SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Methyl salicylate

EC No 204-317-7

CAS No 119-36-8

Evaluating Member State(s): France

Dated: March 2021

Evaluating Member State Competent Authority

French Agency for Food, Environmental and Occupational Health Safety (ANSES) on behalf of French Ministry of Environment
14 rue Pierre et Marie Curie
94701 Maisons-Alfort cedex
France
Email: reach@anses.fr

Year of evaluation in CoRAP: 2015

Before concluding the substance evaluation a Decision to request further information was issued on: 10 March 2016

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Methyl salicylate was originally selected for substance evaluation in order to clarify concerns about:

- Suspected CMR
- Consumer use
- High (aggregated) tonnage

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Not applicable

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	x
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	x
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

- A RMOA is planned in order to :
    - Analyse the relevant RMM to properly manage the risks related to **skin sensitisation** for workers and consumers. It should be noted that uses of sensitizing substances by consumers is an issue not only for methyl salicylate but also for other salicylates (see below).
    - Analyse the relevant RMM to properly manage the risks related to systemic effects – including developmental toxicity - for workers and consumers which arise when lower DNELs are considered.
- <u>Regarding potential additional harmonised classification and labelling</u>:

A harmonised classification is justified for Eye Dam. 1 based on additional data obtained during this substance evaluation. However, since it is not an endpoint of high priority in

regard to C&L process, the evaluating MSCA advises all registrants to self-classify the substance accordingly.

Moreover, an update of the CSR by registrants is strongly recommended to account for this hazard in the chemical risk assessment and communicate adequate risk management measures to downstream users.

- Regarding the assessment of the ED properties of the substance:

Since an ED concern has been identified during the evaluation, there is a need to pay attention to new publications on this topic or to promote toxicovigilance for this substance to ensure that no further concern will arise.

Additionally the evaluating MSCA is waiting for the outcome of the ED assessment of salicylic acid by NL in the Biocidal Product Regulation framework. Indeed, further investigation could be required for methyl salicylate depending on this outcome.

- Additionally, a document is under preparation by ECHA ("GMT" work) for a regulatory action for a specific group of salicylates. This work may be closely linked with the RMOA to be prepared for methyl salicylate.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
RMOA	2022 at the earliest	France

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Methyl salicylate was originally selected for substance evaluation in order to clarify concerns about:
- Suspected CMR (suspected R)
- Consumer use
- High (aggregated) tonnage

During the evaluation also other concerns were identified. The additional concerns were:
- Acute toxicity by oral route
- Eye irritation
- Skin sensitisation
- Mutagenicity
- Endocrine disruption

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Identity	No concern identified on this endpoint
Physico-chemical properties	No concern identified on this endpoint
ADME	Absorption: 100% for oral, dermal and inhalation routes. Methyl salicylate is metabolized into salicylic acid and methanol. The substance is not bioaccumulable and is mainly excreted in the urine.
Acute toxicity	Harmful if swallowed A proposal for a harmonised C&L was submitted by evaluating MSCA to ECHA in June 2018 to classify the substance as Acute Tox. 4 – H302; ATE = 580 mg/kg bw Committee for Risk Assessment (RAC) opinion in September 2019: Acute Tox 4 – H302; ATE = 890 mg/kg bw No acute toxicity by dermal and inhalation routes. No further action.
Irritation	Not a skin irritant. No further action. Eye Dam. 1: A harmonized classification is justified for this endpoint. However, since it is not an endpoint of high priority in regard

	to C&L process, we advice all registrants to self-classify the substance accordingly. Moreover, an update of the CSR by registrants is strongly recommended to account for this hazard in the chemical risk assessment and communicate adequate risk management measures to downstream users. No concern for respiratory irritation. No further action.
Sensitisation	Skin sensitiser A proposal for a harmonised C&L was submitted by eMSCA to ECHA in June 2018 to classify the substance as Skin Sens. 1B – H317. This proposal was agreed by the RAC in September 2019. No concern for respiratory sensitisation. No further action.
Repeated-dose toxicity	Target organ in rats: bone Target organ in dogs: liver NOAEL = 50 mg/kg bw/day (rats and dogs; 2 years)
Mutagenicity	Additional concern identified during the 12-month evaluation period but clarified by additional information provided by the lead registrant during their commenting period. No further action.
Carcinogenicity	No concern. No further action.
Reproductive toxicity	Toxicity on fertility: additional concern identified during the 12-month evaluation period but clarified by additional information provided by the lead registrants during their commenting period. No further action. Toxicity on development: methyl salicylate is teratogenic to rats. A proposal for harmonised C&L was submitted by eMSCA to ECHA in June 2018 to classify the substance as Repr. Cat 1B – H360D. RAC opinion in September 2019: Repr. 2 – H361d
Endocrine disruption	There is a need to pay attention to new publications on this topic or to promote toxicovigilance for this substance to ensure that no further concern will rise. eMSCA waits for ED assessment of salicylic acid by NL in Biocidal Product Regulation

	framework. Further investigation could be required for methyl salicylate depending on the outcome of the ED assessment for salicylic acid.
Environment	All scenarios were assessed and it was concluded that many scenarios are unacceptable (RCR > 1), mainly for the following life cycle stages: manufacture, formulation and some uses at industrial sites. A RMOA is necessary to investigate the possible risk management options.
Exposure	Both human health and environment were assessed. It was an initial ground for concern and therefore further action identified: requests in a DD. Based on the updated exposure scenarios and considering that DNELs should be lower and the classification as skin sensitiser, risks are identified for workers and for consumers (local and systemic effects). A RMOA is necessary to investigate risk management options.

7.2. Procedure

- The evaluation of the methyl salicylate was a comprehensive one, meaning all the hazard's endpoints and all the uses were assessed.
- The aggregated dataset and the literature available were used for the evaluation.
- eMSCA met the lead registrant and had also contacts with the joint submitter. Both provided additional information on the exposure part.
- An eye irritation study (short time exposure (STE) *in vitro* test (OECD TG 491) was requested in 2018.
- The discussion at the RAC concerning the classification of the salicylic acid (SA) were followed by eMSCA since the SA is the main metabolite (with methanol) of methyl salicylate.
- A CLH proposal was submitted by eMSCA to ECHA in June 2018 for methyl salicylate related to acute toxicity by oral route, skin sensitization, developmental toxicity and aquatic toxicity.
- The RAC provided its opinion on methyl salicylate in September 2019, with the following classification: Acute Tox 4 – H302; ATE = 890 mg/kg; Skin Sens 1B – H317; Repr. 2 – H361d and Aquatic Chronic Cat. 3 – H412.

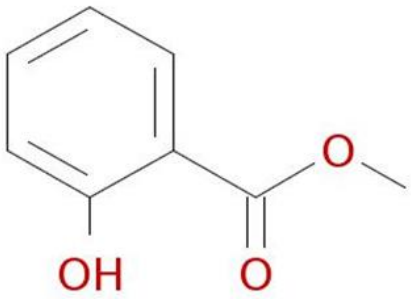
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Methyl salicylate
EC number:	204-317-7
CAS number:	119-36-8
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	$C_8H_8O_3$
Molecular weight range:	152.1473 $g.mol^{-1}$
Synonyms:	Benzoic acid, 2-hydroxy-, methyl ester Methyl 2-hydroxybenzoate

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



The compositions submitted by the registrants are in the range that allows (according to REACH guidance for identification and naming of substances) to consider methyl salicylate as a mono-constituent (content of the substance is higher than 80% and impurities content is less than 20%).
The two provided compositions are synthetic sources.
The analytical information provided by the registrants confirm the structure and the composition of their substance. Analytical data (IR, UV/VIS, NMR) are consistent among the two registrants.

7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	**Value used for SEV:** Colourless to slightly yellow liquid with characteristic of aromatic compounds. This data based on the observation and manipulation of the substance is provided by the lead registrant and is consistent with the data found in the CRC Handbook 86th (2005-2006) and the Merck Index 14th (2006).
Vapour pressure	The vapour pressure values reported in the peer reviewed handbook (CRC Handbook 86th) are between 10 Pa at 22 °C and 100 Pa at 51 °C. **Value used for SEV:** 10 Pa at 22 °C and 100 Pa at 51 °C Data is also available in literature such as the Vershueren Handbook and the BGIA Gestis which give close values: 13 Pa at 20 °C, 130 Pa at 54 °C and 133.3 Pa at 54 °C. Differences may be due to the differences of purity of the test materials and to the precision of methods used which is not always specified. Other literature data provide consistent values.
Water solubility	Value found in a peer reviewed handbook (Merck Index 14th) gives a water solubility of 0.67 g.L^{-1}. **Value used for SEV:** 0.67 g.L^{-1} at ambient temperature. This value is supported by values at 30 °C: 0 74 g.L^{-1} and 0.625 g.L^{-1} found in literature. The value of 0.625 g.L^{-1} originates from a publication where solubility has been measured with a titration method. Slight differences may be due to the differences of purity of the test materials and to the accuracy of methods used which are not always specified.
Partition coefficient n-octanol/water (Log Kow)	The known logKow database (Sangster) gives a value of 2.55. **Value used for SEV:** Log Kow (Log Pow): 2.55 This value is consistent with the values found in other databases such as the Gangolli's dictionary or the Pomona College Medchem Database: 2.55 and 2.546-2.98. Differences may be due to the differences in purity of the test materials and in the methods used which are not specified.
Dissociation constant	Secondary literature data gives values of 9.8 and 9.87. **Value used for SEV**: 9.8-9.9 at 20 °C As only secondary data source have been provided with very close values, they have been used in a WOE approach.
Melting / freezing point	The melting point values reported in the literature (peer reviewed handbooks: Merck Index 14th and CRC Handbook 86th) gives values of -8.6°C and -8 °C. **Value used for SEV:** -8.6 °C at 101.3 kPa

	Data is available in Handbooks: the Merck Index, CRC handbook and Vershueren Handbook which give values of -8.6 °C and -8 °C. Other literature data give values consistent with this data: -8.6 °C and between -7 and -8 °C.
Boiling point	Data is available in peer reviewed handbooks: the Merck Index 14th and CRC handbook 86th which give consistent values of 220-224 °C and 222.9°C. **Value used for SEV:** 220-224 °C at 101.3 kPa Other literature data give values consistent with this data: 222 °C, 226 °C, 222.9 °C, 223.3 °C and 221.2 – 221.5 °C.
Relative density	A publication with the pycnometer method on a substance with 99.3% of purity gives a result of 1.1782 at 25 °C. **Value used for SEV:** 1.1782 at 25 °C Data is available in peer reviewed Handbook (CRC Handbook 86th and Merck Index 14th) which report a consistent value of 1.185 and 1.184. Two other publications with the pycnometer method report consistent value also: 1.1798 and 1.1782 at 25 °C.
Henry's law constant	The Henry's law constant is calculated from vapour pressure, molecular weight and water solubility: 10*152.48/(0.67*100) = 2.28 Pa.m^3.mol^{-1} **Value used for SEV:** 2.28 Pa.m^3.mol^{-1} at ambiant temperature Based on the key study data for vapour pressure and water solubility, the result is 2.28 Pa.m^3/mol at ambient temperature.
Solubility in organic solvents	CRC Handbook 86th specifies that methyl salicylate is very soluble in chloroform, ether and ethanol. **Value used for SEV:** Very soluble in chloroform, ether and ethanol This data is consistent with the data provided in the peer reviewed Merck Index 14th and by Rhodia.
Stability in organic solvents	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.
Flash point	Value found in a peer reviewed handbook (Merck Index 14th) gives a flash-point of 99 °C. **Value used for SEV:** 99 °C Data is available in literature such as the BGIA Gestis which gives close value: 101.1 °C. Other literature data gives values of 95.5 °C and 96 °C.
Flammability	**Value used for SEV:** not flammable The flash point of the substance (i. e. 99 °C) is above the upper limit set in the classification and labeling criteria (i. e. 60 °C).

	Moreover, based on experience handling the substance, the substance is not pyrophoric, and is not flammable in contact with water.
Autoflammability / self-ignition temperature	Literature data from the BGIA Gestis gives a value of 450 °C. **Value used for SEV:** 450 °C This value is consistent with the values of 454.4 °C found in literature.
Explosive properties	**Value used for SEV:** not explosive In accordance with column 2 of REACH Annex VII, the study on explosive properties, required in section 7.11, does not need to be conducted as the substance is a simple organic liquid, with no structural alerts for explosiveness. Indeed it does not contain groups associated with explosive properties as described in the table R.7.1-28 of the ECHA guidance R.7A (May 2008).
Oxidising properties	**Value used for SEV:** no oxidizing properties In accordance with column 2 of REACH Annex VII, the study on oxidising properties, required in section 7.13, does not need to be conducted as the substance is incapable of reacting exothermically with combustible materials. Indeed, it is an organic substance which does not contain halogen atoms and contains oxygen not chemically bonded to nitrogen.
Viscosity	A publication with the capillary method on a substance with 99.3% of purity gives a result of 1.535 mPa.s at 25.15 °C. **Value used for SEV:** 1.535 mPa.s at 25 °C Data is available in peer reviewed Handbook (CRC Handbook 86th) which reports consistent values of 1.102 mPa.s at 75 °C and 0,815 mPa.s at 100 °C.
Surface tension	In accordance with column 2 of REACH Annex VII, the study on surface tension, required in section 7.6, is to be performed only for those substances that have structural alerts for surface tension reducing properties (i. e. presence of both hydrophilic and hydrophobic parts in the molecule/foaming), and/or if the molecule was designed to be used as a surfactant. Methyl salicylate is not this kind of substance.
Granulometry	Not relevant In accordance with column 2 of REACH Annex VII, the study on granulometry, required in section 7.14, does not need to be conducted as the substance is marketed or used in a non solid or granular form.

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)

☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☒ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

The substance is registered in a full registration (16 registrants) and as an intermediate only (4 registrants).

7.5.2. Overview of uses

Information below is provided as available in the disseminated registration dossier in February 2020.

