Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Methyl salicylate

EC Number: 204-317-7
CAS Number: 119-36-8

CLH-O-0000006716-67-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

20 September 2019

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),

Annex VI, Part 2

International Chemical Identification: Methyl salicylate

EC Number: **204-317-7**

CAS Number: **119-36-8**

Index Number: **-**

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)
14 rue Pierre Marie Curie
F-94701 Maisons-Alfort Cedex
classification.clp@anses.fr

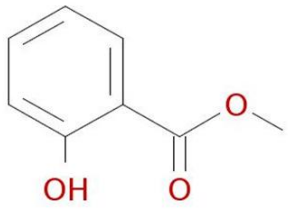
Version number: v1 **Date: June 2018**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	methyl 2-hydroxybenzoate
Other names (usual name, trade name, abbreviation)	Benzoic acid, 2-hydroxy-, methyl ester Methyl salicylate Wintergreen oil
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	204-317-7
EC name (if available and appropriate)	methyl salicylate
CAS number (if available)	119-36-8
Other identity code (if available)	
Molecular formula	$C_8H_8O_3$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	152.1473
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	na
Description of the manufacturing process and identity of the source (for UVCB substances only)	na
Degree of purity (%) (if relevant for the entry in Annex	> 99%

VI)	

To be noted that a substance including >80% (w/w) of methyl salicylate may have been identified by the name "Wintergreen oil".

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Methyl salicylate	> 99%	None	Acute Tox. 4 – H302 Skin Irrit. 2 – H315 Eye Irrit. 2 – H319 STOT SE3 – H335 Repr. 2 – H361 Repr. 1B – H360 Lact. H362

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Only confidential data (see IUCLID file)

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Not applicable

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**

For the physico-chemical properties, data have been provided on pure methylsalicylate (synthetic substance).

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No existing Annex VI entry										
Dossier submitters proposal	To be determined	Methyl salicylate	204-317-7	119-36-8	Acute Tox 4 Skin Sens. 1B Repr. 1B Aquatic Chronic 3	H302 H317 H360D H412	GHS07 GHS08 Danger	H302 H317 H360D H412		ATE = 580 mg/kg (oral)	
Resulting Annex VI entry if agreed by	To be determined	Methyl salicylate	204-317-7	119-36-8	Acute Tox 4 Skin Sens. 1B Repr. 1B	H302 H317 H360D	GHS07 GHS08 Danger	H302 H317 H360D		ATE = 580 mg/kg (oral)	

RAC and COM					Aquatic Chronic 3	H412		H412			

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	No
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	data conclusive but not sufficient for classification	No
Flammable solids	hazard class not applicable	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not applicable	No
Self-heating substances	data conclusive but not sufficient for classification	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	data conclusive but not sufficient for classification	No
Oxidising solids	hazard class not applicable	No
Organic peroxides	hazard class not applicable	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	**harmonised classification proposed**	Yes
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	**harmonised classification proposed**	Yes
Germ cell mutagenicity	hazard class not assessed in this dossier	No

Hazard class	Reason for no classification	Within the scope of public consultation
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	**harmonised classification proposed**	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	**harmonised classification proposed**	Yes
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no current harmonized classification for methyl salicylate (MeS).

For information, methyl salicylate was assessed in 2015 by France in the framework of the CoRAP. The status is still ongoing.

RAC general comment

Chemical structure and pharmacological action

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 (Kucera & Bullock, 1969). Binding fractions also depend on the experimental conditions, e.g. the salicylate concentration. At a drug level of 150 mg/L, plasma binding fraction 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.

Production and use

MeS is an ingredient used in many fragrance mixtures. It is manufactured in and imported into the European Economic Area in quantities of 1 000-10 000 tonnes per year (ECHA website, 2018). It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level for classifying as reprotoxic. Available data show that methyl salicylate has CMR property, i.e. reproductive toxicity that is not currently harmonised and justify a harmonised classification and labelling according to article 36 of CLP.

C&L inventory (checked on 30th November 2017) reported that

- 55/1680 notifiers classify methyl salicylate as Repr. 2 – H361;
- 3/1680 notifiers classify methyl salicylate as Repr. 1B – H360;
- 3/1680 notifiers classify methyl salicylate as lact. H362.

Concerning classification for acute toxicity and skin sensitisation, justification that action is needed at Community level is required.

Differences in self-classification

For acute toxicity, unconsistent self-classifications are reported in the ECHA inventory database (60/1681 notifiers not classifying as Acute Tox 4 – H302) (ECHA website, 2018) whereas the available data with methyl salicylate show acute toxicity by oral route.

Disagreement by DS with current self-classification

For skin sensitisation, no self-classifications are reported in the ECHA inventory database (ECHA website, 2018). Human data supported by animal data show cases of skin sensitisation in different studies. Considering the identified uses of methyl salicylate (especially in cosmetic products), an action at Commmunity level is judged needed regarding classification as skin sensitiser.

5 IDENTIFIED USES

Methyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents (Lapczynski et al. 2007). According to ECHA website, the substance is manufactured and/or imported in the European Economic Area at 1 000 - 10 000 tonnes per year. This substance is used by consumers, by professional workers (widespread uses) and by industrial workers. It is used to formulate mixtures and as an intermediate to manufacture other substances. This substance is used in the following end-products: air care products, washing and cleaning products, cosmetics and personal care products, biocides (e.g. disinfectants, pest control products), polishes and wax blends, fuels and other (unspecified) fragranced products (ECHA website, 2018). Finally, methyl salicylate can also be used as topical medication due to its anti-inflammatory properties (Vidal, 2018).

6 DATA SOURCES

Information described in this CLH report are based on the REACH registration dossier and bibliographic search.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid at 20°C and 101.3 kPa Colourless to slightly yellow. Characteristic odour of aromatic compounds	Rhodia 2010 (Registration dossier, IUCLID)	Observation and manipulation, purity not given
Melting/freezing point	-8.6 °C at 101.3 kPa	Merck Index 2006 (Registration dossier, IUCLID)	Reliable handbook data, purity not given
Boiling point	220-224 °C at 101.3 kPa	Merck Index 2006 (Registration dossier, IUCLID)	Reliable handbook data, purity not given

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Relative density	1.1782 at 25°C	Aminabhavi T.M. & Phayde H.T.S, 1996 (Registration dossier, IUCLID)	Pycnometer method, purity 99.3%
Vapour pressure	10 Pa at 22°C and 100 Pa at 51°C	CRC Hanbook 2005-2006 Registration dossier, IUCLID)	Reliable handbook data, purity not given
Surface tension	Not surface active	(Registration dossier, IUCLID)	Based on chemical structure, no surface activity is to be expected
Water solubility	0.67 g/L at ambient temperature	Merck Index 2006 (Registration dossier, IUCLID)	Reliable handbook data, purity not given
Partition coefficient n-octanol/water	Log Kow: 2.55	Sangster Research Laboratories, 1994 (Registration dossier, IUCLID)	Experimental data, purity not given
Flash point	99°C	Merck Index 2006 (Registration dossier, IUCLID)	Reliable handbook data, Closed-cup method, purity not given
Flammability	non flammable	(Registration dossier, IUCLID)	Based on its flash-point, methylsalicilate is not flammable
Explosive properties	non explosive	(Registration dossier, IUCLID)	Based on the chemical structure, the substance has no explosive properties
Self-ignition temperature	450°C	BGIA Gestis, 1999 (Registration dossier, IUCLID)	No data
Oxidising properties	no oxidising properties	(Registration dossier, IUCLID)	Based on the chemical structure, the substance has no oxidising properties.
Granulometry	not applicable	(Registration dossier, IUCLID)	The substance is a liquid

Property	Value	Reference	Comment (e.g. measured or estimated)
Stability in organic solvents and identity of relevant degradation products	In accordance with REACH Annex IX, the study on stability in organic solvent, required in section 7.15, does not need to be conducted as stability of the substance is not considered to be critical	(Registration dossier, IUCLID)	-
Dissociation constant	9,8-9,9 at 20°C	Scully F.E. and Hoigné J., 1987 Serjeant, E.P. and Dempsey, 1979 (Registration dossier, IUCLID)	Only secondary data sources have been provided with very close values, they have been used in a WOE approach.
Viscosity	1.535 mPa.s at 25°C	(Registration dossier, IUCLID)	Capillary method, purity 99.3%

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Based on the chemical structure, the substance has no explosive properties.

8.2 Flammable gases (including chemically unstable gases)

Not applicable

8.3 Oxidising gases

Not applicable

8.4 Gases under pressure

Not applicable

8.5 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Closed-cup method	99°C	-	Merck Index 2006

Based on its flash-point (99°C), methylsalicilate is not flammable.

8.6 Flammable solids

Not applicable

8.7 Self-reactive substances

Not assessed

8.8 Pyrophoric liquids

Not assessed

8.9 Pyrophoric solids

Not applicable

8.10 Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
No data	450°C	-	BGIA Gestis, 1999

8.11 Substances which in contact with water emit flammable gases

Not assessed

8.12 Oxidising liquids

Based on the chemical structure, the substance has no oxidising properties.

8.13 Oxidising solids

Not applicable

8.14 Organic peroxides

Not applicable, the substance does not contain the bivalent O-O structure and is not derivatives of hydrogen peroxide where one or both of the hydrogen atoms have been replaced by organic radicals

8.15 Corrosive to metals

Not assessed

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 11: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
mouse (Hairless HRS/J (hr)) female oral: gavage in corn oil Exposure regime: Single gavage dose followed by samplings from 15 minutes to 48 hours Doses/conc.: ADE (absorption, distribution, elimination): 97 mg/kg (2.62 mg mixture of [14C]MeS & unlabelled MeS, radioactivity 1.25 uCi) Whole body autoradiography: 68 mg/kg (1.90 mg mixture of [14C]MeS & unlabelled MeS, radioactivity 4.77 µCi) Not guideline; not GLP	Absorption: close to 100%. Tissue and blood level reached a maximum at 30 minutes. Distribution: radioactivity was high in the liver, kidney and adrenals and low in the lungs, uterus, heart, spleen, ovaries and pancreas, with the lowest level in the brain. After 48 hours, radioactivity was still present in the liver and kidney, and only traces in other organs. Metabolites identified: not measured Elimination: almost exclusively in the urine. Less than 3 % in the faeces. Total recovery: 98-104% (between 15 minutes to 48 hours) No bioaccumulation potential: after 48 hours, radioactivity was mostly found in the urine and faeces, and low levels in tissues (0.03%) and GI tract & contents (0.05%)	2 (reliable with restrictions) key study experimental result Test material (EC name): methyl salicylate	Yamagata *et al.* (1976)
rat (Wistar) male (10 animals) oral: gavage in methyl cellulose Exposure regime: only once. Doses/conc.: 500 mg/kg,	Absorption: MeS, NaS and ASA are all rapidly absorbed, with NaS being the most rapid. Distribution: not performed Metabolites identified: yes	Klimisch score = 2 (reliable with restrictions) key study	Davison C *et al.* (1961)

Method	Results	Remarks	Reference
calculated as free salicylic acid Plasma analyses in rats after oral administration of methyl salicylate (MeS), sodium salicylate (NaS) and acetylsalicylic acid (ASA). Not guideline; not GLP	MeS does not produce any higher plasma or brain concentrations than NaS and ASA, and is completely hydrolyzed to free salicylate in as little as 20 minutes. Elimination: not performed. The authors showed that the major site of hydrolysis is the liver (*in vitro* assay with rat, dog, rabbit and monkey livers).	experimental result Test material (EC name): methyl salicylate, NaS and ASA	
Oral absorption and hydrolysis in humans 4 men, 2 women 0.42 ml of MeS orally, equivalent to 0.6 g of ASA (calculated as averaged of 7 mg/kg SA) Plasma analysis for salicylate level. Not guideline; not GLP	After 15 min, the mean MeS and free salicylate values were 4.9 and 7.9 mg/l (total salicylates = 12.8 mg/L), respectively. After 90 min, these values were 2.8 and 10.5 mg/l, respectively (total salicylates = 13.3 mg/L). 30% MeS remained unhydrolysed at 15 minutes, and 21% at 90 minutes. In comparison, ASA administration resulted in 18.2 mg/L total salicylates at 15 min and 24.5 mg/L at 90 min. Therefore, total plasma salicylate concentration reached after ASA administration exceeded those obtained with MeS. Hydrolysis of MeS to free salicylate was slower and less complete than that of ASA.	Klimisch score = 2 (reliable with restrictions) supporting study Test material (EC name): methyl salicylate, ASA	Davison C *et al.* (1961)
Dermal absorption in humans 28 healthy male volunteers with mean age 29 (18-36) years 0.5 mg MeS in acetone solution. The tape was removed together with the foil immediately and 4 hours after application. % absorption was calculated as 1 – (recovery at 4 h / recovery after application) * 100	MeS absorption (0-4h): 92.9+/-2.5% In comparison, other salicylates were tested : SA = 70.8 +/- 2.5% Ethyl salicylate = 58.6 +/- 6.6% n-propylsalicylate = 37.7 +/- 5.7% n-butylsalicylate = 17.1 +/-	Klimisch score = 2 (reliable with restrictions) supporting study Test material (EC name): methyl salicylate, ASA, salicylic acid (SA), ethyl salicylate, n-propylsalicylate, n-butylsalicylate, ethylene glycol	Yano T *et al.* (1986)

Method	Results	Remarks	Reference
UV analysis of the compound on the foil surface and on skin at the applied site. Not guideline; not GLP	5.3% Ethylene glycol monosalicylate = 87.8 +/- 2.3% ASA = 16.9 +/- 2.0%	monosalicylate	

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Oral route

Radiolabelled MeS (methyl salicylate) was administered to female HRS/J (hr) hairless mice once by gavage and samplings were done from 30 minutes to 48 hours (Yamagata *et al*., 1976). An absorption closed to 100% was observed. Blood level of radioactivity reached a peak after 30 minutes (7.68% of radioactivity), then rapidly decreased and only traces of radioactivity was found after 48 hours. In tissues and carcass, a peak of radioactivity was also observed at 30 minutes with 9.14% and 43.1% of radioactivity, respectively. Radioactivity was high in the liver, kidney and adrenals and lower in the lungs, uterus, heart, spleen, ovaries and pancreas, with the lowest level in the brain. After 48 hours, radioactivity was still present in the liver and kidney, and only traces of radioactivity were found in other organs. Considering the low levels in tissues (0.03%) and GI tract & contents (0.05%) at 48 hours, no bioaccumulation is expected for MeS. Metabolism was not investigated in this study. Elimination was almost exclusively found in the urine and less than 3% was in the faeces. Total recovery ranged from 98 to 104%.

Plasma analysis was performed in male rats after oral administration of methyl salicylate, sodium salicylate (NaS) and acetylsalicylic acid (ASA) (Davison *et al.*, 1961). MeS was rapidly absorbed and completely hydrolyzed to free salicylate in as little as 20 minutes. No parent methyl salicylate was detected. The authors stated that the major site of hydrolysis is the liver based on *in vitro* assays. In male dogs receiving MeS once at 300 mg/kg bw, plasma analysis showed that hydrolysis of MeS to free salicylate was 95% complete within one hour (Davison *et al.*, 1961). Distribution and elimination were not investigated in these studies.

Plasma analysis was also performed in humans receiving MeS or ASA orally (Davison *et al.*, 1961). Thirty percent of MeS remained unhydrolysed at 15 minutes, and 21% at 90 minutes.

Dermal route

The skin permeability of MeS was investigated in human volunteers receiving 0.5 mg MeS applied topically to the intact skin of the forearm and occluded for 4 hours (Yano *et al.*, 1986). Approximately 93% of the applied dose was absorbed mainly into the epidermis and less through the skin. The percentage of absorption was calculated as 1 – (recovery at 4 h / recovery after application) * 100.

Several other studies assessing dermal penetration of methyl salicylate are available *in vivo* (animals or humans) or *in vitro*. They are mostly summarized in published reviews (CIR, 2003, RIFM, 2007, Lapczynski *et al.*, 2007). From all these studies, various dermal absorption values were obtained and varied from 1% (human *in vivo* study with undiluted MeS; open application 6h to the chest and back) to 93% (human *in vivo* study with MeS applied to the forearm; 4h occlusion). All the values are not easily comparable considering the various protocols used (different tested materials, duration, skin system, method of application and absorption estimation …). According to RIFM review (2007), human *in vivo* data support a dermal absorption in the range of 2 to 43%.

Further evidence of dermal absorption of MeS can be anticipated from physicochemical data. According to the REACH guidance document 7c, the physicochemical properties of MeS are in favour of a significant absorption. Indeed, with a water solubility of 670 mg/L, absorption is anticipated to be moderate to high. The Log P between 2 and 3 also favours dermal absorption.

Inhalation route:

No information on toxicokinetics after inhalation is available. According to the REACH guidance document 7c, the vapour pressure, log P and oral toxicity data of MeS are in favour of a respiratory absorption.

Conclusion:

MeS is well absorbed by oral route and an oral bioavailability of 100% is assumed. For dermal route, very different values were obtained ranging from 1 to 93%, depending on the protocol used. No data is available after inhalation exposure.

MeS is widely distributed via blood and no bioaccumulation is expected after oral and dermal administrations. The substance is rapidly and extensively hydrolyzed to SA (salicylic acid) and corresponding alcohol (methanol). After oral administration, 80% of MeS were hydrolyzed in 90 minutes in humans; in dogs, hydrolysis is 95% complete in 1 hour and in rats, MeS is completely hydrolyzed to free salicylate within 20 minutes. After dermal administration, free salicylate rapidly appears in blood and level of unhydrolized MeS is low. *In vitro* data showed lower percentage of hydrolysis (25% in rat skin and up to 36% in guinea pig skin). SA obtained is then conjugated with either glycine or glucuronide and excreted in the urine as salicyluric acid and acyl and phenolic glucuronides. Methanol is also formed from MeS during hydrolysis. The alcohol is metabolized to corresponding aldehyde and acid and ultimately to CO_2 (RIFM, 2007).

MeS is mainly and rapidly excreted in the urine after oral and dermal administration. Low level is found in the faeces.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Table 12: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference Reliability**
Oral: gavage Guideline and GLP unspecified – low level of details	Rat Osborne-Mendel (5/sex)	Methyl salicylate (no further specified)	Not reported	LD_{50}: 887 mg/kg bw (male/female) (715-110) →**Acute Tox. 4**	Jenner PM *et al.* (1964) Lapczynski A *et al.* (2007) Klimisch score = 4
Oral: gavage Guideline and GLP unspecified – low level of details	Guinea pig male/female (no further specified)	Methyl salicylate (no further specified)	Not reported	LD_{50}: 1060 mg/kg bw (male/female) (873-1300) →**Acute Tox. 4**	Jenner PM *et al.* (1964) Lapczynski A *et al.* (2007) Klimisch score = 4
Oral: no further information Guideline and GLP unspecified – secondary litterature	Rat Sprague-Dawley 5/sex/dose	Methyl salicylate (no further specified)	2.50; 3.15; 3.97; 5.00 g/kg	LD_{50}: 2820 mg/kg bw (male/female) LD_{50} males: 3050 mg/kg bw LD_{50} females: 2640 mg/kg bw	RIFM (1982) cited in Lapczynski A *et al.* (2007) & RIFM (2007) Klimisch score = 4
Oral: no further information Guideline and GLP unspecified – secondary litterature	Rats (no further specified)	20% suspension of methyl salicylate (w/v) in a gum syrup and water mixture.	1, 1.25, 1.50, 2, 2.25, 2.50, or 3 g/kg	LD_{50}: 1250 mg/kg bw →**Acute Tox. 4**	Giroux J *et al.* (1954) cited in Lapczynski A *et al.* (2007) Klimisch score = 4
Oral: unspecified Guideline and GLP unspecified – secondary litterature	Rats, rabbits, guinea pigs, mice (no further information)	Methyl salicylate (not further specified)	Not reported	LD_{50} = 2800 mg/kg bw for rabbits LD_{50} = 700 mg/kg bw for guinea pigs →**Acute Tox. 4** LD_{50}: 1220 mg/kg bw for male rats →**Acute Tox. 4** LD_{50}: 1060 mg/kg bw for female rats →**Acute Tox. 4**	Rumyantsev GI *et al.* (1992) cited in CIR (2003) Klimisch score = 4

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference Reliability**
				LD_{50} = 580 mg/kg bw for mice **→Acute Tox. 4**	
Oral: unspecified Guideline and GLP unspecified - – low level of details	Mice C3H male	Methyl salicylate in 2% methyl cellulose	Not reported	LD_{50}: 1100 mg/kg bw **→Acute Tox. 4**	Davison C *et al.* (1961) Lapczynski A *et al.* (2007) Klimisch score = 3
Oral: unspecified Guideline and GLP unspecified – secondary litterature	Mouse (ddY) male	Methyl salicylate (not further specified)	1.0; 1.2; 1.3; 1.5; 1.7 g/kg	LD_{50}: 1390 mg/kg bw (male) **→Acute Tox. 4**	Ohsumi T *et al.* (1984) cited in Lapczynski A *et al.* (2007) Klimisch score = 4
Oral: unspecified Guideline and GLP unspecified – secondary litterature	Rabbit	Methyl salicylate (not further specified)	Not reported	LD_{50}: 1300 mg/kg bw **→Acute Tox. 4**	Castagnou *et al.*, (1952) cited in Opdyke (1978) Klimisch score = 4
Oral: unspecified Guideline and GLP unspecified – secondary litterature	Rabbit	Methyl salicylate (not further specified)	Not reported	LD_{50}: 2800 mg/kg bw	Fasset (1978) cited in Industrial Hygiene and toxicology (1958) Klimisch score = 4
Oral: unspecified Guideline and GLP unspecified – secondary litterature	Dog	Methyl salicylate (not further specified)	Not reported	LD_{50}: 2100 mg/kg bw	Bisesi (1994) cited in Opdyke (1978) Klimisch score = 4

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

No fully reliable study was available with methyl salicylate. Only the publications from Jenner *et al.* (1964) and Davison (1961) were made completely available. Results from RIFM (1982), Giroux *et al.* (1954), Rumyantsev *et al.* (1992) and Ohsumi *et al.* (1984) were only reported from reviews, such as Lapczynski *et al.* (2007) or CIR (2003). Results from the three last studies cited in the table above were only available as IUCLID summaries. All these studies are old and poorly detailed. The available LD_{50} ranged from 580 mg/kg bw/day to doses higher than 2000 mg/kg bw in various species (rats, mice, guinea pigs and dogs).

Acute salicylate poisoning was reported after overdose of acetylsalicylic acid (aspirin), excessive application of topical agents, ingestion of salicylate containing ointments, use of keratolytic agents or agents containing

MeS (e.g. wintergreen oil). For example, in 2004, poison control centers in the US reported 40,405 human exposures to salicylates. Of these, MeS was involved in 12,005 cases (30%). The typical symptoms of salicylate toxicity are hematemesis, tachypnea, hyperpnea, dyspnea, tinnitus, deafness, lethargy, seizures or confusion (Chika *et al.*, 2007).

