin penetration of methyl salicylate of 50% (see section 3.2.1) 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 for passage across the oral mucosa. This enables an SED *via* the dermal route to be calculated for each product in mg/kg/day and also an aggregate systemic exposure dose for the 19 products (see Table 5 for exposure in adults).

Table 5: Deterministic worst-case systemic exposure dose calculation for the dermal route using maximum % levels. (NB. dermal exposure to spray/aerosol products are taken into account in the section on inhalation below, taken from the applicant dossier).

Category of Product	Examples of Product	Maximum use (w/w%) in the finished product	$E_{product}$ normalised by body weight [1] (mg/kg bw/day) per product	$E_{product}$ normalised by body weight [1] (mg/kg bw/day) Subtotal for the category	Total dermal exposure (mg/kg bw/day) Per product	% absorbed dermally	Calculated SED[2] (mg/kg bw/day) Per product	Calculated SED[2] (mg/kg bw/day) Per category
Hydroalcoholic-based fragrances		0.60	4.67	4.67	0.0280	50	0.0140	0.0140
Rinse-off skin & hair cleansing products (except hand wash)	Shower gel	0.06	2.79	4.97	0.0017	50	0.0008	0.0015
	Hair conditioner		0.67		0.0004		0.0002	
	Shampoo		1.51		0.0009		0.0005	
Hand wash soap		0.6	3.33	3.33	0.0200	50	0.0100	0.0100
Leave on skin & hair products	Body lotion	0.06	123.20	207.86	0.0739	50	0.0370	0.0623
	Face cream		24.14		0.0145		0.0072	
	Hand cream		32.70		0.0196		0.0098	
	Deodorant non-spray		22.08		0.0132		0.0066	
	Hair Styling		5.74		0.0034		0.0017	
Face Make-up products	Liquid foundation	0.05	7.90	7.90	0.0040	50	0.0020	0.0020
Lip products	Lipstick, lip salve[3]	0.03	0.90	0.90	0.0003	100	0.0003	0.0003
Eye make up & all Make up remover	Make-up remover for face	0.002	8.33	9.16	0.0002	50	0.0001	0.00009
	Eye make-up		0.33		0.000007		0.000003	
	Mascara		0.42		0.000008		0.000004	
	Eyeliner		0.08		0.000002		0.000001	
Oral care products	Toothpaste[3]	2.52	2.16	2.16	0.0544	100	0.0544	0.0544
	Mouthwash[3]	0.60	32.54	32.54	0.1952	100	0.1952	0.1952
	Aggregate				**0.43**		**0.34**	**0.34**

1.According to values in Table 4 of the SCCS 2018 notes of guidance; 2. Total external dermal exposure x 50% 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 4 of the SCCS 2018 notes of guidance, and it is assumed all of the retained MeSal can enter the systemic circulation via dermal and oral routes.

SCCS comment

Exposure *via* the oral route (lipstick, toothpaste and mouthwash with 100% absorption) is also included in this table. It is noteworthy that around 75% of the aggregated exposure is due to these 3 categories of products, and more than 84% when exposure to methyl salicylate *via* body lotion is considered in addition as a 4th category. Exposure to MeS *via* mouthwash already contributes to more than 57% of the total exposure.

Mouth spray products

In addition to toothpaste and mouthwash, methyl salicylate is proposed by the applicant to be used at up to 0.65% in mouth sprays. Mouth sprays are not habitually used products for breath freshening, but products that are used only occasionally.

Table 6: Estimation of systemic exposure dose from mouth spray products

Mouth spray	Adult
Methyl salicylate Concentration (%)	0.65
Amount (g)/day	1.60
Retention %	100
Gastrointestinal Absorption %	100
Conversion factor	1000
Body weight (kg)	60
Systemic Exposure Dose (SED) (mg/kg/day)	**0.173**

SCCS comment
An aggregate exposure assessment should be considered for adults because toothpaste, mouth wash and mouth spray may all be used on the same day. Therefore, the SED of 0.173 mg/kg/day should be added to the aggregated exposure from Table 5 (0.340 mg/kg/day), **leading to an aggregate exposure to MeS in products used in the mouth of 0.513 mg/kg/day.**

Children

Toothpaste

In general, children are expected to ingest more toothpaste and mouthwash than adults. Therefore, the SCCS made a separate SED calculation for children.

Intakes for 1-6 years of age:

The use of toothpaste starts with first erupted teeth and occurs with a high percentage of dentifrice ingestion. Therefore, the amount of toothpaste to be used by children age 6 and under, as implemented for fluoride toothpastes, is generally set at a pea-size amount. The SCCNFP (2003) defined this as 0.25 grams when assessing the safety of fluoridated oral care products for children. Furthermore, a retention factor of 40% for children 7 months-8 years of age was explicitly stated to be "already an overestimate" when these exposure calculations were revisited (SCCP 2005).

Taking the above intake values from product-use scenarios, and dividing by the EFSA default body weights (EFSA 2012b)(P5; lowest 5th percentile body weight) for specific age ranges, the following conservative intakes in mg/kg/day are calculated in Table 6.

Table 7: Intake of methyl salicylate by children 1-6 years old using toothpaste

1-6 years of age: Toothpaste			
Max Methyl Salicylate Concentration	IC	2.52	%
Amount used	A	0.25	g/use
Frequency	FQ	2	uses/day
Retention Factor	RF	40	%
Conversion Factor	CF	1000	mg/g
Systemic Exposure (mg/person/day) =		(IC)(A)(FQ)(RF)(CF)	
Systemic Exposure (mg/person/day) =		(2.52/100) (0.25 g/use) (2 uses/day) (40/100) (1000 mg/g)	
Intake (mg/person/day) =		**5.04**	

For the other age categories, the applicant provided analogous calculations: 7-10 years, 10-14 years, 14-18 years of age.

Taking the above intake values from product use scenarios (Table 7), and dividing by the EFSA default body weights (EFSA 2012b)(P5; lowest 5th percentile body weight) for specific age ranges, the following conservative intakes in mg/kg/day are calculated in Table 8.

Table 8: Intake in mg/kg/day of methyl salicylate in toothpaste

Age range (y)	Estimated Intake of product (mg/person/day)	P5 body weight (kg)	Mean body weight (kg)	Intake at P5 bw (mg/kg/day)	Intake at mean bw (mg/kg/day)
1-3	5.04	8.7	11.6	0.579	0.434
3-6	5.04	14	21.7	0.360	0.232
7-10	3.47	14	21.7	0.248	0.160
10-14	3.47	29.4	42	0.118	0.083
14-18	3.47	45	60	0.077	0.058

Mouthwash

The use of mouthwash potentially starts at age 6 (it is generally recommended that children under 6 should not use mouthwash). The usage volume of 21.62 ml/day and retention factor of 10 % from SCCS's 2018 Notes of Guidance is used. This is appropriate, considering published literature on the ingestion of mouthwash by children age 6, with a reported 8% retention (Zuanon, 2005). An industry-wide usage survey was conducted, and it was determined that currently marketed mouthwash contains up to 0.6 % methyl salicylate, and assuming roughly 1 ml mouthwash, is equivalent to 1g.

During the consultation period, to take into account the concerns raised by SCCS concerning the use of methyl salicylate up to 0.6% in mouthwash for age 6-10 years, the Applicant has proposed to reduce the concentration of methyl salicylate in mouthwash for children aged 10 or below from 0.6% to 0.1%.

Revised Table 9.1: Intake of methyl salicylate by children 6-10 years old using mouthwash

Mouthwash			
Max Methyl Salicylate Concentration	IC	0.10	%
Amount used	A	21.62	g/day
Retention Factor	RF	10	%
Conversion Factor	CF	1000	mg/g
Systemic Exposure Dose (mg/person/day) =	(IC)(A)(RF)(CF)		
Systemic Exposure (mg/person/day) =	(0.1 %)/100 (21.62 g/day) (10 %)/100 (1000 mg/g)		
Methyl Salicylate Intake (mg/person/day) =	**2.16**		

Table 9.2: Intake of methyl salicylate by children 10-18 years old using mouthwash

Mouthwash			
Max Methyl Salicylate Concentration	IC	0.6	%
Amount used	A	21.62	g/day
Retention Factor	RF	10	%
Conversion Factor	CF	1000	mg/g
Systemic Exposure Dose (mg/person/day) =	(IC)(A)(RF)(CF)		
Systemic Exposure (mg/person/day) =	(0.6 %)/100 (21.62 g/day) (10 %)/100 (1000 mg/g)		
Methyl Salicylate Intake (mg/person/day) =	**13.0**		

Taking the above intake values from product use scenarios (Table 9.1 and 9.2), and dividing by the EFSA default body weights (EFSA, 2012b)(P5; lowest 5th percentile body weight) for specific age ranges, the following conservative intakes in mg/kg/day are calculated in Table 10.

Table 10: Intake in mg/kg/day of methyl salicylate in mouthwash

Age range (y)	Estimated intake of product (mg/person/day)	P5 body weight (kg)	Mean body weight (kg)	Intake at P5 bw (mg/kg/day)	Intake at mean body weight (mg/kg/day)
6-10*	2.16	14	21.7	0.154	0.100
10-14	13.0	29.4	42	0.442	0.310
14-18	13.0	45	60	0.289	0.217

*Mouthwash is not recommended for under 6 years of age, the body weights chosen are from the range 3-10 years, and therefore very conservative.

SCCS comment
The SCCS considers that toothpaste and mouthwash have to be aggregated. For mouth spray, these are not habitually used products for breath freshening, and they are just used occasionally by children. Therefore, mouth sprays were not considered for the calculation of exposure to methyl salicylate for children.

The SED and MoS calculations presented in the Opinion were then done accordingly. The SCCS has made new calculations including toothpaste and mouth wash in aggregate exposure oral for children above 10 years old and selected the more conservative values for the calculation of the MoS, meaning:
- For children between 1 and 6y: only toothpaste containing 2.52% methyl salicylate : 0.579 mg/kg bw /d
- For children between 6 and 10y: 0.248 mg/kg bw/d from toothpaste containing 2.52% methyl salicylate and 0.155 mg/kg bw/d from mouthwash containing 0.1% methyl salicylate: aggregate exposure is 0.403 mg/kg bw /d
- For children above 10 years old: 0.118 from toothpaste containing 2.52% methyl salicylate) and 0.442 mg/kg bw/d from mouth wash containing 0.6% methyl salicylate: aggregate exposure is 0.560 mg/kg bw /d

For children, exposure to dermally-applied products expressed in mg/kg bw/day may be higher for some categories due to higher ratio between amounts of products used and body weights for some age categories. As in the SCCS Notes of Guidance (SCCS/1628/21), when no specific values were given for the amount of products used by children, the results from other studies such as Ficheux *et al.* (2017) can be used. SCCS has therefore made new calculations for the products that were identified in this study such as: Shower gel (g), Deodorant non spray, Fragrance, Hand wash soap, Face soap, Body lotion (sunscreen), Face cream, Hand cream, Shampoo, Conditioner, Lip Balm. The aggregated SED dermal and oral was calculated leading to 0.066 0,094 (P50) or 0.235 0,291 (P90) mg/kg bw/day (see Annex 1 for the calculations).

- Inhalation

According to the applicant, there are no measured inhalation exposure data for methyl salicylate. The usual practice is to estimate exposure either by one-box or two-box modelling as described below.

i) General considerations

Two exposure models for inhalation can be constructed: a one-box model and a two-box model.

For inhalation exposure, two exposure scenarios need to be assessed for methyl salicylate. The first one is due to the high volatility of this compound (vapor pressure = 13 Pa at 20°C). Therefore, evaporation of methyl salicylate retained on the skin needs to be taken into account for inhalation exposure and thus, concerns all dermally applied products. The second one is due to the exposure through the airborne fraction of sprayed products.
As a first-tier exposure assessment by inhalation, because it is often more conservative, the common one-box model is usually used. Indeed, the calculation is simple to pursue and does not require any mathematical modelling, in contrast to the two-box model. For the inhalation exposure related to the evaporation of methyl salicylate from the skin, the one-box model is considered to be the most appropriate model. In contrast, for spray products for the body, the 2-box model is more relevant.

Nevertheless, in the exposure assessment of spray products, both the two models were run to compare their results.

Based on this information, the exposure scenario for spray products has been performed by the applicant by using two models, briefly described as:

- One-box-model based on the room volume, the time spent in the room, i.e., time elapsed from the start of the emission and staying in this room and the respiration rate of adults. A more conservative assumption of absence of air exchange ("sealed room") is used in the scenario.

- Two-box model based on the volume and the time spent in each box of the room, as well as the air exchange between these different "boxes" and the respiration rate of an adult. The Research Institute of Fragrance Materials (RIFM) two-box model with adjustments has been used.

In developing these two models for inhalation exposure, the parameters used have been carefully considered and are described in detail below.

The default room volume of a bathroom, i.e. 10 m^3, has been chosen (Bremmer *et al.*, 2006; Biesebeek *et al.* 2014).

The duration of inhalation exposure may be assumed to be 10–20 min, in a worst-case scenario (Rothe *et al.*, 2011). The default parameters of the RIFM two-box model are 1 min, in the first box and 20 min. in the second box (the room). Therefore, the time spent in the room applied is 21 min. in the two models.

For the respiration rate, 0.009 m^3/min is used assuming rest to light activity as documented in US EPA (2011) and also similar to data reported in Biesebeek *et al.*, 2014.

ii) *Inhalation exposure of non-sprayed products dermally applied*

As described previously, the inhalation exposure to methyl salicylate from the retained fraction on the skin of dermally applied products is assessed by using the one-box model. The fraction retained on the skin is the fraction not dermally absorbed, i.e., 50%. Therefore the selected value for skin absorption in the dermal exposure risk assessment is directly linked to this calculation for inhalation. For the dermal exposure of lip products, 100% of bioavailability is considered through dermal/mucosal exposure and also implicitly covers potential ingestion (see Table 5). Nevertheless, for completeness, the inhalation exposure of methyl salicylate from this product category is also included to consider the potential inhalation just after application.

As a worst-case scenario, it is considered for this assessment that methyl salicylate volatilizes rapidly and thus, that the exposure takes place during time spent in the bathroom.

Concentrations are calculated as a function of the amount retained on the skin and considering 100% of methyl salicylate retained on the skin available for inhalation, the room volume, the respiration rate and the time spent in the room. Absence of air exchange ("sealed room") is considered in the scenario. In addition, it is considered that 100% of the methyl salicylate inhaled is bioavailable and will reach the systemic circulation.

The following calculations are made to calculate SED of potential maximum volatilised for inhalation from residual material on skin from non spray products in µg/kg/day:

SED by inhalation from non spray products (µg/kg/day) =

Residual µg/kg MeS/day not dermally absorbed on skin surface/10 m^3 x 21 min x 0.009 m^3/min

In order to calculate the full systemic exposure dose following the use of the dermally applied non-sprayable products, the exposure to methyl salicylate through the inhalation route is added to the dermal exposure.