Table 7

USES	Use(s)
Manufacture	ERC 1 PROC 1, 2, 3, 4, 5, 7, 8a, 8b, 9, 10, 11, 13, 15, 16
Uses as intermediate	4 registrations as intermediate only (strictly controlled conditions) Some other registrants report uses as intermediate as part of their full registration dossier (not under strictly controlled conditions) ERC 6a PROC 1, 2, 3, 8a, 8b, 9, 15 SU 1, 4, 9 Use as such
Formulation	Formulation of fragrance compounds (perfumes, fragrances (PC 28)) Formulation of fragranced end-products: - air care products (PC 3) - biocidal products (PC 8) - perfumes, fragrances (PC 28) - polishes and wax blends (PC 31) - washing and cleaning products (PC 35) - cosmetic products (PC 39) - personal care products (PC 39) Formulation of fuels (PC 13) Formulation of laboratory chemicals (PC 21) Formulation of pharmaceuticals (PC 29) ERC 2 PROC 1, 2, 3, 4, 5, 7, 8a, 8b, 9, 10, 11, 13, 14, 15, 16 Use as such and in a mixture
Uses at industrial sites	Industrial end-use of washing and cleaning products (PC 35) ERC 4 PROC 1, 2, 4, 7, 8a, 8b, 10, 13 Use as such and in a mixture Industrial use of fuels (PC 13) ERC 7 PROC 8a, 8b, 16 Industrial use of other fragranced products (PC 35, 39)

	ERC 5 PROC 10 Multi-purpose substance (PC 3, 8, 13, 21, 28, 29, 31, 35, 39) ERC 4, 5, 6a, 7 PROC 1, 2, 3, 4, 5, 7, 8a, 8b, 9, 10, 11, 13, 15, 16 SU 4, 8, 9, 24 Use as such
Uses by professional workers	Professional end-use of polishes and wax blends (PC 31) ERC 8a PROC 2, 8a, 8b, 10, 11 Use as such and in a mixture Professional end-use of washing and cleaning products (PC 35) ERC 8a, 8d PROC 1, 2, 3, 4, 8a, 8b, 10, 11, 13, 19 Use as such and in a mixture Professional use of fuels (PC 13) ERC 9a, 9b PROC 8a, 8b, 16 Professional end-use of cosmetics (PC 39) ERC 8a PROC 5, 8a, 10 Use in a mixture Professional use (PC 3, 8, 13, 21, 28, 29, 31, 35, 39) ERC 8a, 8d, 9a, 9b PROC 1, 2, 3, 4, 5, 7, 8a, 8b, 9, 10, 11, 13, 15, 16 Use as such and in a mixture
Consumer Uses	Consumer end-use of air care products (PC 3) ERC 8a, 8d Aerosol sprays, solid, liquid, diffusers, candles Use in a mixture Consumer end-use of biocides (PC 8) ERC 8a, 8d Insecticides (spray, electric diffuser) and repellents Use in a mixture Consumer end-use of polishes and wax blends (PC 31) ERC 8a, 8d Wax/cream for floor, furniture, shoes; spray for furniture and shoes Use in a mixture Consumer end-use of washing and cleaning products (PC 35) ERC 8a, 8d Laundry products, dish-washing products, cleaners (all purpose, sanitary, floor, glass, carpet, metal, toilets, oven), wipes, high-pressure washers, automotive care (including sprays) Use as such and in a mixture Consumer end-use of cosmetic products (PC 28, 39) ERC 8a, 8d Use as such and in a mixture
Uses advised against	None

- **Environmental release categories:**
  - ERC 1: Manufacture of the substance
  - ERC 2: Formulation into mixture
  - ERC 4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article)
  - ERC 5: Use at industrial site leading to inclusion into/onto article
  - ERC 6a: Use of intermediate
  - ERC 7: Use of functional fluid at industrial site
  - ERC 8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)
  - ERC 8d: Widespread use of non-reactive processing aid (no inclusion into or onto article, outdoor)
  - ERC 9a: Widespread use of functional fluid (indoor)
  - ERC 9b: Widespread use of functional fluid (outdoor)

- **Process categories:**
  - PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions
  - PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions
  - PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment condition
  - PROC 4: Chemical production where opportunity for exposure arises
  - PROC 5: Mixing or blending in batch processes
  - PROC 7: Industrial spraying
  - PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities
  - PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities
  - PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)
  - PROC 10: Roller application or brushing
  - PROC 11: Non industrial spraying
  - PROC 13: Treatment of articles by dipping and pouring
  - PROC 14: Tabletting, compression, extrusion, pelletisation, granulation
  - PROC 15: Use as laboratory reagent
  - PROC 16: Use of fuels
  - PROC 19: Manual activities involving hand contact

- **Sectors of end-use:**
  - SU 1: Agriculture, forestry, fishery
  - SU 4: Manufacture of food products
  - SU 8: Manufacture of bulk, large scale chemicals (including petroleum products)
  - SU 9: Manufacture of fine chemicals
  - SU 24: Scientific research and development

- **Product categories:**
  - PC 3: Air care products
  - PC 8: Biocidal products (e.g. disinfectants, pest control)
  - PC 13: Fuels
  - PC 21: Laboratory chemicals
  - PC 28: Perfumes, fragrances
  - PC 29: Pharmaceuticals
  - PC 31: Polishes and wax blends
  - PC 35: Washing and cleaning products
  - PC 39: Cosmetics, personal care products

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonized classification is currently available for methyl salicylate.
A CLH report was submitted by eMSCA to ECHA in June 2018 with the following proposals:
- Acute Tox. 4 – H302; ATE = 580 mg/kg bw;
- Skin Sens. 1B – H317;
- Repro. 1B – H360D;
- Aquatic Chronic Cat. 3 – H412.

In September 2019, the classification agreed by the RAC was the following:
- Acute Tox. 4 – H302; ATE = 890 mg/kg bw;
- Skin Sens. 1B – H317;
- Repro. 2 – H361d;
- Aquatic Chronic Cat. 3 – H412.

7.6.2. Self-classification

- In the registrations dossier(s):

Acute Tox 4 – H302

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

Skin Irrit 2 – H315
Eye Dam 1 – H318
Eye Irrit 2 – H319
Repr 1B – H360
Lact – H362
STOT SE 3 – H335

7.7. Environmental fate properties

General discussion of environmental fate and pathways:

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

Considering the vapor pressure of 10 Pa and a water solubility of 670 mg/L, the Henry's Law Constant of methyl salicylate should be 2.28 Pa.m3/mol; then the substance is considered as moderately volatile from water at 25°C.

Based on a weight of evidence approach, considering the available data (i.e. one supportive study and the QSAR predictions (BIOWIN 4.10)), methyl salicylate was found to be readily biodegradable and not bioaccumulative.

Since the substance is readily biodegradable, a Koc study can be waived. A Koc value of 222 L/kg has been extrapolated from the log Kow.

7.7.1. Degradation

7.7.1.1 Abiotic degradation

- **Hydrolysis:**

In accordance with column 2 of REACH Annex VIII, the study on hydrolysis, required in section 9.2.2.1., does not need to be conducted 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).

- **Phototransformation/photolysis**

- **Phototransformation in air**

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 correspond 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.

- Phototransformation in water

No relevant information available

- **Phototransformation in soil**

No relevant information available

7.7.1.2 Biodegradation

7.7.1.2.1 Biodegradation in water

- **Screening tests**

The studies on biodegradation in water (screening tests) are summarised in the following table:

Table 8: Screening tests

Method	Results	Remarks	Reference
Test type: ready biodegradability activated sludge, non-adapted equivalent or similar to OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)	readily biodegradable % Degradation of test substance: 98.4 after 28 d (inorg. C analysis) (95% Confid. Interval : 94.4 - 102.4)	3 (not reliable) Supporting study experimental result **Test material (EC name): methyl salicylate**	Unpublished study Report#9, 1993

7.7.1.2.2. Simulation tests (water and sediments)

No available data

7.7.1.2.3. Summary and discussion of biodegradation in water and sediment

Discussion (screening testing)

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 biodegration 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 degradation values of each 4 samples as well as the raw data are not available. Due to the lack of data, it is not possible to check the validity criteria and the Study Report#9 (1993) 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 Salycilate. 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.

The following information is taken into account for any hazard / risk / persistency assessment regarding the Biodegradation in water: readily biodegradable.

7.7.1.2.2. Biodegradation in soil

No available data

7.7.1.3. Summary and discussion of degradation

Abiotic degradation

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

Biotic degradation

Considering the available data (i.e., one supportive study, data from the US EPA report (2010) and the QSAR predictions (BIOWIN 4.10)), Methyl Salicylate was found to be **readily biodegradable** based on a weight of evidence approach. Therefore, it is not considered to be persistent in water, sediment and soil.

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

The studies on adsorption/desorption are summarised in the following table:

Table 9: Studies on adsorption/desorption

Method	Results	Remarks	Reference
Study type: QSAR calculation QSAR calculation according to equation from Sabljic and Güsten (1995), as reported in the EU TGD (2003), concerning non hydrophobic chemicals: log Koc = 0.52 log Kow + 1.02, with Log Kow=2.55	Adsorption coefficient: Koc: 222 log Koc: 2.346	2 (reliable with restriction) key study (Q)SAR **Test material (EC name): methyl salicylate**	Technical guidance document on risk assessment: Part III, European Chemicals Bureau (2003)

Value used for CSA: Koc of 222 L/kg.

7.7.2.2. Volatilisation

The studies on volatilisation are summarised in the following table:

Table 10: Studies on volatilisation

Method	Results	Remarks	Reference
Calculation according to the equation R16-10 of the guidance on information requirements and chemical safety assessment - chapter R16: environmental exposure assessment (ECHA 2010)	Henry's Law constant H: 2.27 Pa m³/mol at 25 °C	2 (reliable with restrictions) key study estimated by calculation **Test material (EC name): methyl salicylate**	R16-ECHA (2010)

Discussion

The Henry's Law constant of methyl salicylate has been calculated according to the equation R16-10 of the guidance on information requirements and chemical safety assessment - chapter R16: environmental exposure assessment (ECHA 2010).

The equation used is the following:

HENRY = (VP x MOLW) / SOL

With (as vapour pressure and water solubility values have not been measured at the same temperature, these values have been extrapolated to the data at 25°C using EUSES calculation):

VP = 10 Pa at 22°C

MOLW = 152.13 g/mol

SOL = 670 mg/L at 25°C

The following information is taken into account for any environmental exposure assessment:

The Henry's Law Constant has been calculated to be 2.27 Pa. m3/mol. Therefore, Methyl Salicylate is considered as moderately volatile from water at 25°C

Value used for CSA: Henry's law constant (H) at 20°C: 2.27 (in Pa m³/mol or dimensionless) at 25 °C.

7.7.2.3. Distribution modelling

No relevant information available

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

Methyl Salicylate is rapidly metabolised and eliminated from organisms (cf ADME data). This was confirmed in the screening level hazard characterization made on benzyl derivatives category. Benzoic acid derivatives are expected to have low bioaccumulation potential. Furthermore, the QSAR predictions with BCFBAF 3.01 indicate that all 2-hydroxybenzoate esters subcategory III (including Methyl salicylate) have BCF < 500 L/kgww.

As a consequence, based on a weight of evidence approach, Methyl Salicylate could be considered as not bioaccumulative.

7.7.3.2. Terrestrial bioaccumulation

No relevant information available

7.7.4. Secondary poisoning

Based on the available information, there is no indication of a bioaccumulation potential and, hence, secondary poisoning is not considered relevant (see CSR chapter 7.6 "PNEC derivation and other hazard conclusions").

7.8. Environmental hazard assessment

For the aquatic compartment, short-term toxicity data are available for the three trophic levels (i.e. fish, invertebrates and algae). No long-term toxicity values are available for fish and invertebrate. The PNECaqua for freshwater can be determined by applying an assessment factor of 1000 on the lowest L(E)C50, corresponding to the most sensitive species. Using this approach, a safety factor of 1000 should be applied to the worst case EC50 value obtained with methyl salicylate on algae, as follows: **PNECaqua (freshwater) = 1.6 mg/L / 1000 = 1.60 µg/L.** The **$PNEC_{sed,\ EPM}$ = 0.041 mg/kg dw**.

The toxicity of Methyl salicylate to bacteria was tested in a growth inhibition test using Pseudomonas putida. The EC10 and EC50 values were 140 and 380 mg/L respectively. The PNEC for micro organisms in STP is set equal to the NOEC or EC10 (Assessment Factor = 1), then **$PNEC_{STP}$ = 140 mg/L**

Only supportive data are available for terrestrial toxicity of Methyl salicylate on earthworms and one species of plant. The **$PNEC_{soil,\ EPM}$ = 0.007 mg/kg dw**.

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1. Short-term toxicity to fish

The results are summarised in the following table:

Table 11: Short-term effects on fish

Method	Results	Remarks	Reference
Pimephales promelas freshwater flow-through equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test) – no GLP	LC50 (96 h): 19.8 mg/L (Meas. (arithm. Mean between 0 and 72h)) based on: mortality	2 (reliable with restrictions) weight of evidence read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): Ethyl Salicylate (See endpoint summary for justification of read-across)**	Geiger D.L., Northcott C.E., Call D.J. and Brooke L.T. (1985)
Pimephales promelas freshwater flow-through equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test)	LC50 (96 h): 1370 mg/L test mat. (meas. (geom. mean)) based on: mortality (confidence limit 1270-1470 mg/L) LC50 (72 h): 1501 mg/L test mat. (meas. (geom. mean)) based on: mortality LC50 (48 h): 1591 mg/L test mat. (meas. (arithm. mean)) based on: mortality LC50 (24 h): 1853 mg/L test mat. (meas. (arithm.	2 (reliable with restrictions) weight of evidence read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): sodium salicylate CAS n° 54 -21 -7 (See endpoint summary for justification of read-across)**	Geiger DL, Northcott CE, Call DJ and Brooke LT editors (1985)

Method	Results	Remarks	Reference
	mean)) based on: mortality		

Discussion

No reliable studies are available for **Methyl Salicylate** for this endpoint. **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 LC50 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 LC50 (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 both substances have a similar chemical structure**. Both substances are 2-hydroxybenzoate, one being a methyl ester (i. e. methyl salicylate) and the second one being an ethyl ester (i. e. ethyl salicylate). 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 logKow 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. 48hEC50 = 28 mg/L for Ethyl Salicylate and 24hEC50 = 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 logKow). 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), fish from the species *Pimephales promelas* were exposed under flow-through condition s 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 LC50 (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.