10.1.2 Comparison with the CLP criteria

Among the 10 publications available, 7 reported LD_{50} leading to Acute Tox. 4 classification (LD_{50} between 300 and 2000 mg/kg bw). More precisely, there is a total of 13 LD_{50} obtained in different species, with 9 leading to the classification Acute Tox. 4.

According to the CLP guidance version 5.0 (July 2017), in general, classification is based on the lowest ATE (acute toxicity estimate) value available, i.e. the lowest ATE in the most sensitive appropriate species tested. Since there is no robust justification allowing proposing a higher LD_{50}, an harmonized ATE of 580 mg/kg bw is retained.

Methyl salicylate should therefore be classified for acute oral toxicity:

Acute toxicity Cat. 4, H302 with an ATE of 580 mg/kg bw (lowest LD_{50} available)

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Methyl salicylate should be classified for acute oral toxicity: **Acute toxicity Cat. 4, H302 with an ATE of 580 mg/kg bw (lowest LD_{50} available).**

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

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 according to the DS.

***Table**: LD_{50} and resulting classification in different species administered with methyl salicylate.*

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) **3**

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 LD_{50} 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.

The DS proposed to classify MeS as Acute Tox. 4; H302 with an ATE of 580 mg/kg bw (lowest LD_{50} available).

Comments received during consultation

One MSCA stated that Acute Tox. 4; H302 is justified but preferred an ATE value of 500 mg/kg bw as LD_{50} values of 300 mg/kg bw < Category 4 ≤ 2 000 mg/kg bw lead to a converted ATE value of 500 mg/kg bw.

Another MSCA agreed to the ATE of 580 mg/kg bw.

Assessment and comparison with the classification criteria

Among the 13 studies available, seven reported LD_{50} values lead to a classification as Acute Tox. 4 ($300 \leq LD_{50} \leq 2\,000$ mg/kg bw). In the remaining studies, the LD_{50} values reported were above the Guidance value for classification. The lowest LD_{50} value in these studies was reported in a Russian publication to be 580 mg/kg bw in mice, however, it only cited another source without presenting any experimental details. Because the origin of this value remains obscure and a detailed description of the experimental conditions is not available, this publication is not considered sufficiently reliable to derive the ATE value.

A review published by Lapczynski *et al.* (2007) provides a list of seven acute toxicity studies in three species after oral dosing. LD_{50} values range from 890 to 2 820 mg/kg bw. The lowest LD_{50} value of these studies was published in 1964 (Jenner *et al.*, 1964). An LD_{50} value of 887 mg/kg bw was reported in rats and experimental details are available. RAC notes that this

study is considered the key study in the REACH registration dossier (ECHA website, 2019)

RAC concludes that MeS meets the criteria (300 ≤ ATE ≤ 2 000 mg/kg bw) and should be classified as **Acute Tox. 4; H302** (Harmful if swallowed) with an ATE of 890 mg/kg bw.

10.2 Skin sensitisation

Table 13: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference Reliability
Local lymph node assay equivalent or similar to OECD Guideline 429 GLP unspecified	mouse (CBA), sex not indicated 4/group	Methyl salicylate	25, 50 or 100% daily for 3 consecutive days	Negative (SI <3) Stimulation index: 25%: 0.9 50%: 1.0 100%: 2.6 Dose-response relationship noted	Basketter DA *et al.* (1998) Klimisch score = 2
Local lymph node assay equivalent or similar to OECD Guideline 429 GLP unspecified	mouse, sex and strain not indicated	Methyl salicylate	5, 10, 25% in acetone/olive oil 80/20 v/v daily for 3 consecutive days	Negative (SI <3) Stimulation index: 5%: 0.9 10% : 1.4 25% : 2.2 Dose-response relationship noted	Ashby J *et al.* (1995) Klimisch score = 2
Local lymph node assay equivalent or similar to OECD Guideline 429 GLP unspecified	mouse (CBA/Ca) female	Methyl salicylate	10, 20, 25, 50, 100 % (experiment 1) 10, 25, 50% (experiment 2) 12.5, 25, 50, 100% (experiment 3) Neat or diluted in DMF or MEK Daily for 3 consecutive days	**Positive** Stimulation index (relative to vehicle controls): First experiment (DMF as vehicle): 10% : 1.2 20% : 1.6 25% : 2.4 50% : 2.6 100% : 4.0 EC3 = 65% Second experiment (MEK as vehicle): 10% : 1.8 25% : 5.3 50% : 10.7 EC3 = 15% Third experiment (3a) (DMF as vehicle):	Montelius J *et al.* (1998) Klimisch score = 2

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference Reliability
				12.5% : 1.5 25% : 1.7 50% : 5.9 100% : 7.1 EC3 = 33% Third experiment (3b) (MEK as vehicle): 12.5%: 2.0 25%: 2.4 50%: 7.6 100%: 9.4 EC3 = 28%	
Local lymph node assay equivalent or similar to OECD Guideline 429 GLP unspecified	mouse (CBA/Ca) female	Methyl salicylate	1, 5, 25% (experiment 1) 5, 10, 25% (experiment 2) Vehicle used: DMF or MEK daily for 3 consecutive days	**Positive** Stimulation index: Experiment 1 (DMF as vehicle): 1.0%: 1.0 5.0%: 1.2 25.0%: 3 Experiment 2 (MEK as vehicle) : 5.0%: 2.3 10.0%: 2.5 25.0%: 7.5	Montelius J *et al.* (1994) Klimisch score = 2
Local lymph node assay equivalent or similar to OECD Guideline 429 GLP unspecified	mouse (CBA/Ca or CBA/JHsd) female	Methyl salicylate	1, 2.5, 5, 10, 20% Acetone/olive oil 4:1 v/v daily for 3 consecutive days	Negative in 5 laboratories (SI <3) Stimulation index (laboratory A; B; C; D; E): 1%: 1.1 - 1.8 - 1.0 - 1.2 - 1.1 2.5%: 1.4 - 2.0 - 1.1 - 1.1 - 1.4 5.0%: 1.4 - 1.5 - 1.6 - 1.3 - 1.2 10.0%: 1.4 - 2.2 - 1.4 - 1.9 - 1.2 20%: 2.0 - 1.8 - 0.9 - 1.2 - 0.9	Kimber I *et al.* (1998) Klimisch score = 2
Local lymph node assay Deviation from OECD Guideline 429: exposure for 4 days. GLP unspecified	mouse (CBA) female	Methyl salicylate Purity = 90-95%	1, 2.5, 5% in acetone Daily for 4 consecutive days	Negative (SI <3) Stimulation index: 1%: 0.8 2.5% :0.8 5%: 0.8	Gerberick *et al.* (1992) Klimisch score = 2
Local lymph node assay Deviation from OECD Guideline 429:	mouse (CBA) male/female 4/dose	Methyl salicylate	5, 10, 25% in acetone/olive oil 4:1 v/v daily for 3	Negative (SI <3) Stimulation index: 5%: 1.3 10%: 1.0	Basketter DA *et al.* (1992) Klimisch score = 2

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results**	**Reference Reliability**
injection of 3H-TdR on day 4 GLP unspecified			consecutive days	25%: 0.8	
Local lymph node assay Equivalent or similar to OECD Guideline 429 **Maximisation assay** Deviation from OECD Guideline 406: few number of animals GLP unspecified	CBA/Ca mice; 4/group (LLNA) Dunkin/Hartley albino guinea pigs; 9-10 per treatment group and 4 in the control group (Maximisation assay)	Methyl salicylate	1, 2.5, 5% in AOO for LLNA daily for 3 consecutive days 2.5% in 0.01% DOBS/saline for intradermal induction and 100% for topical induction.	Negative (SI <3) LLNA: Stimulation index (laboratories A, B, C, D) 1% : 1.1 – 1.3 – 1.8 – 1.0 2.5% : 1.0 – 1.0 – 2.7 – 0.7 5% : 1.1 – 0.8 – 2.6 – 1.2 0/10 positive reaction in Maximisation assay (one laboratory)	Kimber I *et al.* (1991) Klimisch score = 2
Local lymph node assay Equivalent or similar to OECD Guideline 429 GLP unspecified	female BALB/c mice 9/group	Methyl salicylate Purity ≥ 99%	20, 40, 80% in 4:1 acetone/ olive oil (AOO) daily for 3 consecutive days	**Positive** SI > 3 EC3 = 48.15% No excessive local irritation	Adenuga *et al.* (2012) Klimisch score = 2
LLNA:BrdUELISA According to OECD TG 442B GLP unspecified	BALB/c mouse 4/group	Methyl salicylate	50% in AOO daily for 3 consecutive days	Negative (SI < 1.6) SI = 1.5	Hou *et al.* (2015) Klimisch score = 2
Open epicutaneous test (OET) – no harmonized guideline Induction: topical exposure daily for 21d Challenge: topical exposure on days 21 and 35 (minimal irritating concentration and at some lower nonirritant concentration) **Draize test** – no harmonized guideline Induction: intradermally injection on day 0 (0.05 ml 0.1%) + on 9 alternate days (0.1 ml) Challenge: intradermally injection on days 35 and 49	Male and female outbred Himalayan white-spotted guinea pigs (6-8/group)	Methyl salicylate	OET: Methyl salicylate undiluted and diluted Draize: 0.1% Maximisation test: 5% (in FCA) for intradermal induction, 25% (in petroleum) for topical induction, subirritant concentration (in petroleum) for challenge FCAT: undiluted	**OET: positive** (criterion: ≥ 2/8 animals with positive reaction with non irritant concentrations used at challenge) Minimum sensitising concentration: 30%; minimum eliciting concentration: 1% Daize test, maximization test and FCAT: negative	Klecak *et al.* (1977) Klimisch score = 4

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference Reliability
(0.05 ml) **Maximisation test** Deviation from OECD Guideline 406: few number of animals **Freund's complete adjuvant test (FCAT)** – no harmonized guideline 0.05 ml intradermally injection on days 0, 2, 4, 7 and 9 and epicutaneously on days 21 and 35. GLP unspecified					
Local lymph node assay – not followed OECD guideline Standard procedure but using rats instead of mice and using BrdU (without following guideline 442B) instead of tritiated thymidine **Serum IgE measurement** – no harmonized guideline GLP unspecified	Female Wistar and Brown Norway rats	Methyl salicylate Purity ≥ 99%	LLNA: 5, 12.5, 25% in acetone/olive oil (4:1 v/v) IgE measurement: 25% on day 0 and 12.5% on day 7	Negative SI = 0.8, 0.4, 0.8 in Wistar rats at 5, 12.5, 25% SI = 1.0, 1.0, 1.2 in Brown rats at 5, 12.5, 25% No increase in serum IgE concentration.	Arts *et al.* (1997) Klimisch score = 3
Local lymph node assay **Ex-vivo method** – no harmonized guideline **Standard method** Equivalent or similar to OECD Guideline 429 ***In vitro* BrdU incorporation** – no harmonized guideline GLP unspecified	Female BALB/c mice (4-6/group)	Methyl salicylate Purity = 99.7%	25% in acetone Ex-vivo LLNA: lymph node cells were incubated with 3H-thymidine *in vitro* after 3 days of topical application	Ex-vivo LLNA: - First experiment: negative (SI < 3) - Second experiment: **positive** (SI = 3) Standard LLNA: negative (SI = 2.5) *In vitro* BrdU incorporation: negative No skin irritation noted.	Picotti *et al.* (2006) Klimisch score = 2 (for standard LLNA) Klimisch score = 3 (ex-vivo LLNA & *in vitro* BrdU incorporation)

Table 14: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Induction studies				
Human maximisation with 25 healthy volunteers	12% Wintergreen oil (containing 80-99% methyl salicylate) Methyl salicylate	Application of 12% wintergreen oil in petroleum under occlusion for 5-alternate-day 48h-period after pretreatment of patch site for 24h with 5% aqueous SLS under occlusion. After 10-14 day rest period, 5% SLS was applied under occlusion for 30 min on the left side of the back prior to challenge patch of methyl salicylate under occlusion for 48h on the right side.	0/25 positive reactions	Lapczynski A, *et al.* (2007a)
Human maximisation with 27 healthy volunteers	8% methyl salicylate	Application of 8% methyl salicylate in petroleum under occlusion for 5-alternate-day 48h-periods after pre-treatment with 5% aqueous SLS under occlusion. After a 10-14 day rest period, 10% aqueous SLS solution under occlusion was applied under occlusion prior to challenge consisting on a 48h patch of methyl salicylate under occlusion. Reactions were read at patch removal and 24 h later.	0/27 positive reactions	Lapczynski A, *et al.* (2007b)
Human repeated insult patch test (HRIPT) with 13 males and 26 females	1.25% methyl salicylate	Nine applications of 1.25% methyl salicylate over a 3 week-period. 24h-challenge patch test on the 6th week. Reactions were scored at 24 and 72 h after patch removal	0/39 positive reactions	Lapczynski A, *et al.* (2007c)
Diagnostic studies				
Patch test in 4600 patients - 2784 patients with contact dermatitis - 189 patients with dermatitis of hands - 135 patients with photoallergy - 1491 healthy patients	2% methyl salicylate in petrolatum	**Unselected patients** A total of 4600 patients were patch tested in the 5-year period 1973-1977 in the Allergy Department of Barcelona University Patch test with ICDRG series including 2% Methyl salicylate in petrolatum	0.13% (6/4600) positive reactions It is not specified in which group of patients the positive results were found.	Romaguera & Grimalt (1980) Cited in Lapczynski A *et al.* (2007)
Patch test in 183 patients	2% methyl salicylate	**Selected patients** Patch test of the North American Contact Dermatitis Group from 1 July 1975 to 30	1.6% of positive reactions	Rudner, 1977

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		June 1976. Al Test® strips or Finn Chambers® were used. Tests were read at 48 and 96h.		Cited in Lapczynski A, et al (2007)
Patch test in 241 patients (61 males ; 180 females)	2% methyl salicylate in PMF	**Selected patients** Patch tests from October 1981 and June 1983 in Scotland. Perfume screening series including methyl salicylate	1.2% of positive reactions = 3 females with grade 2 (definite erythema) and above	Fergurson & Sharma (1984) Cited in Lapczynski A, et al (2007)
Patch test in 585 eczema patients	2% methyl salicylate in petroleum	**Selected patients** Standard patch tests on eczema patients in North America. 2 periods: 1978-1979 with 301 patients 1979-1980 with 284 patients	1% positive reaction for the period 1978-1979 2% positive reactions for the period 1979-1980	Mitchell (1982) Cited in Lapczynski A, et al (2007)
Patch test in 89 patients: - 19 with eyelid dermatitis - 70 with dermatitis at other sites	1% methyl salicylate in petroleum	**Selected patients** Patch tests between January 1980 and May 1987 in the Contact Dermatitis Clinic of St Michael's Hospital in North America. A1 Test® strips or Finn Chambers® secured with Scanpor tape for a period of 48 – 72h. Reactions read after removal and re-examined 48 or 72h after the first reading. Responses scored as 1+, 2+ or 3+ were determined to be positive; doubtful responses were scored as negative. Irritant responses were scored as negative.	0% positive reaction among the 19 patients with eyelid dermatitis 1.4% positive reactions among the 70 patients with dermatitis at other sites	Nethercott *et al.*(1989) Cited in Lapczynski A, et al (2007)
1825 patients	2% methyl salicylate in petroleum	**Unselected patients** Multicenter study conducted from September 1998 to April 1999. Test procedures were carried out according to internationally accepted criteria. Potential irritancy was excluded in a pilot study involving 200 patients	0.4% positive reactions (7/1825)	de Groot, A.C. *et al.* (2000) Cited in Lapczynski A, et al (2007)
Patch test in patients - with photosensitivity dermatitis with actinic reticuloid syndrome (50) - with	2% methyl salicylate in PMF	**Selected patients** A1 Test® patch for 48h. Any reactions read at patch removal, and at 72 h after the application	2% (1/50) positive reactions in patients with photosensitivity dermatitis with actinic reticuloid syndrome 0% positive reaction in other groups	Addo *et al.* (1982) Cited in Lapczynski A, et al (2007)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
polymorphic eruption (32) - with contact dermatitis (457)				
Work place study				
Patch test in 267 health care employees with contact dermatitis (82 males and 194 females)	2% methyl salicylate in petroleum	**Epidemiological study with selected workers** Patch test among health care employees in Italian hospital. GIRDCA standard series, "health" series and, when necessary, a "rubber" series. Patches removed after 2 days. Reading on days 2 and 3.	0% positive reaction	Stingeni *et al.* (1995) Cited in Lapczynski A, et al (2007)
Case reports				
Case report	2% methyl salicylate in olive oil	A 79 year-old woman had a rectangular pruritic erythematous macule on the hip following the use of a compress containing methyl salicylate.	1 case Patch test positive to methyl salicylate at 2% on Day 2 (+) and Day 3 (+).	Oiso *et al.* (2004)
Case report	2% methyl salicylate in arachis oil	A 63 year-old Iraqi businessman developed an acute dermatitis of the neck, upper back, shoulders and dorsa of the hands after applying a analgesic ointment.	1 case Patch test positive to methyl salicylate at 2% (grade 2 at 48h)	Hindson (1977)

10.2.1 Short summary and overall relevance of the provided information on skin sensitisation

Animal data:

Several studies were available to assess skin sensitisation property of methyl salicylate, including LLNA and maximization assays. Methyl salicylate was tested neat or diluted in various solvents (acetone/olive oil, DMF, MEK or acetone) to reach concentrations between 1 and 100%.

The substance was negative in the 2 Maximisation assays summarized in the table above (Kimber *et al*., 1991; Klecak *et al*., 1977). However, fewer animals than recommended in the OECD guideline were used. This deviation can decrease the sensitivity of the test in particular for substances with low sensitising potential. Two other Maximisation assays, showing negative results, were identified (Anonymous (2001) and RIFM (1981) cited in Lapczynski *et al*. (2007)). However, Anonymous (2001) study was judged as not reliable considering the very low number of animals used (5 in the treatment group and 3 in the control group), the lack of justification for the concentration tested (5% for intradermal induction; 50% for dermal induction and 20% for challenge) and the lack of information on the presence or not of an adjuvant. In the

RIFM (1981) study (cited in the review from Lapczynski *et al*. (2007)), negative result was reported when methyl salicylate was tested at 1% (intradermal injection) and 40% (topical administration) for induction and 10% for challenge, but without any justification on the concentrations selected.

Regarding the 12 available LLNA, the majority of the studies summarizes testing of sets of chemicals, including methyl salicylate, during the validation of the LLNA as a regulatory test protocol. Methyl salicylate was negative at concentrations up to 20% (Kimber *et al*., 1998; Gerberick *et al*., 1992; Kimber *et al*., 1991). These negative results can be explained by the too low doses tested. At higher concentrations, contradictory results were found. Methyl salicylate was negative in 4 studies at concentrations up to 25 % in acetone or acetone/olive oil (Picotti *et al*., 2006; Ashby *et al*., 1995; Basketter *et al*., 1992; Arts *et al*., 1997), in one study at 50% in acetone/olive oil (Hou *et al*., 2015) and in one study at concentrations up to 100% (Basketter *et al*., 1998, vehicule not stated). However, some limitations can be noted from these studies. For example, Arts *et al.* study (1997) highly deviate from OECD guideline in term of species and protocol used without any justification that the protocol is still sensitive to skin sensitisers. In addition, only one concentration was tested by Picotti *et al.* (2006), that does not allow obtaining a dose-response relationship. It can also be noted that in two of these studies (Ashby *et al.,* 1995 and Basketter *et al.,* 1998) a clear dose response was observed with a SI (stimulation index) closed to 3 (up to 2.2 in the first study and up to 2.6 in the second study). Methyl salicylate was positive in 3 LLNA. In the 2 studies performed by Montelius *et al.* (1994, 1998), the stimulation index was higher than 3 from 25% with MEK (methyl ethyl ketone) as vehicle and from 50% with DMF (dimethylformamide) as vehicle. In the study performed by Adenuga *et al* (2012), the EC_3 was set at 48% (methyl salicylate in acetone/olive oil. SI and concentration not stated).

Finally, studies not following current harmonized guideline are reported. No sign of sensitisation was noted in a Draize assay, in a Freund's complete adjuvant test (FCAT); both tests performed by Klecak *et al*. (1977). In contrast, an Open epicutaneous test (OET) also carried out by Klecak *et al.* (1977) was positive. No increase of IgE in serum was detected after dermal application of methyl salicylate by Arts et al. (1997). Picotti *et al.* (2006) evaluated the validity of an *ex vivo* LLNA using methyl salicylate (purity: 99.7%) among other substances. Contradictory results were obtained with a SI = 1.5 in the first experiment (negative) and 3 (positive) in the second experiment.

Overall, methyl salicylate is negative in Maximisation studies presenting some limitations, in particular a low number of animals tested that can decrease the sensitivity of the tests. LLNA is known to be more sensitive than Maximisation assay to detect skin sensitizer. At concentrations below or equal to 20%, methyl salicylate is clearly negative in all LLNA available. At higher concentrations, conflicting results are obtained. However, **positive results are found with methyl salicylate at concentrations above 25% in different LLNA of adequate quality.** The differences of results can be explained by some variations in the protocols used, the different solvent vehicle and the concentrations tested.

Human data:

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

No sign of sensitisation to methyl salicylate was reported in the 2 maximisation studies and in one HRIPT (human repeated insult patch test) study (Lapczynski *et al.* 2007). In these studies, the number of volunteers tested ranged from 25 to 39. The concentrations used ranged from about 1.25 to 8% of methyl salicylate or 12% of wintergreen oil (containing 80-99% of methyl salicylate). Robust study information are not available for these studies. Only summaries from Lapczynski *et al*. (2007) have been found.

In contrast, positive reactions were noted after patch testing in 7/8 diagnostic studies. A distinction must be made between patch testing "unselected/consecutive" patients, i.e. all patients who are patch tested for suspected contact sensitisation, and "aimed/selected" patch testing, i.e. application of allergens only in the subset of patients in whom exposure to the particular allergens of the applied "special series" is suspected. For any given allergen, the latter "aimed" approach will usually yield higher sensitisation prevalences than the testing of not-further-selected "consecutive" patients. Thus, information on the inclusion of an allergen either in a baseline series (tested in virtually all patients) or in a special series (applied in an aimed fashion) must be considered. Among the diagnostic studies available with methyl salicylate, there was 2 studies with unselected patients (Romaguera & Grimalt, 1980; de Groot *et al.* 2000) and 6 with selected patients (Rudner, 1977; Fergurson & Sharma, 1984; Mitchell *et al.*, 1982; Nethercott *et al.*, 1989; Addo *et al*., 1982; Stingeni *et al.*, 1995). The concentrations used ranged between 1 and 2% of methyl salicylate. Diagnostic studies with unselected patients included 1825 or 4600 patients and showed a frequency of positive reactions of 0.13% or 0.4% respectively. Diagnostic studies with selected patients included 19 to 585 patients and a frequency of positive reactions between 0 and 2%.