Table 11: Results of 1-box modelling of inhalation exposure from non-spray dermal products, taken from the applicant dossier

Type of cosmetic product NON SPRAY PRODUCTS DERMALLY APPLIED	Examples of products	Methyl salicylate max. conc. in finished product % (w/w)	Exposure to the product normalized by body weight (mg/kg/day)		Amount of MeSal available for inhalation from residual product on skin[1] (µg/kg/day)	SED of MeSal by inhalation from residual product on skin[2] (µg/kg/d)		SED of MeSal by dermal exposure[3] (µg/kg/d)	TOTAL SED (dermal + inhalation) of MeSal (µg/kg/d)
Hydroalcoholic-based fragrances		0.6	4.67	4.67	14.0	0.26	0.26	14.0	**14.3**
Rinse-off skin & hair cleansing products (except hand wash)	Shower gel	0.06	2.79	4.970	0.84	0.016	0.028	1.49	**1.52**
	Hair conditioner		0.67		0.20	0.0038			
	Shampoo		1.51		0.45	0.009			
Hand wash soap		0.6	3.33	3.33	9.99	0.19	0.19	9.99	**10.2**
Leave on skin & hair products	Body lotion	0.06	123.2	207.9	37.0	0.70	1.18	62.4	**63.5**
	Face cream		24.14		7.24	0.14			
	Hand cream		32.7		9.81	0.19			
	Deodorant non-spray		22.08		6.62	0.13			
	Hair Styling		5.74		1.72	0.033			
Face Make-up products	Liquid foundation	0.05	7.9	7.90	1.98	0.037	0.037	1.98	**2.01**
Lip products[4]	Lipstick, lip salve	0.03	0.900	0.90	0.14	0.0026	0.0026	0.27	**0.27**
Eye make up & all Make up remover	Make-up remover for face	0.002	8.330	9.16	0.08	0.0016	0.0017	0.092	**0.093**
	Eye make-up		0.330		0.0033	0.0001			
	Mascara		0.42		0.0042	0.0001			
	Eyeliner		0.08		0.0008	0.00002			
	TOTAL			**238.8**	**90.1**		**1.70**	**90.2**	**91.9**

[1] Calculation based on the fraction of Methyl salicylate retained on the skin which is the fraction not absorbed, i.e., 50% of the applied amount. 100% of of Methyl salicylate retained on the skin is considered available for inhalation.
[2] Parameters used for 1-box model: room volume = 10 m^3; time in the room = 21 min; ventilation = sealed room = 0. Respiration rate 0.009 m^3/min (US EPA, 2011; Biesebeek *et al.* 2014). 100% of inhaled Methyl salicylate is considered bioavailable.

SED (inhalation) = Residual µg/kg MeSal/day not dermally absorbed on skin surface/10 m^3 x 21 min x 0.009 m^3/min
[3] SED (dermal) = µg/kg MeSal/day dermally applied x dermal absorption (50% except lip products 100%)
[4] For lip products, as for the other dermally applied products, 50% of Methyl salicylate is considered available for inhalation. For the SED dermal calculation, 100% of dermal absorption is considered; therefore, the TOTAL SED (dermal + inhalation) includes only the SED dermal.

SCCS comment
As the exposure *via* inhalation is limited compared to dermal absorption, the SCCS decided not to consider it for children.
For adults, instead of 0.009 m^3/min as taken by the applicant, the SCCS recommends using the respiration rate of 0.013 m^3/ min based on Rothe *et al.* (2011). Therefore, a factor of 1.44 has been be applied to the SED of MeS by inhalation, leading to 2.46 µg/kg/d for the aggregated exposure by inhalation and to 92.6 µg/kg/d for dermal + inhalation.

iii) <u>Inhalation exposure of sprayed products dermally applied</u>

The sprayed products considered by the applicant in this assessment are:

- Hydroalcoholic-based fragrance spray
- Deodorant spray
- Hair leave-on spray
- Body lotion spray (i.e referring to body lotion products formulated in emulsion).

For sprayed products, the daily amount use (g/d) for hydroalcoholic fragrance spray is 0.28 g/day, reported as the weight loss after use (Ficheux and Roudot, 2017).

For deodorant sprays, the amount that is used in the assessment is 6.1 g/d, reported as the weight loss after use of spray by Hall *et al.* (2007).

Regarding the daily amount of product use for hair spray, Steiling *et al.* (2014) reported 6.8 g/d for hair spray (aerosol) from Bremmer *et al.* (2006) and 3.6 g/d for hairspray (pump spray) from Loretz *et al.* (2006). In this assessment, the worst-case amount for hair spray is used for aerosol and pump sprays, which is the one corresponding to aerosol, i.e. 6.8 g/d. For body lotion spray, the amount used is 7.82 g/day as reported in the SCCS notes of Guidance 10th version (2018).

To calculate the full systemic exposure dose of methyl salicylate following the use of the sprayable product, the inhalation route through the airborne fraction and the fraction retained on the skin that can volatilize need to be added to the dermal exposure.

In this regard an experimental study conducted by Steiling *et al.* (2012) showed the fraction of the dose in ethanol-based deodorant sprays to be 23.5% for dermal exposure and consequently 76.5% available for inhalation, whereas for non ethanol-based deodorant sprays it was 11.4% for dermal exposure and 88.6% available for inhalation. To consider the worst-case scenario for inhalation exposure, the fraction of 88.6% available for inhalation has been applied. This is highly conservative since a significant fraction of the 88.6% fraction will be deposited on surfaces and will not be available for inhalation.

For hairspray and hydroalcoholic fragrance products, as a first-tier exposure assessment and as a worst-case scenario, 100% of the sprayable product has been considered as available for inhalation. In reality, only a fraction of this is available for inhalation. For example, in Bremmer *et al.* (2006) the assumption for dermal exposure is that 85% is retained on the skin and the product fraction available for inhalation is assumed to be similar (15%) for all the spray products.

For the dermal exposure, the assumption made by Bremmer *et al.* (2006) is that 85% is retained on the skin is used for hydroalcoholic fragrance sprays and hair sprays. For body lotion spray, based on the galenic of these products, it is assumed that the fraction available for inhalation is small. Therefore, the Bremmer *et al.* (2006) assumption is retained, and it is considered that 15% of the sprayed product is available for inhalation and 85% is retained in the skin.

Lung exposure would mostly result from inhalation of product droplets <10 µm that can reach the deep lung and of which the delivered fraction is very low with pump sprays (≈ 1% for hydro alcoholic-based fragrances and hair styling pump sprays) and rather low (≈10%) with aerosol hairsprays and of about 20% for aerosolized deodorants (Delmaar and Bremmer, 2009). Aerosol droplets will also settle on to clothes and furniture, which will reduce the amount available for inhalation. Furthermore, the nature or use of the product will also have an impact of the level of exposure; whether it is sprayed close to the face or on the body distant from the face, for example. However, in this exposure assessment, as a first-tier exposure assessment and as a worst-case scenario, it is assumed that for all sprays, the fraction of the sprayed product available for inhalation (88.6% for deodorant sprays; 100% for hydroalcoholic fragrance sprays and hairsprays and 15% for body lotion spray) is entirely inhaled and it reaches the systemic circulation.

As described previously, the inhalation exposure to Methyl salicylate from the retained fraction on the skin of the sprayed products is assessed by using the one-box model. The fraction retained on the skin is the fraction not dermally absorbed, i.e., 50%.

For the inhalation exposure to methyl salicylate from the airborne fraction of the sprayed products, if the two-box model is considered the most relevant for products sprayed at the body, the two models have been run and both results are shown (Table 12).

The one-box model is based on the assumption that particles/droplets are homogeneously distributed in an exposure room of known volume.

Concentrations are calculated as a function of the sprayed amount, the room volume and the respiration rate as well as the time spent in the room. Absence of air exchange ("sealed room") is considered in the scenario.

The RIFM two-box model is a conservative approach, yet more realistic than the one-box model, using the concept of "room within a room" model to calculate peak air concentrations from multiple sources under typical consumer use conditions. The two-box model and its related parameters are described in the Appendix 1. The current assessment of methyl salicylate exposure from sprayable products is based on the use of near-field RIFM two-box model (Steiling *et al.* (2014); FEA (2013)) including some modifications related to the exposure of the sprayable products taken into account in this risk assessment (Appendix 1).

The following calculations were made by the applicant to calculate SED of methyl salicylate in µg/kg/day:

SED (potential maximum for dermal absorption) from spray/aerosol products =

product use (g/day) x 1000/60kg x retention factor x % level MeSal x % proportion on skin x 50% dermal absorption x 1000 = SED in µg/kg/day

SED (potential maximum volatilised for inhalation from residual material on skin) from spray/aerosol products =

Residual µg/kg MeSal/day not dermally absorbed on skin surface/10 m^3 x 21 min x 0.009 m^3/min = SED in µg/kg/day

SED (inhalation for airborne substance using the 1-box model) from spray/aerosol products =

(product use (g/day) x % level MeSal x % airborne/10 m^3 x 21 min x 0.009 m^3/min)/60kg = SED µg/kg/day

Table 12: Total exposure by dermal and inhalation from sprayed products with one-box and two-box inhalation model, taken from the applicant dossier

Product type SPRAY	Methyl salicylate max. conc. in finished product % (w/w)	Daily amount of product use[1] (g/d)	SED of MeSal by dermal absorption from spray products[2] (µg/kg/d)	SED of MeSal by inhalation from residual product on skin (µg/kg/d)	SED of MeSal by inhalation from airborne product[3] (µg/kg/d)		Total SED of MeSal for potential inhalation[4] (µg/kg/d)	TOTAL SED of MeSal (dermal + inhalation) (µg/kg/d)
Hydroalcoholic fragrance spray	0.6	0.28	11.90	NA[3]	1-box model	0.529	0.529	12.43
					2-box model	*0.467*	*0.467*	*12.37*
Deodorant spray	0.003	6.1	0.174	0.0033	1-box model	0.051	0.054	0.228
					2-box model	*0.045*	*0.048*	*0.222*
Hair leave-on spray	0.009	6.8	0.434	NA[3]	1-box model	0.193	0.193	0.627
					2-box model	*0.184*	*0.184*	*0.617*
Body lotion Spray	0.04	7.82	22.16	0.419	1-box model	0.148	0.567	22.72
					2-box model	*0.130*	*0.549*	*22.71*
TOTAL			34.66	0.421	**1-box model**	**0.921**	**1.34**	**36.01**
					2-box model	***0.825***	***1.25***	***35.91***

[1]Hydroalcoholic fragrance spray (0.28 g/d = 4.67 mg/kg/d (Ficheux and Roudot, 2017, SCCS/1599/18). Deodorant spray (6.1 g/d sprayed; Hall *et al.* 2007). Hair leave-on spray (6.8 g/d; Steiling *et al.*, 2014 / Bremmer *et al.*, 2006). Body lotion spray (7.82 g/d; SCCS NoG 10th);
[2]85% of total amount of the sprayed product is considered available for dermal exposure (as per Bremmer 2006). 50% dermal bioavailability; dermal retention factor 1.0 for Hydroalcoholic fragrance spray, deodorant spray and body lotion spray, and 0.1 for hairspray as per SCCS Notes of Guidance Revision 10;
[3]Deodorant spray: inhalable fraction 88.6% (Steiling *et al.* 2012). Body lotion spray: inhalable fraction 15% (Bremmer *et al.* 2006). Hydroalcoholic fragrance spray and hair leave-on spray: 100% product available for inhalation; based on worst case scenario, the inhalable fraction from the product retained on the skin is not considered. 60 kg body weight. Respiratory tract exposure is equal SED as 100% of lung absorption is considered. Parameters used: room volume = 10 m3; time in the room = 21 min; ventilation = sealed room = 0. Respiration rate 0.009 m3/min (US EPA, 2011; Biesebeek et al 2014).
[4]Total SED of MeSal for potential inhalation is the sum of SED of MeSal by inhalation from residual product on skin and of the SED MeSal from airborne product.

These results show that even by considering the worst-case scenario in terms of the bioavailabililty of the methyl salicylate for sprayed products, the inhalation exposure is well below the dermal exposure. In this instance, the 2-box modelling did not have a significant quantitative impact on the outcome for the risk assessment. Considering the 2-box model more relevant for the products sprayed at the body (Steiling *et al.*, 2014), the results of the 2-box model are retained for use in the risk assessment of the aggregate inhalation exposure dose (Table 16).

SED Dermal from spray products = 34.66 µg/kg bw/d

SED Inhalation from spray products (2-box) = 1.25 µg/kg bw/d

SED from spray products dermal plus inhalation = 35.91 µg/kg bw/d

SCCS comment
Instead of 0.009 m^3/min as taken by the applicant, the SCCS recommends using the respiration rate of 0.013 m^3/ min based on Rothe *et al.* (2011). Therefore, a factor of 1.44 should be applied to the SED, leading to:
SED Dermal from spray products = 34.66 µg/kg bw/d

SED Inhalation from spray products (2-box) = 1.80 µg/kg bw/d

SED from spray products dermal plus inhalation = 36.46 µg/kg bw/d

iv) Aggregate Inhalation exposure of products dermally applied (sprays and non-sprays)

For the aggregate exposure calculation in the deterministic approach, there can be no duplication of similar products. Table 13 shows the exposure to methyl salicylate for the product categories duplicated in spray and non-spray products.

Table 13: Comparison of SED by dermal exposure and inhalation exposure for products available as spray and non-spray, taken from the applicant dossier**:**

Type of cosmetic product	Methyl salicylate max. conc. in finished product % (w/w)	External Exposure to MeSal normalized by body weight (µg/kg/day)	SED of MeSal by dermal exposure (µg/kg/d)	SED of MeSal by inhalation (µg/kg/d)	TOTAL SED (dermal + inhalation) of MeSal (µg/kg/d)
Hydroalcoholic fragrances non spray	0.6	28.0	14.0	0.260	14.3
Hydroalcoholic fragrance spray	0.6	28.0	11.9	0.467	12.4
Deodorant non spray	0.06	13.2	6.62	0.125	6.75
Deodorant spray	0.003	0.348	0.174	0.048	0.22
Hair leave-on non spray	0.06	3.444	1.722	0.033	1.75
Hair leave-on spray	0.009	0.867	0.434	0.184	0.62
Body Lotion non spray	0.06	73.9	37.0	0.699	37.7
Body Lotion Spray	0.04	44.3	22.2	0.549	22.7

The data in Table 13 show that dermal exposure is always higher for non-spray products than for sprayed products.

For inhalation exposure, only hydro alcoholic fragrance spray and hair spray show a higher exposure than the related non-spray products. Therefore, the risk assessment of inhalation exposure will be based on the total inhalation exposure of non-sprayed products, except for hydro alcoholic-based fragrance and hair spray leave-on for which spray data will be used.

Finally, for the total systemic exposure including both routes, the exposure through the non-spray products is higher. Therefore, for systemic aggregate exposure calculation, only the product categories of dermally non-sprayed products will be taken into account.

SCCS comment
Instead of 0.009 m^3/min as taken by the applicant, the SCCS recommends using the respiration rate of 0.013 m^3/ min, based on Rothe *et al.* (2011). Therefore, a factor of 1.44 should be applied to the SED of MeS by inhalation. Even in these conditions, the exposure through the non-spray products would be higher compared to spray products of the same categories. Therefore, the SCCS agrees that the risk assessment of inhalation exposure will be based on the total inhalation exposure of non-spray products.

Table 14: Aggregate exposure of all dermally and orally applied products containing methyl salicylate, calculations performed by SCCS

Products	Concentration %	SED dermal mg/kg bw/d	Sed inhal mg/kg bw/d	SED oral mg/kg bw/d	SED Tot mg/kg bw/d
Hydroalcoholic - based Fragrances	0.600	0.014	0.00038	0	0.01438
Shower gel	0.060	0.0008	0.000023	0	0.00082
Shampoo	0.060	0.00045	0.00013	0	0.00058
Hair conditioner	0.060	0.0002	0.0000054	0	0.00021
Hand wash soap	0.600	0.0099	0.001	0	0.01090
Body lotion	0.060	0.03696	0.001	0	0.03796
Face cream	0.060	0.007242	0.0002016	0	0.00744
Hand cream	0.060	0.00981	0.0002736	0	0.01008
Deodorant non-spray	0.060	0.006624	0.0001872	0	0.00681
Hairstyling products	0.060	0.001722	0.00004752	0	0.00177
Liquid foundation	0.050	0.001975	0.00005328	0	0.00203
Make up remover	0.002	0.0000833	0.000002304	0	0.00009
Eye make up	0.002	0.0000033	0.000000144	0	0.00000
Mascara	0.002	0.0000042	0.000000144	0	0.00000
Eyeliner	0.002	0.0000008	0.0000000288	0	0.00000
Lip products	0.030	0.00027	0.000003744	0	0.00027
Toothpaste*	2.520	0	0	0.0544	0.0544
Mouthwash*	0.600	0	0	0.19524	0.19524
Mouth Spray*	0.650	0	0	0.173	0.173
Total		0.0900446	0.003307965	0.42264	0.51599

* 100% oral/mucosal absorption as a worse-case scenario

Other sources

The JECFA evaluated a group of hydroxyl- and alkoxy-substituted benzyl derivatives, including methyl salicylate, at the 57th meeting of the JECFA (WHO, 2002). As part of that evaluation, the Committee estimated a per capita intake (PCI) of the substance via its use as a food flavouring agent based on reported annual production volumes. The annual production of wintergreen oil was 2733 lb and 44,000 kg for methyl salicylate for flavouring use in the U.S. as reported in the 57th report by JECFA. Since the annual production values for methyl salicylate are higher than for wintergreen oil, the PCI for methyl salicylate can be used as a conservative surrogate to estimate a PCI for wintergreen oil, since the JECFA has not yet established a PCI for wintergreen oil. JECFA estimated the PCI for methyl salicylate in the U.S. to be 44 mg/day (approximately 0.7 mg/kg bw/day, for a 60 kg adult). While JECFA usually calculates the PCI on the basis of 10% proportion of eaters, a survey of intake showed that over 50% of the population would be expected to consume methyl salicylate, therefore the calculated intake was refined from 0.7 mg/kg bw/day to 0.1 mg/kg bw per day (based on

an average body weight of 60 kg for the general population). JECFA concluded "no safety concern" at the current levels of intake when used as a flavouring agent for methyl salicylate (WHO 2002).