Value used for CSA:

LC_{50} for freshwater fish: 19.8 mg/L.

7.8.1.2. Long-term toxicity to fish

No available data

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 12: Short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static equivalent or similar to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): 870 mg/L test mat. (nominal) based on: mobility (773-953 mg/L) EC50 (24 h): 1060 mg/L test mat. (nominal) based on: mobility (898-1215 mg/L)	2 (reliable with restrictions) weight of evidence read-across from supporting substance (structural analogue or surrogate) **Test material (IUPAC name): 2-Hydroxybenzoic acid (See endpoint summary for justification of read-across)**	Kamaya Y, Fukaya Y and Suzuki K (2005)
Daphnia magna freshwater static	EC50 (24 h): 58 mg/L DOC (meas. (initial)) based on: mobility	2 (reliable with restrictions)	Unpublished Study Report#10, 2001

Method	Results	Remarks	Reference
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) EU Method C.2 (Acute Toxicity for Daphnia)	EC50 (48 h): 28 mg/L DOC (meas. (initial)) based on: mobility	weight of evidence read-across from supporting substance (structural analogue or surrogate) **Test material (EC name): Ethyl salicylate (See endpoint summary for justification of read-across)**	

Discussion

No reliable studies are available for Methyl Salicylate for this endpoint.

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 (Unpublished Study Report#10, 2001), the acute immobilization (EC50) of the test item Ethyl salicylate to daphnia (STRAUS) 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 over a duration of 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-EC50 values were calculated by probit analysis in the tested concentration range. Exposure of daphnids to Ethyl salicylate resulted in a 48h-EC50 value of 28 mg/L (95% confidence interval = 27 to 29 mg/L). Based on the results of this study, Ethyl salicylate is considered **as harmful to the aquatic organisms tested** in accordance with the Directive 67/548/EC.

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 main assumption to justify the read-across approach is that both substances have a similar chemical structure**. Both substances are 2-hydroxybenzoate, one being a methyl ester (i. e. methyl salicylate) and the second one being an ethyl ester (i. e. ethyl salicylate). 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 logKow 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 fish of both substances show that Ethyl Salicylate is more toxic than Methyl Salicylate (i. e. 96hLC50 = 19.7 mg/L for Ethyl Salicylate and 96hLC50 > 100 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-hydroxubenzoic 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 logKow). 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 ambient 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.

A 48 hours acute toxicity study of salicylic acid to *Daphnia magna* is available. This study 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**. Based on the results of this study, **2-hydroxybenzoic acid was not classified as harmful to *Daphnia magna*** in accordance with the EC classification criteria.

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.

Value used for CSA:

EC50/LC50 for freshwater invertebrates: 28 mg/L.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

No available data

7.8.1.3. Algae and aquatic plants

The results are summarised in the following table:

Table 13: Effects on algae and aquatic plants

Method	Results	Remarks	Reference
Desmodesmus subspicatus (algae) freshwater static OECD Guideline 201 (Alga, Growth Inhibition Test) EU Method C.3 (Algal Inhibition test)	EC50 (72 h): 27 mg/L test mat. (nominal) based on: growth rate (95% CL : 24 - 31 mg/L) EC50 (72 h): 13 mg/L test mat. (nominal) based on: biomass (95% CL: 12 - 14 mg/L) EC50 (72 h): 1.6 mg/L test mat. (meas.	1 (reliable without restriction) key study experimental result **Test material (EC name): methyl salicylate**	Unpublished Study Report#11, 2010

Method	Results	Remarks	Reference
GLP study	(geom. mean)) based on: growth rate (95% CL: 1.5 - 1.7 mg/L) EC50 (72 h): 1.1 mg/L test mat. (meas. (geom. mean)) based on: biomass (95% CL: 1.1 - 1.2 mg/L) NOEC (72 h): 6.25 mg/L test mat. (nominal) based on: growth rate NOEC (72 h): 6.25 mg/L test mat. (nominal) based on: biomass NOEC (72 h): 0.79 mg/L test mat. (meas. (geom. mean)) based on: growth rate NOEC (72 h): 0.79 mg/L test mat. (meas. (geom. mean)) based on: biomass LOEC (72 h): 12.5 mg/L test mat. (nominal) based on: growth rate LOEC (72 h): 12.5 mg/L test mat. (nominal) based on: biomass LOEC (72 h): 1.1 mg/L test mat. (meas. (geom. mean)) based on: growth rate LOEC (72 h): 1.1 mg/L test mat. (meas. (geom. mean)) based on: biomass		

Discussion

Effects on algae / cyanobacteria

One reliable key study is available for this endpoint (Unpublished Study Report#11, 2010). In the study of Cerbelaud (2000), the validity criteria cannot be verified due to the lack of measurements, there were no reference substance and no analytical monitoring, and the inhibition of the growth rate has not been reported. Then, this study is not reliable.

In the Vryenhoef and Mulleer study (2010), the effect of the test item 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 dependant decline in measured concentrations in the range of less than the limit of quantitation (LOQ) of the analytical method employed to 24% of nominal (see the table below)**. This decline was in line with the preliminary stability analyses conducted which indicated slight instability over the test period. The further decline in measured test concentrations was considered by the author to be due to adsorption of the test item to the algal cells present.

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 below, 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.

[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 EC50 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-NOEC = 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-NOEC = 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 was only an unverified hypothesis that is contradicted by the substance water solubility and log Kow that 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.

Therefore, the results obtained with the geometric mean of the measured concentrations instead of nominal concentrations have to be used as a worst case for the risk assessment.

- **Value used in the risk assessment:**

72h-ErC50 = 1.6 mg/L (growth rate)
72h-EbC50 = 1.1 mg/L (biomass)
72h-NOEC = 0.79 mg/L (growth rate and biomass)

As algae is the most sensitive species among the three trophic levels, the 72h-ErC50 = 1.6 mg/L will be used to determine the aquatic PNEC.

Based on the results obtained with the geometric mean of the measured concentrations (NOEC = 0.79 mg/L), Methyl salicylate should be classified as toxic to the aquatic organisms tested in accordance with the Directive 67/548/EC.

7.8.1.4. Sediment organisms

No available data

7.8.1.5. Other aquatic organisms

No relevant information available

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to soil macro-organisms

The results are summarised in the following table:

Table 14: Effects on soil macro-organisms

Method	Results	Remarks	Reference
Eisenia fetida (annelids) short-term toxicity (laboratory study) Substrate: artificial soil The method to determine the toxicity of Methyl salicylate in soil to the earthworm was adapted from the one developed by Neuhauser et al., 1985. Principles of method is similar to the OECD Guideline 207 (Earthworm, Acute Toxicity Tests), but some differences are remarkable in experimental conditions: See details on test conditions.	NOEC (14 d): 50 mg/kg soil dw test mat. (nominal) based on: growth (The NOEC value was not reported in the pubication but deduced from data analysis.) NOEC (14 d): 200 mg/kg soil dw test mat. (nominal) based on: mortality (The NOEC value was not reported in the pubication but deduced from data analysis.) EC50 (14 d): 350 mg/kg soil dw test mat. (nominal) based on: mortality (No EC50 value is reported in the publication. This EC50 value is based on data analysis, discussed and proposed in the endpoint study summary for the determination of the PNEC value.)	3 (not reliable) Supporting study experimental result **Test material (EC name): methyl salicylate**	Unpublished Study Report#13 (1993)
Helicoverpa zea (Lepidoptera) Application method: oral & contact short-term toxicity (laboratory study) Helicoverpa zea were fed with Cotton plants, sprayed with 100 µM Methyl salicylate in 0.1 % ethanol (treated group) or with 0.1% ethanol (vehicle control group) in order to assess the effect of this substance on the weight gain or survivorship of neonates after a 96h feeding period and on third-instar H. zea growth	NOEC (96 h): NOEC test mat. (nominal) based on: Larval weight & Survivors (This value is the concentration of the solution which was sprayed onto Cotton plants, and therefore is not the effective exposure concentration of Helicoverpa zea) NOEC (48 h): NOEC test mat. (nominal) based on: Relative growth rate (This value is the concentration of the solution which was	3 (not reliable) Disregarded study experimental result **Test material (EC name): methyl salicylate**	Bi J.L., Murphy J. B. & Felton G. W. (1997)

Method	Results	Remarks	Reference
rate at both 48 and 64h feeding periods.	sprayed onto Cotton plants, and therefore is not the effective exposure concentration of Helicoverpa zea) NOEC (64 h): NOEC test mat. (nominal) based on: Relative growth rate (This value is the concentration of the solution which was sprayed onto Cotton plants, and therefore is not the effective exposure concentration of Helicoverpa zea)		

Discussion of effects on soil macro-organisms except arthropods

One reliable key study is available for this endpoint. In this study (Unpublished study Report#13, 1993), tests were conducted by ERDEC in 1993 to determine if Methyl salicylate, a component of HL simulant, was responsible for the toxicity exhibited by earthworms (*Eisenia foetida*) in an earlier test. The method used to assess the hazards of Methyl salicylate to these non-target organisms was adapted from the one developed by Neuhauser*et al*., 1985. The test was run with six concentrations of test substance at 13 ± 0.2°C. **Nominal concentrations of Methyl salicylate were 0 (control), 50, 100, 200, 350, and 500 mg/kg dry weight**. For each concentration, **five earthworms** were randomly added to beaker containing a non-sterile artificial soil. Three replicates per concentration were used for each experiment. After 14 days, weight and survival rate of earthworm were examined. The Methyl salicylate produced **no lethal effects on earthworms up to the 200 mg/kg level**. However, **earthworms lost weight at the 100 mg/kg level.** This weight loss continued up to the 350 mg/kg level with no earthworms surviving at the 500 mg/kg level. Two NOEC values may thus be derived from these data, one (200 mg/kg) based on mortality and another (50 mg/kg) based on growth. As this study is a short term toxicity test, the mortality is considered as a more appropriate criteria for the determination of the effect value. No LC50 value has been determined in this study. At the concentration of 200 mg/kg no mortality has been observed. At the concentrations of 350 mg/kg and 500 mg/kg, 14 % and 100% of mortality has been observed. Based on these results, it can be concluded that the **14d LC50 is between 350 and 500 mg/kg dry weight.**

It should be noted that the composition of the soil tested was described as follows: The components of soil used in the earthworm toxicity test were finely ground sphagnum peat (10% by weight), kaolinite clay (20%), fine sand (69%), and calcium carbonate (1%). During mixing, 50 mL of distilled water was added to each 200g batch of soil to provide a moisture level of 25%. The organic matter content is not stated in the publication, therefore, it is not possible to check the compliance with the standardised generic environment defined in the ECHA guidance R16.

eMSCA disagrees to use this LC_{50} for terrestrial PNEC derivation as the reliabilty of the study is low (the study was not performed according to standard method and is partially

GLP (GLP for soil preparation stage); it is not clear if the values are expressed in dry weight or wet weight of soil and there is no organic matter standardization; the organic matter content is not stated in the study report then it is not possible to check the compliance with the standardized generic environment defined in the ECHA guidance R16). Furthermore, if the PNECsoil is based on only one test result, then the PNECsoil should also be derived by applying the equilibrium partitioning method and the lowest one should be used for the risk characterization.

Consequently, the study may be used only as supporting information with a reliability of 3 and the lower terrestrial PNEC EPM should be used for the risk assessment.

Discussion of effects on soil dwelling arthropods:

One study is available for this endpoint (Bi, J. L. *et al.*, 1997). Initially, the aim of the study was to evaluate the effect of exogenous application of methyl salicylate on cotton resistance to *H. zea*. However, effects of this substance on the weight gain or survivorship of neonates H. zea after a 96h feeding period and on third-instar H. zea growth rate at both 48 and 64h feeding periods were evaluated. Thus, *H. zea* were fed with Cotton plants, sprayed with 100 µM Methyl salicylate in 0.1 % ethanol. Applying exogenous Methyl salicylate did not affect the weight gain or survivorship of neonates after a 96h feeding period. The effect of Methyl salicylate on third-instar H. zea growth rate was also insignificant (P>0.05) at both 48 and 64h feeding periods.

Even if this study has not been performed according to standard guideline, it provides information on the absence of toxicity at the concentration tested. As the media of exposure is plant contaminated by spray, it is not possible to determine the effective concentration tested. Therefore, the test is waived for this endpoint.

The following information is taken into account for effects on soil dwelling arthropods for the derivation of PNEC: Helicoverpa zea were fed with Cotton plants, sprayed with Methyl salicylate. Methyl salicylate did not affect the weight gain or survivorship of neonates after a 96h feeding period but it is not possible to determine the effective concentration tested.

This study on *H. zea* is considered as not reliable. This test is not a regulatory test, there are not sufficient information in the iuclid summary.