Finally, two individual cases of skin sensitisation to methyl salicylate are reported in the literature (Oiso *et al*, 2004; Hindson, 1977).

It should be noted that the available human data are somewhat old. However, methyl salicylate is not currently included neither in standard battery (such as Fragrance Mix I or II) or in perfume battery. Therefore, it is difficult to make a clear and definitive conclusion on actual frequency of skin sensitisation to methyl salicylate.

In conclusion, methyl salicylate has shown to be a skin sensitiser in **diagnostic studies with incidence < 1% in unselected patients and ≤ 2% in selected patients.**

Differentiation between sensitising or irritative reactions:

Contradictory results were found in both animal and human studies. In animals, positive effects were reported only in some LLNA at concentrations above 25%. In humans, a frequency of positive reactions up to 2% was noted only in diagnostic studies. Special caution has thus been paid to differenciate if the positive results are linked to irritative or real sensitising effects.

From the literature, contradictory results were found regarding irritative properties of methyl salicylate (Lapczynski *et al*. 2007; Belsito *et al.* 2007).

Table 15: Summary table of animal data on skin irritation

Material	Method	Concentration	Species	Results	References
Methyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1973a)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in water	Rabbits (3/group)	1%: no irritation 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in PEG 400	Rabbits (3/group)	1%, 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in 70% ethanol	Rabbits (3/group)	1%, 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in 70% ethanol plus emollients	Rabbits (3/group)	1%, 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Pre-test for an open epicutaneous test (OET) (24-h primary irritation)	0.03–100% as a single application (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%, 0.1%, 1%: no irritation 3%: minimal irritating concentration 10–100%: irritation observed	Klecak et al. (1977)
Methyl salicylate	Pre-test for an OET (24 h primary irritation)	0.03–100% applied daily for 21 days (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%, 0.1%, 1%: no irritation 3%: considered as the minimal irritating concentration 10–100%: irritation observed	Klecak et al. (1977)
Methyl salicylate (wintergreen oil, 80–99% methyl salicylate)	Irritation evaluated as part of a photoirritation study	100%	6 Mice (hairless)	Irritation observed	RIFM (1976e)
Methyl salicylate (wintergreen oil, 80–99% methyl salicylate)	Irritation evaluated as part of a photoirritation study	100%	Miniature swine	Irritation observed	RIFM (1976e)
Methyl salicylate	Irritation studied as part of a mouse ear swelling test	1%, 2.5%, 10%, and 20% in 4:1 acetone to olive oil	Mice	1%, 2.5%, 10%: no irritation 20%: established as the minimal irritating concentration producing significant increase in ear swelling	Howell et al. (2000)
Methyl salicylate	Irritation studied as part of a mouse ear swelling test	2.5, 5.0, 7.5 and 10% in ethanol	Mice	Irritation observed	Patrick et al. (1985, 1987) and Patrick and Maibach (1986)

Table extracted from Belsito *et al.* 2007

Additionnally to this literature search, no to slightly irritation (not fulfilling CLP criteria) was reported in an OECD guideline 404 study (Anonymous, 1999). In this study, female rabbits were exposed for 4 hours to methyl salicylate unchanged or diluted with ethanol/diethyl phthalate 1:1 (0-1-5-10-25-100% of methyl salicylate). At concentrations up to 10%, the mean erythema and oedema scores (24, 48, 72 hours) were 0. At 25%, the mean erythema score was 0.2 and the oedema score was 0. For the undiluted substance, the mean erythema score was 1.3 and the oedema score was 0.6. All reactions were reversible within the 14-day recovery period.

Table 16: Summary table of human data on skin irritation

Skin irritation studies in humans

Material	Method	Concentration	Subjects	Results	References
Methyl salicylate (wintergreen oil; 80–99% methyl salicylate)	Maximization pre-test (48-h occluded patch)	12% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1976b)
Methyl salicylate	Maximization pre-test (48-h occluded patch)	8% (vehicle not specified)	27 volunteers	No irritation (0/27)	RIFM (1973b)
Methyl salicylate	24-h occluded patch test	25 ml of 30% or 60% solutions	9 volunteers	Irritation observed	Green and Shaffer (1992)

Table extracted from Belsito *et al.* 2007

Information on irritative potential can also be obtained from the available sensitisation assays with methyl salicylate. In the two positive LLNA performed by Montelius et al. (1994, 1998), it is not specified if irritative properties of methyl salicylate were assessed. In the LLNA performed by Adenuga *et al.* (2012), no excessive irritation was noted (mean erythema scores between 0 and 1.6) when the positive reactions were observed. From human studies, potential irritancy was excluded in the de Groot *et al.* (2000) study in which a incidence of positive reactions of 0.4% was reported. In the Nethercott *et al.* (1989) study showing 1.4% of positive reactions, it is clearly specified that irritation was scored as negative reaction. Finally, in Fergurson *et al.* (1984) and Hindson (1977) publications, the positive reactions consisted in clear sensitising effects as characterized by score 2 or above.

In this context, there is no sufficient information to discount the effects reported in both LLNA and human studies. Thus, the positive reactions should be considered as a sensitising effect.

10.2.2 Comparison with the CLP criteria

The decision logic for classification of substance described in the CLP guidance version 5.0 (July 2017) has been followed:

" *Are there data and/or information to evaluate skin sensitization?"*

Yes: there are both experimental studies and human data assessing skin sensitisation properties of methyl salicylate

a) *Is there evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons*

Yes: positive reactions were reported in diagnostic studies with incidence < 1% in unselected patients and ≤ 2% in selected patients.

b) *Are there positive results from an appropriate animal test or in vitro / in chemico test?*

Yes: positive results were obtained in different LLNA performed with methyl salicylate at concentrations from 25%.

Are data sufficient for sub-categorisation?

According to CLP, *"Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test.*

Sub-category 1A*: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.*

Sub-category 1B*: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered."*

Non-human and human data have been analysed to determine if they are sufficient for sub-categorisation:

Non-human data:

Three types of animal tests can be used directly for classification purpose: LLNA, guinea pig maximisation test and Buehler assay.

Classification criteria according to CLP are the following:

Classification	Assay	Criteria
Subcategory 1A	LLNA	EC3 value ≤ 2%
	Maximisation test	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal

		induction dose
	Buehler assay	≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose
Subcategory 1B	LLNA	EC3 value > 2%
	Maximisation test	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose Or ≥ 30 % responding at > 1 % intradermal induction dose
	Buehler assay	≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

With EC_3 values > 2% in LLNA assays where this information is provided, methyl salicylate fulfills criteria for classification Skin Sens. 1B according to the CLP guidance.

Human data:

The frequency of occurrence of skin sensitisation should be considered as a first step to conclude on classification for skin sensitisation:

Table 3.2 Relatively high or low frequency of occurrence of skin sensitisation*

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies:		
1: all or randomly selected workers	≥ 0.4 %	< 0.4 %
2: selected workers with known exposure or dermatitis	≥ 1.0 %	< 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

* Only one or two types of information may be sufficient for sub-categorisation.

The key evidence for classification proposal is mainly based on diagnostic patch tests. Low frequencies of positive reactions were seen in unselected patients (2 studies: incidence of positive reactions = 0.13 and 0.4%). In selected patients, the frequencies were between 0 and 2%. However, the frequency of 2% reported by Addo *et al.* (1982) consisted in only 1/50 patient with positive reaction. Therefore, it is considered that only low to moderate frequency of skin sensitisation is found in selected patients. These tests represent about 30 cases with positive patch test reactions. In addition, two published individual cases were reported.

In addition to the frequency of occurrence of skin sensitisation, the level of exposure to the substance should be considered:

Table 3.3 Relatively high or low exposure *

Exposure data	Relatively low exposure (weighting)	Relatively high exposure (weighting)
Concentration / dose	< 1.0% < 500µg/cm²	≥ 1.0% ≥ 500µg/cm²
	(score 0)	(score 2)
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	<100 exposures (score 0)	≥100 exposures (score 2)

Methyl salicylate is a fragrance ingredient used in many fragrance compounds. This substance is manufactured and/or imported in the European Economic Area in 1 000 - 10 000 tonnes per year (ECHA

website, 2018). It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents.

The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.13% (IFRA, 2002 cited in Lapczynski *et al.* 2007). This is consistent with data submitted by industry (CTFA, 2000) stating that methyl salicylate was used at concentrations of ≤ 0.6% (CIR, 2003).

Ingredient usage as a function of product type *(Continued)*

Product type (Total number reported to FDA 1998)	Number of formulations with the ingredient (FDA 1998)	Concentration of use (CTFA 2000) (%)
Methyl Salicylate		
Dentifrices (38)	4	0.03
Mouthwashes and breath fresheners (49)	10	0.08–0.2
Other oral hygiene products (6)	—	0.2
Bath soaps and detergents (385)	—	0.0001
Bath oils, tablets, and salts (124)	1	—
Body and hand preparations (excluding shaving) (796)	1	0.05
Skin cleansing (653)	1	—
Douches (5)	2	—
Foot powders and sprays (35)	—	0.02
Hair conditioners (636)	1	—
Shampoos (noncoloring) (860)	1	—
Tonics, dressings, and other hair-grooming aids (549)	1	—
Paste masks (mud packs) (255)	1	0.6
Skin fresheners (184)	1	0.1
Other skin care preparations (692)	1	0.02
Suntan gels, creams, and lotions (136)	—	0.2
Total Methyl Salicylate uses and concentration ranges	**25**	**0.0001–0.6**

Table extracted from CIR (2003).

However, higher concentrations were identified in topical medication uses. For example, the Food and Drug Administration (FDA) assessed in 2006 a patch containing 10% of methyl salicylate used for arthritis, backache or strains, sprains and bruises (FDA, 2006). Moreover, in the human case of skin sensitisation reported by Hindson (1977), the patient used an ointment containing 12% of methyl salicylate. This concentration for topical uses is also found from the Vidal database (ex. Inongan cream) (vidal website, 2018).

Overall, according to table 3.3 of the CLP guidance, the following scores can be attributed related to exposure data:

- **Concentration / dose: score = 2**
    - Even if relatively low exposure is expected for cosmetic uses, relatively high exposure (e.g 12%) has been identified for other uses, such as for topical medication.
- **Repeated exposure: score = 2**

- Considering the products in which methyl salicylate can be included (ex. dentifrice, soap etc), a repeated exposure ≥ once/daily is expected.

- **Number of exposure: score = 2**
  - Considering the uses of products containing methyl salicylate, it is anticipated that exposure is at least more than 100 times.

In conclusion the total score for exposure data is set at 6 which corresponds to a relatively high exposure.

Resulting from the results obtained according to tables 3.2 and 3.3 of the CLP guidance, a subcategorization for methyl salicylate can be proposed.

Table 3.4 Sub-categorisation decision table

	Relatively low frequency of occurrence of skin sensitisation	Relatively high frequency of occurrence of skin sensitisation
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	Sub-category 1A

Based on this table and considering human data, methyl salicylate fulfills criteria for classification Skin Sens. 1B.

Overall conclusion :

Based on animal data, methyl salicylate fulfils criteria for classification Skin Sens. 1B.

Based on human data, methyl salicylate fulfils criteria for classification Skin Sens. 1B.

Therefore, methyl salicylate should be classified Skin sens. 1B – H317 according to CLP regulation.

It can also be noted that methyl salicylate is listed by the SCCS as *established contact allergen in human* based on de Groot *et al.* (2000) study, on the cases reported by Oiso *et al.* (2004) and Hindson (1997) and on the RIFM review by Lapcynski *et al.* (2007) (SCCS, 2012).

10.2.3 Conclusion on classification and labelling for skin sensitisation

Based on animal data, methyl salicylate fulfils criteria for classification Skin Sens. 1B.

Based on human data, methyl salicylate fulfils criteria for classification Skin Sens. 1B.

Therefore, methyl salicylate should be classified Skin sens. 1B – H317 according to CLP regulation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitising potential of MeS was mainly investigated in several local lymph node assays (LLNA) and guinea pig maximisation tests (GPMT). MeS was applied in concentrations between 1 and 100 %. A concentration dependency was recognised in the LLNA, where concentrations of MeS of > 25 % showed positive results.

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

Animal data

The DS presented a total set of 12 LLNA. At low concentrations, no relevant lymph node stimulation index (SI < 3) has been observed. Therefore, studies performed with maximum concentrations below 20 % and/or not in compliance with the test guideline, are not presented in this modified table.

Method, guideline	**Species**	**Dose levels**	**Results**
LLNA (similar to OECD TG 429)	Mice	5, 10, 25 % in acetone/olive oil for 3 consecutive days	**Negative** (SI < 3) 5 %: 0.9 10 %: 1.4 25 %: 2.2
LLNA (similar to OECD TG 429)	Mice (CBA/Ca)	10, 20, 25, 50, 100 % (experiment 1) 10, 25, 50 % (experiment 2) 12.5, 25, 50, 100 % (experiment 3) Neat or diluted in (Dimethyl formamide (DMF) or Methylethylketone (MEK) Daily for 3 consecutive days	**Positive** Experiment 1 (DMF) 10 %: 1.2 20 %: 1.6 25 %: 2.4 50 %: 2.6 100 %: 4.0 EC3 = 65 % Experiment 2 (MEK) 10 %: 1.8 25 %: 5.3 50 % 10.7 EC3 = 15 % Experiment 3a (DMF) 12.5 %: 1.5 25 %: 1.7 50 %: 5.9 100 %: 7.1 EC3 = 33 % Experiment 3b (MEK) 12.5 %: 2.0 25 %: 2.4

			50 %: 7.6 100 % 9.4 EC3 = 28 %
LLNA (similar to OECD TG 429)	Mice (CBA/Ca)	1, 5, 25 % (exp. 1) 5, 10, 25 % (exp. 2) Diluted in DMF or MEK	**Positive** Experiment 1 (DMF) 1.0 %: 1.0 5.0 %: 1.2 25 %: 3 Experiment 2 (MEK) 5.0 %: 2.3 10 %: 2.5 25 %: 7.5
LLNA (deviation from OECD TG 429: injection of 3H-TdR on day 4)	Mice (CBA), male/female, 4/dose	5, 10, 25 % in acetone/olive oil Daily for 3 consecutive days	**Negative** (SI < 3) 5 %: 1.3 10 %: 1.0 25 %: 0.8
LLNA (similar to OECD TG 429)	Mice (BALB/c)	20, 40, 80 % in 4:1 acetone/olive oil daily for 3 consecutive days	**Positive** EC3 = 48.15 %, No excessive local irritation

Human data

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

Report	**Test substance**	**Relevant information about the study**	**Observations**
Induction studies			
Human maximisation with 25 healthy volunteers	12 % wintergreen oil (containing 80-99 % MeS)	Application of 12 % wintergreen oil in petroleum under occlusion for 5-alternate-day 48 h period after pre-treatment of patch site for 24 h with 5 % aqueous SLS under occlusion. After 10 to 14 day rest period, 5 % SLS was applied under occlusion for 30 min on the left side of the back prior to challenge patch of MeS under occlusion for 48 h on the right side.	0/25 positive reactions
Human maximisation with 27 healthy volunteers	8 % MeS	Application of 8 % MeS in petroleum under occlusion for 5-alternate-day 48 h periods after pre-treatment with 5 % aqueous SLS under occlusion. After a 10 to 14 days rest period, 10 % aqueous SLS solution under occlusion was applied prior to challenge consisting on a 48 h patch	0/27 positive reactions

		of MeS under occlusion. Reactions were read at patch removal and 24 h later.	
Human repeated insult patch test (HRIPT) with 13 males and 26 females	1.25 % MeS	Nine applications of 1.25 % MeS over a 3 week-period. 24 h challenge patch test on the 6th week. Reactions were scored at 24 and 72 h after patch removal	0/39 positive reactions
Diagnostic studies			
Patch test in 4 600 patients - 2 784 patients with contact dermatitis - 189 patients with dermatitis of hands - 135 patients with photoallergy - 1 491 healthy patients	2 % MeS in petrolatum	**Unselected patients** A total of 4 600 patients were patch tested in the 5-year period 1973-1977 in the Allergy Department of Barcelona University, Spain. Patch test with ICDRG (International Contact Dermatitis Research Group) series including 2 % MeS in petrolatum	0.13 % (6/4 600) positive reactions It is not specified in which group of patients the positive results were found
Patch test in 183 patients	2 % MeS	**Selected patients** Patch test of the North American Contact Dermatitis Group from 1 July 1975 to 30 June 1976. A1 Test® strips or Finn Chambers® were used. Tests were read at 48 and 96 h.	1.6 % positive reactions
Patch test in 241 patients (61 males, 180 females)	2 % MeS in PMF (yellow soft parafin)	**Selected patients** Patch tests from October 1981 and June 1983 in Scotland. Perfume screening series including MeS	1.2 % positive reactions = 3 females with grade 2 (definite erythema) and above
Patch test in 585 eczema patients	2 % MeS in petroleum	**Selected patients** Standard patch tests on eczema patients in North America. 2 periods: 1978-1979 with 301 patients 1979-1980 with 284 patients	1 % positive reaction for the period 1978-1979 2 % positive reactions for the period 1979-1980
Patch test in 89 patients: - 19 with eyelid	1 % MeS in petroleum	**Selected patients** Patch tests between January 1980	0 % positive reaction among the 19 patients

dermatitis - 70 with dermatitis at other sites		and May 1987 in the Contact Dermatitis Clinic of St Michael's Hospital in North America. A1 Test® strips or Finn Chambers® secured with Scanpor tape for a period of 48-72 h. Reactions read after removal and re-examined 48 or 72 h after the first reading. Responses scored as 1+, 2+ or 3+ were determined to be positive; doubtful responses were scored as negative. Irritant responses were scored as negative.	with eyelid dermatitis 1.4 % positive reactions among the 70 patients with dermatitis at other sites
1 825 patients	2 % MeS in petroleum	**Unselected patients** Multicenter study conducted from September 1998 to April 1999. Test procedures were carried out according to internationally accepted criteria. Potential irritancy was excluded in a pilot study involving 200 patients.	0.4 % positive reactions (7/1 825)
Patch test in patients - with photosensitivity dermatitis with actinic reticuloid syndrome (50) - with polymorphic eruption (32) - with contact dermatitis (457)	2 % MeS in PMF	**Selected patients** A1 Test® strips for 48 h. Any reactions read at patch removal, and at 72 h after the application.	2 % (1/50) positive reactions in patients with photosensitivity dermatitis with actinic reticuloid syndrome 0 % positive reaction in the two other groups (0/489)
Work place study			
Patch test in 267 health care employees with contact dermatitis (82 males and 194 females)	2 % MeS in petroleum	**Epidemiological study with selected workers** Patch test among health care employees in Italian hospital. GIRDCA standard series (Gruppo Italiano Ricerca Dermatiti da Contatto ed Ambientali), "health" series and, when necessary, a "rubber" series. Patches removed after 2 days. Reading on days 2 and 3.	0 % positive reactions (0/276)
Case reports			
Case report	2 % MeS in olive oil	A 79 year-old woman had a rectangular pruritic erythematous macule on the hip following the use	1 case Patch test positive to MeS

		of a compress containing MeS.	on day 2 (+) and day 3 (+)
Case report	2 % MeS in arachis oil	A 63 year-old Iraqi businessman developed an acute dermatitis of the neck, upper back, shoulders and dorsa of the hands after applying an analgesic ointment.	1 case Patch test positive to MeS (grade 2 at 48 h)

Animal data

The substance was negative in both Guinea pig maximisation assays. However, fewer animals than recommended in the OECD test guideline were used. This might decrease the sensitivity of the test for substances with low sensitising potential.

Regarding the 12 LLNAs, most studies summarise results of sets of chemicals, including MeS, during the validation of the LLNA as a regulatory test protocol. MeS was negative at concentrations up to 20 % or 25 %, while at higher concentrations positive results were found in the majority of experiments following the guideline protocol. Some studies deviate from the guideline protocol, e.g. using the rat instead of mice.

Human data

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

Of the 8 diagnostic patch testing studies, 7 provide positive results. A distinction must be made between patch testing "unselected/consecutive" patients, i.e. all patients who are patch tested for suspected contact sensitisation, and "aimed/selected" patch testing, i.e. application of allergens only in the subset of patients in whom exposure to the particular allergens of the applied "special series" is suspected. In general, the latter "aimed" approach will usually yield higher sensitisation prevalence than the testing of not-further-selected "consecutive" patients. Thus, information on the inclusion of an allergen either in a baseline series (tested in virtually all patients) or in a special series (applied in an aimed fashion) must be considered. Among the diagnostic studies available with MeS, there were 2 studies with unselected patients and 6 with selected patients. The concentrations used ranged from 1 to 2 % MeS. Diagnostic studies with unselected patients included 1 825 or 4 600 patients and showed a frequency of positive reactions of 0.13 % or 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 % MeS in olive or arachis oil were reported.

In summary, MeS has shown to be a skin sensitiser in diagnostic studies with an incidence < 1 % in unselected patients and ≤ 2 % in selected patients.

The DS proposed that the positive reactions should be considered as sensitising effects, and MeS should be classified as Skin Sens. 1B.

Comments received during consultation

Two downstream users were of the opinion that MeS should not be classified as a skin sensitiser as there are studies indicating that MeS is not a skin sensitiser, but at concentrations above 25 % may induce false positive results due to its irritation properties. They claimed that there is no indication for a skin sensitisation concern from the use of MeS when used for local pharmaceutical treatment at concentrations up to 10 % based on pharmacovigilance data. Additionally, assessment of MeS in guinea pig maximisation assays using optimal conditions for maximal stimulation of the skin immune system did not result in skin sensitisation.

Similarly, one manufacturer and one importer concluded that MeS should not be classified as a skin sensitiser, as the cellular proliferation effects observed in the local lymph node are likely to be an effect of the irritation properties and not an indication for skin sensitisation. In addition, the human data also does not reveal a clear indication that the substance is a skin sensitiser.

Two MSCAs supported the proposal to classify MeS as a skin sensitiser 1B; H317.

Assessment and comparison with the classification criteria

According to CLP guidance version 5.0 (July 2017), *"Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or (b) if there are positive results from an appropriate animal test.*

Sub-category 1A: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.

Sub-category 1B: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered."

Non-human data

Three types of animal tests can be used directly for classification purpose according to CLP: LLNA, GPMT and Buehler assay. The criteria for LLNA are as follows:

1A: EC3 value ≤ 2 %

1B: EC3 value > 2 %

In an LLNA using four concentrations of MeS (vehicle: MEK, methylethylketone) the following results for the stimulation index and EC3 value were obtained:

12.5 %: 2.0; 25 %: 2.4; 50 %: 7.6; 100 % 9.4; EC3 = 28 %.

Corresponding results were obtained in a parallel experiment under identical conditions, but with DMF as a vehicle:

12.5 %: 1.5; 25 %: 1.7; 50 %: 5.9; 100 %: 7.1; EC3 = 33 %

Since these guideline-conform LLNA assays in female CBA mice yielded EC3 values > 2 %, RAC is of the opinion that MeS fulfils criteria for classification as Skin Sens. 1B.