MeS exposure *via* wintergreen oil

Wintergreen oil may contain up to 99% methyl salicylate. Based on notifications under the Cosmetic Regulations, wintergreen oil – which is found in cosmetics such as bath products, cleansers, fragrance products, massage products and moisturizers, among others. The concentration of wintergreen oil in these products ranged from less than 0.1% to 100%. The substance is also reported in the LNHPD as a non-medicinal ingredient in topical, oral, and nasal natural health products. Concentrations of wintergreen in dermally-applied natural health products ranged from less than 1% to 20% and the types of products were analgesic creams. Orally administered natural health products that contain wintergreen oil as a non-medicinal ingredient include antacids and an herbal tea for therapeutic effects (Health Canada, 2017, 2020).

3.3 TOXICOLOGICAL EVALUATION

A number of reviews covering the available safety data for methyl salicylate are provided in the peer review literature: a Cosmetics Ingredient Review (2003, 2018, for all salicylates); two from the Research Institute for Fragrance Materials (RIFM) (Belsito et al 2007; Lapczynski *et al.* (2007) and a review covering oral toxicity by Greene *et al.* (2017). The CIR concluded in their review in 2018 that methyl salicylate, and all salicylates, are safe in the current practice of use in cosmetics. A new literature search has also been conducted by the applicant for this dossier to include all up-to-date references to January 2020.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2002), United States Environmental Protection Agency Biopesticide and Pollution Prevention Division (US-EPA 2005), Norwegian Food Safety Authority (NFSA (Mattilsynet) 2012), and the European Food Safety Authority (EFSA 2012a) currently define an acceptable daily intake (ADI) for methyl salicylate of 0.5 mg/kg/day. This ADI is based on using the no observed adverse effect level (NOAEL) of 50 mg/kg/day as the point of departure reported in the selected 2-year oral study in dogs and rodents (Webb & Hansen, 1963).

Since then, studies by the US National Toxicology Programme (NTP 1984a, 1984b) and high quality OECD guideline reproductive and developmental studies have also been performed in 2006 (US-FDA, 2012), which were not available when JECFA set the ADI, and have not been reviewed comprehensively in other foods and cosmetic safety dossiers.

In 2013, methyl salicylate was proposed by the French authorities (ANSES) for selection as a candidate CoRAP (community rolling action plan) substance under the REACH regulation (EC) No 1907/2006, as a suspected CMR substance based on hints of embryo-foetotoxicity in some fairly dated studies for methyl salicylate. Methyl salicylate is also 'under assessment' in the EU for endocrine disrupting (ED) potential, as a result of it being regarded as structurally similar to salicylic acid, which is also set for review as part of the European Commission call for data on list B of suspected EDs. During the CoRAP process, a call for further *in vitro* data on methyl salicylate for eye irritation was made, namely an OECD 491 study. The dossier provided by the applicant now contains this new study.

Methyl salicylate has no entry in Annex VI of the Classification, Labelling and Packaging (CLP) Regulation and therefore it was reviewed under CLP and a Harmonised Classification and Labelling report was drafted and reviewed (ECHA-CLH report, June 2018).

A CMR category 2 classification has been agreed by the RAC for methyl salicylate. This then activates the need for a SCCS review of a full cosmetics products safety dossier to support continued use of methyl salicylate in cosmetics products. The RAC Opinion on the proposed harmonised classification and labelling was adopted on 20 September 2019 by consensus.

The CMR category 2 classification for methyl salicylate is consistent with the 2016 CMR category 2 classification decision for salicylic acid. Salicylic acid is the principal primary metabolite of methyl salicylate via the dermal and oral routes: systemically the body is exposed to more salicylic acid metabolite than to the parent compound.
A SCCS Opinion was already published for salicylic acid in 2019 concluding that its use was safe when used as a preservative in all cosmetic products up to a maximum concentration of 0.5%. In addition, the SCCS found salicylic acid safe for non-preservative uses to a maximum concentration of 3.0% for cosmetic rinse-off hair products, 2.0% for non-cosmetic use in other product types except for body lotion, eye shadow, mascara, eyeliner, lipstick and roll-on deodorant applications, which are limited to salicylic concentrations up to 0.5%. The SCCS position however excluded oral care products, except lipstick, because exposure estimates were based on a use survey showing that salicylic acid is not directly added in oral care products such as toothpaste and mouthwash due to its bitter taste. However, the SCCS noted that if salicylic acid were to be used as an ingredient in oral care products up to 0.5%, the resulting increase in exposure would likely be very low.
https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_223.pdf

The applicant confirms that the information contained in this dossier complies with the provisions on animal testing as laid down in Article 18(1) of Regulation (EC) No 1223/2009.

Chemical structure and pharmacological action (taken from ECHA-CLH Dossier 2018)

Methyl salicylate (MeS) and acetylsalicylic acid (ASA, aspirin) are related substances, both are esters of SA (ortho-hydroxy benzoic acid), which is characterised by a carboxyl group and a hydroxyl group. Salicylic acid (SA) is the common hydrolysis product of both substances.
Both esters are hydrolysed in the mammalian organism. Besides salicylic acid, acetic acid is a hydrolysis product of ASA. The pharmacological effects of ASA are largely caused by its capacity to acetylate (and inactivate) cyclooxygenase and inhibit prostaglandin synthesis. Methanol is set free from MeS by hydrolysis.
After administration of SA or its esters, the principal metabolite circulating in plasma at comparable concentrations is salicylate. Therefore, RAC considers that in the absence of data for MeS, data from the acetyl ester of SA is acceptable for read across to the methyl ester. Possible effects of the acetyl or methyl moieties generated from acetyl or MeS by hydrolysis are not taken into consideration in such an approach.
Differences in protein-binding fractions of salicylate have been described in various species. Binding fractions also depend on the experimental conditions, e.g. the salicylate concentration. At a drug level of 150 mg/L, the plasma binding fractions were 60 % (human), 58 % (monkey), 55 % (rabbit) and 36 % (rat). Other authors found slightly different binding fractions, however, in all studies binding fractions in humans, primates and rabbits were higher than in rats.

3.3.1. Irritation and corrosivity

3.3.1.1 Skin irritation

Animal data

The available irritation studies in animals are listed in Table 19.

Table 15: Skin irritation studies in animals

Test material % & pH	Test conditions	Results	Reference
Rabbit			
1%, 3%, and 6% in water	Primary irritation study (24- and 72-h occluded patch); n=3 per group	1%: no irritation; 3% and 6%: irritation	Yankell, 1972
1%, 3%, or 6% in PEG 400		1%, 3% and 6%: irritation	
1%, 3%, and 6% in 70% ethanol		1%, 3% and 6%: irritation	
1%, 3%, and 6% in 70% ethanol plus emollients		1%, 3% and 6%: irritation	
100%	Irritation evaluated during an associated LD50 study; n = 10 animals	Irritation observed	RIFM (1973a)
1, 5, 10, 25, 100% in ethanol/diethyl phthalate 1:1	Albino Mol:Russian rabbits. OECD Guideline 404 (Acute Dermal Irritation/Corrosion). GLP certified.	For 100% test substance, animals showed from very slight to well-defined erythema and/or oedema from the 1-hour to the 72-hour observations. At the 7-day observation all animals showed small white scales on the test site. these had resolved by the 14-day observation. For the 25% solution, only very slight erythema was noted in one animal at the 24- and 48-hour observations. No reactions were noted for the 1, 5 or 10% concentrations.	Unpublished (Schreiter 1999) study report cited in EU REACH dossier

Guinea-pig			
0.03–100% (vehicle not specified)	Himalayan whitespotted guinea pigs (6–8/ sex/group); as a single application or as a pre-test for an OET (24 h primary irritation); applied daily for 21 days	0.03%, 0.1%, 1%: no irritation. 3%: minimal irritating concentration; 10–100%: irritation observed	Klecak et al. (1977)
Mouse			
100%	6 Mice (hairless); irritation assessed as part of a photoirritation study	Irritation observed	RIFM (1976c)
2.5, 5.0, 7.5 and 10% in ethanol	Irritation studied as part of a mouse ear swelling test	Irritation observed	Patrick et al. (1985, 1987); Patrick and Maibach (1986)
1%, 2.5%, 10%, and 20% in 4:1 acetone to olive oil	Irritation studied as part of a mouse ear swelling test	1%, 2.5%, 10%: no irritation. 20%: established as the minimal irritating concentration producing significant increase in ear swelling	Howell et al. (2000)
Mini-Pig			
100%	Irritation evaluated as part of a photoirritation study	Irritation observed	RIFM (1976c)

In primary irritation studies, methyl salicylate is observed to have skin-irritating properties that is influenced by vehicle and concentration. In rabbits, with 70% ethanol + emollient, methyl salicylate is a moderate irritant starting at 1%. Conversely, in propylene glycol as a vehicle, there was little or no irritation up to 6% methyl salicylate (Yankell, 1972). Although irritation was often seen in animal studies, it was only slight to moderate.

In the study from Schreiter (1999), four female albino rabbits were exposed to the test article and the vehicle at two skin sites on the back. After 4 hours of exposure, the test article was removed and the skin was examined at 1, 24, 48 and 72 hours, as well as 7 and 14 days after the termination of exposure. Slight to well-defined skin reactions were observed. The mean scores reported were: undiluted methyl salicylate: 1.3 for erythema and 0.6 for oedema; 25% solution: 0.2 for erythema and 0.0 for oedema. No reactions were reported for 1, 5 or 10% solutions. Slight to moderate skin irritation is observed in animal studies and the extent of irritation is vehicle dependent.

Human data

A range of skin irritation tests in humans are presented in Table 16.

Table 16: Skin irritation studies with methyl salicylate in humans

Test material % & pH	Test conditions	Results (classified as)	Reference
12% wintergreen oil in petrolatum; 80–99% methyl salicylate	Maximization pre-test (48-h occluded patch)	No irritation (0/25 volunteers)	RIFM (1976b)
8% (vehicle not specified)	Maximization pre-test (48-h occluded patch)	No irritation (0/27 volunteers)	RIFM (1973b)
8% in petrolatum	Closed 48h patch test	No irritation	Opdyke, 1978
4% in petrolatum	Closed 48h patch test	No irritation	Opdyke, 1979
12 to 50%	Dermal absorption study n=5 subjects	Reported pain and erythema at the application site	Roberts et al 1982
25 ml of 30% or 60% solutions	24-h occluded patch test	Irritation observed (9 volunteers)	Green and Shaffer (1992)
3.75, 15, 38, 40, 67%	20 patients with eczema or contact dermatitis.	Irritation in 8 subjects at 67% and in 2 subjects at 40%	Lee & Lam, 1990
12.5% in an ointment	Dermal absorption study (6 male; 6 female). Applied twice daily for 4 days.	All subjects reported burning, stinging and erythema at the application site, and prolonged erythema for up to 7 days after the last dose.	Morra et al 1996

Overall, there is no evidence of a skin irritation potential of methyl salicylate in humans at concentrations up to 12%. Relevant signs of irritation may only be observed at higher doses.

Skin Irritation and Corrosivity Conclusion from the applicant

Some studies in both animals and humans in the peer-reviewed literature suggest that methyl salicylate may be slightly irritating to the skin at doses present in commercial products. While irritation can occur at high does, the OECD guideline 404 study performed to GLP by Schreiter (1999) indicates that methyl salicylate does not require classification as a skin irritant according to the criteria of the Regulation (EC) No 1272/2008 on classification and labelling of chemicals. Sufficient evidence is also available from human tests that shows methyl salicylate is not an irritant up to doses of 12%.

3.3.1.2 Mucous membrane irritation / eye irritation

***In vitro* Data**

As per the request in the EC CLH 2018 report, an *in vitro* OECD guideline 491 eye irritation assay was performed following GLP (Roth, 2019: Envigo study number 1948700).

The Short Time Exposure *In Vitro* Test Method (STE) was performed using the rabbit corneal cell line SIRC. Pharmaceutical grade methyl salicylate was used at 0.05 and 5% in physiological saline solution. A 0.01% solution of Sodium Lauryl Sulfate (SLS) in physiological saline was used as the positive control. It induced an expected distinct reduction in cell viability in all tests within the range of the acceptance criteria. The test medium and physiological saline were used as negative controls.
Both concentrations (0.05 and 5%) of the test item were tested three times with three replicates per test. The cells were incubated for 5 minutes at room temperature.

The medium control showed an OD of ≥ 0.3 after subtraction of blank OD in all tests. The test results are shown in Table 17:

Table 17: Summary of results from the OECD Guideline 491 study on methyl salicylate (Roth 2019).

	Cell viability (%) per Test			Mean cell viability (%)	Standard deviation (%)
	Test 1	Test 2	Test 3		
Medium control	109.8	97.7	115.2	107.6	9.0
Solvent control (0.9% NaCl)	100.0	100.0	100.0	100.0	0
Positive control	34.9	19.6	13.7	22.7	10.9
Test item 0.05%	11.6	3.9	19.9	11.8	8.0
Test item 5%	25.5	26.8	31.0	27.8	2.9
Solvent control (0.9% NaCl) compared to medium control	91.1	102.4	86.8	-	-

Toxic effects were observed following incubation with the two tested concentrations of 0.05% and 5% in all of the runs. The cell viabilities were reduced below 70% for the 0.05% test item treated cells in the range between 3.9% and 19.9% and for the 5% test item treated cells in the range between 25.5% and 31.0%. The slightly higher measured cell viability of the 5% test item treated cells compared to the cell viability of the 0.05% treated cells was possibly due to changes of the microtiter well surfaces only observed in the wells of the 5% test item treated cells (possibly reactivity of the test item with plastic of the microtiter plate). This could have led to a higher absorption for the 5% test item treated cells. However, since both test item concentrations induced a decrease of the cell viability below 70%, the test item methyl salicylate is classified as "Category 1" according to the UN GHS classification category of the OECD Guideline 491 and to the EU CLP regulation criteria (Classification Labelling and Packaging).

***In vivo* Data**

No *in vivo* data were available.

Eye Irritation Conclusion from the applicant:

As both concentrations (0.05 and 5%) of test items reduced viability to below 70%, methyl salicylate is classifiable as Category 1 for eye irritation or serious eye damage according to the Regulation (EC) No 1272/2008 on classification and labelling of chemicals (GHS) criteria. However, considering that methyl salicylate in cosmetic products close to the eye zone such as face make-up remover, eye make-up, mascara and eyeliner is used only at concentrations up to 0.002%, it can be concluded that there is no risk of eye irritation for the consumer from these exposures.

SCCS comments
SCCS considers that methyl salicylate is non irritating to the skin at a concentration up to 12%, but it may cause severe eye damage.

3.3.2 Skin sensitisation

Animal data

The skin sensitising potential of methyl salicylate was investigated in several local lymph node assays (LLNA) and guinea pig maximisation tests (GPMT).

The results of the two maximisation assays were negative. However, fewer animals were used than recommended in the OECD test guideline.

A total of 12 LLNA have been reviewed in the classification dossier. In general, methyl salicylate was negative in the LLNA when tested up to 20%. At higher concentrations (up to 25-100%), methyl salicylate exposure resulted in both negative as well as positive LLNA studies. In the four positive LLNA studies, EC3 values ranging from 15-65% were found for methyl salicylate.

Human data

Several human data are available, including 3 human volunteer induction studies, 8 diagnostic studies and 2 case reports.