7.8.2.2. Toxicity to terrestrial plants

The results are summarised in the following table:

Table 15 Effects on terrestrial plants

Method	Results	Remarks	Reference
Cucumis sativus (Dicotyledonae (dicots)) short-term toxicity (laboratory study) early seedling growth toxicity test Substrate: natural soil	*Cucumis sativus*: NOEC (14 d): 200 mg/kg soil dw test mat. (nominal) based on: growth (Plant heights) (The NOEC value was not reported in the pubication but deduced from data analysis)	3 (not reliable) Supportive data experimental result **Test material (EC name): methyl salicylate**	Unpublished study Report#13 (1993)

Method	Results	Remarks	Reference
equivalent or similar to EPA OTS 560/6-82-002 (Early Seedling Growth Toxicity Test)	*Cucumis sativus*: NOEC (14 d): 350 mg/kg soil dw test mat. (nominal) based on: growth (Biomass measurements for the fresh weights (fw)) (The NOEC value was not reported in the pubication but deduced from data analysis.) *Cucumis sativus*: NOEC (14 d): 200 mg/kg soil dw test mat. (nominal) based on: growth (Biomass measurements for the dry weights (dw)) (The NOEC value was not reported in the pubication but deduced from data analysis.) *Cucumis sativus*: EC50 (14 d): 350 mg/kg soil dw test mat. (nominal) based on: growth (No EC50 value is reported in the publication. This EC50 value is based on data analysis, discussed and proposed in the endpoint study summary for the determination of the PNEC value)		

Discussion

One reliable study is available for this endpoint (Unpublished study Report#13, 1993). In this study, tests were conducted by ERDEC in 1993 determine if Methyl salicylate, a component of HL simulant, was responsible for the toxicity exhibited by **cucumbers (*Cucumis sativus*)** in an earlier test. **Seeds were placed in contact with soil treated with the Methyl salicylate and evaluated for effects following 14 days after 50 % emergence of the seedlings in the control group**. Endpoints measured were height, fresh and dry weights of the plants. These measurements and observations were compared to those of untreated control plants. Methyl salicylate was tested at 0, 50, 100, 200, 350, and 500 mg/kg dry weight. The test method used was adapted from the U. S. Environmental Protection Agency's (US-EPA) Environmental Effects Test Guidelines EPA OTS 560/6-82-002 (Early Seedling Growth Toxicity Test).

The Methyl salicylate produced sublethal effects on cucumbers at the 350 and 500 mg/kg levels. The NOEC value deduced from data analysis is 200 mg/kg by weight. The study results indicated that MS was responsible for the effects exhibited by cucumber in the earlier test. No LC50 value has been determined in this study. At the concentrations of 350 mg/kg and 500 mg/kg, significant differences in the height and dry weight of the plants have been determined by statistical evaluation. Based on the comparison of the mean plant height and the mean dry weight between the control and the doses 350 mg/kg and 500 mg/kg, it can be observed that around 20 % of effect is observed at 350 mg/kg and 50 % of effect at 500 mg/kg. **As a worst case, the value of 350 mg/kg dry weight is used for the derivation of the PNEC value.**

Natural soil has been used in this study, with the following characteristics:

- % sand: 87

- % silt: 9

- % clay: 4

-% organic matter: 0.3

- CEC (meq/100g): 2.2

Therefore, the organic matter content is lower than the one of the standardised generic environment defined in the ECHA guidance R16, which contains 3.4% of organic matter. Considering that it can be assumed that the binding behavior of the substance is predominantly driven by its log Kow, and that organisms are exposed predominantly via pore water, the bioavailability of the substance should be higher in this natural soil than in artificial standard soil. Consequently, the value of 350 mg/kg is considered to be a worst case value.

Value used for CSA:

Short-term EC50 or LC50 for terrestrial plants: 350 mg/kg soil dw

Nevertheless, the study was not performed according to standard method and is partially GLP (GLP for soil preparation stage). Only one plant species has been observed. Consequently, the study may be used only as supporting information with a reliability of 3.

7.8.2.3. Toxicity to soil micro-organisms

No relevant information available

7.8.2.4. Toxicity to other terrestrial organisms

No relevant information available

7.8.3. Microbiological activity in sewage treatment systems

The results are summarised in the following table:

Table 16: Effects on micro-organisms

Method	Results	Remarks	Reference
Pseudomonas putida	EC10 (16 h): 140 mg/L test mat. (nominal) based on:	2 (reliable with restrictions)	Unpublished study

Method	Results	Remarks	Reference
freshwater static Draft standard ISO/CD 10712 (1990-10-10)	growth inhibition (freshly prepared solutions) EC10 (16 h): 162 mg/L test mat. (nominal) based on: growth inhibition (Solutions were prepared 120 hours prior to beginning the assay) EC50 (16 h): 380 mg/L test mat. (nominal) based on: growth inhibition (freshly prepared solutions) EC50 (16 h): 500 mg/L test mat. (nominal) based on: growth inhibition (Solutions were prepared 120 hours prior to beginning the assay)	key study experimental result **Test material (EC name): methyl salicylate**	Report#12 (1992)

Discussion

The toxicity of Methyl salicylate to bacteria was tested (Unpublished Study Report #12, 1992) in a growth inhibition test using *Pseudomonas putida*, according to the draft standard ISO / CD 10712 (1990 -10 -10). EC values were determined by comparing the turbidity (standard ISO 7027 / 1984 -07 -01) of the test and control cultures after 16 ± 1 hours incubation. The EC10 and EC50 values were 140 and 380 mg/L respectively.

Since the product is hydrolysable, it was verified from Methyl salicylate solutions beforehand maintained for 120 hours under magnetic stirring at 24°C and in the dark, that their toxicity decreased slightly. In this case, EC10 and EC50 values were 162 and 500 mg/L respectively.

The EC10 and EC50 values of 3,5 -dichlorophenol were 16 and 22 mg/L respectively. These EC values are within the confidence limits selected by the subcommittee ISO/TC 147/SC5.

Value used for CSA:

EC50/LC50 for aquatic micro-organisms: 380 mg/L
EC10/LC10 or NOEC for aquatic micro-organisms: 140 mg/L

7.8.4. Non compartment specific effects relevant for the food chain (secondary poisoning)

7.8.4.1. Toxicity to birds

No relevant information available

7.8.4.2. Toxicity to mammals

No relevant information available

7.8.5. PNEC derivation and other hazard conclusions

Table 17

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC aqua (freshwater): 1.6 µg/L	Assessment factor: 1000 Extrapolation method: assessment factor Short-term toxicity data are available for the three trophic levels (i.e. fish, invertebrates and algae). No long-term toxicity values are available for fish and invertebrate. According to the guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation of dose [concentration]-response for environment (ECHA 2008), the PNECaqua for freshwater can be determined by applying an assessment factor of 1000 on the lowest L(E)C50, corresponding to the most sensitive species. Using this approach, a safety factor of 1000 should be applied to the worst case EC50 value obtained with methyl salicylate on algae, as follows: PNEC aqua (freshwater) = 1.6 mg/L / 1000 = 1.6 µg/L.
Marine water	PNEC aqua (marine water): 0.16 µg/L	Assessment factor: 10000 Extrapolation method: assessment factor Only short-term toxicity data from freshwater representatives of the three taxonomic groups (algae, crustaceans and fish) are available. So, according to the guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation of dose [concentration]-response for environment (ECHA 2008), a conservative and protective factor of 10’000 should be applied on the lowest L(E)C50 to ensure that substances with the potential to cause adverse effects are identified in the hazard assessment. Using this approach, a safety factor of 10000 should be

		applied to the worst case EC50 value obtained with ethyl salicylate in algae, as follows: PNEC aqua (marine waters) = 1.6 / 10000 = 0.16 µg/L.
Intermittent releases to water	PNEC aqua (intermittent releases): 16 µg/L	Assessment factor: 100 Extrapolation method: assessment factor According to the guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation of dose [concentration]-response for environment (ECHA 2008), if intermittent release is identified for a stage of the life cycle, only short-term effects need to be considered for risk characterisation of that stage (only for the aquatic compartment); in this case, the assessment factor can be reduced from 1000 to 100.
Sediments (freshwater)	PNEC sediment (freshwater): 0.041 mg/kg sediment dw 0.009 mg/kg wwt	Extrapolation method: partition coefficient Log Kow is 2.55, so extrapolation by partition coefficient from PNECaqua has been used because log Kow is not far from the validity field for this type of calculation.
Sediments (marine water)	PNEC sediment (marine water): 0.0041 mg/kg sediment dw 0.0009 mg/kg wwt	Extrapolation method: partition coefficient Log Kow is 2.55, so extrapolation by partition coefficient from PNECaqua has been used because log Kow is not far from the validity field for this type of calculation.
Sewage treatment plant	PNEC STP: 140 mg/L	Assessment factor: 1 Extrapolation method: assessment factor No sludge inhibition respiration test is available, but a standard inhibition test performed with Pseudomonas putida is available which provided 16h-EC10 and EC50 values of 140 and 380 mg/l respectively. According to the guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation of dose [concentration]-response for environment (ECHA 2008), the PNEC for micro organisms in STP is set equal to the NOEC or EC10 (Assessment Factor = 1).

Soil	PNEC soil: 0.007 mg/kg soil dw 0.0062 mg/kg wwt	Equilibrium partitioning method Only supportive studies on soil organisms Log Kow is 2.55, so extrapolation by partition coefficient from PNECaqua has been used because log Kow is not far from the validity field for this type of calculation.
Air	No hazard identified:	A weight of evidence approach has concluded that methyl salicylate is not hazardous by inhalation
Secondary poisoning	No potential for bioaccumulation	As there is no indication for bioaccumulation potential of methyl salicylate, no secondary poisoning assessment is performed and therefore no PNECoral has been defined.

7.8.6. Conclusions for classification and labelling

Based on the available data, methyl salicylate is readily biodegradable, not potentially bioaccumulable and the lowest L(E) C50 obtained is between 10 and 100 mg/L. The only NOEC available is in algae < 1 mg/L (NOEC = 0.79 mg/L)

Therefore, according to the Regulation (EC) No 1272/2008 (CLP), methyl salicylate should be classified as Aquatic Chronic Cat. 3, H412 which was agreed by RAC in its opinion in September 2019.

7.9. Human Health hazard assessment

Read-across assessment

In the registration dossier, the lead registrants make a read-across from data on salicylic acid (SA), acetylsalicylic acid (ASA) or sodium salicylate (NaS) to methyl salicylate (MeS), in particular for genotoxicity and reproductive/developmental toxicity endpoints.

This read-across is not considered as particularly useful in the present assessment since the available data are mostly performed with methyl salicylate itself and toxicological information available with SA, NaS and ASA is rather of limited quality. It can be noted that consistent findings are reported with salicylates, in particular regarding their toxicity on development (see Annex I for further details). Read-across between salicylic acid and methyl salicylate is judged as acceptable by NL in the Biocidal Product Regulation framework for the approval of salicylic acid. However, some uncertainties remains. A possible specific effect of the parent molecule can be highlighted by the fact that 21% of methyl salicylate remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans (Davison *et al*., 1961). A potential toxicity of methanol (the other metabolite) cannot be neither excluded. Furthermore, regarding a possible read-across between ASA and methyl salicylate, biological differences can be suspected since their commercial uses are quite different (even if methyl salicylate is used topically for its anti-inflammatory properties, its major use is as a fragrance and that is not the case for ASA). Secondly, ASA and methyl salicylate present some differences in prostaglandin inhibition. In conclusion, without other robust considerations, it is not known to what extent data with ASA are extrapolable to methyl salicylate.

7.9.1. Toxicokinetics

Absorption

Methyl salicylate is well absorbed by ***oral route***; thus an oral bioavailability of 100% is assumed (Unpublished Study Report #7, 1976).

No relevant data is available for absorption by inhalation.

Several studies are available *in vivo* (animals and humans) and *in vitro* to assess ***dermal absorption*** of methyl salicylate, either undiluted or in formulations. 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 methyl salicylate; open application 6h to the chest and back) to 93% (human *in vivo* study with methyl salicylate 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 ranging from 2 to 43%.

Further evidence of dermal absorption of methyl salicylate can be anticipated from physico-chemical data. Indeed, according to the REACH guidance document 7c, the physico-chemical properties of methyl salicylate 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.

From all the above data and according to the R7C guidance document, a default dermal absorption of 100% can be considered for methyl salicylate. This value may be further revised during the RMOA based on current approach used by the SCCS in its note of guidance (2018) for cosmetic ingredients.

Distribution and metabolism

Methyl salicylate is widely distributed in the body and no bioaccumulation is expected after oral and dermal administrations (Unpublished Study Report #7, 1976).

The substance is hydrolyzed into salicylic acid (SA) and methanol. According to Davison *et al*. (1961), after an oral administration, methyl salicylate is hydrolyzed at 95% in 1 hour in dogs and is completely hydrolyzed to free salicylate within 20 minutes in rats. Metabolism seems less rapid and less extensive in humans with 21% of methyl salicylate remaining non hydrolysed at 90 minutes. After dermal administration of a cream containing 20% methyl salicylate to rats, free salicylate rapidly appears in blood (with a peak at 30 minutes) and level of unhydrolyzed methyl salicylate is low (Cross *et al*., 1998).

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 methyl salicylate during hydrolysis. The alcohol is metabolized to corresponding aldehyde and acid and ultimately to CO_2 (RIFM, 2007).

Figure 1 Biotransformation of salicylic acid (issued from RIFM, 2007).

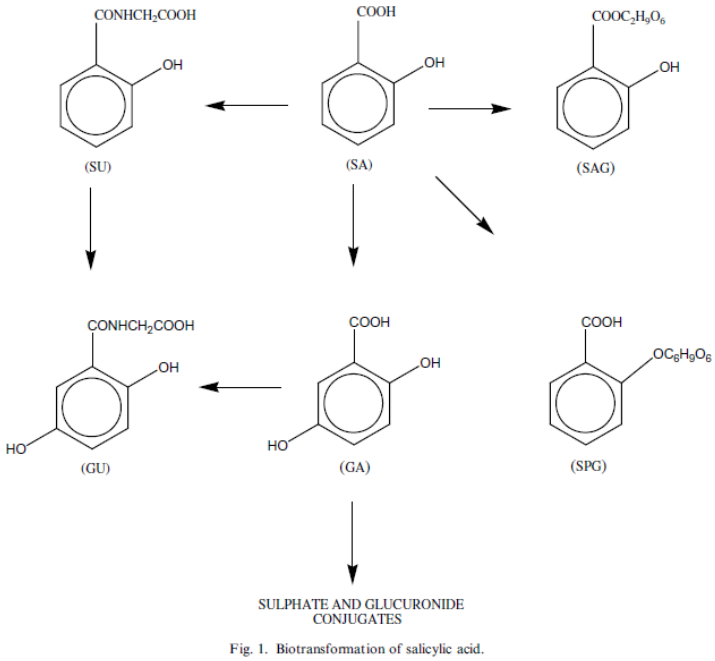


Fig. 1. Biotransformation of salicylic acid.