Human data

According to CLP, the frequency of occurrence of skin sensitisation should be considered as a first step to conclude on classification for skin sensitisation. Data show that only low to moderate frequency of skin sensitisation is found in selected patients. These tests represent about 30 cases with positive patch test reactions. In addition, two case reports are available.

Overall, based on animal data, MeS fulfils criteria for classification Skin Sens. 1B. Based on human data, MeS also fulfils criteria for classification Skin Sens. 1B.

RAC agrees with the DS that MeS should be classified as **Skin Sens. 1B; H317 (May cause an allergic skin reaction)**.

10.3 Reproductive toxicity

10.3.1 Adverse effects on sexual function and fertility

Table 17: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference Reliability
Study of fertility and early embryonic development to implantation Crj:CD(SD)IGS rats male/female Subcutaneous administration GLP and ICH guidelines	Methyl salicylate (purity: 100.1%) 0, 30, 100, 300 mg/kg/day in corn oil From 2 weeks prior to mating until sacrifice (total of 52 days) for males and until gestation day 6 for females (total of 30 days). Sacrifice of females on GD13.	NOAEL for general toxicity: 100 mg/kg/day based on one mortality in males, decreased body weight gain and food consumption at 300 mg/kg bw/day. NOAEL for fertility and early development: 300 mg/kg/day (no effect). Increased plasmatic salicylic acid concentration dependent on the dose ratio but scarcely affected by repeated dosing. No clear sexual difference.	FDA (2006a) Klimisch score : 1 Key study
Two-generation study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 4 (offspring assessment) of the NTP continuous breeding protocol Limited examination	Methyl salicylate (purity ≥ 99%) 0, 25, 50 and 100 mg/kg/day. (nominal conc.) 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).	NOAEL (reproductive effects): 100 mg/kg bw/day – no adverse effect NOAEL (developmental effects): 100 mg/kg bw/day – no adverse effect	NTP (1984a) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Lamb *et al.*, (1997) Klimisch score : 2 Supporting

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference Reliability**
NTP protocol, GLP	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 mated at about 74 days of age.		study
One generation study + crossover mating study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 3 (crossover mating) of the NTP continuous breeding protocol Limited examination NTP protocol, GLP	Methyl salicylate (purity ≥ 99%) 100, 250 and 500 mg/kg/day. (nominal conc.) 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). Task 3: high-dose animals of each sex were mated to control mice of the opposite sex.	500 mg/kg bw/day – no effect on fertility index NOAEL (developmental effect): 100 mg/kg bw/day based on a reduction in pup weight from 250 mg/kg bw/day. At 500 mg/kg bw/day, a significant decrease in the mean number of litter and in the average of pups per litter, the proportion of pups born alive was observed. Task 3: due to fertility problem in the control groups (26% in the first task 3 and 41% in the second task 3) and lack of significant results in the litter analysis, an affected sex cannot be determined.	NTP (1984b) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Klimisch score : 2 Supporting study
Three-generation study Rat (Osborne-Mendel); male/female (20/sex/dose) Oral: feed (no vehicle) A supplementary study was performed with adding calcium carbonate to MeS diet with the same examination. Examination very limited Several deficiencies from OECD 416, not GLP	Methyl salicylate 0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw as MeS) (nominal in diet) Exposure: 100 days before the first mating and then throughout the experiment (until weaning of the 3rd generation).	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 MeS alone.	Collins TFX *et al.* (1971) Gross MA, Fitzhugh OG (1977) Klimisch score : 3 Supporting study
Two-generation study Rat (Wistar) male/female 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD	Methyl salicylate 0.25% and 0.5% (2500 ppm and 5000 ppm equivalent to 125 and 250 mg/kg bw MeS/day) (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (weaning of the F2b litters).	No adequate NOAEL can be set based on the low quality of the reported results. Decreased litter size at all doses. Higher number of unsuccessful matings for the first generation and decreased reproduction index for both generations at the highest dose. Higher number of death between birth and day 5 at 250 mg/kg	Anonymous (1978a) Klimisch score : 3 Supporting study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference Reliability
416, not GLP		bw/day.	
Two-generation study Mouse male/female (no data on strain); 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	Methyl salicylate 0.25% and 0.5% (2500 ppm and 5000 ppm, equivalent to 375 and 750 mg/kg bw MeS/day) (nominal in diet) Exposure: 30 days before the first mating and then throught the experiment (weaning of the pups).	No adequate NOAEL can be set based on the low quality of the reported results. Litter size slightly smaller in test groups only in the first generation.	Anonymous (1978b) Klimisch score : 3 Supporting study
One-generation study Rat (Sprague-Dawley); male/female; 24-27 animals/dose Oral: feed (no vehicle) Guideline and GLP not stated – secondary litterature	Methyl salicylate 4000 ppm and 6000 ppm equivalent to 200 and 300 mg/kg bw/day (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (until weaning of offspring on day 20-21)	NOAEL (F1): 300 mg/kg bw/day (male/female) based on no effect No abnormalities. Neonate survival at weaning was greater in the test group than in control.	FDA (1966) CIR (2003) Klimisch score : 4 Disregarded study

10.3.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

<u>Animal data:</u>

In the first study (summarized in FDA (2006a)), 20/sex rats were exposed subcutaneously to methyl salicylate (MeS) at 0, 30, 100 or 300 mg/kg/day 2 weeks prior to mating until sacrifice of males and until gestation day 6 for females. Females were sacrificed on gestation day 13. One male at 300 mg/kg/day showed hypoactivity, bradypnea, hypothermia and blanching on day 3 and died on day 4. Crust on the treated site and/or loss of hair were observed in 2 females at 300 mg/kg/day from day 9 of administration to day 13 of gestation. A significant lower body weight, body weight gain and food consumption was observed in males and females at the highest dose. There was no significant difference in the weights of the testes or epididymides. There was no significant difference in the count of oestus or estrous cycle. The copulation indices were 100, 100, 95.00, 94.74% for each group, respectively. The male and

female fertility indices[1] were 100, 90.00, 94.74, 94.44% for control, 30, 100 and 300 mg/kg/day respectively. There was no significant difference between control and methyl salicylate groups in the sperm form anomalies index, sperm count or sperm motility. There was no significant difference in the numbers of implants or live embryos, pre-implant low index or dead embryo index. A significant decrease in the number of corporea lutea was observed at 100 mg/kg/day (1.84% versus 4.81% in control) but not at 300 mg/kg/day (3.25%). Plasma salicylic acid concentration was measured on day 0 and day 13 of administration. The increase was nearly dependent on increases in the dose ratio and was scarcely affected by repeated dosing. No sexual difference was observed. In conclusion, the NOAEL for general toxicity is 100 mg/kg/day and the NOAEL for fertility and early development was 300 mg/kg/day.

Two studies have been conducted on MeS in CD-1 mice by gavage according to the NTP continuous breeding protocol (NTP, 1984a, 1984b).

In the first study (NTP, 1984a), male and female mice were exposed to 25, 50 and 100 mg/kg bw/day of MeS for 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 mated at about 74 days of age. Examinations were rather limited in parental animals (clinical signs and body weight, sperm measures (F1), fertility and mating index, limited examination of organ weight, gross and histopathology) and offsprings (number, sex, live and dead, body weight). There was no treatment related effect on parental survival, body weight and food consumption. No adverse effects were reported on fertility, number of pups per litter, percentages of live pups or pup weight. Necropsy of F1 mice revealed no adverse effects on body or organ weights or sperm motility, density or morphology. In task 4, mating and fertility indices were decreased at 100 mg/kg bw/day but it was not statistically significant (76% vs 95% for mating index and 65% vs 89% for fertility index). Based on the absence of statistically significant effect on fertility and development, the NOAEL were set at 100 mg/kg bw/day. It should be noted that this study does not permit a full assessment of reproductive

[1] **Male fertility index = number of pregnant females/number of males with confirmed copulation**

Female fertility index = number of pregnant females/number of females with confirmed copulation

and developmental toxicity considering the limited numbers of parameters assessed in this study compared to OECD test guidelines.

In the second NTP study (NTP, 1984b), male and female mice were exposed to 100, 250 and 500 mg/kg bw/day of MeS for 7 days prior to mating, during 100 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). Examinations were rather limited: only clinical signs in parents, fertility index, number of litter produced, number of live/died pups and body weight were reported. No effect on fertility index (number of fertile/cohabite x 100) was observed (94-100%). No treatment related effect on parental mortality, clinical signs and body weight was reported. Reduced pup viability was reported at the high dose with decrease in the mean number of litter, in the average of live pups per litter and the proportion of pups born alive. At 250 mg/kg bw/day, a reduction in pup weight (about -4%) was reported in females. Based on the absence of effect on fertility index, the NOAEL for reproduction was 500 mg/kg bw/day. The NOAEL for development was 100 mg/kg bw/day based on a decrease of pup body weight. In order to discriminate which sex (or sexes) may be affected by the chemical exposure, a cross-over mating trial (task 3) was carried out where high-dose animals of each sex were mated to control mice of the opposite sex. An affected sex cannot be determined due to fertility problem in the control groups (29% in the first task 3 and 41% in the second task 3 versus 41-72% in the treated groups). It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the limited numbers of parameters assessed in this study compared to OECD test guidelines.

In a 3-generation study (Collins *et al.,* 1971), methyl salicylate (MeS) was administered to male and female Osborne-Mendel rats in the diet at 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw as MeS). Parental generation rats were fed MeS for 100 days prior to mating, then throughout two mating, gestation and lactation periods (until weaning of the F3 offspring). Each generation of rats was mated twice. Examinations performed in this study were very limited and consisted on fertility index, litter size, viability at birth, on day 4 and at weaning, external examination of newborn and weanling rats (all generations, all matings), histopathological examination of liver and kidney (for the 3rd generation only). No examination of reproductive tract (including histopathology, sperm and oestrus cycle measures…) was performed in both parents and offspring animals. Furthermore, peri- and post-natal development (including functional development, sexual maturation…) were not assessed. No significant effect was reported in fertility index at any dose for any generation.

According to the authors, "*appreciable decreases can be seen, however, in the second and third generation matings at 5000 ppm level*". Indeed, fertility indices (number of litters casts/number of females exposed to mating) were 85% and 77% for the first and second matings of the 2nd generation at 5000 ppm versus 100% in control. In the third generation, fertility indices were 89% vs 100% in the first mating and 84% vs 90% in the second mating. Adverse effects were reported on offspring, such as decreases in average litter size, number of liveborn progeny per female, viability (liveborn), survival (survivors on day 4) and weaning survival. These effects are only statistically significant in the 2nd generation, with a dose-related decrease starting from 1500 ppm. Decreases in weight at the weaning appeared consistently from 3000 ppm. There was no external abnormality or histopathological effect on the liver and kidney of offspring of the 3rd generation at weaning. Based on the absence of statistically significant effect on fertility, the NOAEL for fertility was set at 250 mg/kg bw/day. The NOAEL for development was 75 mg/kg bw/day based on pup mortality and decreased weight. It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the very limited numbers of parameters assessed in this study compared to OECD test guidelines.

In a 2-generation study (Anonymous, 1978a), male and female Wistar rats received MeS in the diet at 2500 and 5000 ppm (equivalent to 125 and 250 mg/kg bw MeS) for 60 days prior to mating, then throughout the study (until weaning of the offspring). Each generation of rats was mated twice. Examinations performed were very limited: mating performance, number of pups, stillbirths, live birth, postnatal mortality, gross abnormalities, physical and behavioural abnormalities. An increase of unsuccessful mating for the first generation (21.7% vs 8% in control with no mating) and a decrease in reproduction index for both generations [number of weaned 21 days/number of liveborn * 100] (76.2% vs 82.7% in the first generation and 76.9% vs 89.8% in the second generation) were reported at the highest dose. A decrease of litter size was noted at all doses. Higher number of deaths between birth and day 5 was also observed at 500 mg/kg bw/day. Only results of statistical analysis for total born, live born and total weaned/female were reported but not statistical significant effect was found. No gross abnormalities were observed in young born. All young surviving to weaning appeared normal in respect to body growth, appearance and behavior. Considering the low quality of the study and results, no adequate NOAEL can be set. It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the very limited numbers of parameters assessed in this study compared to OECD test guidelines.

In a further 2 generation study (Anonymous,1978b), male and female mice were exposed to MeS at 2500 and 5000 ppm (equivalent to 375 and 750 mg/kg bw MeS) from 30 days prior to mating until weaning of offspring. Examinations performed were very limited: mating performance, number of pups, stillbirths, live birth, postnatal mortality, gross abnormalities, physical and behavioural abnormalities. The only effect reported is a "slightly smaller litter size" in test groups at birthhowever no statistical analysis was performed. Thus the relevance of this effect cannot be adequately assessed. Considering the low quality of the study and results, no adequate NOAEL can be set. It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the very limited numbers of parameters assessed in this study.

A last study was summarized in the CIR (2003) review. Groups of 24 to 27 SD rats were fed a diet containing 4000 or 6000 ppm of MeS and calcium carbonate for 60 days prior to mating (FDA, 1966). The dams were fed the test diets until the neonates were weaned at day 20 or 21, and the procedure was repeated with a second mating. No abnormalities were observed in the offspring of test animals. Neonate survival at weaning was greater in the test group than in the control group. This study cannot be adequately assessed due to the very limited level of details available.

As conclusions on above studies, no statistically significant effect on fertility and mating was reported in rats at doses up to 250 mg/kg bw/day by oral route and 300 mg/kg bw/day by subcutaneous application and in mice at doses up to 750 mg/kg bw/day.

<u>Human data</u>

No human data has been found with methyl salicylate. However, many human data are available for an analoguous compound, acetyl salicylic acid (ASA or aspirin). ASA and methyl salicylate were both rapidly and almost completely metabolized into salicylic acid. Although most of the data did not show an increased risk of adverse effect on pregnancy at low salicylate (acetyl salicylic acid) doses in humans (review by Bard (2012)), some indications of effects on maternal bleeding, pregnancy duration and labour are nevertheless reported in the literature (Lewis *et al.* (1973), Collins and Turner (1975), Golding (1998) cited in the Bard review (2012). **Overall, due to limitations (such as misclassification of exposure and lack of**

quantitative data), human data are considered non-conclusive, even if some of them report some effects.

10.3.3 Comparison with the CLP criteria

Even if most of the fertility studies show a number of deficiencies compared to OECD guidelines in term of parameters studied, none reported any significant and/or consistent effect on fertility. Therefore, there is insufficient evidence that methyl salicylate exhibits adverse effect on sexual function and fertility.

No classification is justified for methyl salicylate for adverse effects on sexual function and fertility.

10.3.4 Adverse effects on development

Table 18: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference/ reliability
Prenatal developmental assay (GD6-18) Rabbit New Zealand White (18-20 females/group) Subcutaneous administration Study performed according to ICH guidelines and GLP	Methyl salicylate (purity: 100.1%) 0, 30, 100, 300 mg/kg bw/day in corn oil Exposure: day 6 to 18 (daily)	NOAEL (development): 300 mg/kg/day based on no effect. NOAEL (maternal): 100 mg/kg/day based on abortion in one dam and on decreased body weight gain at 300 mg/kg/day. Increase of the plasma salicylic acid concentration nearly dependent of increases in the dose ratio and scarcely affected by repeated dosing.	FDA (2006b) Klimisch score : 1 Key study
Prenatal developmental assay (GD6-17) Rat Crj:CD(SD)IGS (20 females/group) Subcutaneous administration Study performed according to ICH guidelines and GLP	Methyl salicylate (purity: 100.1%) 0, 50, 100, 200 mg/kg bw/day in corn oil Exposure: day 6 to 17 (daily)	NOAEL (development): 100 mg/kg bw/day based on decreased body weight, external and skeletal anomalies at 200 mg/kg bw/day. NOAEL (maternal): 100 mg/kg bw/day based on depression of the body weight gain and decrease in food consumption at 200 mg/kg bw/day.	FDA (2006c) Klimisch score : 1 Key study
Study for effects on pre and postnatal development including maternal function Crj:CD(SD)IGS pregnant	Methyl salicylate (purity: 100.1%) 0, 20, 60, 200 mg/kg/day in corn oil Exposure: from gestation day 6 to	NOAEL maternal: 60 mg/kg/d based on decreased body weight, food consumption and mortality at 200 mg/kg bw/day. NOAEL development < 60 mg/kg/day	FDA (2006d) Klimisch score : 1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference/ reliability**
female rats (20/group) Subcutaneous administration. Groups of offspring sacrificed on lactation day 22 for organ weight and skeletal examination. Remaining males and females were mated to assess reproductive performance. Females sacrificed on gestation day 13. GLP and ICH guidelines	lactation day 21	based on skeletal variations at 60 mg/kg bw/day. Decreased birth index, delayed balanopreputial separation, delayed incisor eruption and skeletal anomalies and variations at 200 mg/kg/day.	Key study
Two-generation study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 4 (offspring assessment) of the NTP continuous breeding protocol NTP protocol, GLP	Methyl salicylate (purity ≥ 99%) 0, 25, 50 and 100 mg/kg/day. (nominal conc.) 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 mated at about 74 days of age.	NOAEL (reproductive effects): 100 mg/kg bw/day – no adverse effect NOAEL (developmental effects): 100 mg/kg bw/day – no adverse effect	NTP (1984a) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Lamb *et al*., (1997) Klimisch score : 2 Supporting study
One generation study + crossover mating study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 3 (crossover mating) of the NTP continuous breeding protocol NTP protocol, GLP	Methyl salicylate (purity ≥ 99%) 100, 250 and 500 mg/kg/day. (nominal conc.) 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). Task 3: high-dose animals of each sex were mated to control mice of the opposite sex.	500 mg/kg bw/day – no effect on fertility index NOAEL (developmental effect): 100 mg/kg bw/day based on a reduction in pup weight from 250 mg/kg bw/day. At 500 mg/kg bw/day, a significant decrease in the mean number of litter and in the average of pups per litter, the proportion of pups born alive was observed. Task 3: due to fertility problem in the control groups (26% in the first task 3 and 41% in the second task 3) and lack of significant results in the litter analysis, an affected sex cannot be determined.	NTP (1984b) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Klimisch score : 2 Supporting study
Three-generation study	Methyl salicylate	NOAEL (fertility): 250 mg/kg bw/day (male/female) based on no statistically	Collins TFX *et al.* (1971)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference/ reliability**
Rat (Osborne-Mendel); male/female (20/sex/dose) Oral: feed (no vehicle) A supplementary study was performed with adding calcium carbonate to MeS diet with the same examination. Examination very limited Several deficiencies from OECD 416, not GLP	0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw as MeS) (nominal in diet) Exposure: 100 days before the first mating and then throughout the experiment (until weaning of the 3rd generation).	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 MeS alone.	Gross MA, Fitzhugh OG (1977) Klimisch score : 3 Supporting study
Two-generation study Rat (Wistar) male/female 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	Methyl salicylate 0.25% and 0.5% (2500 ppm and 5000 ppm equivalent to 125 and 250 mg/kg bw MeS/day) (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (weaning of the F2b litters).	No adequate NOAEL can be set based on the low quality of the reported results. Decreased litter size at all doses. Higher number of unsuccessful matings for the first generation and decreased reproduction index for both generations at the highest dose. Higher number of death between birth and day 5 day at 250 mg/kg bw/day.	Anonymous (1978a) Klimisch score : 3 Supporting study
Two-generation study Mouse male/female (no data on strain); 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	Methyl salicylate 0.25% and 0.5% (2500 ppm and 5000 ppm, equivalent to 375 and 750 mg/kg bw MeS/day) (nominal in diet) Exposure: 30 days before the first mating and then throught the experiment (weaning of the pups).	No adequate NOAEL can be set based on the low quality of the reported results. Litter size slightly smaller in test groups only in the first generation.	Anonymous (1978b) Klimisch score : 3 Supporting study
One-generation study Rat (Sprague-Dawley); male/female; 24-27 animals/dose Oral: feed (no vehicle) Guideline and GLP not stated – secondary litterature	Methyl salicylate 4000 ppm and 6000 ppm equivalent to 200 and 300 mg/kg bw/day (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (until weaning of offspring on day 20-21)	NOAEL (F1): 300 mg/kg bw/day (male/female) based on no effect. No abnormalities. Neonate survival at weaning was greater in the test group than in control.	FDA (1966) CIR (2003) Klimisch score : 4 Disregarded study

10.3.5 Short summary and overall relevance of the provided information on adverse effects on development

Animal data

In the first study (summarized in FDA, 2006b), pregnant New Zealand White rabbits (18-20/group) were exposed to methyl salicylate by subcutaneous administration from gestation day 6 to gestation day 18 at the doses of 0, 30, 100 or 300 mg/kg/day. The highest dose was selected based on a preliminary study showing mortality from the dose of 500 mgkg/day and pre-implant loss index from 250 mg/kg/day. In the main study, one dam at the highest dose had an abortion on gestation day 18, with a complete late resorption. Slight not significant depression in body weight gain (without impact on body weight) was observed throughout the administration period at 300 mg/kg/day. A NOAEL of 100 mg/kg/d is set for maternal toxicity based on these effects. There was no treatment related effect on the numbers of corporea lutea, implants or live fetuses, dead embryo / foetus indices or body weight of live fetuses. A significant, but not dose-related, decrease in the pre-implant loss index (66.7%) as compared with the control group was observed in the 30 mg/kg group. Since implantation occurs before treatment to methyl salicylate, this effect is not considered related to treatment. There was a significant difference in sex ratio, with a larger number of male fetuses (↑ 44.4%) in the 300 mg/kg/d as compared with the control group. However, sex determination occurs genetically on day 6 of gestation or before. There was no placental anomaly, no external, visceral or skeletal anomalies related to methyl salicylate treatment. The NOAEL for development toxicity is 300 mg/kg/day. The degree of elevation of the plasma salicylic acid concentration was nearly dependent on increases in the dose ratio. Plasma concentration of salicylic acid was scarcely affected by repeated dosing.