In the three human volunteer induction studies (2 maximisation studies and one human repeated insult patch test – HRIPT study) no signs of sensitisation to methyl salicylate was reported. The number of volunteers ranged from 25 to 39, with concentrations ranging from 1.25 to 8 % methyl salicylate or 12 % wintergreen oil, containing 80 to 99 % methyl salicylate. These studies lack detailed information as the cited reference is a review article.

Diagnostic studies with unselected patients suspected of contact allergy included 1825 and 4600 patients and showed a frequency of positive reactions of 0.13% and 0.4% respectively. Diagnostic studies with selected patients included 19 to 585 patients and report a frequency of positive reactions between 0 and 2 %.
Finally, two case reports with positive results of skin sensitisation after exposure to 2 % methyl salicylate in olive or arachis oil were reported.

SCCS comment
Methyl salicylate is identified as a skin sensitiser in different LLNA studies using high (>25%) concentrations. This is further supported with clinical data showing that methyl salicylate is a skin sensitiser in humans. The incidence in unselected and selected patients is low. Taken all data together, methyl salicylate is a weak skin sensitiser in the LLNA and in humans, which is in line with the classification as a 1B skin sensitiser.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

Several acute oral toxicity studies in five species (rats, mice, rabbits, guinea pigs, dogs) were available in the CLH report and are summarised in the Table below. The oldest studies were published in the 1950s. Studies according to current guidelines are not available. For most of the studies, detailed information on the experimental conditions is lacking and thus, reliability is poor.

Table 18: LD50 and resulting classification in different species administered with methyl salicylate (taken from the RAC opinion, 2019)

Species (n/sex)	Doses (g/kg)	LD_{50}
Rats (5/sex)	n/a	887 mg/kg → Acute Tox. 4
Rats (5/sex/dose)	2.5, 3.15, 3.97, 5.0	3 050 mg/kg (males) 2 640 mg/kg (females) 2 820 mg/kg (combined)
Rats	1.0, 1.25, 1.5, 2.0, 2.25, 2.5, 3.0	1 250 mg/kg → Acute Tox. 4
Rats	n/a	1 220 mg/kg (males) → Acute Tox. 4 1 060 mg/kg (females) → Acute Tox. 4
Mice	n/a	580 mg/kg → Acute Tox. 4
Mice (male)	n/a	1 100 mg/kg → Acute Tox. 4
Mice (male)	1, 1.2, 1.3, 1.5, 1.7	1 390 mg/kg → Acute Tox. 4
Rabbits	n/a	1 300 mg/kg → Acute Tox. 4
Rabbits	n/a	2 800 mg/kg
Rabbits	n/a	2 800 mg/kg
Guinea pigs	n/a	700 mg/kg → Acute Tox. 4
Guinea pigs (male/female)	n/a	1 060 mg/kg → Acute Tox. 4
Dogs	n/a	2 100 mg/kg

n/a = not available

The available LD50 values range from 580 mg/kg bw (mice) to doses higher than 2 000 mg/kg bw in rats, rabbits and dogs.

Human data on salicylate poisoning are available due to overdoses of ASA, excessive application of topical agents, ingestion of salicylate containing ointments, use of keratolytic agents or agents containing MeS (e.g. wintergreen oil). In 2004, US poison control centres reported 40 405 human exposures to salicylates, with 12 005 (30 %) cases of MeS. Typical symptoms of salicylate toxicity are hematemesis, tachypnoea, hyperpnoea, dyspnoea, tinnitus, deafness, lethargy, seizures or confusion.

RAC concludes that MeS should be classified as Acute Tox. 4; H302 (Harmful if swallowed) (ECHA-CLP, 2018).

SCCS comment
SCCS concurs with the RAC conclusion.

3.3.3.2 Acute dermal toxicity

The acute dermal data is summarised in Table 19.

Table 19: Acute dermal toxicity study for methyl salicylate

Reference	Species	Dosing	Dermal LD_{50} (mg/kg bw/day)	Observed effects
RIFM, 1973; Opdyke 1978	Rabbit	Neat MeSal, 24 h under occlusion	>5000	1/10 deaths

The dermal LD50 in rabbits exceeded 5 g MeS/kg bw based on 1/10 deaths at that dose. A single dermal application of neat methyl salicylate at 5 g/kg was applied for 24h under occlusion. Animals were observed for a 14-day period. No clinical signs were observed (RIFM, 1973a). This was also in agreement with the LD50 reported as >5 g/kg by Opdyke (1978).

3.3.3.3 Acute inhalation toxicity

The acute inhalation data is summarised in Table 20. No further details for these studies are available other than those tabulated.

Table 20: Acute inhalation data for methyl salicylate

Reference	Species	Dosing	Inhalation LD_{50} (mg/kg bw/day)	Observed effects
Gage 1970	Rat	N=4, 20 x 7h exposures; 120ppm in a saturated atmosphere of 700 mg/m³.	NR	No toxicity and all organs appeared normal.
Rumiantsev et al 1992	Rat and mice	MeSal heated to 80C and unknown duration	>400 mg/m³	No effects
Rumiantsev et al 1992	Rats	18, 69 and 114 mg/m3 for unknown duration	NR	Unspecified changes in nervous system functioning at all doses. High dose decreased orientation, increased LDH and ALT activity in serum decrease in blood coagulation time

3.3.3.4 Acute subcutaneous toxicity

The acute subcutaneous data is summarised in Table 21. No further details for these studies are available other than those tabulated.

Table 21: Acute intraperitoneal data for methyl salicylate

Reference	Species	Dosing	Subcutaneous LD_{50} (mg/kg bw/day)	Observed effects
LaWall & Harrisson research labs (1964)	Rats	400, 600, 900 and 1200 mg/kg/day for 2 weeks; n =2	NR	One animal died in each of the 900 and 1200 mg/kg groups. No bone lesions.
Opdyke 1978	Guinea-pig	NR	2700 to 2750	NR
	Rabbit	NR	4250 to 4350	NR
	Dog	NR	2250	NR

3.3.3.5 Acute intraperitoneal toxicity

The acute intraperitoneal data is summarised in Table 22. No further details for these studies are available other than those tabulated.

Table 22: Acute intraperitoneal data for methyl salicylate

Reference	Species	Dosing	Intraperitoneal LD_{50} (mg/kg bw/day)	Observed effects
Giroux et al (1954)	Guinea-pig	0.5, 0.75, or 1 g/kg in alcohol	750-1000	Slight palsy, shaking, lateral decubitus, slower breathing or difficultly in breathing
	Rat	0.5, 0.75, and 1 g/kg in alcohol	750-1000	Drowsiness, nervousness, mydriasis, changes in breathing (rhythm and amplitude), paralysis of the hind legs and rapid and violent shaking. Congested liver, kidneys, and lungs.
LaWall & Harrisson research labs (1964)	Rat	0.025 mg/day, with and without a calcium diet n=5, no duration specified.	NR	No bone lesions observed

SCCS comment
By routes of exposure other than the oral one, MeS does not warrant classification for acute toxicity.

3.3.4 Repeated dose toxicity

Since the early 1960's, MeS has been studied in repeated dose toxicity studies of varying duration in different species. Repeated dose toxicity studies ranging in duration from 4 weeks to 2 years have been conducted in rats, rabbits and dogs.

Oral studies

Table 23: Repeated dose toxicity studies by oral route in animals

Study	Species	Duration	Route	Doses#
Packman et al (1961)	Rat	2 years	Oral, diet	700 or 2100 ppm (0.07 or 0.21%)
Webb & Hansen (1963)	Rat	17 weeks	Oral, diet	0, 0.1 and 1% (approx. 0, 50, 500 mg/kg bw/day)
	Rat	2 years	Oral, diet	0, 0.1, 0.5, 1, 2% (approx. 0, 50, 250, 500 and 1000 mg/kg bw/day)
	Rat	Supplemental study on bone (71-days)	Oral, diet	2% (approx. 1000 mg/kg/day)
	Dog	Up to 59 days (6 days per week)	Oral, capsule	50, 100, 250, 500, 800, 1200 mg/kg/day
	Dog	2 years (6 days per week)	Oral, capsule	0, 50, 150, 350 mg/kg/day
	Rabbit	Up to 96 days (5 days per week; 6.5h daily)	Dermal	0.5, 1, 2 ml/kg/day (assume neat liquid MeSal and no vehicle)
LaWall and Harrisson Research Laboratories Inc (1964)*	Rat	1,2,3,4 or 5 days	Oral, diet	20000 ppm
	Rat	7 weeks	Oral, diet	0, 6000, 9000, 12000 ppm
	Rat	7 weeks	Oral, protein enriched diet	0.6, 0.9, 1.2, 2%
	Rat	8 weeks	Oral diet + coadministered substances	a) 12000 ppm or 20000 ppm MeSal alone vs 12000 ppm MeSal + 1 unit/day parathyroid hormone i.p.

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

When MeS was fed to rats at a dietary level of 1% (~500 mg/kg bw/day) for 13 days, no effects on body weights, liver weights, or functional changes in liver cells were observed (Hruban *et al.* 1966, as cited in Opdyke 1978).

In a separate range-finding study, 50 to 1000 mg/kg bw/day of methyl salicylate (in corn oil, ≥99% purity) was administered by gavage for 2 weeks in CD-1 mice (n=8/sex/group) (NTP 1984b). A maximum tolerated dose (MTD) of 500 mg/kg bw/day was established as the highest dose without significantly reducing body weight or depressing weight gain by more than 10% or resulting in significant mortality (≤10%).

In another short-term study, methyl salicylate was given orally in capsules to dogs (n=1/sex/group) at doses of 0, 50, 100, 250, 500, 800, 1200 mg/kg/day, 6 days/week for 59 days (Webb and Hansen, 1963). No treatment-related, adverse effects were noted up to 250 mg/kg bw/day. Higher doses were associated with diarrhea, weakness, vomiting, body weight loss, and morphological changes in the liver.

The effects of subchronic administration of methyl salicylate have been investigated in weanling Osborne-Mendel rats (n=10/sex/group) fed diets containing 0, 0.1, 1% synthetic methyl salicylate (99% purity) for 17 weeks (Webb and Hansen, 1963). This was equivalent to approximately 0, 50, and 500 mg/kg bw/day (Health Canada, 1994). In this study, all major organs and one hind leg were subjected to gross pathology. Histopathology was

performed on the liver, kidney, spleen, testes, adrenal, and thyroid of a limited number of animals in the control and high-dose groups. No treatment-related, adverse effects were observed up to the highest dose tested.
The effects of subchronic administration of methyl salicylate have also been investigated in beagle dogs (n=3/sex/group) (Abbott and Harrisson 1978). Synthetic methyl salicylate was administered to the dogs by gelatin capsules at doses of 0, 150, 300, 500, and 800 mg/kg/day for 7.5 months. Clinical signs, hematology, urinalyses, gross pathology, and histological examination were performed on all major organs. At 150 mg/kg bw/day, mean relative liver and kidney weights were in excess of those for negative control, but were within normal variation. At higher-dose levels, these parameters exceeded normal variation values while other effects such as reduced body weights and mortality were observed.

A subsequent study was conducted by the same authors, whereby beagle dogs (n=4-6/sex/group) were given 0, 50, 100, and 167 mg/kg bw/day methyl salicylate in capsules for 6 months. Clinical signs, hematology, food consumption and body weight was monitored. At the end of the study, the animals were subjected to comprehensive macroscopic examination with the weights of the liver and kidneys being determined. Only the liver and kidneys, the prime organs of interest, were subjected to histological examination. No treatment-related, adverse effects were identified up to the highest dose tested (167 mg/kg nw/day).

3.3.4.3 Chronic (> 12 months) toxicity

Oral route

The effects of chronic exposure to methyl salicylate have also been examined in multiple species being treated for a period of 2 years (Webb and Hansen 1963). Weanling Osborne-Mendel rats (n=25/sex/group) were administered at 0, 0.1, 0.5, 1, 2% methyl salicylate in the diet. This was equivalent to approximately 0, 50, 250, 500, and 1000 mg/kg bw/day (Health Canada, 1994). Hematologic examinations, gross pathology, histopathology, and microscopic examination of the leg bones and muscles were performed. At levels greater or equal to 0.5% (250 mg/kg bw/day), there were findings of gross pituitary lesions, significant growth inhibition, rough hair coats, significantly increased heart and kidney weights, and mortality. In addition, there was a dose-dependent increase in the number of animals with an increased amount of cancellous bone, along with decreased length of certain bones (femur, humerus, tibia, and radius).

The effects of methyl salicylate administration on bones have been reproduced in rats in a series of short-term experiments conducted by Abbott and Harrisson (1978). In these experiments, Sprague-Dawley rats were administered 0.2 – 2% methyl salicylate in the diet (equivalent to 100-1000 mg/kg bw/day) for 11-12 weeks, and then subjected to X-ray examinations. At the highest level, there were observations of increased density in the femur, humerus, tibia and radius. The NOAEL for bone effects was determined to be 0.9%, which was equivalent to approximately 863 mg/kg bw/day. The authors noted that the effects on bone appeared to be species-specific as they were only observed in rats and not in other experimental animals.

Webb and Hansen (1963) also investigated the effects of chronic exposure in beagle dogs (n=2/sex/group), whereby methyl salicylate was administered as oral capsules containing 0, 50, 150, 350 mg/kg/day, 6 days a week for 2 years. The animals were weighed weekly and hematologic examinations were made throughout the study. Necropsies were done and the major organs weighed. From the surviving high-dose animals, microscopic examinations were made on the brain, pituitary, salivary gland, thyroid, parathyroid, thymus, lymph nodes, lung, heart, stomach, spleen, pancreas, kidney, adrenal, liver, gallbladder, small intestine, large intestine, urinary bladder, rib, skeletal muscle, bone marrow, and testis/prostate or

ovary/uterus. The administration of 150 and 350 mg/kg bw/day resulted in reduced body weight gains and enlarged livers. Microscopically, these livers had larger hepatic cells than those in control. The NOAEL was determined to be 50 mg/kg bw/day on the basis of reduced body weight and liver effects observed at the next dose level (150 mg/kg bw/day).

No treatment-related, adverse effects were observed when weanling albino rats were administered methyl salicylate derived from the oil of sweet birch, which can comprise up to 90.4% of the extract (Tisserand and Young, 2014).

In a two-year chronic study, the experimental animals (n=25/sex/group, unknown strain) were administered 700 and 2100 ppm of oil of sweet birch through the diet (Packman *et al.*, 1961). Growth, survival, food usage, general physical condition of the test animals, routine blood examinations, urinalyses, gross and histopathological findings were comparable with the controls.

Dermal route

With respect to the dermal route of administration, Webb and Hansen (1963) conducted a study whereby 0.5, 1, 2, 4 mL/kg/day of methyl salicylate (99% purity) was applied to the skin of rabbits (n=3/group), 5 days per week, 6.5h per day, for 96 days. This was equivalent to approximately 585, 1170, 2360, and 4680 mg/kg bw/day (assuming a density of 1.17 g/mL). Microscopic examinations were made on the heart, liver, gallbladder, kidney, skin, bone marrow, thyroid, testis or uterus/ovary from each rabbit. Additional tissues (spleen, pancreas, adrenal, lymph nodes, small intestine, voluntary muscle, brain) from 5 animals were also examined. At 2 mL/kg bw/day, slight sloughing of epidermal scales was observed in 2/3 rabbits. At 4 mL/kg bw/day, all of the animals died within 28 days, accompanied by signs of anorexia, weight loss, and decreased activity. Microscopic examination of one rabbit revealed lesions such as dilatation, desquamation, formation of new atypical epithelium of the renal tubules, small foci of superficial necrosis and sloughing of skin, foci of moderate necrosis and slight calcification of voluntary muscles, marked vacuolation of pancreatic acinar cells, slight myeloid hyperplasia of the left of bone marrow, and slight hepatitis. In the surviving animals, there were reduced weight gains and the incidence of spontaneous nephritis and mild hepatitis appeared to be increased compared to historical data. There was also slight to very slight dermatitis. The LOAEL for systemic effects was determined to be 0.5 mL/kg bw/day (585 mg/kg bw/day) on the basis of reduced weight gains, nephritis, and mild hepatitis observed in the treated animals.