Elimination

MeS is mainly and rapidly excreted in the urine after oral and dermal administration. Low levels are found in the faeces (Unpublished Study Report #7, 1976).

7.9.2. Acute toxicity and Corrosion/Irritation

Acute toxicity

The substance is acutely toxic by oral route (LD_{50} of 580 mg/kg bw in mice) based on several studies (RIFM, 1982 ; Giroux *et al.,* 1954 ; Ohsumi *et al.,* 1984, cited in Lapczynski *et al.,* 2007; Rumyantsev *et al.,* 1992 cited in CIR, 2003 ; Castagnou *et al*., 1952 ; Fassett (1963) ; Bisesi, 1994; Jenner *et al.,* 1964; Davison *et al.,* 1961)

Methyl salicylate is not acutely toxic by dermal (Bisesi, 1994; Moreno, 1973) or inhalation routes (Rumyantsev *et al.,* 1992 cited in CIR, 2003)

Based on the available information, the eMSCA submitted in June 2018 a CLH report for classifying methyl salicylate as Acute Tox 4 – H302; ATE = 580 mg/kg bw. The RAC concluded in September 2019 a classification as Acute Tox 4, with an ATE of 890 mg/kg bw.

Corrosion/ Irritation

The substance is not a skin irritant based on an experimental study in rabbits (Unpublished Study Report #5, 1999).

The substance is not expected to be a respiratory irritant, based on the lack of reported local effect on respiratory tract in acute and repeated dose toxicity studies of low quality.

During the initial Substance Evaluation, the evaluating MSCA had considered that further information was needed to appropriately conclude on the eye irritation potential of the substance. Details and conclusion outcome are provided below.

Table 18: Studies on eye irritation

Method	Results	Remarks	Reference
rabbit (New Zealand White) – 1 animal Vehicle: unchanged (no vehicle)	not irritating Conjunctivae score: 1 of max. 1 (animal #1) observed at 1 hour (fully reversible within: 24 hours) Results in one animal; not confirmed by the use of additional animals.	2 (reliable with restrictions) key study experimental result **Test material (EC name): methyl salicylate**	Unpublished Study Report#3 (2001)
STE assay Rabbit corneal cell line SIRC Vehicle: physiological saline OECD TG 491	Category 1 Cell viability: 3.9-19.9% for 0.05% MeS 25.5-31.0% for 5% MeS	1 (reliable without restrictions) key study experimental result **Test material (EC name): methyl salicylate; 99.3% purity**	Unpublished Study Report #8 (2019)

During the initial evaluation of the substance, one eye irritation study (Unpublished Study Report#3, 2001) was provided by the registrant in the registration dossier as a key study. This study consisted in the application of unchanged methyl salicylate into the eye of one rabbit. Chemosis, discharge and redness of the conjunctiva (score of 1) were reported at 1 hour. No further irritation was observed at later observation times. Since this result was not confirmed in 2 additional animals as recommended in the OECD test guideline, no final conclusion was possible from this study.

In contrast, additional data (Carpenter, 1946 ; Sax and Lewis, 1992 ; Fassett, 1963 ; Unpublished study report#14, 1963), submitted as IUCLID summaries only, reported

ocular irritation but were assigned with a reliability of 3 or 4 due to very low level of details on study design and results and/or protocol with major deficiencies. The limitations consisted for example in: study not performed according to current recognized test guideline, application of methyl salicylate to the center of the cornea rather than in the conjunctival sac, tested material consisting in mixture etc. Contradictory results are also reported for methyl salicylate in the Cosmetic Ingredient Review (CIR, 2003).

Furthermore, methyl salicylate is rapidly and mostly metabolized into salicylic acid, which is classified as Eye Damage 1 (ATP13). However, it is not known to what extent this classification applies to methyl salicylate.

Considering the available information described above, no final conclusion could be made on the eye irritation properties of methyl salicylate. Therefore, as an outcome of the initial substance evaluation, a new eye irritation study (short time exposure (STE) *in vitro* test (test method OECD TG 491) was requested.

The STE study was submitted in 2019 (Unpublished Study Report#8). Solutions of methyl salicylate with concentrations of 0.05% and 5% were prepared using physiological saline. After 5-min exposure, cell viability ranges from 3.9 to 19.9% (mean = 11.8%) for 0.05% methyl salicylate and from 25.5 to 31.0% (mean = 27.8%) for 5% methyl salicylate. Since cell viabilities for each concentrations were below 70%, the substance should be classified as Eye Damage 1. Positive, medium and solvent controls were included. Acceptability criteria were met.

In conclusion, based on the new STE study, it is considered that methyl salicylate should be classified as Eye Damage 1 according to CLP Regulation. Since it is not an endpoint of high priority for C&L process, <u>all</u> registrants are advised to self-classify the substance accordingly. Update of the CSR by the registrants is also strongly recommended to take into account this hazard in the chemical risk assessment and communicate adequate risk management measures to downstream users.

7.9.3. Sensitisation

<u>Skin sensitisation</u>:

Several animal studies are 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 to 100%. Overall, methyl salicylate is negative in Maximisation studies with methodological limitations, in particular a low number of animals tested that can decrease the sensitivity of the tests (Unpublished Study Report#3, 2001; RIFM, 1981, cited in Lapczynski *et al*., 2007; Kimber *et al*., 1991; Klecak *et al*., 1977). At concentrations below or equal to 20%, methyl salicylate is clearly negative in all LLNA available (Kimber *et al*., 1998; Gerberick *et al*., 1992; Kimber *et al*., 1991). At higher concentrations, conflicting results are obtained. However, positive results are found with methyl salicylate at concentrations above 25% in 3 LLNA of adequate quality (Montelius *et al*., 1994 and 1998 and Adenuga *et al.,* 2012). The differences of results can be explained by some variations in the protocols used, the different solvent vehicles and the concentrations tested.

Human data are available including 3 human volunteer induction studies, 8 diagnostic studies and 2 case reports. No sign of skin 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 contrast, positive reactions were noted after patch testing in 7/8 diagnostic studies (Romaguera & Grimalt, 1980; de Groot *et al.,* 2000; Rudner, 1977; Ferguson & Sharma, 1984; Mitchell *et al.,* 1982; Nethercott *et al.,* 1989; Addo *et al*.,

1982; Stingeni *et al.,* 1995). Finally, two individual cases of skin sensitisation to methyl salicylate are reported in the literature (Oiso *et al.*, 2004; Hindson, 1977).

In conclusion, the evaluating MSCA considers methyl salicylate as a skin sensitiser. In this context, evaluating MSCA submitted in June 2018 a C&L proposal to classify methyl salicylate as Skin Sens. 1B based on animal and human data. This classification was agreed by the RAC in September 2019.

Respiratory sensitization:

Methyl salicylate is not expected to be a respiratory sensitizer based on a respiratory LLNA (Arts *et al*., 2008).

7.9.4. Repeated dose toxicity

Oral route - Animal data

Chronic toxicity studies:

Chronic/carcinogenicity toxicity studies were conducted on methyl salicylate in rats and dogs for two years (Webb and Hansen, 1963). Nevertheless, these studies are limited in endpoints evaluated (no biochemical, urinalysis and ophthalmological examination, limited histopathological examination).

Webb and Hansen (1963) administered methyl salicylate to groups of male and female Osborne-Mendel rats (50 animals/dose) at concentrations of 0, 0.1%, 0.5%, 1.0% or 2.0% in the diet equivalent to approximately 0, 50, 250, 500, and 1000 mg/kg bw/day for two years. All rats in the 1000 mg/kg bw/day group died by the 49th week. Body weight of both sexes was significantly decreased in the 500 and 1000 mg/kg bw/day groups. An increased amount of cancellous bone was present in the metaphysis in rats treated at either 500 or 1000 mg/kg bw/day. Bone effects were moderate in 5/9 and marked in 4/9 bones examined at the highest dose. There were of slight degree in 2/11 bones examined in the 500 mg/kg bw/day group and in 1/11 bone in the 250 mg/kg bw/day group. The relative testis weight of males was significantly increased as were the relative weights of the heart and kidneys of females in the 500 mg/kg bw/day group (this was not examined at 1000 mg/kg bw/day). Gross pituitary gland lesions were found in 10 rats at 250 mg/kg bw/day compared to 4 animals in the control groups. No gross lesion of the pituitary in rats from 500 mg/kg bw/day was mentioned in the publication. Based on this study, the NOAEL is 50 mg/kg bw/day.

The authors performed a supplemental study to understand the bone changes observed in rats. Six rats were given 0% or 2% of methyl salicylate until they died (the last one on day 71). In this study, it was reported that chondroclastic and especially osteoclastic activity was virtually completely blocked. Chondroblastic and osteoblastic activity was somewhat diminished. Other effects included slight to moderate lung damage (4/6 animals) and focal gastric hemorrhages (3/6 animals).

The same authors gave methyl salicylate in capsule form to beagle dogs (2/sex/dose) at doses of 0, 50, 150, or 350 mg/kg body weight/day, 6 days/week for 2 years. One high-dose animal died of hepatitis apparently unrelated to methyl salicylate. Dogs treated at 150 and 350 mg/kg bw/day presented reduced body weight and had enlarged livers, seen microscopically as enlarged hepatic cells. Based on this chronic oral study, the NOAEL is 50 mg/kg bw/day.

Subchronic toxicity studies:
Subchronic toxicity studies have been conducted on methyl salicylate with durations between 12-17 weeks in rats and from 59 days to 7.5 months in dogs.

In a 17-week study (Webb & Hansen, 1963), Osborne-Mendel rats (10/sex/dose) were fed with methyl salicylate at 0%, 0.1% or 1.0% (equivalent to 0, 50 and 500 mg/kg bw/day) in the diet for 17 weeks. The endpoints evaluated were limited since no hematological or biochemical examination were performed and histopathology was done only on some organs (excluding bone) of a few numbers of rats. The high dose was associated with reduced bodyweight gain. No effects were reported in the other dose groups. The results of this study support a NOAEL value of 0.1% in the diet, equivalent to 50 mg/kg bw/day.

A set of six mechanistic studies of 6 to 12 week duration examined the effects of methyl salicylate on bone metabolism and growth (Unpublished Study Report #1, 1978). Sprague-Dawley rats fed a diet containing between 0.2 and 2% (equivalent to 100 to 1000 mg/kg bw/day) depending on the study. Bone lesions were reported in all studies at doses equal or higher than 1.13% (equivalent to 560 mg/kg bw/day) leading to an overall NOAEL of 450 mg/kg bw/day (0.9%) for this effect. Growth retardation was also found in all studies at doses equal or higher than 0.63% (320 mg/kg bw/day) leading to the lowest NOAEL of 180 mg/kg bw/day. Similar effects on body weight, food consumption and on bones were observed with Acetylsalicylic acid (ASA) and Sodium salicylate (NaS) when tested in one of the mechanistic studies.

In a subchronic study (Webb and Hansen, 1963), groups of two beagle dogs (1/sex) were given 50 to 1200 mg/kg of methyl salicylate orally in capsule form daily 6 days per week for up to 59 days. In this old study, the endpoints evaluated were limited since no hematological or biochemical examination was performed and histopathology was not done on all organs, as recommended in current validated guidelines. Dogs receiving 500 mg/kg/day or more lost weight and died or were killed due to moribund condition within 1-month of study initiation. Moderate to marked fatty changes were observed in the liver of one animal at 800 mg/kg and both at 1200 mg/kg. Animals given 500 mg/kg had diarrhea and weakness during their last 3 days. No adverse effects were observed in animals given 50, 150 and 250 mg/kg/day methyl salicylate. A NOAEL of 250 mg/kg bw/day was identified.

Two other studies were available in dogs (Unpublished Study Report#1, 1978). In the first study, a NOAEL of 300 mg/kg bw/day was stated based on mortality, decreased body weight, increased relative liver and kidney weight and effects in the liver (increase in liver cell size and alteration in cytoplasmic granulometry) were found after exposure of Beagle dogs for 6.5-7.5 months to 500 mg/kg bw/day. In the second study, no treatment-related effects were observed after exposure of dogs for 6 months to doses up to 167 mg/kg bw/day.

Oral route – human data

Abbott & Harrisson, 1978 conducted a retrospective evaluation of clinical cases which had been treated with salicylates and where periodic X-rays were available to establish any possible changes in the density of the metaphyses approximating the effected joints. A total of 155 cases involving young actively growing children which had received salicylate therapy for various forms of juvenile rheumatoid arthritis was included. Various forms of salicylates were given in daily doses ranging from 100 to 3240 mg for durations between several months to intermittent dosage over a 14-year period. Prolonged salicylate therapy did not elicit bone pathology in these children.
Study Report #1 (1978) conducted a retrospective study to ascertain whether or not hepatomegaly was associated with massive daily dosages of salicylates used in the management of juvenile rheumatoid arthritis. A total of 218 cases with records of liver palpation was reported. Salicylate dosages ranged up to 4800 mg/day over periods of up to 8 or 10 years. The authors concluded that salicylate therapy did not cause hepatomegaly or interfere with growth or weight gain. However, hepatomegaly was occasionally seen upon admission or during course of treatment.

Dermal route

Webb and Hansen (1963) applied methyl salicylate dermally to groups of three rabbits of mixed sex at 0.5, 1.0, 2.0 and 4.0 mL/kg (590, 1180, 2360, and 4720 mg/kg) body weight/day, 5 days per week for up to 96 days. No control group was included. All 3 high-dose animals had died by day 28 of exposure, following weight loss and depressed activity. At 2.0 mL/kg, slight sloughing of epidermal scales was observed in 2/3 rabbits. No dermal effects were noted in rabbits exposed to 0.5 or 1.0 mL/kg. It is further indicated in the publication that the incidence of spontaneous nephritis and mild hepatitis appeared increased. The NOAEL for local effects was 1.0 mL/kg/day (1180 mg/kg bw/day). No NOAEL can be determined for systemic effects since it was not clear at which concentration the effects on liver and kidney occurred.