In the second study (summarized in FDA, 2006c), pregnant Crj:CD(SD)IGS rats (20/group) were exposed to methyl salicylate by subcutaneous administration from gestation day 6 to gestation day 17 at the doses of 0, 50, 100 or 200 mg/kg/day. The highest dose was selected based on a preliminary study showing mortality at the dose of 400 mgkg/day and decreased maternal body weight gain and embryolethality at the dose of 300 mg/kg/day. In the main study, no mortality or clinical signs occurred in the treated groups. Statistically significant depression of body weight (< 5%), body weight gain (≥ 10%) and food consumption was reported in dams at 200 mg/kg/day. A transient statistically significant decreased body weight

gain was also observed at 100 mg/kg/day without any significant impact on the body weight. A NOAEL of 100 mg/kg/day is set for maternal toxicity based on the decreased body weight. There was no effect of the treatment on the number of corporea lutea, implants, live and dead fetuses, sex ratio or placental anomalies. Lower body weight of live fetuses (- 22%) was observed at 200 mg/kg/day. In the highest dose group, there was an increase of external anomalies (3.21% *versus* 0.36% in the control), characterized principally by craniorachischisis (8 foetuses in 3 litters equivalent to 2.86% *versus* 0% in the control group) and gastroschisis (1 foetus). Even not clearly indicated in the report, these anomalies should be considered as malformations (Devtox.org). Although not statistically significant, it should be noted that, based on historical control data for development and reproductive toxicity studies using the Crl:CD®BR rat compiled by MARTA (1993), craniorachischisis and gastroschisis are rarely observed in rats (both with average fetal incidence of 0.01%). In this context, the incidence of 2.86% reported in the FDA (2006c) study is clearly above the MARTA historical controls in rat experiments (1993). In addition, these effects are considered by the authors as related to methyl salicylate treatment because they are consistent with the results of the preliminary study and with available data reported in the literature with methyl salicylate and salicylic acid. Visceral anomalies (ventricular septal defect (considered as malformation according to Devtox.org) in one foetus, dilatation of the ureter (unilateral) in 2 foetuses and thymic remnant in the neck in 8 foetuses) were also increased at 200 mg/kg/day (7.75% *versus* 3.52% in the control) but not statistically significant. A statistically significant increase of skeletal variations was also observed at the highest dose (75.19% versus 10.61% in the control group)**, with short and full supernumerary ribs, splitting of the thoracic and lumbar vertebral bodies, 7 lumbar vertebrae and incomplete ossification of the thoracic centrum. In addition, there was a delay of ossification of the vertebrae, sternebra, metacarpus, metatarsus and phalanges. In conclusion, methyl salicylate is considered teratogenic in rats. The NOAEL for developmental toxicity is 100 mg/kg/day based on external malformations, visceral anomalies, decreased fetal body weight, skeletal variations and delayed ossification.**

In the third study (summarized in FDA (2006d)), 20 pregnant female rats per group were exposed subcutaneously to methyl salicylate at 0, 20, 60 or 200 mg/kg/day from gestation day 6 to lactation day 21. Dams were sacrificed on day 22 after delivery. The highest tested dose was selected based on a preliminary study showing mortality in almost all dams at 500 mg/kg/day, no live delivery at 300 mg/kg/day and slight effect on birth index and body weight at 80 and 200 mg/kg/day.

Two dams at 200 mg/kg/day died on gestation day 23. These death were considered to have been induced by aggravation of their general condition attributable to methyl salicylate. There was a significantly lower mean body weight (-3.7% on GD12 and -4.6% on GD20) and body weight gain (between -4.08% on GD9 and -15.7% on GD20) during gestation at 200 mg/kg/day. The food consumption was significantly decreased on day 9 of gestation (-10.2%) and during lactation (-42.9% on day 1 and -21.9% on day 21) at this same dose. A significant prolongation of gestational days was observed in the 60 mg/kg/day group (with no dose-response relationship and within background data of the institution).

In male offspring, a significant decrease in the birth index (-6%) and a lower body weight (-9.2%) were observed in live newborn in the 200 mg/kg/day group. A trend toward a decrease in the number of litter (215 litters at 200 mg/kg bw/day versus 270 in the control group) and live newborns and a trend toward an increase in the stillbirth index (7 stillborns at 200 mg/kg bw/day versus 2 in the control group) were also observed in the 200 mg/kg/day group. These effects were considered attributable to methyl salicylate administration. No abnormality was reported in the external examination of the live newborn but craniorachischisis was noted in 4 stillborns (among 6 stillborns reported in 4 females; there is no indication in how many litters craniorachischisis occurred) in the 200 mg/kg/day group. A trend toward a decrease in the viability index (92.79%) on day 4 was observed at the highest dose compared to control (98.13%) but was within the range of the background data (91.32-99.28%). Excessive elongation of the maxillary incisors (1 female; 2 males), corectopia and dycoria (1 male; 1 female) were reported at 200 mg/kg/day. A significant lower mean body weight with decreased food consumption was noted during lactation and maturation in the 200 mg/kg/day group. A significant decrease in the differentiation indices of incisor eruption in both sexes (64% in males and 56% in females versus 100 % in controls of both sexes on PND12), eyelid separation in the females (85% versus 100% on PND15 in controls) and cleavage of the balanopreputial gland in the males (67% versus 100% in controls on PND46) were reported at the highest dose. In the males at weaning, a significant decrease in the absolute and relative weights of the liver and kidneys, in the absolute weights of the brain, adrenals and testes and a significant increase in the relative weights of the brain and lungs was observed at 200 mg/kg/day. In females, a significant decrease in the absolute weights of the brain, heart, lungs, liver, kidneys, adrenals and ovaries and a significant increase in the relative weight of the brain were noted at 200 mg/kg/day.

Skeletal anomalies, especially fusion of the cervical vertebra and misshapen sternebra, were significantly increased at 200 mg/kg/day (32.26% versus 3.90% in the control). Skeletal variations slightly increased at 60 mg/kg bw/day (cervical ribs, accessory sternebra, incomplete ossification of thoracic and caudal vertebrae) and was significantly increased at 200 mg/kg/day (93.55% versus 25.97% in the control), with full supernumerary ribs, accessory sternebra, lumbarization, 7 lumbar vertebrae and incomplete ossification of the cervical, thoracic and lumbar centrum. No historical control data was presented. Considering that these effects were also identified in other prenatal developmental toxicity studies, it could not be ruled out that the variations occurring at 60 mg/kg/day are treatment-related. A significant decrease of the number of rearing occurred in F1 female offspring at 200 mg/kg/day (8.1 versus 12.6) but was within laboratory control (6.0-8.7).

Regarding reproductive ability of the offspring, there was no significant difference in the copulation indices (95%, 85%, 95%, 94%), fertility indices (100%, 100%, 94.74%, 80%) and in the numbers of days required for copulation. A significant lower body weight was observed in F1 dams on gestation day 13 at the highest dose. At necropsy of the males after mating, excessive elongation of the maxillary was observed in 1 male and corectopia and dyscoria in another male at 200 mg/kg/day. In the necropsy of the females on gestation day 13, corectopia and dyscoria were observed in 1 female at 200 mg/kg/day. There was an increase of pre-implantation losses (7.18 versus 1.99) at the highest dose but not statistically significant. The NOAEL for maternal toxicity is set at 60 mg/kg/day based on decreased body weight and food consumption. According to the authors, the NOAEL for developmental toxicity is < 60 mg/kg/day based on the slight and non-statistically significant increased incidence of skeletal variations at 60 mg/kg bw/day. An increase in lethality and skeletal anomalies / variations and a decrease in differentiation indices and body weight were noted in the highest tested group.

Developmental effects are also reported in fertility studies:

Additional information on developmental toxicity can be obtained from the fertility studies (details are provided on section 10.3.1).

In a study of fertility and early embryonic development to implantation in rats (FDA, 2006a), there was no significant effect on early development of embryos (numbers of implants or live embryos, pre-implant low index or dead embryo index) at doses up to 300 mg/kg bw/day of methyl salicylate by subcutaneous route.

In a continuous breeding protocol study (NTP, 1984a), including task 2 and 4, in mice, there was no adverse effects reported in the number of pups per litter, percentages of live pups or pup weight and at necropsy of F1 at doses up to 100 mg/kg bw/day of methyl salicylate in the diet. There was also no treatment-related effect on parental survival, body weight and food consumption.

In a second continuous breeding protocol study (NTP, 1984b), including task 2 and 3, in mice exposed to methyl salicylate in the diet, reduced pup viability was reported at the high dose of 500 mg/kg bw/day with decrease in the mean number of litter (-8%), in the average of live pups per litter (-31%) and the proportion of pups born alive (-6%). At 250 mg/kg bw/day, a reduction in pup weight (about -4%) was reported in females. The NOAEL for development was set at 100 mg/kg bw/day based on the decrease of pup body weight. No treatment-related effect on parental mortality, clinical signs and body weight was reported.

In a 3-generation study performed by Collins *et al.* (1971) in rats exposed to methyl salicylate in the diet, adverse effects were reported on offspring such as decreases in average litter size (2nd generation: mating 1: 10.8, 10.2, 10.3, 8.4, 6.2 at 0, 500, 1500, 3000 and 5000 ppm respectively; mating 2: 11.9, 10.2, 10.5, 9.4, 6.6 at 0, 500, 1500, 3000 and 5000 ppm respectively), number of liveborn progeny per female, viability (liveborn) (2nd generation: mating 1: 10.8, 10.2, 10.2, 8.2, 5.6 at 0, 500, 1500, 3000 and 5000 ppm respectively; mating 2: 11.8, 10.2, 10.5, 9.1, 6.3 at 0, 500, 1500, 3000 and 5000 ppm respectively), survival (survivors on day 4) (2nd generation: mating 1: 9.4, 9.0, 9.5, 6.2, 4.3 at 0, 500, 1500, 3000 and 5000 ppm respectively; mating 2: 11.1, 9.4, 10.3, 8.2, 4.7 at 0, 500, 1500, 3000 and 5000 ppm respectively) and weaning survival (2nd generation: mating 1: 8.8, 8.4, 9.4, 6.0, 3.9 at 0, 500, 1500, 3000 and 5000 ppm respectively; mating 2: 10.5, 8.6, 9.9, 8.0, 3.7 at 0, 500, 1500, 3000 and 5000 ppm respectively). These effects are only statistically significant in the 2nd generation, with a dose-related decrease starting from 1500 ppm (75 mg/kg bw/day). Decreases in weight at the weaning (up to – 21%) appeared consistently from 3000 ppm (equivalent to 150 mg/kg bw/day). There was no external abnormality or histopathological effect on the liver and kidney of offspring of the 3rd generation at weaning. The NOAEL for development was 75 mg/kg bw/day based on pup mortality and decreased weight. It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the limited numbers of parameters assessed in this study. In addition, there was no information on general parental toxicity reported in the publication.

In a 2-generation study (Anonymous, 1978a) performed in rats exposed to methyl salicylate in the diet, a decrease of litter size was noted at both doses tested (10.61, 9.00, 9.74 at 0, 2500 and 5000 ppm, respectively – equivalent to 125 and 250 mg/kg bw/day). Higher number of deaths between birth and day 5 was also observed at 500 mg/kg bw/day (alive at 5 days: 8.46 versus 10.04 in control group). Only results of statistical analysis for total born, live born and total weaned/female were reported but not statistical significant effect was found. No gross abnormalities were observed in young born. All young surviving to weaning appeared normal in respect to body growth, appearance and behavior. Considering the low quality of the study and results, no adequate NOAEL can be set. It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the very limited numbers of parameters assessed in this study. In addition, there was no information on general parental toxicity reported in the report.

In a further 2 generation study (Anonymous,1978b) performed in mice exposed to methyl salicylate in the diet, the only effect reported is a "slightly smaller litter size" in test groups (2500 and 5000 ppm equivalent to 375 and 750 mg/kg bw/day) at birth (11.53, 10.95, 10.35 at 0, 2500 and 5000 ppm, respectively), however no statistical analysis was performed. Thus the relevance of this effect cannot be adequately assessed. Considering the low quality of the study and results, no adequate NOAEL can be set. It should be noted that this study does not permit a full assessment of reproductive and developmental toxicity considering the very limited numbers of parameters assessed in this study. In addition, there was no information on general parental toxicity reported in the report.

In a one-generation study summarized in the CIR (2003) review, no abnormalities were observed in the offspring of test animals exposed to 4000 ppm and 6000 ppm (equivalent to 200 and 300 mg/kg bw/day) of methyl salicylate. Neonate survival at weaning was greater in the test groups than in the control group. This study cannot be adequately assessed due to the very limited level of details available.

Studies of lower quality are also available:

Pregnant rats received dermal application of undiluted MeS or diluted in a petroleum based grease. Undiluted MeS was initially applied at 2000 mg/kg bw/day from GD6 but due to severe toxicity (dermal irritation and 25% mortality), the dose was reduced to 1000 mg/kg

bw/day from GD10 to GD15. At this dose, a 100% resorption was reported, but there was no information on maternal toxicity after reduction of the dose (Infurna *et al.*, 1990 – only abstract available).

MeS was administered topically at 3500 and 5250 mg/kg bw to pregnant LVG hamsters on day 7 and teratogenic results were compared with those obtained following oral treatment at 1750 mg/kg bw. After dermal exposure for 2 hours, the skin was thoroughly washed with running water. Blood samples were obtained at regular intervals to monitor salicylate. Most embryos were recovered at GD9, few survived to the age of 12 days. Both treatments produced neural tube defects, especially in the area of the developing brain. Percentage of neural tube defect was 72% at 1750 mg/kg bw/day after oral exposure versus 11% in control. After dermal exposure, the percentage of neural tube defect was 0% in control, 6% at 3500 mg/kg bw/day and 53% at 5250 mg/kg bw/day. Analysis of serum showed that salicylate levels reached a peak of 125 mg/100 ml at about 2 hours after oral administration and 50 mg/100 ml at 5-6h after dermal application. Comparison of maternal and fetal salicylate levels in older fetuses showed that salicylate was reaching the foetus in some fraction of the concentration found in the mother (Overman & White, 1983).

Other studies were available and described in different reviews (RIFM, 2007; Lapczynski *et al.,* 2007 and CIR, 2003).

Female rats received 0.05 or 0.1 mL MeS by intraperitoneal route on days 10 and 11 of pregnancy. The young were obtained on GD21 or postnatally at 1, 6, 12 or 24 days of age. They were counted, weighted and examined for viability and external malformations. Kidneys were removed, weighted and examined. At 0.1 mL, females gained less weight, had fewer and smaller offspring and more resorptions and malformed young than in the control group. Fetal kidneys weighted significantly less than those of the controls and lengthening of the renal papilla was inhibited by MeS, suggesting that MeS can induce renal growth retardation. Additionally, there was a significantly higher frequency of kidneys with absent papillae. Retarded renal development recovered on PND6, but persistent hydronephrosis (11/138 kidneys) was still observed at weaning. It is not clear from the publication if these effects are only observed at the highest tested dose or at both doses (Woo *et al.*, 1972).

Daston *et al* (1988) performed several experiments where MeS was given by intraperitoneal route to pregnant rats from 200 to 450 mg/kg bw/day, on different gestation days and for different durations. Malformations, reduction of fetal weight and some increase in the

incidence of resorption were reported. On this basis, a further study was performed to study postnatal renal function of offspring. Pregnant rats were exposed to 200-300 mg/kg bw/day MeS on GD 11-12. Increased mortality during the first 2 days after birth was noted from 250 mg/kg bw/day. Increase in kidney/body weight ratio was observed on day 15 but not by 4 weeks of age.

In a last study performed in rats by intraperitoneal route at 200 and 400 mg/kg bw/day on GD9 and 10, decreased fetal weight, reduction of fetal body weight index and malformations were reported at both tested doses in the presence of maternal toxicity (Kavlock *et al.*, 1982).

As conclusions, developmental effects, mainly characterized by lethality, external malformations, visceral and skeletal anomalies and effects on differentiation indices, were reported in developmental and reproductive studies with methyl salicylate. The lowest developmental NOAEL are < 60 mg/kg bw/day in rats exposed subcutaneously from gestation day 6 to lactation day 21 (FDA, 2006b) and 75 mg/kg bw/day in a 3-generation study in rats by oral route (Collins *et al.* 1971).

Human data

No human data has been found with methyl salicylate. However, many human data are available for an analoguous compound, acetyl salicylic acid (ASA or aspirin). ASA and methyl salicylate were both rapidly and almost completely metabolized into salicylic acid. Although most of the data did not show an increased risk of adverse effect on development at low salicylate (acetyl salicylic acid) doses in humans (review by Bard (2012)), some indications of effects on intra-uterine fetal growth retardation, lethality and malformation are reported in the litterature. Even if not exhaustive, the following observations can be reported.

Effects on intra-uterine fetal growth retardation, stillbirth and infant mortality

Findings of a survey performed in 144 regular takers of salicylates (including ASA) reported that salicylate consumption was associated with perinatal mortality, decreased intra-uterine growth and birth weight (Collins & Turner, 1975 cited in the Bard review).

Low dose aspirin (75 mg per day from 5 weeks of amenorrhoea until delivery) significantly improves the livebirth rate among women with a previous late miscarriage but not in women with unexplained recurrent early miscarriage. In contrast, significantly higher number of late miscarriages was observed amongst women who took aspirin compared those who did not. An

explanation proposed by the authors was the development of placental intervillous blood flow. They hypothesized that the daily dose of 75 mg of aspirin is too low to maintain pregnancies after 14 weeks of gestation (Rai *et al.*, 2000).

Li *et al.* (2003) (cited in the Bard (2012) review) reported a significant increase of miscarriage in women using aspirin from conception. This is consistent with the hypothesis that prostaglandin inhibition by aspirin interferes with implantation. A reevaluation of this study was performed by Nielsen *et al.* (2004) (cited in the Bard (2012) review) who showed a positive association between NSAIDS (nonsteroidal anti-inflammatory drugs; not further specified) use and miscarriage. However, it was not statistically significant when gestation age was included in the calculation.

Finally, it has also been shown from cases reports that salicylate overdose during pregnancy can result in fetal distress and *in utero* or post-natal deaths (Farid *et al*., 2011).

The following studies reported some indications of malformations

Richards (1969) (cited in the Bard (2012) review) reported a strong association between salicylates taken during the first trimester and defects on central nervous system, alimentary tract, miscellaneous defects (such as mongolism, defects on eye, ear, urogenital, skin and other) and talipes in a retrospective study on 833 cases with an equal number of controls. When salicylates were taken during the second and third trimester, only miscellaneous defects were statistically significant.

Significant association was found between aspirin use during the 1st trimester of pregnancy and all types of abnormalities and between major abnormalities and aspirin consumption during the whole pregnancy in a retrospective study consisting in 458 mothers giving birth to infants with congenital abnormalities and 911 mothers with normal babies (Nelson & Forfar, 1971 (cited in the Bard (2012) review)). Aspirin use during the first 28 days of gestation was also associated with higher incidence of achondroplasia, hydrocephalus, congenital heart disease, mongolism, congenital dislocation of the hip, hydrocele, talipes and papilloma of the forehead.

An about 2-fold increase in the frequency of defects in septation of the truncus arteriosis was associated with aspirin use in early pregnancy (Zierler & Rothman, 1985 (cited in the Bard (2012) review)).

A prospective survey assessing the prenatal use of prescription drugs and congenital malformations in Tasmania was performed by Correy *et al.* (1991) (cited in the Bard (2012) review). A significant association was found between the use of aspirin during the first trimester and hypospadias. However, the authors noted that the statistical significance of this association is marginal.

Lynberg *et al*. (1994) (cited in the Bard (2012) review) observed an increased risk of anencephaly, spina bifida and encephalocele in women reporting taking aspirin for episodes of flu with fewer from 3 months before pregnancy through the first 3 months of pregnancy.

The relation between maternal use of cough/cold/analgesic medications and risks of gastroschisis and small intestinal atresia (SIA; such as atresia, stenosis, or webbing of the duodenum, jejunum, or ileum without gastroschisis) was assessed in a retrospective study (Werler *et al.*, 2002 (cited in the Bard (2012) review)). The mothers of 206 gastroschisis cases, 126 SIA cases and 798 controls in the United States and Canada from 1995-1999 were considered. Risks of gastroschisis were elevated for use of aspirin alone.

Kozer *et al*. (2002) (cited in the Bard (2012) review) reviewed the published studies reporting exposure to aspirin during the first trimester of pregnancy and congenital malformations. Twenty-two studies met the inclusion criteria. No evidence of an overall increase in the risk of congenital malformations that could be associated with aspirin was found. However, exposure to aspirin may be associated with an increased risk of gastroschisis. An increased risk of other specific malformations (such as NTDs (neural tube defect), CNS (central nervous system) malformations, and cleft lip and palate) cannot be excluded and should be investigated further in studies of more rigorous design.

A large multi-site population-based case control study was carried out by Hernandez *et al.* (2012). Data from the US National Birth Defect Prevention Study (NBDPS) were used to collect cases of infants with major birth defect born between 1997 and 2004. Among the women who reported their exposure frequency, 703 reported using aspirin and 218 women reported using aspirin as needed. There were 15,836 women who were not exposed to NSAIDS at any time during pregnancy. Significant association was found between aspirin consumption and anencephaly/craniorachischisis (total and isolated), anophthalmia/microphthalmia (total), cleft palate (total and isolated) and amniotic bands/limb body wall (total and isolated).

Kristensen *et al*. (2011) found an increase of cryptorchid sons in mothers who reported the use of aspirin during the first and second trimester in Denmark; however, this was not found in Finland.

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. These effects seem consistent with those reported in experimental studies with methyl salicylate. However, due to some limitations (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.

10.3.6 Comparison with the CLP criteria

According to CLP: *"Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.*

***The classification of a substance in this Category 1A** is largely based on evidence from humans."*

There is no human data with methyl salicylate. Human data are however available with an analoguous substance, acetyl salicylic acid. Even if most of the epidemiological do not reported an increased risk of adverse effect on development at therapeutic dosage, some indications of fetal lethality and malformations were suggested. **These data are judged unconclusive and not sufficient to justify a classification for adverse effects on development as category 1A for methyl salicylate.**

***"The classification of a substance in this Category 1B** is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non specific consequence of other toxic effects."*

Although methyl salicylate did not induce any developmental effect in a well conducted prenatal developmental toxicity study in rabbits (FDA, 2006b), there is clear evidence of developmental effects in two well-conducted studies performed in rats (FDA, 2006 c, d).

In the first study, methyl salicylate administrated by subcutaneous route from gestation day 6 to lactation day 21. In this study, several developmental effects were reported including lethality (decreased birth index), growth retardation (lower body weight), external malformation (craniorachischisis), delay in post-natal differentiation indices (incisor eruption, eyelid separation, cleavage of the balanopreputial gland), skeletal anomalies (**fusion of the cervical vertebra and misshapen sternebra), skeletal variations** and incomplete ossification at 200 mg/kg bw/day. Skeletal variations were already slightly increased at 60 mg/kg bw/day. Effects in dams occurred at

200 mg/kg bw/day and consisted on a lower body weight (< -5%), a lower body weight gain **(between -4.08% on GD9 and -15.7% on GD20)** and 2 mortalities. Although some of the developmental effects (such as skeletal variation, decreased body weight, delay in post-natal differentiation indices) may be secondary to maternal toxicity, it is not possible to explain the other effects such as offspring lethality and external/skeletal anomalies by the observed maternal toxicity (FDA, 2006d). Indeed, the maternal toxicity is considered rather slight in view of the severity of the developmental effects as lethality and anomalies.