Inhalation

With respect to the inhalation route of administration, two studies examining the effects of methyl salicylate were identified.

In the first study, female Alderley Park specific-pathogen-free rats (n=4/group) were exposed to 700 mg/m^3 (120 ppm) of saturated vapour of methyl salicylate. The animals were exposed 20 times, each with a duration of 7h per day, over a period of approximately 3 to 4 weeks (Gage, 1970). The rats were weighed each morning and their general condition and behaviour recorded throughout the exposure period. Urine and blood were collected for further analyses. After gross examination of the organs, the following organs were also taken for microscopic examination: lungs, liver, kidneys, spleen, adrenals, occasionally heart, jejunum, ileum, and thymus. The authors reported "no toxic signs" (i.e., the animals remained in good condition) and "organs normal" (i.e., histopathological examinations revealed no changes that could be attributed to the treatment). A NOAEL of 700 mg/m^3 (120 ppm) was determined.

In another inhalation study, male rats were exposed to 1.2, 8, or 40 mg/m^3 methyl salicylate, 4h/day for 4 months (Rumyantsev *et al.*, 1992, as cited in CIR 2003). In the report by CIR (2003), it was noted that the high-dose resulted in changes in nervous system functioning, a decrease in hemoglobin content, the number of erythrocytes, a change in serum leucine aminopeptidase and urinary creatinine content. At microscopic examination, pulmonary focal hemorrhages and hyperplasia were observed in the peribronchial lymphoid tissue and the number of plasmatic cells in the lymphoid follicles had increased. In the kidneys, scaling of the epithelium of the convoluted tubules, focal infiltration, and focal hemorrhages were seen. The original article by Rumyantsev *et al.* (1992) was in Russian and thus the results could not be interpreted. However, the English abstract indicated a "threshold level of 8 mg/m3."

SCCS comments
SCCS notes that the repeated dose toxicity studies are for most of them, old studies that were not performed following the current guidelines. Moreover, it should be noted that limited endpoints were evaluated and a limited number of animals were examined. Furthermore, it is not indicated if a statistical analysis was performed on histopathological findings. Therefore, it cannot be excluded that effects can occur at a lower doses in organs that were not examined. In addition, considering the small number of animals examined at histopathology, only the effects occurring at a high incidence can be detected in these studies.
Based on the data available for the calculation of the MoS; the following value could be identified:

For oral exposure: **a NOAEL of 50 mg/kg bw/day**

For dermal exposure: **a LOAEL of 585 mg/kg bw/day**

For exposure by inhalation: **A NOAEL of 700 mg/m^3 (120 ppm)**

3.3.5 Reproductive toxicity

Human data

No human data are available on MeS. However, because human data are available for another salicylate ester, ASA, several – mostly retrospective – publications with aspirin during pregnancy could be used as supportive information to assess the reproductive toxicity of MeS.

It is difficult or even impossible to estimate the causal relationship between the effects observed in retrospective studies and the salicylate exposure, because the drug had been taken for certain diseases – such as fever or viral infections – which might pose a risk to pregnancy on their own.
The studies by Richards (1969), Nelson & Forfar (1971), Lynberg *et al.* (1994), Kozer *et al.* (2002) reported defects on central nervous system, alimentary tract, talipes, achondroplasia, anencephaly, spina bifida and encephalocele and other congenital malformations (ECHA-CLP, 2018).

In conclusion, even if most of the epidemiological studies with ASA do not report an increased risk of adverse effect on development at therapeutic dosage, there are some indications of fetal lethality and malformations with this compound. However, due to limitations of the retrospective studies, such as misclassification of exposure, confounding factors and lack of quantitative data, human data are considered inadequate to firmly conclude on the developmental toxicity of salicylates.

Animal studies

All *in vivo* animal reproductive and developmental studies that are available for methyl salicylate are summarised in Table 24 (oral route). Studies via other routes of exposure are reported in Annex 2. There are no studies by the inhalation route.

Table 24: Reproductive and developmental animal studies with methyl salicylate – oral route.

Species	Method	Route of exposure	Dosage	Results	Reference
Rat, Sprague-Dawley 24–27/ group	Animals were fed test diet with calcium carbonate for 60 days prior to mating through weaning at day 20 or 21	Oral, diet	4000 or 6000 ppm (0.4 and 0.6%)	No abnormalities noted in offspring; neonate survival at weaning was greater in the test than the control groups	FDA 1966 (only cited in CIR 2003)
10 Osborne-Mendel rats/sex/group	Three generation study: F0 animals mated after 100 days of dosing; F1a animals were	Oral, diet	500, 1500, 3000, or 5000 ppm in feed (0.05, 0.15, 0.3 and 0.5%) equivalent to	No gross abnormalities were observed; various effects were seen, especially in the 2nd	Collins et al 1971

	killed at weaning; 20 littermated F1b animals were mated; procedure was Repeated.		25, 75, 150 and 250 mg/kg	generation and at doses >3000ppm (see text below for further details). **NOAEL 75 mg/kg/day; LOAEL 150 mg/kg/day**	
F0: 25 Wistar rats/sex/group F1b: 30/sex//group	**Animals were dosed for 60 days** prior to mating. F0 animals were mated twice. F1a animals maintained through weaning. F1b animals mated twice → F2a and F2b	Oral, diet	0.25 or 0.5% (2500 or 5000 ppm) in feed	No gross abnormalities were observed with any litter; all surviving neonates appeared normal; mating performance and reproduction and viability indices were decreased. Number of deaths between birth and day 5 were increased in the 0.5% group; litter size was decreased in both test groups	Abbott & Harrisson, 1978 as cited in CIR2003
F0: 25 mice/sex/ group F1b: 30 males/30 females/group	**Animals were dosed for 30 days** prior to mating. F0 animals were mated twice. F1a animals maintained through weaning. F1b animals mated twice → F2a and F2b	Oral, diet	0.25 or 0.5% (2500 or 5000 ppm) in feed	Results are only from females in each generation that mated twice. No gross abnormalities were observed with any litter; all surviving neonates appeared normal; no reproductive abnormalities were seen	Abbott & Harrisson 1978 as cited in CIR 2003
LVG Hamsters	Animals were given a single high dose on GD7 and killed on GD 9	Oral	1750 mg/kg	72% of 35 litters had neural defects; Salicylate reached the fetus at this high dose.	Overman & White, 1983
CD1 mice, 40/sex/group (control) and 20/sex/group (treated)	Reproductive assessment by continuous breeding RACB protocol; control and high-dose F1 offspring	Oral	25, 50, or 100 mg/kg/day	Reproductive and fertility parameters were generally not affected; also no significant effect on mating behavior, fertility rate, or	NTP (1984a) Research Triangle Institute; Lamb et al; further details in Greene et al 2017

	reproductive and fertility performance was evaluated due to lack of effect in F0 mice			reproductive performance was seen. **Top dose NOAEL 100 mg/kg/day**	
CD1 mice, 40/sex/group (control) and 20/sex/group (treated)	Reproductive assessment by continuous breeding RACB protocol; 7 day (premating); 100 day (cohabited).	Oral, gavage	0, 100, 250, 500 mg/ kg/day	Decreased number of litters, number of pups per litter, proportion of pups born alive and decreased mean pup weight. **NOAEL 100 mg/kg/day BMDL 220 mg/kg/day (95%ile, one SD below the control mean)**	NTP (1984b) Environmental Health Research Testing Lb Inc. also referred to as Gulati et al 1984; further details in Greene et al 2017.

Reproduction and fertility assessment in CD-1 mice when administered by gavage. NTP, NIEHS Report No. NTP-85-022, November 1984 (PB85-164283)

Task 1: 14 days preliminary study

Guidelines/Guidances:	NTP Dose-Finding study
GLP:	Yes
Test item:	Methyl salicylate (MeS)
Purity:	≥ 99%
Vehicle:	corn oil
Positive control:	/
Route of exposure:	Oral gavage
Duration of exposure:	14 days
Doses:	0, 50, 100, 250, 500 or 1000 mg/kg bw/day for 2 weeks
Experimental animals:	
Species:	Mice
Strain:	CD-1, (ICR) BR outbred albino mice
Sex:	male and female
Animal numbers:	

In this preliminary study, mice received 0, 50, 100, 250, 500 or 1000 mg/kg bw/day for 2 weeks. There was no discernible effect on body weight. Seven animals died during the study: 2 in the control group, 2 in the 50 mg/kg bw/day group and 3 at the highest dose.

Two-generation study in mice (NTP continuous breeding protocol: tasks 2 + 4)

Guidelines/Guidances:	NTP continuous breeding protocol
GLP:	Yes
Test item:	Methyl salicylate (MeS)
Purity:	≥ 99%
Vehicle:	corn oil
Positive control:	17α-Ethinyl estradiol (EE)
Purity	/

Vehicle:	Peanut Oil
Route of exposure:	Oral - gavage
Duration of exposure:	7 days prior to mating, during 98 days of cohabitation (allowing the production of about 4 litters) and then during a separation period of 21 days during which final litters were delivered (task 2). A second generation was then produced only for the highest dose group (task 4): the mothers were dosed through weaning and F1 mice were dosed until
Doses:	0, 25, 50 and 100 mg/kg/day.
Experimental animals:	
Species:	Mice
Strain:	CD-1, (ICR) BR outbred albino mice
Sex:	male and female
Animal numbers:	20/sex/dose for MeS groups and 40/sex for vehicle group.
Study period:	Before 1984

Note: task 3 was performed in another NTP study (1984c) Study reference: Morrissey et al., 1989

Description of test design:

• Task 2: Male and female mice were exposed to the chemical during a 7-day premating period, after which they were randomly paired (1 male: 1 female) within each dose group. Cohabitation was continued for 100 days. Newborn litters were evaluated and immediately sacrificed. Parameters assessed: mortality, body weight, body weight gain, clinical signs, fertility index, litter per pair, live pup per litter, proportion of pups born alive, sex of pups born alive, live pup weight.

• Task 3: animals from the 500 mg/kg bw/day group were tested in a crossover mating trial to determine whether the males or females or both sexes had compromised reproductive performance when matched with control animals. Animals did not receive any treatment between days 127 (week 19) and day 155 (week 23) of the study. Parameters assessed: mating index, fertility index, live pup per litter, proportion of pups born alive, sex of pups born alive, live pup weight.

Results and discussion

TASK 2

- Eleven animals died during Task 2; 3 in the control, 2 each in the 100 and 250 mg/kg dose groups and 4 in the 500 mg/kg dose group. The cause varied from case to case but was neither chemical nor dose related. No distinct treatment related symptoms of toxicity were observed during routine health surveillance
- Methyl salicylate had no apparent effect on male or female body weights.
- The fertility index in the control and various treatment groups varied between 94 to 100%; all breeding pairs except 1 in the 100 mg/kg group delivered at least one litter. Data from breeding pairs in which one or both animals died during task 2 were excluded when computing the average number of litters per pair, live pups per litter, proportion of pups born alive, sex ratio and live pup weight.
- There was a significant decrease ($p<0.05$) in the mean number of litters at the highest dose. The average number of pups per litter, the proportion of pups born alive, and mean live pup weight values were also significantly reduced ($p<0.05$) in the 500 mg/kg group compared to the corresponding controls.

Table 25: reproductive parameters of fertile mouse pairs during a RACB protocol on MeS (Gulati *et al.*, 1984)(reproduced from Green *et al.*, 2017).

	Treatment group (methyl salicylate concentration)			
Reproductive parameter[a]	Control	100mg/kg	250mg/kg	500mg/kg
Litters per pair	4.92 ± 0.044 (38)[b]	4.82 ± 0.128 (17)	4.78 ± 0.129 (18)	4.50 ± 0.258 (16)*
Proportion of pups born alive	0.97 ± 0.010 (38)	0.98 ± 0.010 (17)	0.96 ± 0.017 (18)	0.91 ± 0.033 (16)*
Live pups per litter				
Male	5.73 ± 0.209 (38)	5.41 ± 0.249 (17)	4.75 ± 0.384 (18)	4.32 ± 0.364 (16)**
Female	5.55 ± 0.206 (38)	5.05 ± 0.228 (17)	4.83 ± 0.313 (18)	3.46 ± 0.351 (16)**
Combined	11.29 ± 0.364 (38)	10.47 ± 0.348 (17)	9.58 ± 0.648 (18)	7.78 ± 0.635 (16)**
Live pup weight (g)				
Male	1.65 ± 0.014 (38)	1.64 ± 0.020 (17)	1.64 ± 0.018 (18)	1.60 ± 0.023 (16)
Female	1.60 ± 0.014 (38)	1.59 ± 0.021 (17)	1.57 ± 0.020 (18)*	1.53 ± 0.016 (16)*
Combined	1.62 ± 0.013 (38)	1.62 ± 0.021 (17)	1.60 ± 0.017 (18)	1.57 ± 0.021 (16)*
Adjusted live pup weight (g)[c]				
Male	1.67 ± 0.014 (38)	1.65 ± 0.019 (17)	1.63 ± 0.019 (18)	1.57 ± 0.021 (16)**
Female	1.61 ± 0.013 (38)	1.59 ± 0.019 (17)	1.56 ± 0.018 (18)*	1.51 ± 0.021 (16)**
Combined	1.64 ± 0.013 (38)	1.62 ± 0.018 (17)	1.59 ± 0.018 (18)*	1.54 ± 0.020 (16)**

[a]Mean ± SE

[b]Number of pairs providing data indicated in ().

[c]Means adjusted for total number of live and dead pups per litter by analysis of covariance.

*Significantly different $\rho<0.05$ from control group.

**Significantly different $\rho<0.01$ from control group.

TASK 3

- First trial: both the fertility and mating index values in the control were considerably lower than expected. Only 5 out of 17 breeding pairs delivered pups in the control group. These data were considered unacceptable and Task 3 was repeated.
- Second trial: fertility in all three groups, including control, was poor and essentially the same. It was still not possible to determine which sex was affected by the treatment.

Greene *et al* (2017) performed a quantitative dose-response assessment using benchmark dose (BMD) modelling of the Gulati et al data. Data on average pup weight and litters per mating pair were modelling using Benchmark Dose Modelling Software BMDS (USEPA, version available in 2016) and the results are presented in Table 34. The lowest oral BMDL for reproductive effects with the best fit was observed at 220 mg/kg/day. This is approximately double the value of the NOAEL at 100 mg/kg/day determined in Gulati *et al.*

The main effects *via* the oral route were reduced pup size and reduced litter size.

An oral NOAEL of 100 mg/kg/day is established for reproductive and developmental toxicity in mice (Gulati et al (NTP), 1984).

An oral BMDL 1SD of 220 mg/kg/day was calculated from the dose-response data for reductions in the litter size per mating pair.

Collins, 1971

Three-generation study (each generation mated twice) in rats

Guidelines/Guidances: Several deficiencies from OECD 416,
GLP: No

Test item:	Methyl salicylate(MeS)
Purity:	≥ 99%
Vehicle:	No vehicle
Route of exposure:	Diet
Duration of exposure:	100 days before the first mating and then throughout the experiment (until weaning of the 3rd generation).
Doses:	0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw as MeS)
Experimental animals:	
Species:	Rats
Strain:	Osborne-Mendel
Sex:	male and female
Animal numbers:	20/sex/dose
Examination:	fertility index, litter size, viability at birth, on day 4 and at weaning, external examination of newborn and weanling rats (all generations, all mattings), histopathological examination of liver and kidney (for the 3rd generation only). Limited statistical analysis.
Study period:	before 1971

Results:

NOAEL (fertility): 250 mg/kg bw/day (male/female) based on no statistically significant effect reported.

NOAEL (development): 75 mg/kg bw/day based on statistically significant decrease of litter size, viability (D0), survival (D4), weaning data in the second generation and decreased pup body weight at 150 mg/kg bw/day.

The addition of calcium carbonate did not markedly differ from those obtained after administration of methyl salicylate alone.

Summary on fertility and developmental toxicity of methyl salicylate

Fertility and reproductive function

A number of studies have been conducted to investigate adverse effects on sexual function and fertility or on development after exposure to MeS. Fertility studies reported in the classification dossier cover an extensive time period that starts in the 1960s and extends to the early 2000s. None of the studies reported any significant and/or consistent effect on fertility. Therefore, RAC was of the opinion that no classification is justified for MeS for adverse effects on sexual function and fertility.