Inhalation route

Four female rats were exposed to a saturated atmosphere (700 mg/m^3) of methyl salicylate for 7 hours per day, 5 days per week for 4 weeks (20 exposures). Examinations consisted on clinical signs, body weight, urinalysis, hematology, biochemistry, microscopy of some organs. Methyl salicylate at 700 mg/m^3 did not cause any adverse effects (Gage, 1970). A further study was summarized in the CIR review (2003): a NOAEC of 8 mg/m^3 was reported in rats exposed for 4 months based on changes in nervous system functioning, hematological, urinalysis and biochemical changes and effects on lung, lymphoid tissues and kidney at 40 mg/m^3.

Conclusion:

From the current available data, an overall NOAEL of 50 mg/kg bw/day can be set from the 2-year studies in rats and dogs by oral route. The main target organs reported are bones for rats and liver for dogs. However, it should be noted that the studies are limited in endpoints evaluated and in number of animals examined. Furthermore, it is not indicated if a statistical analysis was performed on histopathological findings. Therefore, it cannot be excluded that effects occur at a lower dose in organs not examined. In addition, considering the small number of animals examined at histopathology, only the effects occurring at a high incidence can be detected in these studies.

The available studies performed by dermal route and inhalation are considered not reliable.

7.9.5. Mutagenicity

Based on the data available during the 12-month evaluation period, evaluating MSCA considered that a clastogenic concern had been raised that needs to be clarified.
Methyl salicylate did not show any mutagenicity in bacteria (*S. typhimurium*) (Unpublished Study Report#4, 1986; Ishidate *et al.*, 1984; Kuboyama & Fujii, 1992; Oda *et al*., 1978).

Contradictory results were obtained for induction of chromosomal aberrations in two *in vitro* studies of questionable quality. Methyl salicylate was negative in one *in vitro* mammalian chromosome aberration test without metabolic activation in CHL cells (Ishidate *et al*., 1984). However, due to major deficiencies of this study (no short treatment, no metabolic activation, no information on cytotoxicity and absence of positive control) when compared to current validated test guidelines, no definite conclusion can be made from this study. In contrast, chromosomal aberrations were reported in hamster lung fibroblasts with and without metabolic activation (Kawachi, 1980a & b cited in the JECFA document (serie 48), 2002), without any further details reported in the safety evaluation performed by the JECFA in 2002. No *in vivo* study investigating chromosomal aberrations induction was available for methyl salicylate.

Mixed results were also obtained for chromosomal aberrations with other salicylates considered (SA, ASA and NaS) either in *in vitro* or *in vivo* studies (Stich *et al*., 1981; Unpublished Study Report #6, 2008; Müller *et al*., 1991; Giri *et al.*, 1996; EMEA, 1999; Coffing, 2011). However, it should be noted that many of these tests are old, insufficiently documented or presented major deficiencies.

In conclusion, some *in vitro* and *in vivo* data, although not fully reliable and contradictory, suggested a clastogenic potential of salicylates. In the absence of adequate data with methyl salicylate, an *in vitro* mammalian cell micronucleus test (test method: OECD 487) was requested in the initial draft decision sent to the Registrants.

During the Registrants commenting period, they provided additional existing information, including a pharmacology review and evaluation of a medical patch containing methyl salicylate (the Food and Drug Administration, FDA, 2006) referring, amongst others, to additional data on genotoxicity of methyl salicylate. The FDA (2006) review reports the following results: *In vitro*, an Ames test and a chromosomal aberrations assay in Chinese hamster fibroblast cells reported negative results. *In vivo*, a micronucleus assay in rats did not show genotoxic effects up to the highest tested dose of 1000 mg/kg bw/day administered by subcutaneous route (twice at 24h interval) in male rats. Exposure of the substance to bone marrow was evidenced by a decrease of immature erythrocytes observed from 500 mg/kg bw/day. In addition, at 1000 mg/kg bw/day, toxicity was evidenced by the mortality of 4/10 animals.

These new data provide adequate information to allow a clarification of the initial request related to a concern for genotoxicity (i.e. an *in vitro mammalian cell micronucleus test (test method OECD 487*). No further action is needed at this time for this endpoint in this SEv framework.

7.9.6. Carcinogenicity

Carcinogenic potential of methyl salicylate was assessed in a 2-year study by the oral route in rats exposed to 0, 0.1, 0.5, 1 or 2% methyl salicylate (equivalent to 0, 50, 250, 500, and 1000 mg/kg bw/day) in the diet (Webb and Hansen, 1963). This study suffers of various methodological deficiencies as described in section 7.9.4 of this document. In addition, it should be noted that in the 2% group, carcinogenicity cannot be adequately assessed since all animals died within the first year. Similar kind and numbers of tumors occurred in rats on all diets, mammary tumors of the females being the most common one. Benign pituitary tumors occurred in similar numbers of surviving rats on all diets. Malignant pituitary tumors occurred in one male and two females at 0.5% diet but not in the higher dose groups. These authors also administered methyl salicylate in capsule to dogs at doses of 0, 50, 150 or 350 mg/kg/bw/day for 2 years. No information on tumor was reported.

Other non-standard and not reliable studies were submitted. Methyl salicylate did not induce skin papilloma and malignant tumor in mice after dermal application of 1 mL at biweekly interval for 400 days (Burdette & Strong, 1941). No increase of lung tumor was found in mice exposed to 100 or 500 mg/kg bw of methyl salicylate 3 times per week for 8 weeks by intraperitoneal route (Stoner *et al.,* 1973).

Additional information is available from published reviews. Antitumor activity of wintergreen oil (containing 99% of methyl salicylate) was evaluated in 32 mice of the A strain that commonly develops spontaneous tumors of the mammary gland. The oil was added to the diet at 1, 2, or 3 drops of oil to 1 g of diet daily for an unspecified period after tumors had developed. There was no detectable effect on animal survival or tumor growth rate. In a related study, the effect of wintergreen oil in the diet on the occurrence

of spontaneous mammary gland carcinomas was studied in 45 female D strain mice. Average time to tumor formation was 18 months in treated mice and 12.1 months in controls (Strong, 1932 cited in RIFM, 2007 and Lapczynski, 2007 reviews).

In conclusion, even if not fully adequate carcinogenicity study conducted according to current test guidelines is available, no carcinogenic concern has been raised for methyl salicylate. No further information needs to be requested in the SEv framework.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Methyl salicylate was originally selected for substance evaluation in order to clarify a concern related to reproductive toxicity.

Effects on fertility

Table 19 Studies on fertility with methyl salicylate

Method	Results	Remarks	Reference
Three-generation study (each generation mated twice) in rats rat (Osborne-Mendel) male/female 20/sex/dose oral: feed 0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw as MeS) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 100 days before the first mating and then throughout the experiment (until weaning of the 3rd generation). Examination: 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). Limited statistical analysis. A supplementary study was performed with adding calcium carbonate to methyl salicylate diet with the same examination. Several deficiencies from OECD 416, not GLP	NOAEL (fertility): 250 mg/kg bw/day (male/female) based on no statistically significant effect reported. NOAEL (development): 75 mg/kg bw/day based on statistically significant decrease of litter size, viability (D0), survival (D4), weaning data in the second generation and decreased pup body weight at 150 mg/kg bw/day. The addition of calcium carbonate did not markedly differ from those obtained after administration of methyl salicylate alone.	3 (not reliable) Supportive study No definitive conclusion for all fertility and development endpoints based on the limited examination performed. Experimental result **Test material (EC name): methyl salicylate**	Collins *et al.* (1971)
Two-generation study (each generation mated twice) in rats rat (Wistar) male/female 25/sex/dose (F0); 30/sex/dose (F1) oral: feed	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 mating for the first generation and decreased reproduction	3 (not reliable) Supportive study No definitive conclusion for all fertility and development endpoints based on	Unpublished Study Report #1 (1978)

0.25% and 0.5% (2500 ppm and 5000 ppm equivalent to 125 and 250 mg/kg bw MeS). (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 60 days before the first mating and then throughout the experiment (weaning of the F2b litters). Examination: number of pups, stillbirths, live birth, postnatal mortality, gross abnormalities, physical and behavioural abnormalities. Several deficiencies from OECD 416, not GLP	index for both generations at the highest dose. Higher number of death between birth and day 5 at 250 mg/kg bw/day.	the limited examination performed. experimental result **Test material (EC name): methyl salicylate**	
One-generation study in rats rat (Sprague-Dawley) male/female 24-27 animals/dose oral: feed 4000 ppm and 6000 ppm equivalent to 200 and 300 mg/kg bw MeS. (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 60 days before the first mating and then throughout the experiment (until weaning of offspring on day 20-21) Guideline and GLP not stated – secondary literature	NOAEL (F1): 300 mg/kg bw/day (male/female) No abnormalities. Neonate survival at weaning was greater in the test group than in control.	4 (not assignable) Disregarded study experimental result **Test material (EC name): methyl salicylate**	FDA (1966) cited in CIR (2003)
Two-generation study in mice **(NTP continuous breeding protocol: tasks 2 + 4)** mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. oral: gavage in corn oil 0, 25, 50 and 100 mg/kg/day. (nominal conc.) Purity ≥ 99% 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. NTP continuous breeding protocol (Task 1, dose finding; Task 2, continuous breeding phase, Task 3,	NOAEL (reproductive effects): 100 mg/kg bw/day – no adverse effect NOAEL (developmental effects): 100 mg/kg bw/day – no adverse effect	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): methyl salicylate**	NTP (1984a) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Lamb *et al.*, (1997)

identification of the affected sex and Task 4, offspring assessment). **In this study, only tasks 2 and 4 were performed[2].** Limited examination: For parental generation: clinical signs and body weight, sperm measures (F1), fertility and mating index, limited examination of organ weight (liver, pituitary, brain, reproductive tract), gross and histopathology (pituitary and reproductive tract). For pups: number, sex, live and dead, body weight NTP protocol, GLP			
One generation+ fertility in mice **(NTP continuous breeding protocol: tasks 2 + 3)** mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. oral: gavage 100, 250 and 500 mg/kg/day (nominal conc.) Purity ≥ 99% Vehicle: corn oil 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 task 3 was also carried out: high-dose animals of each sex were mated to control mice of the opposite sex. NTP continuous breeding protocol (Task 1, dose finding; Task 2, continuous breeding phase, Task 3, identification of the affected sex and Task 4, offspring assessment). **In this study, tasks 2 and 3 were performed**. Limited examination: For parental generation: body weight, clinical signs, fertility and mating index. For pups: viability and body weight NTP protocol, GLP	Task 2: 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. No conclusion can be made from this task.	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): methyl salicylate**	NTP (1984b) Chapin & Sloane (1997) Morrissey *et al.,* (1989)

[2] A task 3 was performed in another NTP study (1984b)

Two-generation study in mice mouse male/female (no data on strain) 25/sex/dose (F0); 30/sex/dose (F1) oral: feed 0.25% and 0.5% (2500 ppm and 5000 ppm, equivalent to 375 and 750 mg/kg bw MeS) (nominal in diet) Vehicle: unchanged (no vehicle) Exposure: 30 days before the first mating and then through the experiment (weaning of the pups). Examination: number of pups, stillbirths, live birth, postnatal mortality, gross abnormalities, physical and behavioral abnormalities. Several deficiencies from OECD 416, not GLP	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	3 (not reliable) Supportive study No definitive conclusion for all fertility and development endpoints based on the limited examination performed. experimental result **Test material (EC name): methyl salicylate**	Unpublished Study Report #1 (1978)
Study of fertility and early embryonic development in rats Crj:CD(SD)IGS rats male/female 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. Subcutaneous administration GLP and ICH guidelines	NOAEL for general toxicity: 100 mg/kg/day based on 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.	1 (reliable without restriction) Key study experimental result **Test material (EC name): methyl salicylate**	FDA (2006a) Reference provided during the registrants' commenting period

Effects on fertility

Data on methyl salicylate

The evaluation performed during the initial 12-month evaluation period was based on the following studies: Collins *et al*., 1971; Unpublished Study Report #1, 1978a & b; NTP, 1984a & b; FDA, 1966.

Even if several fertility studies are available for methyl salicylate, the parameters examined in parents and pups are very limited and do not allow to reach a definitive conclusion for this endpoint. For pups, effects on peri- and post-natal development cannot be adequately assessed (e.g. no adequate histopathology, sexual maturation examination…). Parameters examined in parental generation were also very limited.

In addition, although not reported as statistically significant, some effects on reproductive function were found in these studies. Collins *et al.* (1971) reported "*appreciable decreases*" of fertility index in the second and third generations in a three generation study in rats. In the Unpublished Study report #1 (1978), an increase of unsuccessful mating was noted in

the first generation. In the NTP continuous breeding test in mice (NTP, 1984a), mating and fertility indices were decreased in the second generation (task 4). Therefore, these effects found on the reproductive function in different studies in a non-significance manner do not allow a firm conclusion on this endpoint.

Data on other salicylates

Available data with other salicylates (such as salicylic acid and sodium salicylate), whereas not fully adequate, raise additional concerns related to fertility/gestation (CIR, 2003; disseminated registration dossiers) and endocrine disruption potential (see section 7.10.2 for details).