In the second study, methyl salicylate induced significant lower foetal body weight, external malformations (craniorachischisis and gastrochisis), **visceral anomalies (ventricular septal defect, dilatation of the ureter and thymic remnant in the neck)** and skeletal variations at 200 mg/kg bw/day after subcutaneous administration from gestation day 6 to 17. **All these effects occurred at concentrations inducing a slight toxicity in dams as characterized by a decrease of body weight (< 5%) and body weight gain (≥ 10%). However, considering the severity of the observed external malformations and visceral anomalies, these effects cannot be secondary to the slight maternal toxicity (FDA, 2006c).**

Developmental effects, characterized by lethality, are also consistently reported in fertility studies in both mice and rats:

- **decreases in litter size, number of liveborn progeny per female, viability (liveborn), survival (survivors on day 4) and weaning survival at 150 mg/kg bw/day in the Collins *et al.* (1971) study in rats;**
- **higher number of deaths between birth and day 5 at 250 mg/kg bw/day in the Anonymous (1978a) study in rats;**
- **"slightly smaller litter size" from 375 mg/kg bw/day at birth in the Anonymous (1978b) study in rats;**
- **reduced pup viability, decrease in the mean number of litter, in the average of pups per litter and the proportion of pups born alive at 500 mg/kg bw/day in the NTP (1984b) study in mice.**

Studies of low quality also report various developmental effects after *in utero* exposure to methyl salicylate by dermal route in rats and hamsters and by intraperitoneal route in rats. Developmental effects consisted on lethality and malformations (**brain and kidney). They are thus consistent with those reported in the prenatal developmental toxicity studies described above.**

In conclusion, from studies performed in rats, there is a clear evidence that methyl salicylate is toxic for development, with consistent effects reported among the available studies. The observed effects, especially lethality, external malformation and visceral and skeletal anomalies, are not considered secondary to the rather slight maternal toxicity observed and thus can justify a classification as Repr. Cat. 1B.

"*However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."* (CLP guidance version 5.0 (July 2017)).

Malformations and foetal lethality are clearly observed in prenatal developmental toxicity study in rats. No effect on development was observed in the prenatal developmental toxicity study in rabbits. However, it should be noted that the rabbits were exposed only from gestation day 6 to 18 which is rather short considering their length of gestation which is about 30 days. Thus, the lack of observed effect in rabbits could be explained, at least in part, by a insufficient duration of exposure or exposure during inadequate sensitive window. In contrast, lethality at birth and/or during lactation, was consistently reported in fertility studies performed in both rats and mice. In addition, **neural tube defect was reported in hamster. Finally,** even if not conclusive, there are some indications of developmental effects from human data with acetyl salicylic acid, which can support the relevance of the observed effects in experimental studies to humans. In particular, it can be noted that mortality, cranioraschisis and gastroschisis are both reported in rats after methyl salicylate exposure and from human data with acetylsalicylic acid.

The mode of action underlying the developmental effects reported with methyl salicylate has not been particularly investigated. However, it can be noted that salicylic acid, a metabolite of methyl salicylate, is known to inhibit cyclooxygenases leading to a decrease of prostaglandins synthesis. Prostaglandins play many roles in the organism, including in reproductive functions (such as uterine contractility, platelet function, fetal vascular structure) (Greene *et al.*, 2017). Based on this hypothesis and in the absence of information on any other possible mode of action, it is assumed that the developmental effects reported in animals following methyl salicylate exposure are relevant to humans.

In conclusion, based on a clear evidence of developmental effects which are considered of biological relevance for humans, **methyl salicylate fulfills criteria for classification as Repr. 1B – H360D.**

10.3.7 Conclusion on classification and labelling for reproductive toxicity

Available experimental data with methyl salicylate do not report significant effect on fertility. Thus, it is concluded that no classification is justified for methyl salicylate for adverse effects on sexual function and fertility.

Regarding developmental toxicity, methyl salicylate induces lethality, external malformations, visceral / skeletal anomalies and growth retardation in rats exposed *in utero*. These effects are observed in the presence of slight toxicity in dams, which is not sufficient to explain the reported developmental effects. Since these effects are considered relevant to humans, it is concluded that classification Repro. 1B – H360D is justified for methyl salicylate for developmental toxicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility and reproductive function

The DS presented a number of studies conducted to investigate adverse effects on sexual function and fertility or on development after exposure to MeS. Fertility studies reported by the DS cover an extensive time period that starts in the 1960s and extends to the early 2000s. According to the DS, none of the studies reported any significant and/or consistent effect on fertility. Therefore, the DS was of the opinion that no classification is justified for MeS for adverse effects on sexual function and fertility.

Table 17 of the CLH report provides an overview of the animal studies used for assessing the classification of MeS regarding reproductive toxicity. They are briefly summarised in the background document under "Supplemental information – In depth analyses by RAC".

Effects on Development

In addition to the reproductive toxicity studies presented above, the DS evaluated several developmental toxicity studies in rats and rabbits summarised below.

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

In the rabbits 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. Skeletal variations were also observed to have a statistically significant increase 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).

Human data

The DS did not present any human data on MeS. However, because human data are available for another salicylate ester, ASA, the DS presents several – mostly retrospective – publications with aspirin during pregnancy.

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.

A large multi-site population-based case control study was carried out by Hernandez *et al.* (2012). Significant association was found between aspirin consumption and anencephaly/craniorachischisis (total and isolated), anophthalmia/microphthalmia (total), cleft palate (total and isolated) and amniotic bands/limb body wall (total and isolated).

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 foetal 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 by the DS.

Comments received during consultation

Three downstream users considered that MeS should not be classified as Repr. 1B as some studies were conducted by the subcutaneous route. According to them, findings at 200 mg/kg (FDA, 2006c) occurred at maternally toxic doses and the rabbit study did not show any potential for reprotoxic effects when MeS was given during the period of organogenesis (GD6-18). They also argued that the hamster study was performed at doses well above the human toxic level and salicylic acid, as the relevant metabolite, was evaluated by RAC as Repr. 2.

One downstream user, two manufacturers and one importer stated that Repr. 1B is not justified. Another downstream user proposed to classify MeS as Repr. 2; H361d based on the RAC opinion on SA in 2016.

One MSCA supported the classification for developmental effects as Repr. 1B.

Another MSCA proposed to discuss if classification as Repr. 2; H361 or Repr. 1B is appropriate, because the available animal and toxicokinetic data delivered clear evidence of developmental effects, independently of the route of administration. The similarity of teratogenic effects of MeS and SA, the lack of human data on MeS, the generation of methanol as an additional hydrolysis product and the toxicokinetic differences (possibility of distribution of intact parent MeS into target tissues and toxic action of either parent and/or hydrolysis products of SA and methanol in the target tissue) point to different and possibly additional effects of MeS in humans when compared to SA. In case the same classification strategy is applied as for SA, MeS would need to be classified as Repr. 2; H361d. In case not, a harmonised classification as Repr. 1B; H360D has to be discussed.

Assessment and comparison with the classification criteria

According to CLP guidance version 5.0 (2017), "*Substances are classified in **Category 1** for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)*."

*Substances are classified in **Category 2** for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification (*"Suspected human reproductive toxicant"*).*

Fertility and reproductive function

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 do not allow to draw a clear conclusion.

There is insufficient evidence that MeS exhibits adverse effects on sexual function and fertility.

RAC concurs with the proposal by the DS that no classification is justified for MeS for adverse effects on sexual function and fertility.

Development

With respect to developmental toxicity, RAC is 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.

In a cohort of 50 282 gravidas and their offspring in the U.S.A., malformation rates were similar in the children of 35 418 women not exposed to aspirin, 9 736 with intermediate exposure, and 5 128 women heavily exposed during the first four lunar months of pregnancy. After controlling a wide range of potential confounding factors using multi-variate analysis, the observed and expected numbers for a variety of malformation categories were similar in all three comparison groups. The data suggest that aspirin is not teratogenic (Slone *et al.*, 1976).

Nowadays, professional associations recommend the prophylactic daily use of low-dose aspirin in pregnant women who are considered to be at high risk for pre-eclampsia. More than 30 trials have investigated the benefit of ASA at doses of 50 to 150 mg per day for the prevention of pre-eclampsia. These studies showed that such therapy resulted in a 10 % lower incidence of pre-eclampsia. A recent trial showed that among women who were at high risk for preterm pre-eclampsia, the administration of ASA at a dose of 150 mg per day from 11 to 14 weeks of gestation until 36 weeks of gestation resulted in a significantly lower incidence of preterm pre-eclampsia in comparison to placebo. No significant between-group differences in the incidence of neonatal adverse outcomes or other adverse events were observed between women treated with ASA or placebo (Rolnik *et al.*, 2017).

Salicylic acid has been classified by RAC in 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 considers the following quotes from the RAC (2016) opinion on SA to be relevant for MeS:

"*Neither ASA nor SA are proven human developmental toxicants. There is a lack of evidence to support an increased risk of birth defects following exposure to ASA. Also, the evidence for other developmental effects has uncertainties. Taking that into account, classification in Category 1A is not justified.*

In the study of Wilson et al. (1977), when general embryotoxicity of rats and monkeys to ASA was compared at equivalent dosages, some differences were detected. According to the study author this difference in effects seen can be attributable to the differences in embryonic exposure; since the free (unbound) SA is responsible for the teratogenic potential and the

binding capacity differs between species, the rat embryo is exposed to higher levels and for a longer duration than the monkey embryo.

In rats plasma concentrations of salicylate 20 minutes after oral administration of methyl- or acetylsalicylate at a dose of 500 mg/kg bw were 217 ± 16.1 mg/L (MeS) and 209 ± 18.6 (ASA) and 60 minutes after dosing salicylate concentrations of 278 ± 16.7 mg/L (MeS) and 274 ± 23.5 (ASA) mg/L were measured (Davison et al., 1961) indicating a similar toxicokinetic behaviour of both esters in rats.

In humans, no malformations could be detected; based on the assumption of a similar teratogenic potency in all species, a hypothetical human threshold for malformations around of 200 mg/L of total salicylate in maternal serum was calculated".

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, increased incidence in foetuses of severe neural tube defects, such as craniorachischisis, was not statistically significantly different. At the top dose, incidence of skeletal variations was significantly increased, which can be interpreted as a consequence of maternal toxicity and not substance-related.

Based on the weight of the evidence, RAC is of the opinion that MeS should be classified as **Repr. 2; H361d (Suspected of damaging the unborn child)** based on positive

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Degradation

1.1.1 Ready biodegradation (screening studies)

Summary table of screening test for biodegradation in water

Method, guideline	Test parameter	Test substance	Inoculum	% degradation	Reference Reliability
Ready biodegradability equivalent or similar to OECD Guideline 301B Not GLP	CO_2/DIC	Methyl salicylate 10 mg/L COD	Secondary effluent from unacclimatized activated sludge plant	98.4 after 28 d (inorg. C analysis) (95% Confid. Interval : 94.4 - 102.4)	KING J.M.H. (1993) Reliability 3

Method, guideline	Test parameter	Test substance	Inoculum	% degradation	Reference Reliability
No guideline followed		Methyl salicylate 200 mg/L	A microbial mixture: five Pseudomonas, one Klebsiella, four Rhodococci and two fungal strains.	100 after 168 h (Test mat. analysis) ((ie. 7 days))	GOULDING C., GILLEN C.J. & BOLTON E. (1988) Reliability 3

In the study report of King J.M.H. (1993), the evaluation of the biodegradability of Methyl salicylate was conducted in accordance with the draft Ecotoxicology Section Standard Operating Procedure N° 158 01 (Operation of the Sealed Vessel Test). The sealed vessel test is a CO_2 production test based on OECD Guideline 301 B (Ready Biodegradability: CO_2 Evolution Test). Secondary effluent from an unacclimatized activated sludge plant at URL North was used as inoculum. The samples were incubated for 28 days at 20°C. Analysis of both the headspace and the liquid medium for CO_2/DIC was performed on day numbers: 3, 8, 10, 14, 17, 21, 24, and 28 and the extent of biodegradation determined. The test substance was degraded to 68.8, 89.3 and 98.4% after 3, 8 and 28 days respectively. In this study, the percentage of degradation corresponds to a geometric mean calculated with 4 out of 5 samples (the fifth sample was significantly lower than the mean of the remainder (2.2 % cf. 98.4%) and was omitted after application of Dixon's test). There is no data on the toxicity control and the raw data for the blank and test samples are not available. Do to the lack of data, it is not possible to check the validity criteria and this study could be used only as supportive information.

A second study from literature focuses on the ability of a microbial mixture (five Pseudomonas, one Klebsiella, four Rhodococci and two fungal strains) to degrade a representative sample of methylated and chloro-methylated compounds (Goulding et al, 1988). The percentage removal of these compounds was examined at 24h intervals by HPLC. No guideline was followed: the inoculum used does not correspond to recommendation for ready biodegradability test. Additionnally, only primary biodegradation has been measured and not ultimate biodegradation. This study could be used only as supportive information.

However, a screening-level hazard characterization made on benzyl derivatives category (US EPA, 2010) showed the ready biodegradability for all members of the category which include methyl salicylate. Furthermore, the QSAR predictions with BIOWIN 4.10 indicate that all 2-

hydroxybenzoate esters subcategory III from the US EPA report (among which MeS belongs) are readily biodegradable substances.

Consequently, a weight of evidence approach was applied for considering the readily biodegradability of methyl salicylate.

1.1.2 Abiotic degradation

No data on the potential of methyl salicylate to be hydrolysed and photodegraded in water and soil is available. Indeed, these removal processes are not considered as predominant as the substance is readily biodegradable. However, as any ester, methyl salicylate is subject to hydrolysis to form the corresponding acid and alcohol, that is salicylic acid and methanol. For information, at pH 7.5, an hydrolysis half-life of 14.1 days has been estimated (HSDB, 1996).

According to the AOPWIN v1.92 model, methyl salicylate is considered to have a half life of 0.967 day or 11.6 hours in atmosphere (within the following conditions: 12 -h day and 1.6E06 OH/ cm3). This corresponds to 0.478 day or 11.472 hours within the following conditions: 24h day and 5E05 OH/ cm3. It is therefore not considered to be persistent in air based on this estimated rapid photodegradation potential.

11.1.1 Bioaccumulation

The substance methyl salicylate has a low potential for bioaccumulation (i. e. the substance has a log Kow < 3). As a consequence, Methyl Salicylate could be considered as not bioaccumulative.

11.2 Acute toxicity

11.2.1 Fish

Summary table – acute fish toxicity									
Method, Guideline, GLP status, Reliability	**Species**	**Endpoint**	**Exposure**		**Results (mg/L)**			**Remarks**	**Reference**
			Design	**Duration**	**LC/EC_0**	**LC/EC_{50}**	**LC/EC_{100}**		
equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test) No GLP RI 2	*Pimephales promelas* (fathead minnow)	Mortality	Flow through	96 h	14.9	**19.8**	26.2	Test material (EC name): Ethyl Salicylate Measured concentrations	Geiger D.L., Northcott C.E., Call D.J. and Brooke L.T. (1985)
equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test) No GLP RI 2	*Pimephales promelas* (fathead minnow)	Mortality	Flow through	96 h	-	1370 (confidence limit 1270-1470 mg/L)	-	Test material (EC name): sodium salicylate Measured concentrations	Geiger DL, Northcott CE, Call DJ and Brooke LT editors (1985)
method C.1 of the European Directive 92/69/EC and the OECD guideline 203 No GLP RI 3	*Danio rerio*	Mortality	static	96 h	-	>100	-	Test material (EC name): methyl salicylate Nominal concentrations (no analytical monitoring)	Anonymous. (2000)

One study was performed to assess the acute toxicity of methyl salicylate to freshwater fish (*Danio rerio*) under static conditions in accordance with the method C.1 of the European Directive 92/69/EC and the OECD guideline 203 (Anonymous, 2000). A group of ten fish was exposed to different concentrations of methyl salicylate: nominally 1, 10 and 100 mg/L. Observations were made on the number of dead fish and the incidence of sub-lethal effects after 24, 48, 72 and 96 hours exposure. The 96h-LC_{50} for freshwater fish (*Danio rerio*) was found to be higher than 100 mg/L. However, the test item concentration levels were not checked although oily insoluble droplets were observed in the stock solution. It has not been demonstrated that the concentration of the substance being tested has been satisfactorily maintained through the test. Consequently, this study on *Danio rerio* is considered as not reliable.

Therefore, a weight of evidence approach with results obtained on analog substances is applied for the assessment of the toxicity to fish of methyl salicylate. Ethyl salicylate (CAS RN 118 -61 -6) and salicylic acid (CAS RN 69 -72 -7) are used as analog substances.

One reliable study is available for ethyl salicylate for this endpoint. In this acute toxicity study (Geiger *et al.* 1985), fishes from the species *Pimephales promelas* were exposed under flow-through conditions to ethyl salicylate (CAS 118 -61 -6). The average measured concentrations tested were 0 (control), 2.73, 4.82, 7.70, 14.9 and 26.2 mg/L. Twenty five fish were tested in duplicate at each control and tested concentrations. This study was not performed according to GLP but authors followed a method similar to OECD 203 and gave sufficient details to check all validity criteria, which were all fulfilled. Therefore this study is considered as reliable with acceptable restrictions. At 96h, no mortality was observed at 14.9 mg/L and 100% of fishes exposed to 26.2 mg/L died. Then, an LC_{50} could be estimated using the geometric mean between the highest concentration without effect (14.9 mg/L) and the lowest concentration with 100% effect (26.2 mg/L). The resulting approximate LC_{50} (96h) was 19.8 mg/L, based on measured concentrations.

It is proposed to use this data for the assessment of the toxicity to fish of methyl salicylate as a read-across approach. The main assumption to justify the read-across approach is that methyl and ethyl salicylate have a similar chemical structure. Both substances are 2-hydroxybenzoate, one being a methyl ester and the second one being an ethyl ester. Therefore, both substances have the same functional groups in their chemical structure, and the addition of an alkyl "CH2" in the ester function for ethyl salicylate compared to methyl salicylate is not expected to have a significant impact on the biological and physico-chemical properties of the substance.

This assumption is supported by the physico-chemical information which shows that both substances have very similar physicochemical properties (including water solubility and vapour pressure). The $logK_{ow}$ value of ethyl salicylate is slightly higher than the one of methyl salicylate (i. e. 3.09 and 2.55 respectively). It can therefore be expected that ethyl salicylate has higher effect on the biological cells than methyl salicylate, and therefore applying the read-across approach would be a worst case and protective strategy. Even if not completely comparable due to different test conditions, the toxicity data to *Daphnia magna* of both substances show similar conclusion (i. e. $48hEC_{50}$ = 28 mg/L for Ethyl Salicylate and $24hEC_{50}$ = 50 mg/L for Methyl Salicylate).

To support the fact that methyl salicylate is expected to be less toxic than ethyl salicylate, data on salicylic acid is used to show that the 2–hydroxybenzoic acid is less toxic than the methyl ester, and therefore that the lower the 2-hydroxybenzoic form is substituted, the lower is the toxicity. The read-across approach is supported by the physico-chemical information which

shows that both substances have very similar physicochemical properties (including logK_{ow}). But it should be noted that salicylic acid is more soluble in water than methyl salicylate (i. e. 1.5 - 2.6 g/L at 20°C - 25°C and 670 mg/L at ambiant temperature respectively) and less volatile (i. e. 0.0208 Pa at 25°C and 10 Pa at 22°C respectively), but these differences are not expected to impact the results of the aquatic toxicity test at the concentrations tested.

The aquatic toxicity of salicylic acid is assessed based on its sodium salt to avoid pH effect. In the acute toxicity study for this substance (Geiger et al. 1985), fishes from the species *Pimephales promelas* were exposed under flow-through conditions to salicylic acid sodium salt (CAS n° 54 -21 -7) at average measured concentrations of 0 (in duplicate), <50 (in duplicate), 497, 536, 837, 867, 1238, 1272, 2211, 2217, 3442 and 3573 mg/L. The LC_{50} (96h) was 1370 mg/L (CI: 1270 - 1470 mg/L), based on measured concentrations. Therefore, salicylic acid sodium salt is not dangerous to *Pimephales promelas* in the conditions tested.

In conclusion, the result obtained with ethyl salicylate is used in a worst case read-across approach to assess the fish toxicity of methyl salicylate.

11.2.2 Aquatic invertebrates

Summary table – acute invertebrate toxicity							
Method, Guideline, GLP status, Reliability	**Species**	**Endpoint**	**Exposure**		**Results (mg/L)**	**Remarks**	**Reference**
			Design	**Duration**	**LC/EC$_{50}$**		
equivalent or similar to NF T 90301, 1983 No GLP RI 3	*Daphnia sp*	Mobility	Not specified	24 h	IC_{50} = 50	Test material (EC name): Methyl Salicylate Nominal concentrations (no analytical monitoring)	Dion M. (1983)
OECD Guideline 202, EU Method C.2 GLP RI 2	*Daphnia magna*	Mobility	static	48 h	28	Test material (EC name): Ethyl salicylate Measured initial concentrations via DOC analysis	Noak M. (2001)
equivalent or similar to the OECD guideline 202 No GLP RI 2	*Daphnia magna*	Mobility	static	48h	870	Test material (EC name): 2-Hydroxybenzoic acid Nominal concentrations (no analytical monitoring)	Kamaya Y, Fukaya Y and Suzuki K (2005)

One study is available for methyl salicylate for this endpoint (Dion, 1983). This study is a screening AFNOR Test on daphnids with a test duration of 24h. Toxicity has been observed and result is reported as nominal concentration as no analytical monitoring has been performed during the test. Key information describing this study is lacking. Furthermore, based on the uncertainties of the stability of the test item during the test and the duration of exposure of 24 hours instead of 48 hours as required by OECD Testing Guideline, this study is considered as not reliable.

Therefore, similarly to the assessment of acute toxicity to fish, a weight of evidence approach with results obtained on analog substances is applied for the assessment of the toxicity to aquatic invertebrates of methyl salicylate. Ethyl salicylate (CAS RN 118-61-6) and salicylic acid (CAS RN 69-72-7) are used as analog substances.

One reliable key study is available for ethyl salicylate for this endpoint. In this acute toxicity study (Noack M., 2001), the acute immobilization (EC_{50}) of the test item ethyl salicylate to daphnia was determined according to the method C.2 of the European Directive 92/69/EC and the OECD Guideline 202. The study was conducted under static conditions during 48 hours. 20 test organisms were exposed to each test concentration and control. The test item dilutions were clearly dissolved after filtration of the saturated solution in all tested

concentration levels throughout exposure. The real test concentrations were calculated based on DOC-analysis: 9.2, 19, 40, 84 and 165 mg/L. The 48h-EC_{50} values were calculated by probit analysis in the tested concentration range. Exposure of daphnids to ethyl salicylate resulted in a 48h-EC_{50} value of 28 mg/L (95% confidence interval = 27 to 29 mg/L).

As for fish studies, it is proposed to use this data for the assessment of the toxicity to aquatic invertebrates of methyl salicylate as a read-across approach.

The 48 hours acute toxicity study of salicylic acid (hydroxybenzoic acid) to *Daphnia magna* was conducted under static conditions with nominal concentrations from 276 to 2210 mg/L (pH adjusted to 7.45 +/- 0.05). The 48 hours EC_{50} was determined to be 870 mg/L.

In conclusion, the result obtained with ethyl salicylate is used in a worst case read-across approach to assess the toxicity to aquatic invertebrates of methyl salicylate.