Seven different studies were performed to investigate the effects of MeS on fertility. No statistically significant effects on fertility and mating were reported in rats at doses up to 250 mg/kg bw/d by oral route and 300 mg/kg bw/d by subcutaneous application and in mice at doses up to 750 mg/kg bw/d which were the highest doses tested. Even if most of the fertility studies show a number of deficiencies compared to OECD test guidelines in term of parameters studied, none reported any significant and/or consistent effect on fertility.

Human data are conflicting and no clear conclusion can be drawn from it.

There is insufficient evidence that MeS exhibits adverse effects on sexual function and fertility. Therefore the SCCS concurs with the proposal by RAC that no classification is justified for MeS for adverse effects on sexual function and fertility.

Effects on Development

In addition to the reproductive toxicity studies presented above, several developmental toxicity studies in rats and rabbits were examined and are summarised below.

Two studies are available in rabbits and rats focusing on the period of organogenesis. In both studies, MeS was injected subcutaneously.

In the rabbit study, there was no treatment-related effect on the numbers of corpora lutea, implants or live foetuses, dead embryo / foetus indices or body weight of live foetuses. There was no placental anomaly, no external, visceral or skeletal anomalies related to MeS treatment.

In rat study, no mortality or clinical signs occurred in the treated groups. Statistically significant depression of body weight (< 5 %), bw gain (≥ 10 %) and food consumption was reported in dams at 200 mg/kg bw/d. There was no effect of the treatment on the number of corpora lutea, implants, live and dead foetuses, sex ratio or placental anomalies. External anomalies, characterised principally by craniorachischisis and gastroschisis were detected at levels above HCD (2.86 % versus 0.01 %) but not considered to be statistically significant. Visceral anomalies were also increased but considered to be not statistically significant. A statistically significant increase in skeletal variations was observed at the highest dose and in addition, there was a delay of ossification of the vertebrae, sternebra, metacarpus, metatarsus and phalanges.

In another study, MeS was given subcutaneously to pregnant and lactating rats. There was a statistically significantly lower mean body weight and bw gain during gestation at the top dose of 200 mg/kg bw/d, with significantly decreased food consumption during gestation and lactation. In male offspring, a statistically significant decrease in the birth index (-6 %) index (-6 %) and a lower body weight (-9.2 %) were observed in live newborns in the top-dose group with a trend toward a decrease in the number of litter and live newborns and a trend toward an increase in the stillbirth index. These effects were considered attributable to MeS.

Other effects, such as, statistically significant decrease in the differentiation indices of incisor eruption in both sexes, skeletal anomalies, cleavage of the balanopreputial gland and statistically significant changes in the weights of organs (brain, lungs, testes, ovaries, kidneys) were observed in the top-dose group.

There are also several studies with some shortcomings and unusual routes of administration, such as dermal application or intraperitoneal injection, where the effects varied from severe toxicity and 100 % resorption (Infurna *et al.*, 1990 – only abstract available) to neural tube defects (Overman & White, 1983) and lethality, external malformations, visceral and skeletal anomalies and effects on differentiation indices (Kavlock *et al.*, 1982; Daston *et al.*, 1988).

The lowest developmental NOAEL are < 60 mg/kg bw/d in rats exposed subcutaneously from GD6 to LD21 (FDA, 2006b) and 75 mg/kg bw/d in a 3-generation study in rats by oral route (Collins *et al.*, 1971). This value will be used for the margin of Safety (MoS) calculation.

With respect to developmental toxicity, RAC was of the opinion that MeS should be classified in Cat. 2, mainly due to the weight of evidence put on the human data with ASA which do not indicate that ASA is a human teratogen.

Among the salicylates, the vast majority of human data derive from the use of ASA in pregnant women. The drug is widely used as an analgesic, antipyretic and anti-inflammatory agent. Some older retrospective studies reported malformations in

children from women treated during pregnancy with aspirin for viral infections, fever and other indications.

Larger, prospective studies did not show a teratogenic effect of aspirin and, for women at risk for pre-eclampsia, the drug shows some benefit when given during pregnancy.

Salicylic acid was classified by RAC as Category 2 for developmental toxicity in March 2016. In a weight of evidence approach, this recommendation was mainly based on the lack of birth defects in humans, despite clear teratogenicity in rats and monkeys.

RAC is of the view that, with MeS, the situation is similar to SA and it is a matter of consistency to classify the methylester of SA accordingly.

Several studies showed that MeS is teratogenic in rats, but not in rabbits. This finding is in agreement with salicylic acid, which causes a similar pattern of neural tube defects and other malformations in rats and monkeys, but not in rabbits.

Malformations observed with MeS *via* the subcutaneous route occurred at low incidence at the highest dose only, which caused significant maternal toxicity. In comparison to concurrent controls, there was no statistically significant increase in the incidence of severe neural tube defects in fetuses such as craniorachischisis.At the top dose, the incidence of skeletal variations was significantly increased, which can be interpreted as a consequence of maternal toxicity and not substance-related.

Conclusion from RAC
Based on the weight of the evidence, RAC was of the opinion that MeS should be classified as Repr. 2; H361d (Suspected of damaging the unborn child) based on positive animal experiments with MeS and negative human data with acetylic salicylic acid.

SCCS comment
The SCCS concurs with the RAC opinion that MeS warranted classification as Repr.2.

3.3.6 Endocrine disrupting effects

- Estrogenic pathway

- Molecular docking

A pre-screening computational tool for endocrine activity, Scigress (Ultra Version 2.2.0, Fujitsu, USA)(Kiss and Allen, 2007), was used to dock methyl salicylate in the ligand-binding site of the 3-dimensional structure of the human estrogen-receptor α (hERα-LBD). The active site of chain A was used for docking calculations, which were evaluated with a 15 x 15 x 15 angstrom grid box with 0.375 angstrom grid spacing. The procedure was set to run 60,000 generations with an initial population size of 50, elitism of 5, cross-over rate of 0.8 and mutation rate of 0.2. The binding affinity of methyl salicylate in the active site was scored using a knowledge-based approach called potential of mean force (PMF) that extracts pairwise atomic potentials from structure information of known protein-ligand complexes.
The positive control (natural) ligand for this receptor is 17β-estradiol (E2), which had a PMF of -55.655 kcal/mol. The PMF of bisphenol-A, discussed as being a potent estrogenic compound, was -53.694 kcal/mol, so the assumption was made that substances with similar or higher PMFs than BPA were 'high affinity' substances. The PMF value for methyl salicylate in this experiment was -44.570 and this was not regarded as a high-binding affinity for hERα-LBD. There are no comparators available in this study, however, for low affinity binding.

- *In vitro* assay

A ligand-dependent coactivator recruiting assay with glutathione-S-transferase-tagged hERα-LBD was performed as per the method described in Kanayama *et al.* (2003) to assess estrogen-receptor agonist activity *in vitro*. Stock solutions of methyl salicylate were diluted with DMSO, as a 10-fold serial dilution of eight concentrations in the range 10^{-3} to 10^{-10} M. Binding affinities to the hERa were expressed as the absorbance at 405 nm. The wells with only DMSO added were used as background values. Calculations were performed for the 10% relative effect concentration (REC10 – concentration of methyl salicylate corresponding to 10% of the maximum activity of E2) and the maximal acceptable daily exposure (the concentration of methyl salicylate showing the same agonist activity as 0.05 µg/kg bw of E2, which was determined by the Joint FAO/WHO expert committee on food additives (JECFA) as the maximal acceptable daily E2 intake. In this *in vitro* assay, methyl salicylate showed no estrogenicity and the activity was so negligible, a maximal acceptable daily exposure value could not be calculated.

- Mouse uterotrophic assay

An immature (19 postnatal day) female CD-1 mouse uterotrophic assay was performed according to the OECD guideline method. Each group comprised 12 mice. A dose of 300 mg/kg/day was used as the highest dose of methyl salicylate that would not result in serious toxicity. Stock solutions were subjected to a 3-fold serial dilution in peanut oil to generate doses of 11, 33, 100 and 300 mg/kg/day. E2 was used as a positive control at doses of 10, 50 and 400 µg/kg/day. Intragastric administration (20 ml/kg bw) of controls (vehicle and E2) and methyl salicylate was performed daily for 3 days beginning with PND 21 according to the weight of the mouse. At PND 24, the mice were weighed and sacrificed by cervical dislocation. The uteri were dissected, blotted and weighed.

No mortality was seen in treated animals. A slight reduction in body weight (not statistically significant at this dose) compared to controls was seen for methyl salicylate treated animals), which is consistent with the general observations seen in all *in vivo* studies for methyl salicylate (see earlier sections). There was no significant difference in uterine weights at any of the doses of methyl salicylate tested (Zhang *et al.*, 2012).

SCCS comments

The only endocrine pathway that was investigated by the applicant is the estrogenic pathway. No information on the androgen, thyroid and steroidogenesis (EATS) pathways were provided. However methyl salicylate is not identified at EU level as an SVHC substance for its endocrine properties, either for human health or for the environment.
Methyl salicylate is not on the ED-list (https://edlists.org/the-ed-lists) of endocrine disrupters, meaning that it is not a substance identified as an endocrine disruptor at EU level (List I), a substance under evaluation for endocrine disruption under an EU legislation (List II) or a substance considered, by the evaluating National Authority, to have endocrine disrupting properties (List III).
Therefore, the SCCS has no specific concern regarding the endocrine disrupting potential of methyl salicylate. Moreover, SCCS considers that *in vitro* data provided by the applicant are not useful for calculating a maximum dose.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

From Cosmetic Europe

In vitro studies

The key study in bacteria for this endpoint is a GLP OECD guideline study performed in 2001, as described in the submission to the US FDA in 2006 from the Hisamitsu Pharmaceutical Co Inc. (FDA 2006e). Methyl salicylate lot Y619E 100% pure was evaluated in an Ames test at concentrations of 46.9, 93.8, 187.5, 375, 750, 1500 µg/plate using a vehicle of DMSO. Strains used were S. typhimurium TA1535, TA1537, TA98 and TA100 and E.coli WP2uvrA, with and without metabolic activation. The study was GLP compliant, used a DMSO vehicle and methodology followed OECD guidelines. At concentrations of >750 µg/plate, methyl salicylate was cytotoxic. Under the conditions of this assay, methyl salicylate was not mutagenic.

Older studies also provide further supporting evidence that methyl salicylate is not mutagenic. A Rec assay was conducted by Oda *et al.* (1978), using Bacillus subtilis strains H 17 (rec+) and M 45 (rec -). DMSO was used as a vehicle for a dose of 23 µg methyl salicylate. No effects were observed.

In a reverse gene mutation assay (Ames test) in bacteria (Mortelmans, 1986), Salmonella typhimurium strains TA1535, 1537, 98 and 100 were exposed to methyl salicylate, at concentrations of 1.0 to 333.3 µg/plate with and without metabolic activation by S9 fraction from the livers of male rats and hamsters. Cytotoxicity was seen at the highest concentration. There was no evidence of induced mutant colonies over background either with or without metabolic activation in any tested strain.

In a reverse gene-mutation assay (Ishidate *et al.*, 1984), Salmonella typhimurium strains TA 92, 94, 98, 100, 1535 and 1537 were exposed to methyl salicylate with and without metabolic activation by liver microsomal fraction, S9, from Fischer rats pretreated with polychlorinated biphenyls. Methyl salicylate was non-mutagenic under the assay conditions. It was concluded that methyl salicylate is not mutagenic in bacteria.
In an Ames test, Kuboyama and Fujii (1992) reported a weak positive result for methyl salicylate, but only in the presence of hamster S9. The results of this study are challenging to interpret as no cytotoxicity data was reported.
A Rec assay was conducted by Kuboyama and Fujii (1992) using Bacillus subtilis strains H 17 (rec+) and M 45 (rec -). DMSO was used as a vehicle for a dose of 5000 µg/plate methyl salicylate. No effects were observed.

Table 27: *in vitro* bacterial assays for methyl salicylates

Method	Test Article	Method details	Results	Reference
Ames test	Doses up to 10 mg/plate	*S. typhimurium* TA92, TA1535, TA100, TA 1527, TA94 and TA98, with and without metabolic activation	Not mutagenic	Ishidate et al 1984
Modified Ames test, preincubation assay (NTP protocol)	1 to 333.3 µg/plate	*S. typhimurium* TA1535, TA1537, TA98 and TA100, with and without metabolic activation using hamster S9	Not mutagenic	Mortelmans et al 1986
Ames test	0.1mg/disc (Ames)	*S. typhimurium* TA98 and TA100, with and without metabolic activation (hamster, rat, mouse and guinea-pig S9). NB. No cytotoxicity data	Not mutagenic (without S9) Weakly mutagenic but only with hamster S9.	Kuboyama & Fujii, 1992
Rec assay	5000 µg/plate, in DMSO vehicle	Bacillus subtilis strains H 17 (rec+) and M 45 (rec -)	No DNA damage	Kuboyama & Fujii, 1992
Ames Test	46.9, 93.8, 187.5, 375, 750, 1500 µg/plate	*S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E.coli* WP2*uvrA*. With and without metabolic activation. GLP compliant. DMSO vehicle. OECD guideline method.	Not mutagenic Cytotoxic at >750 µg/plate	FDA (2006e) NDA 022-029 report Study performed 2001

In a mammalian cell chromosome aberration assay (Ishidate *et al.*, 1984), cell cultures were exposed to MeS at three concentrations up to 0.25 mg/ml for 24 or 48 hours without metabolic activation. The cytotoxic concentration was defined as the dose which induced 50% cell growth inhibition. Methyl salicylic acid did not induce a clastogenic effect. No positive controls were reported. However, this study was without metabolic activation.
An OECD guideline chromosome aberration assay was performed to GLP in 2001, as described in the submission to the US FDA in 2006 from the Hisamitsu Pharmaceutical Co Inc (FDA 2006f). Methyl salicylate lot Y619E 100% pure was evaluated at concentrations of 300, 350, 400, 450, 500, 550 µg/ml in DMSO. The study used a Chinese lung hamster fibroblast cell line (CHL/IU), with continuous treatments for 24 and 48h. The top dose of 550 µg/ml was selected on the basis of a cellular growth inhibition test where 50% growth inhibition occurred at 519 µg/ml and 515 µg/ml in continuous treatments of 24 and 28h, respectively. There were no structural abberrations seen in the chromosomes and no signs of clastogenicity in this study.

Table 28: *In vitro* mammalian clastogenicity and DNA damage

Methods	Test Article	Method details	Results	Reference
Mammalian cell Chromosome aberration assay	0-250 µg/ml in DMSO	Chinese hamster fibroblast cells without exogenous metabolic activation	Not clastogenic; no DNA damage	Ishidate et al 1984
Mammalian cell Chromosome aberration assay	0, 300, 350, 400, 450, 500, 550 µg/ml in DMSO	Chinese lung hamster fibroblast cells (CHL/IU), with and without metabolic activation	Not mutagenic	FDA (2006f) NDA 022-029 report Study performed 2001

3.3.6.2 Mutagenicity / genotoxicity *in vivo*

An *in vivo* micronucleus assay was performed as reported in the submission to the US FDA (NDA 022-029 report) by the Hisamitsu Pharmaceutical Co Inc. to support a new topical methyl salicylate product for clinical use in the USA. The details are provided in Tables 29 and 30.