Therefore, based on the data available during the initial 12-month evaluation process described above, it was considered that the quality of the studies performed with methyl salicylate does not allow to reach firm conclusions but is sufficient to raise concerns for methyl salicylate. First, no definitive conclusions on peri- and post-natal development can be drawn because of the current lack of relevant information in the registration dossier or elsewhere. Furthermore, some reproductive and endocrine effects are identified with methyl salicylate or other salicylates. Therefore, based on the data above, the evaluating MSCA considered that the potential impact of methyl salicylate on the reproductive function, including endocrine disruption, needs to be clarified, in particular considering the wide dispersive uses of methyl salicylate leading to exposure of sensitive populations (including pregnant women, newborn or children). In this context, an Extended One Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) was requested in the initial draft decision sent to the registrants. *This study should include a F2 generation (based on the wide dispersive uses and the bone effects suggesting a potential oestrogenic activity of MeS) and cohorts 2A/2B for DNT (based on potential anti-androgenicity and effects on thyroid hormones of ASA). Specific additional endpoints must also be assessed in this study: assessment of bone effect (as the most sensitive target organ reported) and measurement of testosterone, thyroid hormones and cortisol (to identify as early as possible any disturbance of hormone homeostasis). A determination of StAR (steroidogenic acute regulatory protein), PBR (peripheral-type benzodiazepine receptor) and brain GR (glucocorticoid receptor) transcripts or protein levels in parental and offspring generations should also be included in order to conclude on the relevance of the endocrine findings observed in fishes to humans. Finally, one of the hypothesis suggested in the literature to explain the endocrine effects found with acetyl salicylic acid or salicylic acid is the inhibition of prostaglandins synthesis. In this context, a measurement of PGE2 and PGD2 must be undertaken in the required EOGRTS.*

However, during the commenting period, Registrants provided additional existing information, including a pharmacology review and evaluation of a medical patch containing methyl salicylate (the Food and Drug Administration, FDA, 2006) referring, among other, to a study of fertility and early embryonic development. In this study (FDA, 2006a), 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, in the sperm form anomalies index, sperm count or sperm motility. There was no significant difference in the count of oestrus or estrous cycle. Both

copulation and fertility indices[3] were not affected by methyl salicylate treatment. 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 but not at 300 mg/kg/day. Salicylic acid concentration in plasma was measured on day 0 and day 13 of administration. The increase was nearly dependent on increases in the dose ratio and is 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 is 300 mg/kg/day (no adverse effect).**

These new data provide adequate information to clarify the initial concern related to reproductive toxicity and therefore allow to withdraw the request initially formulated in the decision i.e. an *Extended one-generation reproductive toxicity study (test method: OECD 443)*. No further action is needed at this time in this SEv framework. In addition, it can be noted that all these data were evaluated by the RAC in September 2019 which reached the conclusion that no classification is necessary for methyl salicylate regarding its toxicity on fertility.

Developmental toxicity

Table 20 Studies on developmental toxicity with methyl salicylate

Method	Results	Remarks	Reference
Prenatal developmental assay (GD6-18) Rabbit New Zealand White (18-20 females/group) Methyl salicylate (purity: 100.1%) 0, 30, 100, 300 mg/kg bw/day in corn oil Exposure: day 6 to 18 (daily) Subcutaneous administration Study performed according to ICH guidelines and GLP	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 on increases in the dose ratio and is scarcely affected by repeated dosing	1 (reliable without restriction) Key study experimental result **Test material (EC name): methyl salicylate**	FDA (2006b) Reference provided during the registrants' commenting period
Prenatal developmental assay (GD6-17) Rat Crj:CD(SD)IGS (20 females/group) Methyl salicylate (purity: 100.1%) 0, 50, 100, 200 mg/kg bw/day in corn oil Exposure: day 6 to 17 (daily) Subcutaneous administration Study performed according to ICH guidelines and GLP	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.	1 (reliable without restriction) Key study experimental result **Test material (EC name): methyl salicylate**	FDA (2006c) Reference provided during the registrants' commenting period
Study for effects on pre and	NOAEL maternal: 60	1 (reliable without	FDA

[3] 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

postnatal development including maternal function Crj:CD(SD)IGS pregnant female rats (20/group) Methyl salicylate (purity: 100.1%) 0, 20, 60, 200 mg/kg/day in corn oil Exposure: from gestation day 6 to lactation day 21 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 with females sacrificed on gestation day 13. GLP and ICH guidelines	mg/kg/d based on decreased body weight, food consumption and mortality at 200 mg/kg bw/day. NOAEL development < 60 mg/kg/day 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.	restriction) Key study experimental result **Test material (EC name): methyl salicylate**	(2006d) Reference provided during the registrants' commenting period
See also studies described in Table 19.			

Developmental toxicity

Studies on methyl salicylate:

The evaluation performed during the initial 12-month period was based on studies of low quality.

Pregnant rats received dermal application of undiluted methyl salicylate or diluted in a petroleum based grease. Undiluted methyl salicylate was initially applied at 2000 mg/kg bw/day from gestation day (GD) 6 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).

Methyl salicylate 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. Most embryos were recovered at gestation day (GD) 9, few survived to the age of 12 days. Both oral and dermal treatments produced neural tube defects, especially in the area of the developing brain. 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 foetuses 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 methyl salicylate by intraperitoneal route on days 10 and 11 of pregnancy. The pups 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 pups than in the control group. Fetal kidneys weighted significantly less than those of the

controls and lengthening of the renal papilla was inhibited by methyl salicylate, suggesting that methyl salicylate 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 methyl salicylate 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 methyl salicylate 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).

Developmental effects 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 were reported in the Collins *et al.* (1971) study in rats. These effects were 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). The NOAEL for development was 75 mg/kg bw/day based on pup mortality and decreased weight.

- Decrease of litter size and higher number of deaths between birth and day 5 at 250 mg/kg bw/day were observed in the Study Report#1 (1978) study in rats.

- "Slightly smaller litter size" in rats from 375 mg/kg bw/day at birth was cited by Study Report#1 (1978).

- Reduced pup viability, decrease in the mean number of litter, in the average of pups per litter and the proportion of pups born alive were reported at 500 mg/kg bw/day in the NTP (1984b) study in mice. 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.

Based on these results, eMSCA concludes that methyl salicylate is toxic for development of rodents.

During the Registrants commenting period, they provided additional existing information, including a pharmacology review and evaluation of a medical patch containing methyl salicylate (the Food and Drug Administration, FDA, 2006) referring, among other, to additional data on developmental toxicity of methyl salicylate.

In the first study (summarized in FDA, 2006b), pregnant New Zealand White rabbits 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. No developmental effect was reported in this study.

In the second study (summarized in FDA, 2006c), pregnant rats 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. A NOAEL of 100 mg/kg/day is set for maternal toxicity based on decreased body weight and body weight gain. Lower body weight of live foetuses was observed at 200 mg/kg/day. In the highest dose group, there was an increase of external anomalies (characterized principally by craniorachischisis and gastroschisis). Visceral anomalies (ventricular septal defect, dilatation of the ureter (unilateral) and thymic remnant in the neck) were also increased at 200 mg/kg/day but were not statistically significant. A statistically significant increase of skeletal variations was also observed at the highest dose, 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. The NOAEL for developmental toxicity was 100 mg/kg/day.

In the third study (summarized in FDA (2006d)), pregnant female rats 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 maternal NOAEL is set at 60 mg/kg bw/day based on 2 mortalities, lower mean body weight and body weight gain and decreased food consumption. Most of the effects reported in offspring occurred at the highest tested dose.

The following effects were reported in offspring at 200 mg/kg bw/day at birth: significant decrease in the birth index and lower body weight in live male newborn, trend toward a decrease in the number of litter and live newborn and a trend toward an increase in the stillbirth index, craniorachischisis in 4 stillborn.

The following effects were reported in offspring at 200 mg/kg bw/day during lactation: excessive elongation of the maxillary incisors, corectopia and dycoria in few animals, significant lower mean body weight with decreased food consumption, significant decrease in the differentiation indices (incisor eruption in both sexes, eyelid separation in the females and cleavage of the balanopreputial gland in the males).

The following effects were reported on offsprings at 200 mg/kg bw/day at weaning: effects on organ weights (decrease in the absolute and relative weights of the liver and kidneys, in the absolute weights of the brain, adrenals and testes and increase in the relative weights of the brain and lungs in males; decrease in the absolute weights of the brain, heart, lungs, liver, kidneys, adrenals and ovaries and increase in the relative weight of the brain in females), skeletal abnormalities (especially fusion of the cervical vertebra and misshapen sternebra) and variations (full supernumerary ribs, accessory sternebra, lumbarization, 7 lumbar vertebrae and incomplete ossification of the cervical, thoracic and lumbar centrum).

The following effects were reported in offspring at 200 mg/kg bw/day at the time of necropsy of offspring (gestation day 13 for females and after mating for males): significant lower body weight in F1 dams, corectopia and dyscoria in 1 female, excessive elongation of the maxillary in 1 male, corectopia and dyscoria in another male. There was an increase of pre-implantation losses at the highest dose but not statistically significant.

At the lower dose of 60 mg/kg bw/day, the only effect noted is a slightly increase of skeletal variations (cervical ribs, accessory sternebra, incomplete ossification of thoracic and caudal vertebrae). Since there was no historical control data and 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. Thus, the NOAEL for development was set < 60 mg/kg bw/day (but > 20 mg/kg bw/day) based on the skeletal variations.

Finally, there was no significant effect on early development of embryos (numbers of implants or live embryos, pre-implant low index or dead embryo index) when methyl salicylate was administered 2 weeks prior to mating until sacrifice of males and until

gestation day 6 for females at doses up to 300 mg/kg bw/day by subcutaneous route (FDA, 2006a).

Human data

No human data has been found with methyl salicylate. Many human data are available for acetyl salicylic acid (ASA or aspirin). These data were taken into consideration in order to identify possible effects of salicylates (especially, salicylic acid) in humans. 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, some indications of effects on intra-uterine fetal growth retardation, lethality (Study Report#2, 2012; Rai *et al.,* 2000; Farid *et al.,* 2011) and malformations (Study Report#2, 2012; Hernandez *et al.* (2012); Kristensen *et al.* (2011)) are reported in the literature. These effects seem consistent with those reported in experimental studies performed 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 (see also above remarks on the uncertainties related to read-across between ASA and methyl salicylate).

In conclusion, methyl salicylate induces lethality, external malformations, visceral/skeletal anomalies and growth retardation in rats exposed *in utero*. The lowest NOAEL for developmental toxicity can be set at < 60 mg/kg bw/day (but > 20 mg/kg bw/day) based on skeletal variations.

The developmental effects are observed in the presence of slight toxicity in dams, which is not sufficient to explain the reported developmental effects. Therefore, based on these findings, a CLH proposal was submitted by the evaluating MSCA to ECHA in June 2018 in order to classify methyl salicylate as Repr. Cat. 1B for development. In September 2019, the RAC concluded to a classification as Repr. Cat 2 based on a read-across with the classification of salicylic acid.

7.9.8. Hazard assessment of physico-chemical properties

Explosivity

Data waiving: see CSR section 1.3 Physicochemical properties.

Discussion

In accordance with column 2 of REACH Annex VII, the study on explosive properties, required in section 7.11, does not need to be conducted as the substance is a simple organic liquid, with no structural alerts for explosiveness.

Classification according to GHS

Name: methyl salicylate

State/form of the substance: liquid

Reason for no classification: conclusive but not sufficient for classification

Justification for classification or non-classification:

The substance does not contain groups associated with explosive properties as described in the table R.7.1-28 of the ECHA guidance R.7A (May 2008). Therefore, the substance is not considered as explosive.

Flammability

Data waiving: see CSR section 1.3 Physicochemical properties.

Discussion

In accordance with column 2 of REACH Annex VII, the study on flammability, required in section 7.10, does not need to be conducted as the substance is a simple organic liquid, with no structural alerts for explosiveness nor pyrophoric properties.

Flash point

Value found in a peer reviewed handbook (Merck Index 14th) gives a flash-point of 99°C. Data is available in literature such as the BGIA Gestis which gives close value: 101,1°C. Other literature data gives values of 95,5°C or 96°C.

The following information is taken into account for any hazard / risk assessment:

The flash point values of Methyl Salicylate is 99 °C.

Classification according to GHS

Name: methyl salicylate

State/form of the substance: liquid

Reason for no classification (Flammable gases): conclusive but not sufficient for classification

Reason for no classification (Flammable aerosols): conclusive but not sufficient for classification

Reason for no classification (Flammable liquids): conclusive but not sufficient for classification

Reason for no classification (Flammable solids): conclusive but not sufficient for classification

Justification for classification or non-classification:

The substance does not contain functional group as described in section R.7.1.10.3 of the ECHA guidance R.7A (May 2008). Moreover, based on experience handling the substance, the substance is not pyrophoric, and is not flammable in contact with water.

Additionally, the flash point of the substance (i. e. 99°C) is above the upper limit set in the classification and labeling criteria (i. e. 60°C).
Therefore, the substance is not considered as flammable.

Oxidising potential

Data waiving: see CSR section 1.3 Physicochemical properties.

Discussion

In accordance with column 2 of REACH Annex VII, the study on oxidising properties, required in section 7.13, does not need to be conducted as the substance is incapable of reacting exothermically with combustible materials.

Classification according to GHS

Name: methyl salicylate

State/form of the substance: liquid

Reason for no classification (Oxidising gases): conclusive but not sufficient for classification

Reason for no classification (Oxidising liquids): conclusive but not sufficient for classification

Reason for no classification (Oxidising solids): conclusive but not sufficient for classification

Justification for classification or non-classification:

The substance is an organic substance which does not contain halogen atoms and contains oxygen not chemically bonded to nitrogen. Therefore, the substance is not considered as having oxidizing properties

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Table 21. Available dose-descriptor(s) per endpoint as a result of its hazard assessment

Endpoint	Route	Dose descriptor or qualitative effect characterisation; test type	Reference to selected study (see footnotes for justification)
Acute toxicity	Oral	Acute Tox 4; ATE = 890 mg/kg agreed by the RAC (2019)	Rumyantsev *et al.* (1992) cited in the CIR (2003)
Irritation / Corrosivity	Skin	No adverse effect observed (not irritating)	Study Report #5, 1999
Irritation / Corrosivity	Eye	Eye Damage 1	Study Report#8, 2019
Irritation / Corrosivity	Respiratory tract	No specific study but no concern raised.	Weight of evidence
Sensitisation	Skin	Sens. 1B agreed by the RAC (2019)	Weight of evidence
Sensitisation	respiratory tract	No specific study but no concern raised.	Weight of evidence
Repeated dose toxicity	Oral	NOAEL: 50 mg/kg bw/day (chronic; rat) Target organs: bone NOAEL : 50 mg/kg bw/day (chronic ; dog) Target organs: liver	Webb & Hansen, 1963
Repeated dose toxicity	Dermal	No reliable study.	
Repeated dose toxicity	Inhalation	No reliable study.	