11.2.3 Algae and aquatic plants

	Summary table – acute algal toxicity							
Method, Guideline, GLP status, Reliability	**Species**	**Endpoint**	**Exposure**		**Results (mg/L)**		**Remarks**	**Reference**
			Design	**Duration**	**ErC_{50} / EbC_{50}**	**NOEC**		
OECD Guideline 201 (2006), and EU method No 440/2008, C.3 GLP RI 1	*Desmodesmus subspicatus*	Growth rate / Biomasse	Static, closed system	72h	27 / 13 (nominal) **1.6** / 1.1 (geom. mean meas.)	6.25 / **0.79** (nominal / geom. mean meas.) **Aquatic Chronic 3**	Test material (EC name): Methyl salicylate Analytical monitoring	Vryenhoef H. and Mullee D.M. (2010)

Therefore, only one reliable key study is available for this endpoint (Vryenhoef and Mulleer, 2010). The effect of methyl salicylate on the growth of the freshwater green algal species *Desmodesmus subspicatus* was investigated in a 72-hour static test according to OECD Guideline 201 (2006), and the method C.3. of Commission Regulation (EC) No 440/2008, C.3. The study was compliant with the GLP.

Following a preliminary range-finding test, *Desmodesmus subspicatus* was exposed to an aqueous solution of the test item at concentrations of 6.25, 12.5, 25, 50 and 100 mg/L (three replicate flasks per concentration) and a control (six replicate flasks) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C. Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Coulter®Multisizer Particle

Counter. Analysis of the test preparations at 0 hours showed measured test concentrations to range from 97% to 106% of nominal. Analysis of the test preparations at 72 hours showed a concentration dependent decline in measured concentrations in the range of less than the limit of quantitation (LOQ) of the analytical method employed to 24% of nominal.

This decline was in line with two preliminary stability tests performed in aqueous solution and in the same algal conditions test. Those test indicated instability over the 72h test period (see tables 1 and 2 below). In the first preliminary aqueous test, a test sample was tested for stability without prior mixing (sonication) of the test sample bottle to assess for losses due to adsorption and/or insolubility. Since the unsonicated stability results indicated no evidence of insolubility or adherence to glass, the further decline in measured test concentrations was considered by the authors to be due to adsorption of the test item to the algal cells present.

Table 1: Stability of methyl salicylate in aqueous samples:

[MeS]$_{nominal}$ *mg/L*	6.25	25	100
[MeS]$_{72h,\ light}$ *% [C°]$_{initial}$*	**71**	**66**	88
[MeS] $_{72h,\ dark}$ *% [C°]$_{initial}$*	**77**	93	94
[MeS] $_{72h,\ dark,unsonicated\ samples}$ *% [C°]$_{initial}$*	80	-	93

In the table 2, additional stability analyses conducted under identical algal test conditions confirmed the unstable nature of the test item over the 72-Hour exposure period and the losses of the test item below the LOQ (0.19 mg/L) when the algal cells are present.

Table 2: Stability in Aqueous Samples Incubated Under Test Conditions

[MeS]$_{nominal}$ *mg/L*	6.25	25	100
[MeS]$_{72h,\ light}$ **WTHOUT ALGAE** *% [C°]$_{nominal}$*	**8**	**50**	90
[MeS] $_{72h,\ light}$ **WITH ALGAE** *% [C°]$_{nominal}$*	**<LOQ**	**<LOQ**	**77**

According to current regulatory advice that in cases where a decline in measured concentrations is observed, geometric mean measured concentrations should be used for calculating EC_{50} values. Results were not only based on nominal concentrations but also on the geometric mean measured test concentrations in order to give a "worst case" analysis of the data. In cases where the measured concentration was less than the LOQ of the analytical method following current regulatory advice a value of half the LOQ (i. e. 0.095 mg/L) was used to enable calculation of the geometric mean measured concentration.

The results obtained with nominal concentrations were as follows:

72h-ErC50 = 27 mg/L (growth rate)

72h-EbC50 = 13 mg/L (biomass)

72h-NOEbC/ NOErC = 6.25 mg/L (growth rate and biomass)

The results obtained with the geometric mean of the measured concentrations were as follows:

72h-ErC50 = 1.6 mg/L (growth rate)

72h-EbC50 = 1.1 mg/L (biomass)

72h-NOEbC/ NOErC = 0.79 mg/L (growth rate and biomass)

The high level of methyl salicylate decrease observed in this study when algae are present in the assay medium has been attributed by the author, to adsorption of the substance on algal cells. This unverified hypothesis is inconsistent with the substance water solubility and log K_{ow} which do not let predict such a strong adsorption. The moderate volatility of methyl salicylate has been taken into account in the experiment by using flasks plugged with polyurethane foam bungs.

The authors have investigated whether methyl salicylate metabolization could take place in algae in order to explain the instability of the substance. All proposed hypotheses are based on literature data on various algae enzymes able to metabolize a variety of chemical among which esterase like carboxyesterase (review of Takagi, 2010). However, these hypotheses have not been verified in the study of Vryenhoef and Mullee (2010) with *D. subspicatus* exposed to methyl salicylate. The authors argued that decline of methyl salicylate could be due to a combination of both the unstable nature of the test item and also adsorption of the test item to the algal cells present, and that it is not possible to determine precisely the concentrations to which the algal cells were exposed.

Therefore, the results obtained with the geometric mean of the measured concentrations have to be considered as the relevant algal toxicity values.

11.3 Chronic toxicity

No additional chronic data, other than the above algae NOEC of 0.79 mg/L, is available.

11.4 Comparison with CLP criteria

11.4.1 Acute aquatic hazards

The lowest L(E)C_{50} obtained in acute aquatic toxicity studies is 1.6 mg/L, in the algae *Desmodesmus subspicatus.* This value is above the classification threshold value of 1 mg/L. Methyl salicylate does therefore not fulfil the criteria for classification as acute hazard to the aquatic environment.

11.4.2 Chronic aquatic hazards

Based on a **weight of evidence approach,** Methyl salicylate is rapidly degradable in the environment. **This substance has a low potential for bioaccumulation (i. e. the substance has a log Kow < 3).**

Chronic aquatic toxicity information is available for only one trophic level, the lowest NOEC available is 0.79 mg/L obtained in algae study. **Therefore, according to the table 4.1.0 (b) ii,** this value is **between 0.1 mg/L and 1 mg/L.** Methyl salicylate does therefore fulfil the criteria for classification as a chronic hazard category 3, H412 to the aquatic environment.

Nevertheless, as chronic data are available for only one trophic level, the proposed classification should also be compared with the classification based on acute data according to figure 4.1.1 of the CLH Regulation. **table 4.1.0 (b) iii.** The lowest L(E)C_{50} obtained in acute aquatic toxicity studies is 1.6 mg/L, in the algae *Desmodesmus subspicatus*. The substance is rapidly degradable in the environment and has a log Kow <3. Therefore, based on the acute toxicity data, no classification is needed.

However, the most stringent outcome should be retain for classification. Thus, Methyl salicylate fulfils the criteria for classification as a chronic hazard category 3, H412 to the aquatic environment.

11.5 Conclusion on classification and labelling for environmental hazards

Based on the lowest aquatic acute toxicity values of more than 1 mg/L and the lowest aquatic chronic values between 0.1 mg/L and 1 mg/L, methyl salicylate need to be classified as chronic hazard category 3, H412 **with respect to the aquatic environment according to the Regulation (EC) No 1272/2008.**

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as Aquatic Chronic 3; H412. The lowest L(E)C_{50} obtained in acute aquatic toxicity studies was 1.6 mg/L for the algae *Desmodesmus subspicatus*. This value was above the classification threshold value of 1 mg/L and no acute classification was warranted. The substance was considered rapidly degradable based on a weight of evidence approach. Based on the log K_{OW} < 3, MeS had a low potential for bioaccumulation. The lowest NOEC value was 0.79 mg/L for algae which for a rapidly degradable substance was the basis for the proposed Aquatic Chronic 3; H412 classification.

Degradation

There was one non-GLP ready biodegradability study available performed according to a draft Ecotoxicology Section Standard Operating Procedure No 158 01, which is based on OECD TG 301B. The test showed 98.4 % degradation after 28 days with a test substance concentration of 10 mg/L. There were no data on the toxicity control and the raw data for the blank and test samples are not available. Due to the lack of data, this study was only used as supportive information. A second non guideline study focused on the ability of a microbial mixture (five *Pseudomonas*, one *Klebsiella*, four *Rhodococci* and two fungal strains) to degrade a representative sample of methylated and chloro-methylated compounds. MeS at a concentration of 200 mg/L degraded 100 % in 7 days. The inoculum used did not correspond to recommendations for ready biodegradability testing. Additionally, only primary biodegradation was measured and not ultimate biodegradation. This study was used only as supportive information. A screening-level hazard characterisation made on benzyl derivatives category showed the ready biodegradability for all members of the group, which include MeS. Furthermore, the QSAR predictions with BIOWIN 4.10 indicated that all 2-hydroxybenzoate esters subcategory III from the US EPA report (among which MeS belongs) were readily biodegradable substances. Consequently, the DS applied a weight of evidence approach for considering MeS as a rapidly degradable substance.

Bioaccumulation

MeS had a low potential for bioaccumulation based on the experimental log K_{OW} = 2.55, which was below the cut-off value of 4 for bioaccumulation. The purity of the test substance was not given. The REACH Registration Dossier (full, 15/08/2019) indicates that the value originates from the databank of Sangster (1989), which was reported as a key reference in the ECHA IR & CSR Guidance Document. The databank contains experimental log K_{OW} data, retrieved from the literature, on over 20 000 organic compounds. For each compound, whenever possible, the

compiler gives the log K_{OW} value which, in their judgment, is closest to the true value. For MeS, this databank reports 5 experimental log K_{OW} values ranging from 2.08 to 2.98. The recommended value of 2.55 is selected as key value for the assessment of the substance.

Aquatic toxicity

***Table**: Reliable aquatic toxicity data on MeS, ethyl salicylate and sodium salicylate and 2-salicylic acid*

Test substance	Method	Species	Endpoint	Exposure	Results mg/L	Reference
Fish						
Ethyl salicylate	equivalent or similar to OECD TG 203, not GLP	*Pimephales promelas*	Mortality	96 h flow-through	LC_{50}: 19.8 mm (*	Geiger *et al.*, 1985a
Sodium salicylate	equivalent or similar to OECD TG 203, not GLP	*Pimephales promelas*	Mortality	96 h flow-through	LC_{50}:1 370 mm	Geiger *et al.*, 1985b
Invertebrates						
Ethyl salicylate	OECD TG 202, GLP	*Daphnia magna*	Mobility	48 h static	EC_{50}: 28 (im, DOC analysis) (**	Noak, 2001
2-salicylic acid	equivalent or similar to OECD TG 202, not GLP	*Daphnia magna*	Mobility	48 h static	EC_{50}: 870 Nominal concentration, no analytical monitoring	Kamaya *et al.*, 2005
Algae						
MeS	OECD TG 201, GLP	*Desmodesmus subspicatus*	Growth rate	72 h static, closed system	ErC_{50}: 1.6 (1 NOEC: 0.79 (1 ErC_{50}: 1.475 (2 NOEC: 0.79 (2 ECr_{10}: 1.033 (2 geometric mm	Vryenhoef and Mullee, 2010

mm = mean measured concentrations
im= initial measured concentrations
(*the stability of the substance cannot be estimated in regard to nominal concentration that were not mentioned in the publication. But regarding measured concentration, it is clear that in every replicate considered separately the concentration of test substance was satisfactorily maintained during the test. Therefore, the DS considered the validity criteria fulfilled. Mean measured concentrations corrected for percent recovery: 2.73, 4.82, 7.70, 14.9 and 26.2 mg/L. It should be noted that samples were not taken at 96 h (the mean measured concentration was calculated from measurements at t0, 24, 48 and 72 h)
(** test concentrations have only been measured at 0 h
(1 Geometric mean values calculated using data on 0 and 72 h
(2 Information from PC comments, geometric mean values calculated using data on 0, 49 and 72 h

Basis for read-across

The main assumption to justify the read-across approach is that methyl and ethyl salicylate have a similar chemical structure. Both substances are 2-hydroxybenzoate, methyl ester and ethyl ester, respectively. Therefore, both substances have the same functional groups in their chemical structure, and the addition of an alkyl group "-CH_2-" in the ester function for ethyl salicylate compared to MeS was not expected to have a significant impact on the biological and physico-chemical properties of the substance.

This assumption is supported by the very similar physico-chemical properties of the

substances, including water solubility and vapour pressure. The log K_{OW} value of ethyl salicylate was slightly higher than the one for MeS (i.e. 3.09 and 2.55 respectively). Therefore, it could be expected that ethyl salicylate has higher effects on biological cells than MeS, and application of the read-across approach represent a worst case scenario.

To support the assumption that MeS is less toxic than ethyl salicylate, data on SA was used to show that the 2–hydroxybenzoic acid is less toxic than the methyl ester, and therefore that the toxicity of the substituted 2-hydroxybenzoic form is proportional to the length of the substituent.

Acute Aquatic toxicity

Fish

The only acute fish toxicity test with MeS was not reliable because of uncertainty regarding the test substance concentration during the test. The DS used data on the analogous substances ethyl salicylate and SA in a weight of evidence approach. The tests followed a method similar to OECD TG 203. The validity criteria were fulfilled in the test with ethyl salicylate where the measured concentrations were 0 (control), 2.73, 4.82, 7.70, 14.9 and 26.2 mg/L. At 96 h, no mortality was observed at 14.9 mg/L and 100 % of fish exposed to 26.2 mg/L died. The resulting approximate LC_{50} (96 h) was 19.8 mg/L, based on mean measured concentrations. The aquatic toxicity of SA was assessed based on its sodium salt to avoid the pH effect. In the acute toxicity study with *Pimephales promelas*, fish were exposed to SA sodium salt at average measured concentrations of 0, < 50, 497, 536, 837, 867, 1 238, 1 272, 2 211, 2 217, 3 442 and 3 573 mg/L. The LC_{50} (96 h) was 1 370 mg/L based on mean measured concentrations.

In conclusion, the result obtained with ethyl salicylate was used in a worst-case read-across approach to assess the fish toxicity of MeS.

Invertebrates

There was no reliable acute toxicity study available on MeS. Therefore, similar to the assessment of acute toxicity to fish, a weight of evidence approach was applied for the assessment of the toxicity to aquatic invertebrates of MeS. Ethyl salicylate and SA were used as analogous substances. One reliable key study is available for ethyl salicylate for this endpoint. In this acute *D. magna* toxicity study, the acute immobilization of the test item was determined according to OECD TG 202 following GLP. The initial test concentrations were determined based on DOC analysis: 9.2, 19, 40, 84 and 165 mg/L. Exposure of daphnids to ethyl salicylate resulted in a 48 h EC_{50} value of 28 mg/L (initially measured concentration).

The 48 h acute toxicity study of SA to *D. magna* was conducted under static conditions with nominal concentrations from 276 to 2 210 mg/L. The 48 h EC_{50} was determined to be 870 mg/L.

In conclusion, the result obtained with ethyl salicylate is used in a worst-case to assess the toxicity to aquatic invertebrates of MeS.

Algae and aquatic plants

There was one reliable study available for acute algae toxicity using MeS. Its effect on the growth of the freshwater green algae *Desmodesmus subspicatus* was investigated in a 72 h static test according to OECD TG 201 and GLP compliant.

Following a preliminary range-finding test, *Desmodesmus subspicatus* was exposed at concentrations of 0 (control) 6.25, 12.5, 25, 50 and 100 mg/L. The initial concentration were

analytically confirmed, however at 72 hours a concentration dependent decline in measured concentrations was observed. Thus, geometric mean measured concentrations were used for calculating EC_{50} values. The 72 h EC_{50} was 1.6 mg/L for growth rate.

Chronic aquatic toxicity

There was no chronic toxicity data available for fish and invertebrates. The 72 h NOEC for growth rate and biomass of 0.79 mg/L resulted from the only algae test available.

Comments received during consultation

Comments were received from one MSCA and from a leading group of REACH registrants.

The registrants pointed out that the available OECD TG 201 algae study had deficiencies and should not be used for classification. They submitted an assessment of the study report focusing on paragraphs 37 and 40 of OECD TG 201. According to the assessment, the current OECD TG 201 study did not allow a final assessment of chronic toxicity in algae due to failures in the methodology mainly regarding analytical measurement. Moreover, the EC_{10} for growth rate calculated in the assessment was > 1 mg/L indicating that the study most likely overestimates the effects of MeS. More details of the assessment report are described under the Additional Key Elements. The DS answered that they did not include time points at 24- and 48 h following the REACH Guide R7b p. 75: "*For static tests, where the concentrations do not remain within 80-120 % of nominal, the effect concentrations should be expressed relative to the geometric mean of the measured concentrations at the start and end of the test*." They considered that the study should be used for classification. The commenting MSCA agreed with the DS.

The MSCA paid attention to the available fate data. The ready biodegradation test is a non-GLP test with limited reliability due to lack of raw data. In addition, the reported Henry's law constant of 4.76 $Pa \cdot m^3/mol$ indicates the test item may be lost from the aquatic phase meaning the test method may not be the most appropriate. Additional supporting fate data involving non-standard methods and/or inoculum and unknown sample composition were included. While QSAR data were quoted, it is unclear if the test item fell within the model domain and if the QSARs were valid. Additional information would be welcomed to support MeS as rapidly degradable e.g. read-across with reliable analogue data and/or valid QSARs. Without this information a substance would normally be considered not rapidly degradable. In the answer to the consultation comments, the DS presented QSAR calculations made with EPISUITE and the Danish QSAR Database, which showed that MeS was readily biodegradable while being in the applicability domain of each model.

The MSCA also asked if there was analytical verification at study termination in the ethyl salicylate *D. magna* test giving a 48 h EC_{50} of 28 mg/L based on initial measured concentrations. The DS answered that the study was considered of reliability 2. The test item was only analytically verified at the beginning of the test. However, the test item instability during experiment was specifically reported for the algae study with MeS and not in the other assays. The DS was of the view that the stability of ethyl salicylate in other experiments confirmed that the EC_{50} (48 h) value of 28 mg/L for *D. magna* is acceptable and the instability of the test item was specific to the algae study.

The MSCA also thought that it is unclear if algae were the most chronically sensitive trophic level. They welcomed additional toxicity data for relevant analogues with a clear read-across justification. The DS informed that no additional chronic aquatic toxicity data on the level was

available on analogues. Classification based on one toxicity data for one trophic level is allowed. In addition, the DS presented QSAR data from the Danish QSAR database where the acute toxicity for the 3 trophic levels are in the same order of magnitude. According to the DS, MeS was included in the applicability domains.

Additional key elements

Biodegradation

RAC performed a BIOWIN v.4.10 estimation on MeS. The estimation shows that MeS is predicted to be readily degradable.

Probability of Rapid Biodegradation (BIOWIN v.4.10)	
Biowin1 (Linear Model)	0.9651; biodegrades fast
Biowin2 (Non-Linear Model)	0.9971; biodegrades fast
Expert Survey Biodegradation Results	
Biowin3 (Ultimate Survey Model)	3.0595 weeks
Biowin4 (Primary Survey Model)	3.8969 days
MITI Biodegradation Probability	
Biowin5 (MITI Linear Model)	0.7007; readily degradable
Biowin6 (MITI Non-Linear Model	0.8275; readily degradable
Anaerobic Biodegradation Probability	
Biowin7 (Anaerobic Linear Model)	0.6274; biodegrades fast
Ready Biodegradability Prediction: YES	

Algae toxicity, assessment of the original study

The assessment report concerns interpretation of the biological results of Harlan Study No 1975/0003, Algae Growth Inhibition Test with MeS (CAS: 119-36-8) and re-evaluation of EC_X-values and concentration effect curves. Selected details of the report are presented in the BD.

Decline of Test Item Concentrations

During the main test, a strong decline of test item concentrations was observed. From a stability pre-experiment, the most important cause of the substance degradation was identified as absorption/metabolism by the algae cells.

Inhibitory Effect on Algae Growth

In the two lowest concentration a decrease of algae growth inhibition at 72 h was observed which could be explained by the degradation of the test item (measured concentration at 72 h were 0, 1.1, 1.5, 2.2, 50 mg/L). However, this conclusion is partly compromised by the lack of a clear concentration effect relationship at 49 hours, which cannot be explained from a biological point of view. The inhibitions at 49 h were 0, -80.6, 62.7, 39.2, 150, 98.2 % for

nominal concentrations of 0, 6.25, 12.5, 25, 50, 100 mg/L, respectively. Therefore, as no analytical results are available for 25 and 49 hours, an assessment of the decrease of inhibition is not possible for these test concentrations.

Decline of Test Item Concentrations

During the main test, a strong decline of test item concentrations was observed. The test item concentrations measured at the start of test confirmed the correct preparation of the test media. At the end of the test, the test item concentrations were below LOQ at the lower test concentrations and were 24 mg/L at the highest test concentration.
Stability pre-experiments were performed (before and after the main test) for better understanding of the behaviour of the test item in test water.
From the results of these tests, it is concluded that the decrease of the test item concentrations could be caused by hydrolysis (small part), photolysis (more likely) and absorption/metabolism by the algae cells (very likely). Furthermore, adsorption on the surfaces of the test vessels are indicated although the specific properties of the molecule make this unlikely.

Conclusions

In summary, due to the degradation of the test item and the decrease of inhibition of algae growth, the geometric mean concentrations should be used. However, as no analytical measurements were performed at 25 and 49 hours, the decline of test item concentrations is not well documented which affects the calculated endpoints values. In addition, a precise comparison of the decline of test concentrations with the decrease of algae growth inhibition is not possible. Furthermore, a clear interpretation of the biological results is partly compromised by the lack of a concentration effect relationship after 49 hours. This cannot be explained from a biological point of view and is in contrast to the results after 25 and 72 hours where a clear concentration effect relationship was observed.

Algae toxicity, assessment of the original study

Inhibitory Effect on Algae Growth

After analysis of the growth curves (Figure 1 of the Harlan-Report) and the section by section growth rates (see table below), it is generally concluded that there is a decrease of algae growth inhibition after 49 hours (going in line with the degradation of the test item).

Treatment / Dilution	Mean Measured Concentration [µg/L]	Section-by-Section Growth Rates [day^{-1}] and Inhibition I_r [%]					
		0-25 h		25-49 h		49-72 h	
		µ [day^{-1}]	I_r [%]	µ [day^{-1}]	I_r [%]	µ [day^{-1}]	I_r [%]
Control	Control	0.975	0.0	1.466	0.0	1.654	0.0
6.25	0.79	0.848	13.0	0.284	80.6	3.093	-87.0
12.5	1.1	0.678	30.4	0.547	62.7	2.057	-24.4
25	1.5	0.480	50.8	0.891	39.2	0.829	49.9
50	2.2	0.365	62.5	-0.733	150.0	0.158	90.4
100	50	-0.684	17 0.1	0.026	98.2	0.016	99.0

Calculations performed at IES based on data as stated in the Harlan Report

However, this conclusion is partly compromised by the lack of a clear concentration effect relationship after 49 hours, which cannot be explained from a biological point of view.
Nevertheless, a reduction (negative inhibition) of growth rate inhibition during the third section (49-72 hours) is indicated at least for the two lowest test concentrations of nominal 6.25 and 12.5 mg/L. This observation is supposed to be due to the degradation of the test item (test item no longer in the system), but clear evidence for this statement is not provided as no analytical results are available after 25 and 49 hours test duration.