Table 29: *In vivo* micronucleus assay

Methods	Test Article	Results	Reference
Micronucleus assay; GLP; Crj:CD (SD) IGS rate (male)	Methyl salicylate: Dose range finding study: 0, 30, 100, 300 and 1000 mg/kg. Main study doses: 125, 250, 500, 1000 mg/kg; subcutaneous dose administered twice at 24 h intervals. Corn oil vehicle. Cyclosphosphamide (20 mg/kg oral) positive control.	4 deaths occurred in the 1000 mg/kg group on day 2 of the dose-range finding study. Not genotoxic/mutagenic.	FDA (2006f) NDA 022-029 report Study performed 2001

Methyl salicylate did not produce any overt clinical signs or death at 30, 100 and 300 mg/kg/day in the dose-range finding study. However 4/10 deaths occurred in the 1000 mg/kg group on day 2. This is consistent with observations in all *in vivo* repeat dose studies, where a maximum tolerated dose of 500 mg/kg/day was observed. Cyclophosphamide as the positive control at 20 mg/kg significantly increased the number of micronucleated immature erythrocytes. Methyl salicylate did not increase the number of micronucleated immature erythrocytes, but at the 500 mg/kg (IE% = 40.54%) and 1000 mg/kg (IE% = 33.80%) doses the number of immature erythrocytes was significantly lower than the vehicle control (IE% = 49.43%)

Table 30: Detailed results from the *in vivo* micronucleus assay (FDA, 2006f)

Test article	Dose levels (mg/kg)	Treatment frequency	Administration route	Mortality of animals	IE %		MINIE %		
					Mean ± SD	Minimum – maximum value	Total#	Mean ± SD	Minimum – maximum value
Corn oil	-	2	s.c.	0/6	49.43 ± 4.61	41.2 - 53.6	14	012 ± 0.07	0.00 – 0.20
Methyl salicylate	125	2	s.c.	0/10	48.72 ± 1.64	46.8 – 51.2	18	0.09 ± 0.06	0.00 – 0.20
Methyl salicylate	250	2	s.c.	0/10	45.02 ± 2.13	41.6 – 48.2	17	0.08 ± 0.06	0.00 – 0.20
Methyl salicylate	500	2	s.c.	0/10	40.54 ± 1.70▪	36.4 – 42.2	17	0.09 ± 0.05	0.00 – 0.15
Methyl salicylate	1000	2	s.c.	4/10	33.80 ± 3.45*	30.6 – 39.2	11	0.09 ± 0.07	0.00 – 0.20
CP	20	1	p.o.	0/6	39.47 ± 1.48▪	37.2 – 41.2	429	3.58 ± 0.28▪	3.20 – 4.00

IE %: incidence of immature erythrocytes
MINIE %: Incidence of micronucleated immature erythrocytes
CP: Cyclophosphamide monohydrate
▪ significantly different from negative control ($\rho<0.01$, student t-test)
* significantly different from negative control ($\rho<0.05$, Kastenbaum and Bowman's method)
#Total number of MNPCE per group
Remarks: Bone marrow sample were collected at 24 hours following the final treatment with negative control , test article, or positive control.

Mutagenicity and Genotoxicity: conclusion from the applicant

On the balance of evidence from *in vitro* and *in vivo* studies, methyl salicylate is not genotoxic or mutagenic. This agrees with the conclusion of the European Medicines Agency, that salicylic acid and methyl salicylate can be considered not to possess genotoxic properties (EMEA, 1999). The principal metabolite of methyl salicylate, salicylic acid is also not mutagenic (SCCS Opinion, 2019).

Conclusion from Health Canada (2017)
With respect to genotoxicity, wintergreen oil was not mutagenic in an *in vitro* assay incubated with rat neuronal cells (Celik *et al.* 2016). Methyl salicylate was also negative for genotoxicity in Rec-assays (Oda *et al.* 1978, Kuboyama 1992), in bacterial mutagenicity assays (Mortelmans *et al.* 1986, Kuboyama et al 1992, Ishidate *et al.* 1984), and in chromosome-aberration assays conducted on mammalian cells (Ishidate *et al.* 1984).

The SCCS overall comment of mutagenicity
The genotoxicity of methyl salicylate was investigated with valid *in vitro* genotoxicity tests for bacterial gene mutations and chromosomal aberrations with negative results. Although in the study by Kuboyama and Fujii (1992) a weak positive effect was seen in both tested TA98 and TA100 strains, this study is of limited value as only one concentration of methyl salicylate was used without any data on cytotoxicity provided and the effect was observed only in the presence of hamster S9 fraction. A valid *in vivo* micronucleus in rats with a negative result was provided. Based on the results, methyl salicylate can be considered to pose no genotoxic hazard.

3.3.7 Carcinogenicity

In a chronic study investigating the effects of wintergreen oil derived from Gaultheria, D strain mice (n=45) were administered daily treatment in the diet beginning ~11.7 months of age when spontaneous tumours are known to manifest. For 41 days, one drop of oil was added to 10 g of the diet (~2945 mg/kg bw/day): then for 26 days, one drop of oil to 50 g (~590 mg/kg bw/day): and finally one drop to 40 g (~740 mg/kg bw/day) continued to be added until the end of experiment. Historical data on this strain of mice revealed an incidence of 75% for breast carcinomas occurring at approximately 10-12 months of age. The administration of wintergreen oil resulted in 22% of the animals developing spontaneous tumours at approximately 18 months. The authors suggested that the wintergreen oil could delay the time at which tumours would normally develop (Strong, 1932). Similar results were observed in a follow-up study, whereby a larger proportion of oil was used (one drop of oil to 20g of the diet, equivalent to approximately 1475 mg/kg bw/day). After 8 weeks of treatment, the incidence of carcinoma was again lower than control (27.82% compared to 75%) (Strong, 1934). Subsequent studies on survival time revealed that animals fed wintergreen oil lived longer than control after they developed cancer and had better prognosis (Strong, 1935; Strong, 1936).

In another study, oil of wintergreen (derived from Betula lenta) (10 mg/day, equivalent to approximately 333 mg/kg bw/day) was given to Strong A or Little Dilute Brown mice (n=5/group) containing grafted Crocker Sarcoma 180 tumours. The tumours were measured thrice weekly and compared to the growth of control tumours. At the end of the study (undeclared duration), the tumours in the treated animals were not significantly different from those in the control mice (Boyland, 1938).

Chronic treatment with the main constituent of wintergreen oil, methyl salicylate, also did not reveal any carcinogenic effects (Webb and Hansen, 1963).

SCCS comments
Although these aforementioned studies did not follow current carcinogenicity testing guidelines, the overall totality of evidence indicates that wintergreen oil is not likely to be carcinogenic.

3.3.8 Photo-induced toxicity

The UV absorption maximum of a methanol solution of methyl salicylate is 305nm, which indicates that methyl salicylate can undergo direct photolysis. CIR (2003) concluded that salicylic acid is not a photo sensitiser, nor is it phototoxic. There is no evidence from over a century of human use of products containing methyl salicylate that photo-mediated toxicity is an issue.

3.3.9 Human data

See the respective sections above.

3.3.10 Special investigations

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3.4 SAFETY EVALUATION (including calculation of the MoS)

MeS can be used in 18 product categories (the standard 17 product types + hydroalcoholic fragrances) of cosmetic products that could lead to exposure by different routes - dermal, oral and by inhalation – therefore, aggregated exposure has to be taken into consideration.

These products could be used by adults but also by children below 3 years old and above. The SED have been calculated for adults and for children, taking into account the age of children (as the use of cosmetic products may differ due to age-related different behaviors).The exposure to product (Eproduct) value has also been normalised by weight.

To assess the risk of MeS by systemic exposure, the SCCS has used the NOAEL of 75 mg/kg bw/d derived from the 3-generation study in rats by oral route (Collins *et al.*, 1971). Because of the evidence for rapid and almost complete absorption of MeS from the oral route, the SCCS has not applied any adjustment for bioavailability to this NOAEL value.

Details of the calculation of systemic exposure dose (SED) are presented in the Tables in section 3.2.4. A generic maximal value for skin penetration of methyl salicylate of 50% (see section 3.2.1) 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 for passage across the oral mucosa. The calculations of MoS for different product types are given in Tables below.

For the aggregate exposure calculation in the deterministic approach, absence of duplication of similar products is necessary. Table 13 above (Comparison of SED by dermal exposure and inhalation exposure for products available as spray and non-spray) shows the exposure to MeS for the product categories duplicated in spray and non-spray products. Worst-case scenarios of exposure were selected for the calculation of the MoS, considering that if the MoS is above 100 for these scenarios then it would also be safe for the others.

For the total systemic exposure including both routes, the exposure through the non-spray products is higher compared to spray products of the same category. Therefore, for systemic aggregate exposure calculation, only the product categories of dermally non-sprayed products will be taken into account.

For adult

Table 31: MoS calculation for Adults

Products	Concentration %	SED dermal mg/kg bw/d	Sed inhal mg/kg bw/d	SED oral mg/kg bw/d	SED Tot mg/kg bw/d	NOAEL	MOS
Hydroalcoholic - based Fragrances	0.600	0.014	0.00038	0	0.01438	75	5216
Shower gel	0.060	0.0008	0.000023	0	0.00082	75	91130
Shampoo	0.060	0.00045	0.00013	0	0.00058	75	129310
Hair conditioner	0.060	0.0002	0.0000054	0	0.00021	75	365141
Hand soap	0.600	0.0099	0.001	0	0.01090	75	6881
Body lotion	0.060	0.03696	0.001	0	0.03796	75	1976
Face cream	0.060	0.007242	0.0002016	0	0.00744	75	10076
Hand cream	0.060	0.00981	0.0002736	0	0.01008	75	7438
Deodorant non-spray	0.060	0.006624	0.0001872	0	0.00681	75	11011
Hair Styling products	0.060	0.001722	0.00004752	0	0.00177	75	42384
Liquid foundation	0.050	0.001975	0.00005328	0	0.00203	75	36977
Make up remover	0.002	0.0000833	0.000002304	0	0.00009	75	876127
Eye make up	0.002	0.0000033	0.000000144	0	0.00000	75	21777003
Mascara	0.002	0.0000042	0.000000144	0	0.00000	75	17265193
Eyeliner	0.002	0.0000008	0.0000000288	0	0.00000	75	90492278
Lip products	0.030	0.00027	0.000003744	0	0.00027	75	273979
Toothpaste*	2.520	0	0	0.0544	0.0544	75	1379
Mouthwash*	0.600	0	0	0.19524	0.19524	75	384
Mouth Spray*	0.650	0	0	0.173	0.173	75	434
Total		0.0900446	0.003307965	0.42264	0.51599	75	145

* 100% oral/mucosal absorption as a worst case scenario

For systemic effects, considering all products taken individually and also the aggregated exposure, the margin of safety is above 100.

Local effect by inhalation: the NOAEL of 700 mg/m^3 (120 ppm) from Gage (1970) is used to compare with the concentration of MeS in the lungs.

For spray products, for an adult, the dose of MeS would be 108 µg in 10 m^3 equivalent to a concentration of 10.8 µg/m^3, which leads to a MoS of around 65 000.

For dermally applied products, part of the dose will volatilize. Based on the hypothesis described above, the concentration is 14.73 µg/m^3 which leads to a MoS of around 47 000.

For Children

The SCCS considers that for children above 6 years old, toothpaste and mouthwash have to be aggregated.
Besides toothpaste and mouthwash (for children above 6 years old), other skin products used by girls between 4 and 14 years old were considered, based on the study from Ficheux *et al.* (2017) (see above).

As the exposure *via* inhalation is limited compared to dermal absorption, the SCCS decided not to consider it for children.

The MoS was calculated considering the NOAEL of 75 mg/kg/bw /day as for adults (see table 32 below).

Table 32: MoS calculation for children

	SED (mg/kg bw/d) (P95)	NOAEL	MoS
Toothpaste, 1-6 years	0,579	75	130
Toothpaste, 6-10 years	0,248	75	302
Toothpaste, 10-14 years	0,118	75	636
Toothpaste, 14-18 years	0,077	75	974
mouthwash, 6-10 years [MeS = 0.1%]	0,155	75	484
mouthwash, 10-14 years [MeS = 0.6%]	0,442	75	170
mouthwash, 14-18 years [MeS = 0.6%]	0,289	75	260
mouthwash + toothpaste, 6-10 years	0,403	75	186
all other skin products (Total from Ficheux, et al., 2017 4-14 years, girls) P5 body weight 29,4 kg	**0,291**	75	258
Aggregate exposure, 6-10 years, P95	0,694	75	108

For children, considering all products taken individually and also the aggregated exposure, the margin of safety is above 100.

3.5 DISCUSSION

Physicochemical properties

Methyl salicylate (methyl 2-hydroxybenzoate; CAS 119-36-8 as 99% pure) is the ester of methyl alcohol and Salicylic acid. Different studies have shown that Salicylic acid is the main metabolic product of Methyl salicylate by hydrolysis. The SCCS issued an Opinion on the safety of Salicylic acid in 2018 (Corrigendum 2019).

Methyl salicylate is also the main component of the natural 'oil of wintergreen'.

After having reviewed the data provided in the dossier, SCCS considers that salicylic acid and dimethyl 4-hydroxyisophthalate are organic impurities in methyl salicylate. A full report in terms of impurity tests in representative batches of the test substance should be provided and the validity of the analytical methodologies used must be shown. Identity and concentration of any impurities that may be present must also be stated.

Methyl salicylate should be considered as very slightly soluble according to the table in NoG.

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 have to be provided.

Exposure

Methyl salicylate is used in many fragrance mixtures and as a flavouring and soothing agent in oral hygiene products. Methyl salicylate is not listed in the Annexes to the EU Cosmetic Products Regulation n. 1223/2009 on cosmetic products (Cosmetics Regulation) and its use is not otherwise restricted in cosmetic products.

Methyl salicylate is also used at high concentrations in some medical creams.
For the estimation of the exposure to methyl salicylate *via* cosmetic products, values for the maximum % level of methyl salicylate for each of the standard 17 product types that are used

in Europe have been provided by the members of the Cosmetics Europe Methyl Salicylate consortium. These values were used by the SCCS to calculate the total dermal exposure to methyl salicylate (in mg/kg/day) from each product for adults.

Dermal/percutaneous absorption

As no reliable data are available to properly assess skin absorption, the SCCS considers that a default value of 50% skin absorption, based on the data reported in humans and on the physico-chemical properties of methyl salicylate, can be used to estimate systemic exposure following skin application.

Metabolism *via* the dermal route is rapid, with maximal absorption between 1-4 h, and mostly as salicylic acid and its secondary metabolites. Some studies indicate that methyl salicylate conversion to salicylic acid systemically could be assumed to be 50% as it passes through the skin, but then any parent material that enters the blood is hydrolysed rapidly in blood and by the liver such that within only a few hours, no parent substance can be detected except, only free salicylate/salicylic acid.

Toxicokinetics

Limited studies are available on the ADME properties and kinetics of methyl salicylate via the oral route in animals and humans. However, available data provide evidence that methyl salicylate is rapidly and extensively absorbed across the gut and is completely hydrolysed to its primary metabolites salicylic acid and methanol. An oral absorption value of 100% can be used in risk assessment.

Based on the available data, an absorption value by inhalation of 100% can also be used in the risk assessment.

Exposure by dermal and oral route

For adults, as MeS can be used in all kind of cosmetic categories, a SED *via* the dermal route has been calculated for each product in mg/kg/day as well as an aggregate systemic exposure dose including the other products used in the mouth. It is noteworthy that around 75% of the aggregated exposure is due to lipstick, toothpaste and mouthwash. Exposure to MeS via mouthwash already contribute to more than 57% of the total exposure.

For children, as the use of cosmetic products may vary depending on age categories, different scenarios of exposure have been considered:

- Between 1 and 6 years old:

For oral products, the SCCS has only considered toothpastes and values provided in the SCCS NoG have been used for the calculation of the SED.

- Between 6 and 10 years old:

For oral products, the SCCS has considered toothpastes containing MeS at 2.52% and mouth wash at 0.1%; again values provided in the SCCS NoG have been used for the calculation of the SED.

- Above 10 years old:

For oral products, the SCCS has considered toothpastes containing MeS at 2.52% and mouth wash at 0.6%; again values provided in the SCCS NoG have been used for the calculation of the SED.

For mouth spray, these are not habitually used products for breath freshening, and they are just used occasionally by children. Therefore, mouth sprays were not considered further for the calculation of exposure to MeS for children.

Exposure by inhalation

For children, for the reasons given above, the SCCS considers that the exposure is negligible *via* inhalation compared to dermal absorption. Therefore, the SCCS has not considered exposure *via* inhalation in the MoS calculation.

For adults, 2 exposure models for inhalation can be used: a one-box model and a two-box model.

2 exposure scenarios need to be assessed:

- For all dermally applied products: due to the high volatility of this compound (vapour pressure = 13 Pa at 20°C), evaporation of MeS retained on the skin needs to be taken into account for inhalation exposure
- Exposure through the airborne fraction of sprayed products.