At the two highest test concentrations of nominal 50 and 100 mg/L, a high toxic effect on the algae was observed. The algae populations did not increase until the end of the test, i.e. the cell numbers were in the range of the starting values during the whole test period. This may be due to an irreversible toxic effect on the algae at the beginning of the test. Therefore, as no analytical results are available for 25 and 49 hours, an assessment of the decrease of inhibition is not possible for these test concentrations.

Conclusions

In summary, due to the degradation of the test item during the exposure period and due to the decrease of inhibition of algae growth, the geometric mean concentrations should be taken into consideration for calculation of the endpoints (according to OECD TG 201, §40). However, the study presents some methodological issues, which lower the reliability of the study. As no analytical measurements were performed after 25 and 49 hours, the decline of test item concentrations is not well documented, i.e. it is not known at which time point the concentrations reached levels < LOQ. As a consequence, a precise comparison of the decline of test concentrations with the decrease of algae growth inhibition is not possible.

As a further consequence, and as it is assumed that the measured concentrations would be > LOQ at least after 25 hours and at least for the higher test concentrations, the calculation of the geometric mean measured test item concentrations based on analytical measurements at all intervals would have resulted in higher values. This would have resulted in higher endpoint values (NOEC, EC_x-values), considering that these are based on geometric mean concentrations.

A clear interpretation of the biological results is partly compromised by the lack of a clear

concentration effect relationship after 49 hours. This cannot be explained from a biological point of view and is in contrast to the results after 25 and 72 hours where a clear concentration effect relationship was observed.

Summary of results:

Growth rate

	EC10	0.629	0.276	1.033
95%-CL	lower	0.391	0.160	0.930
	upper	0.799	0.381	1.109
	EC20	0.871	0.426	1.167
95%-CL	lower	0.639	0.288	1.083
	upper	1.030	0.539	1.232
	EC50	1.622	0.978	1.475
95%-CL	lower	1.436	0.859	1.416
	upper	1.904	1.079	1.541
Growth rate	LOEC	1.100	<=0.790	1.100
	NOEC	0.790	<0.790	0.790

Aquatic toxicity

RAC has made estimations with ECOSAR v.1.11 to complement the very scarce dataset on aquatic toxicity on MeS. The estimated data and the test data provided by the DS are presented in the table below.

***Table**: Data available for evaluation of aquatic toxicity of MeS*

	MeS (1				Ethyl salicylate (2			
	ECOSAR Class Esters	ECOSAR Class Phenols	ECOSAR Baseline toxicity		ECOSAR Class Esters	ECOSAR Class Phenols	ECOSAR Baseline toxicity	
	Predicted mg/L			Data mg/L	Predicted mg/L			Data mg/L
Fish, 96 h LC_{50}	9.034	9.189	35.874		5.098	4.249	14.191	19.8 (3 mm (*
Daphnids 48 h LC_{50}	17.604	3.620	21.537		9.425	2.083	8.915	EC_{50}: 28 (3 (im) (**
Green algae 96 h EC_{50}	6.809	15.702	20.201	72 h E_rC_{50}: 1.6 (3 1.475 (5 mm	3.372	8.614	10.086	72 h E_rC_{50}: 9.47 (im) (4
Fish ChV	0.599	1.013	3.745		0.305	0.504	1.563	
Daphnids ChV	10.118	0.688	2.453		4.704	0.396	1.152	
Green algae ChV	2.123	7.320	5.989	72 h NOEC: 0.79 (3 EC_{10}:	1.233	3.991	3.308	72 h EC_{10}: 7.89 (im) (4

				1.033 (5 (mm)				
Fish (SW) 96 h LC_{50}	13.244	3.703	-		7.252	1.564	-	
Mysid 96 h LC_{50}	9.675	-	-		4.159	-	-	
Fish (SW) ChV	2.116	-	-		1.266	-	-	
Mysid (SW) ChV	208.065	-	-		32.875	-	-	

(1 Log K_{OW} 2.604, water solubility 700 mg/L
(2 Log K_{OW} 3.095, water solubility 3 317 mg/L
(3 From the CLH Report
(4 REACH Registration
(5 Consultation comments
(im) = measured initial concentration
(mm) = mean measured concentration
(*the stability of the substance cannot be estimated in regard to nominal concentration that were not mentioned in the publication. But in regard to measured concentration, it is clear that in every replicate considered separately the concentration of test substance was satisfactorily maintained during the test. Therefore, the DS considers the validity criteria fulfilled.
(** test concentrations have only been measured at 0 h
ChV = The ChV, or Chronic Value, is defined as the geometric mean of the NOEC and LOEC.

Assessment and comparison with the classification criteria

Degradation

In a ready biodegradability study performed according to OECD TG 301B with adaptations for volatile substances (sealed vessel), the biodegradation was 98.4 % after 28 days. The 10-day window criteria were fulfilled. Consequently, MeS was considered readily biodegradable. The study report lacked information needed for validity checking e.g. information on replicates and CO_2 evolution in the inoculum blank at the end of the test. The study report by King from 1993, The Biodegradability of Perfume Ingredients in the Sealed Vessel Test, refers to study report published in Chemosphere, Vol. 23, No 4, pp. 507-524 in 1991 for development and validation of the method used. This publication by Birch and Fletcher is titled The Application of Dissolved Inorganic Carbon Measurements to the Study of Aerobic Biodegradability. The article was about developing a test that is essentially the same as the Sturm CO_2 Production tests (OECD TG) but with greater simplicity of the technique and the high precision of the data. It did not include any validity criteria as such. This study has been referenced and used as the basis of OECD TG 310.

While the ready biodegradability study report on the does not contain all information needed for checking its validity, on the other hand, the referenced publication strengthens the case. Considering the supporting data provided by the DS together with the BIOWIN v.4.10 estimation, RAC considers MeS as rapidly degradable for classification.

Bioaccumulation

RAC agrees with the DS that MeS has a low potential for bioaccumulation based on the experimental log K_{OW} = 2.55. The KOWWIN v.1.68 in EPISUITE gives an estimated log K_{ow} of 2.60. Both values were below the cut-off value of 4 for bioaccumulation.

Aquatic toxicity

The only data on the aquatic toxicity on MeS is from an OECD TG 201 algae test giving a 72 h E_rC_{50} of 1.6 mg/L and a 72 h NOEC of 0.79 mg/L as geometric mean measured concentrations.

No EC_{10} value is available. The assessment report on the original test report also gave results using measured concentrations at 49 h: 72 h E_rC_{50} of 1.475, 72 h E_rC_{10} of 1.033 mg/L and 72 h NOEC of 0.79 mg/L.

RAC agrees with the DS to read-across aquatic toxicity data from ethyl salicylate. RAC also supports the DS's view that data on SA shows that the 2–hydroxybenzoic acid is less toxic than the methyl ester, and therefore that the toxicity of the substituted 2-hydroxybenzoic form is proportional to the length of the substituent. MeS was expected to be less toxic than ethyl salicylate which is supported by the ECOSAR v.1.11 estimations. For ethyl salicylate, there is information for fish and *D. magna*. For fish, there is an OECD TG 203 fish test available giving a 96 h LC_{50} of 19.8 mg/L. The DS considered the test valid but the test report does not give information on nominal concentrations to allow the comparison with the measured ones. The DS observed that in every replicate considered separately the concentration of test substance was satisfactorily maintained during the test. For *D. magna* there is an OECD TG 202 test giving a 48 h EC_{50} of 28 mg/L based on initial measured concentrations. The concentrations have not been followed during the test.

Assessing the data on methyl and ethyl salicylate together there were reliable acute aquatic toxicity data on algae and fish and reliable chronic aquatic toxicity data on algae.

The DS based their classification proposal on the chronic algae data namely a 72 h NOEC of 0.79 mg/L. RAC agrees with this approach.

RAC has gathered data on methyl and ethyl salicylate via ECOSAR v.1.11 estimations to complement the very scarce database on MeS aquatic toxicity. The data is presented in the BD. The estimations show a great variability depending on the class of the substance and the endpoint in question.

Comparison with CLP criteria

Acute aquatic hazards

RAC agrees with the DS that **no acute aquatic classification is warranted** for MeS. RAC's opinion is based on the algae study result 72 h E_rC_{50} of 1.6 mg/L.

Chronic aquatic hazards

RAC agrees with the DS that MeS warrants classification as **Aquatic Chronic 3; H412** based on the 72 h NOEC of 0.79 mg/L and rapid degradability of the substance. The ECOSAR v.1.11 calculations show that algae are not necessarily the most sensitive trophic level. Thus, the classification might have to be revisited in case of new information.

12 EVALUATION OF ADDITIONAL HAZARDS

13 ADDITIONAL LABELLING

[If relevant, please justify here the reason for supplemental hazard information in accordance with Annex II of the CLP Regulation.]

14 REFERENCES

Anonymous. 1978. Methyl salicylate: studies of osseous changes in the rat, reproduction in the rat and mouse and liver and kidney effects in the dog.

Anonymous. 2000. Methyl salicylate: Acute toxicity for freshwater fish (96 Hours) Danio rerio. Testing laboratory: SEPC (Société d'Ecotoxicologie et de Physico-Chimie, ZAC de Milieux, 42160 Andrezieux Boutheon (France). Report no.: 00-918013-008. Owner company: Rhône Poulenc Industrialisation. Report date: 2000-09-19.

Anonymous. 2001. Methyl salicylate: Safety Assessment Screen Comprising: Acute eye irritation and delayed dermal sensitisation.

Addo HA, Ferguson J, Johnson BE, Frain-Bell W, 1982. The relationship between exposure to fragrance materials and persistent light reaction in the photosensitivity dermatitis with actinic reticuloid syndrome. Br J Dermatol.; 107(3): 261–74.

Adenuga D, Woolhiser MR, Gollapudi BB, Boverhof DR. 2012. Differential gene expression responses distinguish contact and respiratory sensitizers and nonsensitizing irritants in the local lymph node assay. Toxicol Sci.;126(2):413-25. doi: 10.1093/toxsci/kfs071.

Arts JHE, Droge SCM, Spanhaak S, Bloksma N, Penninks AH, Kuper CF. 1997. Local lymph node activation and IgE responses in Brown Norway and Wistar rats after dermal application of sensitizing and non-sensitizing chemicals. Toxicology.; 117(2-3):229-34.

Ashby J, Basketter DA, Paton D, Kimber I. 1995. Structure activity relationships in skin sensitization using the murine local lymph node assay. Toxicology.;103(3):177-94.

Basketter DA, Gerberick GF, Kimber I. 1998. Strategies for identifying false positive responses in predictive skin sensitization tests. Food Chem Toxicol.;36(4):327-33.

Basketter DA, Scholes EW. 1992. Comparison of the local lymph node assay with the guinea pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol., 30(1):65-9.

Chapin RE and Sloane RA. 1997. Reproductive assessment by continuous breeding: Evolving study design and summaries of ninety studies. Environ Health Perspect.;105 (S1):199-205.

Chyka PA, Erdman AR, Christianson G, Wax PM, Booze LL, Manoguerra AS, Caravati EM, Nelson LS, Olson KR, Cobaugh DJ, Scharman EJ, Woolf AD, Troutman WG; American Association of Poison Control Centers; Healthcare Systems Bureau, Health Resources and Services Administration, Department of Health

and Human Services. 2007. Salicylate poisoning: an evidence-based consensus guideline for out-of-hospital management. Clin Toxicol (Phila).;45(2):95-131.

Collins TF, Hansen WH, Keeler HV. 1971. Effect of methyl salicylate on rat reproduction. Toxicol Appl Pharmacol.;18(4):755-65.

Cosmetic Ingredients Review (CIR) Expert Panel. 2003. Safety Assessment of Salicylic Acid, Butyloctyl-, Calcium-, C12-15 Alkyl Salicylate, Capryloyl Salicylic Acid, Hexyldodecyl-, Isocetyl-, Isodecyl-, Magnesium-, MEA-, Ethylhexyl-, Potassium-, Methyl-, Myristyl-, Sodium-, TEA-, and Tridecyl Salicylate. Int J Toxico.; 22(S3):1-108.

Davison C, Zimmerman EF, Smith PK. 1961. On the metabolism and toxicity of methyl salicylate. J Pharmacol Exp Ther.;132: 207-11.

de Groot AC., Coenraads PJ, Bruynzeel DP, Jagtman BA, van Ginkel CJW, Noz K, van der Valk PGM, Pavel S, Vink J, Weyland JW. 2000. Routine patch testing with fragrance chemicals in The Netherlands. Contact Dermatitis.; 42(3):184–5.

DevTox website: http://www.devtox.org/index_en.php

Dion M. (1983). Détermination de la toxicité aiguë à l'égard de la daphnie de seize substances chimiques. Testing laboratory: Centre de recherches de Décines, 24 avenue Jean-Jaurès 69150 Décines - B. P. 5.

ECHA website: https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.003.925

Farid H, Wojcik MH, Christopher KB. 2011. A 19-year-old at 37 weeks gestation with an acute acetylsalicylic acid overdose. NDT Plus.; 4(6): 394–6. doi: 10.1093/ndtplus/sfr104

Fergurson J, Sharma S. 1984. Cinnamic aldehyde test concentrations. Contact Dermatitis.; 10(3):191–2.

Food and Drug Administration (FDA). Center for Drug Evaluation and Research. 2006. Pharmacology / Toxicology review and evaluation. FS-67 Patch (10% Methyl salicylate & 3% l-menthol Topical patch). NDA number 22-029.

Geiger D. L., Northcott C. E., Call D. J. and Brooke L. T. (1985). Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas). Center for Lake Superior Environmental Studies - vol II: 203-206. Testing laboratory: The majority of the tests were conducted at the U. S. EPA Environmental Research Laboratory-Duluth, MN; with the remainder completed at the University of Wisconsin-Superior campus.

Geiger DL, Northcott CE, Call DJ and Brooke LT editors (1985). Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas). Center for Lake Superior Environmental Studies - vol II: 141-144, 330. Testing laboratory: The majority of the tests were conducted at the U. S. EPA Environmental Research Laboratory-Duluth, MN; with the remainder completed at the University of Wisconsin-Superior campus.

Gerberick GF, House RV, Fletcher ER, Ryan CA. 1992. Examination of the Local Lymph Node Assay for Use in Contact Sensitization Risk Assessment. Fundam. & Appl. Toxicol., 19(3):438-45.

Goulding C., Gillen C. J. & Bolton E. (1988). Biodegradation of substituted benzenes. Journal of Applied Bacteriology, 65(1), 1-5.

Greene T, Rogers S, Franzen A & Gentry R. 2017. A critical review of the literature to conduct a toxicity assessment for oral exposure to methyl salicylate. Crit Rev Toxicol., 47(2):98-120. Doi: 10.1080/10408444.2016.1236071

Hernandez RK, Werler MM, Romitti P, Sun L, Anderka M; National Birth Defects Prevention Study. 2012. Nonsteroidal antiinflammatory drug use among women and the risk of birth defects. Am J Obstet Gynecol.; 206(3):228.e1-8. doi: 10.1016/j.ajog.2011.11.019.

Hindson C. 1977. Contact eczema from methyl salicylate reproduced by oral aspirin (acetyl salicylic acid). Contact Dermatitis.; 3(6):348-9.

Hou F, Xing C, Li B, Cheng J, Chen W, Zhang M. 2015. Application of BALB/c mouse in the local lymph node assay:BrdU-ELISA for the prediction of the skin sensitizing potential of chemicals. J Pharmacol Toxicoll Methods; 72: 53-8. doi: 10.1016/j.vascn.2015.01.001.

Patty. 1958. Industrial Hygiene and toxicology. 2nd Ed. Vol. 2 p 1897, Interscience, NY.

Infurna R, Beyer B, Twitty L et al. 1990. Evaluation of the dermal absorption and teratogenic potential of methyl salicylate in a petroleum based grease. Teratology.; 41:566.

Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh. 1964. Food flavourings and compounds of related structure. I. Acute oral toxicity. Fd Cosmet. Toxicol.; 2:327-43. doi: 10.1016/S0015-6264(64)80192-9

Kamaya Y, Fukaya Y and Suzuki K (2005). Acute toxicity of benzoic acids to the crustacean Daphnia magna. Chemosphere 59 255-261.

Kimber I, Hilton J, Dearman RJ, Gerberick GF, Ryan CA, Basketter DA, Lea L, House RV, Ladics GS, Loveless SE, Hastings KL. 1998. Assessment of the skin sensitization potential of topical medicaments using the local lymph node assay: an interlaboratory evaluation. J Toxicol Environ Health A.; 53(7):563-79

Kimber I, Hilton J, Botham PA, Basketter DA, Scholes EW, Miller K, Robbins MC, Harrison PT, Gray TJ, Waite SJ. 1991. The murine local lymph node assay: results of an inter-laboratory trial. Toxicol Lett.;55(2):203-13.

King J. M. H. (1993). The biodegradability of Perfume Ingredients in the Sealed Vessel Test. Testing laboratory: Ecotoxicology Section, Unilever Research, Port Sunlight Laboratory. Report no.: 34961. Owner company: Unilever. Report date: 1993-11-12.

Klecak G, Geleick H, Frey JR. 1977. Screening of fragrance materials for allerginicity in the guinea pig. Journal of the Society of Cosmetic Chemists.; 28(2): 53-64.

Kristensen DM., Hass U., Lesné L., Lottrup G., Jacobsen PR., Desdoits-Lethimonier C., Boberg J., Petersen JH., Toppari J., Jensen TK., Brunak S., Skakkebæk NE., Nellemann C., Main KM., Jégou B., Leffers H.

2011. Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. Human Reproduction. ; 26(1): 235–44.

Lamb J, Gulati D, Choudhury H, Chambers R, Sabharwal. 1997. Methyl salicylate. Environmental Helath Perspectives.; 105 (S1).

Lapczynski A, Jones L, McGinty D, Bhatia SP, Letizia CS, Api AM. 2007. Fragrance material review on methyl salicylate. Food and Chemical Toxicology; 45(S4):28–52

MARTA. 1993. Historical control data for development and reproductive toxicity studies using the Crl:CD®BR rat.

Mitchell JC, Adams RM, Glendenning WE, Fisher A, Kanof N, Larsen W, Maibach HI, Rudner EJ, Schnorr W, Storrs F, Taylor JS. 1982. Results of standard patch tests with substances abandoned. Contact Dermatitis; 8(5): 336–7.

Montelius J, Wahlkvist H, Boman A, Wahlberg JE. 1998. Murine local lymph node assay for predictive testing of allergenicity: Two irritants caused significant proliferation. Acta DermVenereol; 78(6):433-7.

Montelius J, Wahlkvist H, Boman A. 1994. Experience with the murine local lymph node assay: Inability to discriminate between allergens and irritants. Acta Derm-Venereol; 74(1):22-7.

Morrissey RE, Lamb JC 4th, Morris RW, Chapin RE, Gulati DK, Heindel JJ. 1989. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundam Appl Toxicol.;13(4):747-77.

Nethercott JR, Nield G, Holness DL. 1989. A review of 79 cases of eyelid dermatitis. Journal of the American Academy of Dermatology; 21(2 Pt 1):223–30.

Noack M. (2001). Ethyl Salicylate: Acute Immobilisation Test (48h) to Daphnia magna STRAUS. Testing laboratory: Dr. U. Noack-Laboratorium für angewandte Biologie, Käthe-Paulus-Str. 1, D-31157 Sarstedt. Report no.: Project-N° 001212HR / Study-N° DAI77661. Owner company: Sponsor: Haarmann & Reimer Gmbh, Postfach 1253, D-37601 Holzminden. Report date: 2001-03-13.

Oiso N, Fukai K, Ishii M. 2004. Allergic contact dermatitis due to methyl salicylate in a compress. Contact Dermatitis.;51(1):34-5.

Opdyke DLJ. 1978. Monographs on fragrance raw materials. Methyl salicylate. 16S1:821-825.Overman DO, White JA. 1983. Comparative teratogenic effects of methyl salicylate applied orally or topically to hamsters. Teratology.;28(3):421-6.

Piccotti JR, Knight SA, Gillhouse K, Lagattuta MS, Bleavins MR. 2006. Evaluation of an ex vivo murine local lymph node assay: multiple endpoint comparison. J Appl Toxicol.;26(4):333-40.

RAC. 2016. https://echa.europa.eu/fr/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e180653f2e

Rai R. Backos M, Baxter N, Chilcott I, Regan L. 2000. Recurrent miscarriage – an aspirin a day? Hum Reprod.; 15 (10): 2220-3.

RIFM Expert Panel, Belsito D, Bickers D, Bruze M, Calow P, Greim H, Hanifin JM, Rogers AE, Saurat JH, Sipes IG, Tagami H. 2007. A toxicologic and dermatologic assessment of salicylates when used as fragrance ingredients. Food Chem Toxicol.;45 (S1):S318-61.

Romaguera C, Grimalt F. 1980. Statistical and comparative study of 4600 patients tested in Barcelona (1973-1977). Contact Dermatitis.; 6(5):309-15.

Rudner EJ. 1977. North American Group results. Contact Dermatitis.3(4):208-9.

Scientific Committee on Consumer Safety (SCCS). 2012. Opinion on Fragrance allergens in cosmetic products. SCCS/1459/11.

Stingeni L, Lapomarda V, Lisi P, 1995. Occupational hand dermatitis in hospital environments. Contact Dermatitis; 33(3): 172–6 .

[US EPA] United States Environmental Protection Agency. 2010. Screening-level hazard characterization, Benzyl Derivatives Category. Hazard Characterization Document.

Takagi T. 2010. Bioconcentration, bioaccumulation and metabolism of pesticides in aquatic organisms, D. M. Whitacre (ed.), Reviews of Environmental Contamination and Toxicology 204, 1-132.

Vidal website. 2018. https://www.vidal.fr

Vryenhoef H. and Mullee D. M. (2010). MethylSalicylate (CAS: 119-36-8): ALGAL GROWTH INHIBITION TEST - PROJECT NUMBER: 1975/0003. Testing laboratory: Harlan Laboratories Ltd - Shardlow Business Park - Shardlow Derbyshire DE72 2GD UK. Report no.: 1975/0003. Owner company: RHODIA. Report date: 2010-10-25.

Yamagata T, Inoue H, Kodama R et al. 1976. Metabolic fate of methyl salicylate. I. Absorption, distribution and excretion in hairless mice. Oyo Yakuri 12:371-383.Yano T, Nakagawa A, Tsuji M, Noda K. 1986. Skin permeability of various non-steroidal anti-inflammatory drugs in man. Life Sci.;39(12):1043-50.

15 ANNEXES

[Please add ANNEX I to the CLH report and potential other annexes.]