As a first-tier exposure assessment by inhalation, one-box model is used (often more conservative). For spray products, 1-box and 2-box models have been compared: as the 1-box is most conservative, it is the only one described in detail in this Opinion.

The results show that even by considering worst case scenario in terms of bioavailabililty of the methyl salicylate for spray products, the inhalation exposure is strongly below the dermal exposure. In this instance, the 2-box modelling did not have a significant quantitative impact on the outcome for the risk assessment.

Finally, for the total systemic exposure including both routes, the exposure through the non-spray products is higher compared to spray products. Therefore, for systemic aggregate exposure calculation, only the product categories of dermally non-spray products will be taken into account.

For some parameters (e.g. the respiration rate), as the values proposed by the applicant deviate from the values present in the SCCS Notes of Guidance, new calculations were made by the SCCS and used for the calculation of the SED and the MoS (see Table 14).

Toxicological Evaluation

Methyl salicylate (MeS) and acetylsalicylic acid (ASA, aspirin) are related substances, both are esters of SA (ortho-hydroxy benzoic acid), which is characterised by a carboxyl group and a hydroxyl group. Salicylic acid (SA) is the common hydrolysis product of both substances.

Irritation and corrosivity

Based on the data available, the SCCS considers that there is no evidence of a skin irritation potential of methyl salicylate in humans at concentrations up to 12%. Relevant signs of irritation may only be observed at higher doses. The SCCS considers that methyl salicylate is non irritating to the skin at a concentration up to 12%, but it may cause severe eye damage.

Skin sensitisation

Methyl salicylate is identified as a skin sensitiser in different LLNA studies using high (>25%) concentrations. This is further supported by clinical data showing that methyl salicylate is a skin sensitiser in humans. The incidence in unselected and selected patients is low. Taken all

data together, methyl salicylate is a weak skin sensitiser in the LLNA and humans, which is in line with the classification as a 1B skin sensitiser.

Acute toxicity

The available LD50 values by oral route range from 580 mg/kg bw (mice) to doses higher than 2 000 mg/kg bw in rats, rabbits and dogs. Based on the available data, MeS should be considered as Harmful if swallowed (Acute Tox. 4; H302).

By the other routes of exposure, MeS does not warrant any classification for acute toxicity.

Repeated dose toxicity

Since the early 1960's MeS has been studied in repeated dose toxicity studies of varying duration in different species. Repeated dose toxicity studies ranging in duration from 4 weeks to 2 years have been conducted in rats, rabbits and dogs.

SCCS notes that the repeated dose toxicity studies are mostly old studies that were not performed following the current guidelines. Moreover, it should be noted that limited endpoints were evaluated and a limited number of animals were examined. Furthermore, it is not indicated if a statistical analysis was performed on histopathological findings. Therefore, it cannot be excluded that effects can occur at a lower doses in organs that were not examined. In addition, considering the small number of animals examined at histopathology, only the effects occurring at a high incidence can be detected in these studies.

Based on the data available for the calculation of the MoS; the following values could be identified:

- For oral exposure (Webb and Hansen, 1963): a NOAEL of 50 mg/kg bw/day (LOAEL = 150 mg/kg bw/day)
- For dermal exposure (Webb and Hansen, 1963): a LOAEL of 585 mg/kg bw/day
- For exposure by inhalation (Gage,1970): A NOAEL of 700 mg/m^3 (120 ppm)

Reproductive toxicity

Concerning fertility and reproductive function, there is insufficient evidence that MeS exhibits adverse effects on sexual function and fertility. Therefore the SCCS concurs with the proposal by RAC that no classification is justified for MeS for adverse effects on sexual function and fertility.

Concerning effects on development, a CMR category 2 classification has been agreed by the RAC on September 2019 for methyl salicylate. The CMR category 2 classification for methyl salicylate is consistent with the 2016 CMR category 2 classification decision for salicylic acid. Salicylic acid is the principal primary metabolite of methyl salicylate via the dermal and oral routes: systemically the body is exposed to more salicylic acid metabolite than to the parent compound.

The lowest developmental NOAEL are < 60 mg/kg bw/d in rats exposed subcutaneously from GD6 to LD21 (FDA, 2006b) and 75 mg/kg bw/d in a 3-generation study in rats by oral route (Collins *et al.*, 1971). The NOAEL of 75 mg/kg bw/d is used by SCCS for the calculation of the MoS.

Mutagenicity / genotoxicity

The genotoxicity of methyl salicylate was investigated with valid *in vitro* genotoxicity tests for bacterial gene mutations and chromosomal aberrations with negative results. Additionally, a

valid *in vivo* micronucleus in rat with negative result was provided. Based on the results, methyl salicylate can be considered to pose no genotoxic hazard.

Carcinogenicity

The overall totality of evidence, even if limited, indicates that methyl salicylate did not reveal any carcinogenic effects.

Photo-induced toxicity

The UV absorption maximum of a methanol solution of methyl salicylate is 305nm, which indicates that methyl salicylate can undergo direct photolysis. CIR (2003) concluded that salicylic acid is not a photo sensitiser, nor is it phototoxic. There is no evidence from over a century of human use of products containing methyl salicylate c that photo-mediated toxicity is an issue.

Special investigation: endocrine disrupting effects

The only endocrine pathway that was investigated by the applicant is the estrogenic pathway. No information on the androgen, thyroid and steroidogenesis (EATS) pathways were provided.

However methyl salicylate is not identified at EU level as an SVHC substance for its endocrine properties, either for human health or for the environment.

Methyl salicylate is not on the ED-list (https://edlists.org/the-ed-lists) of endocrine disrupters, meaning that it is not a substance identified as an endocrine disruptor at EU level (List I), a substance under evaluation for endocrine disruption under an EU legislation (List II) or a substance considered, by the evaluating National Authority, to have endocrine disrupting properties (List III).
Therefore, the SCCS has no specific concern regarding the endocrine disrupting potential of methyl salicylate. Moreover, the SCCS considers that *in vitro* data provided by the applicant are not useful for calculating a maximum dose.

The SCCS is also aware that benzyl salicylate is 'under assessment' for endocrine disrupting (ED) potential in the EU, as a result of it being regarded as structurally similar to salicylic acid, which is also set for review as part of the European Commission call for data on list B of suspected EDs.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the RAC Opinion on Methyl salicylate and the expected new classification as Toxic for Reproduction Category 2 of Methyl salicylate (to be introduced by an update of Annex VI Reg. 1272/2008), does the SCCS consider Methyl salicylate safe when used up to the maximum concentrations provided in the dossier submitted by the applicant?*

   The SCCS considers Methyl salicylate safe when used in cosmetic products up to the maximum concentrations provided in the dossier submitted by the applicant as detailed in Table 1.

2. *Does the SCCS have any further scientific concerns with regard to the use of Methyl salicylate in cosmetic products, also in relation to the RAC recommended classification of Methyl salicylate as 'Skin sensitizer Category 1B'?*

   Methyl salicylate should be considered as a weak skin sensitiser in humans and as an eye irritant.

   Moreover, the SCCS would like to express other concerns related to the use of methyl salicylate:

   - Methyl salicylate can be used in consumer products other than cosmetic products, such as household cleaning products, air care products, biocides (e.g. disinfectants, pest control products), polishes and waxes, which may increase the systemic exposure dose and possibly exceed the safe level.

   - Methyl salicylate will be metabolised in the body to salicylic acid which is also classified as a reprotoxicant and used in cosmetic products (see Opinion SCCS/1601/18). Therefore the combined exposure to cosmetic products containing various salicylates may increase the systemic exposure dose and possibly exceed the safe level.

   The SCCS also notices that wintergreen oil that is used in cosmetic products may contain up to 99% methyl salicylate. Therefore when calculating the content of methyl salicylate in a cosmetic product, any fraction coming from wintergreen oil should also be considered.

5. MINORITY OPINION

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6. REFERENCES

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- The main US FDA application for SALONPAS cream is at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022029TOC.cfm
- The Pharmacology review includes the repro and developmental data and are in: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2008/022029s000pharmr.pdf
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7. GLOSSARY OF TERMS

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

8. LIST OF ABBREVIATIONS

MeS = methyl salicylate

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

Annex 1

Table A: Deterministic worst-case systemic exposure dose calculation for the dermal and oral route using maximum % levels of MeS in cosmetic products used by French girls between 4 and 14 years old (amount of products used taken from Ficheux *et al.,* 2016).

	Girls 4-14 ans		Efsa : 29 kg P5 10-14 and	Efsa : 42 kg P50 10-14								
	amount used P50	amount used P95	body weight	body weight	NoG retention factor	Calculated relative daily exposure1 Eproduct /bw (mg/kg bw/d) P50	Calculated relative daily exposure1 Eproduct /bw (mg/kg bw/d) P95	Adult Eproduct normalized (mg/kg bw/d)	Maximum conc MeS (w/w %)	Dermal absorption (%)	Tier 1, dermal/oral SED (mg/kg bw /d) P50	Tier 1, dermal/oral SED (mg/kg bw /d) P95
Shower gel (g)	4,5	15	29,4	42	0,01	1,071428571	5,102040816	2,79	0,06	50	0,000321429	0,00153061
Deodorant non spay	0,341	0,615	29,4	42	1	8,119047619	20,91836735	22,08	0,06	50	0,002435714	0,00627551
Fragrance	0,181	0,68	29,4	42	1	4,30952381	23,1292517	4,67	0,6	50	0,012928571	0,06938776
Hand wash soap	1,9	3,4	29,4	42	0,01	0,452380952	1,156462585	3,33	0,6	50	0,001357143	0,00346939
Face soap	2,28	3,5	29,4	42	0,01	0,542857143	1,19047619		0,06	50	0,000162857	0,00035714
Body lotion (sunscreen)	4,8	14,6	29,4	42	1	114,2857143	496,5986395	123,2	0,06	50	0,034285714	0,14897959
Face cream	0,907	3,029	29,4	42	1	21,5952381	103,0272109	24,14	0,06	50	0,006478571	0,03090816
Hand cream	1	2,6	29,4	42	1	23,80952381	88,43537415	32,7	0,06	50	0,007142857	0,02653061
Shampoo	6	13,6	29,4	42	0,01	1,428571429	4,62585034	1,51	0,06	50	0,000428571	0,00138776
Conditionner	6	16,7	29,4	42	0,01	1,428571429	5,680272109	0,67	0,06	50	0,000428571	0,00170408
Lip Balm	0,0063	0,0128	29,4	42	1	0,15	0,43537415	0,9	0,03	100	0,000045	0,00013061
						177,1928571	750,2993197	215,99			0,066015	0,29066122

Annex 2

Table B: Reproductive and developmental animal studies with methyl salicylate – **subcutaneous** route.

116 rats	Animals were dosed SC on GD 9, 10, or 11 and killed on GD 21	Sub-cutaneous	0.1–0.5 cc (equivalent to 118 and 590 mg/kg/day)	26/69 dams died; 47 resorbed their fetuses; external abnormalities in 45/298 fetuses; 75 of the 253 fetuses that appeared normal had skeletal anomalies (no information given as a function of dose)	Warkany & Takacs, 1959
Pregnant Long Evans rats	Animals were dosed SC on GD 10, or 11	Sub-cutaneous	0.1 ml (equivalent to 118 mg/kg/day)	Animals receiving treatment on GD10, 27.3% underwent resorption; 31.4% living foetuses showed congenital malformations. Animals treated on GD11 had 32.7% resorption; 18.2% living foetuses had abnormalities.	Bertone & Monie, 1965

Rats/Crj:CD (SD)IGS 20/sex/group for main study + 3/sex/group for TK (satellite groups)	Once daily from 2 weeks prior to mating, through mating and up to GD6 (f) beginning 10 wks age.	Sub-cutaneous	30, 100, 300 mg/kg/day; dissolved in corn oil, dose volume of 1.0 mL/kg	No mortality or abnormal signs in the 30 and 100 mg/kg/day groups. **Reduced body weight gain** and hair loss was observed at 300 mg/kg/day. No significant differences in testes or epididymides weight (m) or in estrous cycle (f). No significant differences in sperm count, copulation index or fertility or fetal development. Salicylate present in plasma. **NOAEL = 100 mg/kg/day.**	FDA 2006a
New Zealand white rabbit	Administered once daily for 13 days from GD6 to 18	Sub-cutaneous	0, 30, 100, 300 mg/kg/day; dissolved in corn oil, dose volume of 1.0 mL/kg	A depressive trend in body weight gain (not statistically significant) was observed throughout the administration period in the 300 mg/kg group as compared with the control group. No effects on embryo-fetal development. **NOEL = 100 mg/kg/day NOAEL = 300 mg/kg/day**	FDA 2006b
20 female Rats /Crj:CD(SD)IGS	Administered once daily for 12 days from GD6 to 17	Sub-cutaneous	0, 50, 100, 200 mg/kg/day; dissolved in corn oil, dose volume of 1.0 mL/kg	Reduced body weight gain was seen in dams at 200 mg/kg/day. A number of skeletal variations were seen in offspring at 200 mg/kg/day (see main text below for details) **NOAEL = 100 mg/kg/day.**	FDA 2006c

20 female Rats /Crj:CD(SD)IGS	Administered once daily from GD6 to lactation day 21.	Sub-cutaneous	0, 20, 60, 200 mg/kg/day	Reduced body weight gain was seen in dams at 200 mg/kg/day. A number of skeletal anomalies and variations were seen in offspring at 200 mg/kg/day (see main text below for details). **NOAEL = 60 mg/kg/day.**	FDA 2006d

Table 2: Reproductive and developmental animal with methyl salicylate – **intraperitoneal** route.

Animals were dosed on GD 10 and 11 and either killed on GD 21 or allowed to deliver	Intra-peritoneal	0.05 or 0.1 ml	The 0.1-ml group had decreased body weight gain, fewer and smaller neonates, and more resorptions and malformed neonates; fetal kidney weight was decreased (GD 21) but was not different from control on postnatal day 6	Woo and Hoar, 1972
Animals were dosed IP on GD 8-9 and killed on GD 20	Intra-peritoneal	200 or 400 mg/kg	Embryotoxicity was seen at 400 mg/kg; fetal mortality was 2% and 50% in the 200- and 400-mg/kg groups; fetal body weight index was significantly decreased in the 400-mg/kg group; some developmental anomalies were seen in both groups, and dose-related decreases in organ weights were observed	Kavlock et al 1982
Animals were dosed IP with 250–450, 200–300, 300–375, or 200–300 mg/kg on GD 11, 10–11, 11–12, or 11–13 and killed on GD 20	Intra-peritoneal	250–450 mg/kg	Maternal toxicity was observed; fetal weight was significantly decreased (dose dependent); malformations were observed in fetuses of groups dosed with ≥350 mg/kg on GD 11 and ≥300 mg/kg	Daston et al 1988

			on >1 day; incidence of resorptions was significant in the 400 mg/kg gp dosed on GD 11; kidney development was affected	
Animals were dosed IP on GDs 10–13	Intra-peritoneal	200, 250, or 300 mg/kg	A high incidence of maternal mortality was seen in the 300-mg/kg group; neonatal mortality was increased in the 250- and 300-mg/kg groups on days 1–2; no external abnormalities were seen in surviving neonates; some effect on urine-concentrating ability was seen in young neonates	Daston et al 1988

Table C: Reproductive and developmental animal studies with methyl salicylate – **dermal** route.

LVG Hamster	Methyl Salicylate was applied to the back of each animal on GD7, and the skin was washed 2 h after dosing; fetuses were recovered on GD9	Dermal	3500 or 5250 mg/kg	Neural tube defects were seen in 6% and 53% of the low- and high-dose litters, respectively	Overman & White, 1983
Rat 12/group	Applications were made on gestation days (GDs) 6–15; positive controls were dosed dermally with 2 and 1 g/kg Methyl Salicylate on GDs 6–9 and 10–15; dose was changed due to maternal toxicity (tox.)	Dermal	1, 3, or 6 g/kg of a petroleum-based grease using 3% Methyl Salicylate	No maternal toxicity and no changes in reproductive parameters or malformations were seen; positive controls had 100% incidence of total resorptions	Infurna et al 1990