Endpoint	Route	Dose descriptor or qualitative effect characterisation; test type	Reference to selected study (see footnotes for justification)
Mutagenicity	in vitro / in vivo	Not genotoxic	FDA, 2006
Carcinogenicity	Oral	Not carcinogenic NOAEL: 50 mg/kg bw/day (chronic; rat)	Webb & Hansen, 1963
Carcinogenicity	Dermal	No reliable study.	
Carcinogenicity	Inhalation	No reliable study.	
Reproductive toxicity: effects on fertility	Oral	Not toxic for fertility NOAEL =300 mg/kg bw/day	FDA, 2006
Reproductive toxicity: developmental toxicity	Oral	Toxic to prenatal development. Classification proposed by e-MSCA: Repr. 1B – H360f. Classification Repr. 2 agreed by the RAC (2019) NOAEL < 60 mg/kg bw/day (>20 mg/kg bw/day)	FDA, 2006 NTP, 1984

No DNEL/DMEL has been established by the evaluating MSCA during the substance evaluation.

Different DNELs have been set by the lead registrant:

DNELs for Workers:

For workers, DNELs have been set for systemic effects after acute exposure by inhalation and after long-term exposure by inhalation and dermal route.

Acute exposure

The DNEL for acute inhalation exposure is based on the subacute inhalation toxicity study, with a NOAEC of 700 mg/m^3 (Gage, 1970). This study is considered as non-reliable by the evaluating MSCA and thus appears not sufficiently robust, as such, to be used as a point of departure for risk assessment. However, methyl salicylate seems to have a low potential to induce effects after an acute exposure by inhalation based on available data.

Long-term exposure

The DNEL for long-term exposure by *dermal* route was based on the 2-year study in rats by oral route (Webb (1963)), with a NOAEL of 50 mg/kg bw/day. This choice can be justified since it is the lowest NOAEL among the available dataset. In contrast, it should be keep in mind that the Webb (1963) study is an old study which is limited in endpoints evaluated, and thus, it cannot be excluded that effects occur at a lower dose in organs not examined.

A dermal absorption of 40% was considered by the lead registrants, compared to an oral absorption of 100%. However, the available data do not allow to reach a firm conclusion on a specific value for dermal absorption. In this context, the choice of a dermal absorption

value of 40% remains questionable, without further argumentation. The value of the dermal absorption will be further revised if needed when the RMOA will be drafted.

The following assessment factors have been taken into account by the lead registrants:

- Interspecies factor (allometric scaling) = 4
- Interspecies factor (remaining differences) = 1 considering that the NOAELs set from rat and dog studies are similar. However, the NOAELs are not based on the same target organ (liver for dogs and bone for rats). Without any further argumentation, it seems not adequate to reduce the default value of 2.5.
- Intraspecies = 5

Considering the methodological deficiencies related to the repeated-dose toxicity studies (old studies with limited parameters investigated), an additional factor for the quality of the whole database may have been considered.

Two approaches have been followed by the lead registrants for the DNEL for long term exposure by *inhalation*, one based on the subacute inhalation study (Gage, 1970) and the other based on the 2-year oral study (Webb, 1963). Similar remarks on the studies and on the assessment factors as described above are applicable for this DNEL. In addition, in the case of conversion of an oral NOAEL into a corrected NOAEC for inhalation, the methodology for modification of the starting point described in the guidance on information requirements and chemical safety assessment - chapter 8 (figure R8-3) was not followed without any justification.

DNELs for the general population:

For the general population, DNELs have been set for systemic effects by inhalation (acute and long-term exposure), by dermal route (long-term exposure) and by oral route (acute and long-term exposure).

Acute exposure

For inhalation: the same comments as those made for DNEL for workers apply.

For oral route: the point of departure is a subchronic oral study, with a NOAEL of 180 mg/kg bw/day (Study Report #1, 1978). This study is a mechanistic study focusing on the effects of methyl salicylate on bone metabolism and growth. The study, which is an old study, is limited in endpoints evaluated. Thus, it cannot be excluded that effects occur at a lower dose in organs not examined. Same comments as those made on assessment factors above apply here.

Long-term exposure

For dermal route: the point of departure is the 2-year oral study, with a NOAEL of 50 mg/kg bw/day (Webb, 1963). It can be noted that in this case, the lead registrants consider that methyl salicylate is readily absorbed by dermal route (100%) (contrary to the approach followed for the DNEL for workers where a dermal absorption value of 40% was retained). Same remarks on the choice of the NOAEL and the assessment factors listed above apply here. Moreover, an intraspecies factor of 5 instead of 10, as recommended for the general population, was chosen by the lead registrants.

For inhalation: the same approach as that for the DNEL for workers was followed by the lead registrants. Same comments listed above apply here.

For oral route: the point of departure is the 2-year oral study, with a NOAEL of 50 mg/kg bw/day (Webb, 1963). Same comments on the quality of this study and choice of the assessment factors apply here.

Additional remarks

- Developmental toxicity:

No DNEL has been proposed by the lead registrants to cover developmental toxicity since they do not consider methyl salicylate as reprotoxic for human. This is not consistent with the RAC conclusion on classification of methyl salicylate as Repro. Cat. 2 for development. However, the choice of the NOAEL of 50 mg/kg bw/day (Webb, 1963) used to cover chronic toxicity is lower than the NOAELs set for developmental toxicity. Indeed, the lowest conservative NOAEL < 60 mg/kg bw/day (but > 20 mg/kg bw/day) was based on a slight increase of skeletal variations, but clear effects were rather observed at doses ≥ 100 mg/kg bw/day. Thus, the chronic NOAEL can cover this type of effects.

- Local effects (skin sensitisation and eye irritation):

No local DNEL has been proposed by the lead registrant. However, methyl salicylate is a skin sensitiser and provokes severe eye irritation. Therefore, appropriate risk management measures should be implemented to protect workers from skin sensitisation and eye irritation, taking into account the hierarchy of protective measures set out in the occupational health and safety legislation. Where exposure cannot be prevented by other means, personal protective equipments, such as gloves and goggles, should be worn to avoid skin and eye contacts. These would also reduce systemic exposure to methyl salicylate in occupational setting. **Update of the CSR by registrants is strongly recommended** to account for these hazards in the chemical risk assessment and communicate adequate risk management measures to downstream users.

In the case of consumer use, it is necessary to implement appropriate risk management measures to protect consumers from skin sensitisation and eye irritation. However, for consumers, the use of personal protective equipments is not an adequate risk management tool, and therefore other risk management measures have to be implemented. Registrants may need to reconsider whether they wish to still support the uses, and if so, adapt the products design or conditions of use in order to ensure consumer safety.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

Methyl salicylate is acutely toxic by oral route, induces skin sensitisation and teratogenic effects. Thus, a CLH report was submitted by the eMSCA to ECHA in June 2018 with the following proposals:

- Acute Tox 4 – H302; ATE = 580 mg/kg bw
- Skin Sens. 1B – H317
- Repro. 1B – H360D

In 2019, the RAC agreed with the following classification:

- Acute Tox 4 – H302; ATE = 890 mg/kg bw
- Skin Sens. 1B – H317
- Repro. 2 – H361d

The requested short-time exposure (STE) assay, submitted in 2019, confirms that methyl salicylate needs to be classified as Eye Dam. 1. However, since it is not an endpoint of high priority in regard to C&L process, the evaluating MSCA advises all registrants to self-classify the substance accordingly. Moreover, an update of the CSR by registrants is strongly recommended to account for this hazard in the chemical risk assessment and communicate adequate risk management measures to downstream users.

After subchronic and chronic exposures to methyl salicylate by oral route, bones were identified as the target organ in rats and liver as the target organ in dogs. However, the effects observed do not merit a classification.

Based on the overall dataset, no concern is raised regarding mutagenicity, carcinogenicity and fertility endpoints.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Although the validity of the read-across can be questioned based on Miller (2001) and Zhang (2012) showing various estrogenic potencies for the different salicylates tested (see HH section 7.10.2), there are some publications suggesting potential effects of salicylates on hormone release and steroidogenesis in fish involved in the response to stress (van Anholt *et al*., 2003; Gravel and Vijayan, 2006).

Table 22

Method	Results	Remarks	Reference
Tilapia *Oreochromis.mossambicus*, adults *In vivo* study oral: feed 10 and 100 mg/kg bw **Acetyl Salicylic Acid** (nominal in diet) Exposure: 3 days Dosage of plasma concentrations without and with stress (net confinement)	↓ Protaglandin (PGE_2) at 100 mg/kg bw ↓ cortisol levels at 100 mg/kg bw ↓ T3 (T4 not modified) ↑ Prolactin (PRL188) ↓ COX activity at 72 mg/L	2 (reliable with restrictions) Supporting study experimental result **Test material (EC name): Acetyl Salicylic Acid**	Van Anholt *et al.* (2003)
Rainbow trout *O. mykiss*, adults oral: feed *in vitro* Interrenal cells suspension [**Salicylic Acid**] = 1mM – 1000µM – 22h. [ACTH] = 0.5 IU/mL - 2h *In vivo* interrenal tissue [**Salicylic Acid**] = 100 mg/kg bw – 3d.	*in vitro* ↓ ACTH-mediated cortisol production at ≥10^{-5}M *in vivo* ↓ 50% ACTH-mediated cortisol production (↔ plasma cortisol when no stimulation); ↓ 20% StAR and PBR mRNAs (Cholesterol transport); ↓ 50% Glucocorticoid Receptor (GR) protein content in brain; ↔ mRNA for P450scc, 11 β-hydroxylase and GR	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): Salicylic Acid**	Gravel and Vijayan (2006)

In the Van Anholt et al. study (2003), Acetylsalicylic acid (ASA), a cyclooxygenase (COX) inhibitor, was used to characterize the effects of prostaglandins (PGs) on the release of

several hormones and the stress response of tilapia. Cyclooxygenase (COX) pathway converts arachidonic acid (ArA) into prostaglandins, which interact with the stress response in mammals and possibly in fish as well. Prostaglandins control various physiological functions in fish, including respiratory and cardiovascular output, ovulation and spawning behavior, oocyte maturation, nervous system function, osmoregulation, and immune functions.

Tilapia were fed gelatine capsules filled with 100 mg of crushed pellets and the required amount of ASA. Plasma PGE_2 was significantly reduced at 100 mg ASA/kg body wt, and both basal PGE_2 and cortisol levels correlated negatively with plasma salicylate. Basal thyroid hormone T3 (plasma 3,5,3-triiodothyronine) was reduced by ASA treatment, whereas plasma prolactin PRL_{188} increased at 100 mg ASA/kg body wt. ASA depressed the cortisol response to the mild stress of 5 min of net confinement. This is the first time ASA has been administered to fish *in vivo*, and the altered hormone release and the inhibition of the acute stress response indicated the involvement of PGs in these processes. The results of this study indicated that inhibiting the COX pathway by ASA modified the release of several important hormones and inhibited the response of tilapia to an acute stressor.

Gravel (2006) had tested the hypothesis that salicylic acid is an endocrine disruptor in fish by examining its impact on interrenal corticosteroidogenesis in rainbow trout. The authors showed that acute adrenocorticotrophic hormone (ACTH)-mediated cortisol production in trout interrenal cells *in vitro* was significantly depressed (up to 40%).

The authors investigated afterward whether this interrenal dysfunction involved inhibition of the steroidogenic capacity in rainbow trout receiving salicylate-laced feed (100 mg/kg body weight) for 3 days. The transcript levels of key proteins involved in corticosteroidogenesis were assessed, particularly key cholesterol transport proteins (including steroidogenic acute regulatory protein (StAR), peripheral-type benzodiazepine receptor (PBR), cytochrome P450 cholesterol side chain cleavage (P450scc), and 11b-hydroxylase.

Salicylic acid *in vivo* treatment did not affect the resting plasma cortisol or glucose levels, whereas the acute ACTH-stimulated cortisol production was significantly depressed (50%) in the interrenal tissue. This disruption of steroidogenesis by salicylic acid corresponds to a significant drop in the gene expression of StAR and PBR, but not P450scc or 11bhydroxylase, compared to the control fish. The circulating cortisol levels are also tightly regulated by a negative feedback loop, including glucocorticoid receptor (GR) signaling in the brain, inhibiting the release of trophic hormones (CRF and/or ACTH) in response to elevated steroid levels (cortisol). Gravel (2006) showed that brain glucocorticoid receptor (GR) protein content (but not GR mRNA level) was significantly reduced by salicylate (50%). These results suggest that the decrease of corticol production would be due to an impairment of cholesterol transport and of negative feedback regulation of cortisol. The authors concluded that salicylic acid is a corticosteroid disruptor in trout and the targets include the key rate-limiting step in interrenal steroidogenesis and brain glucocorticoid signaling.

7.10.2. Endocrine disruption - Human health

Endocrine disruption was not initially in the scope of this SEv for methyl salicylate. However, these properties have been evaluated by the evaluating MSCA since some effects reported in repeated exposure studies with methyl salicylate can suggest alterations of endocrine pathways.

First, skeletal effects were noted in repeated dose toxicity studies, i.e. a decrease in bone resorption and an increase in metaphysal cancelous bone density. These effects are consistent with those that can be observed in the case of an excess of oestrogens and/or disruption of thyroid hormones. Webb and Hansen (1963) administered methyl salicylate in the diet to rats at concentrations up to 2.0% in the diet

(equal to about 1000 mg/kg bw/day) for two years. All rats in the 1000 mg/kg group died by the 49th week. A significant decrease of body weight associated with an increased amount of cancellous bone was present in the metaphysis in rats treated at either 500 or 1000 mg/kg bw/day. Other effects included gross pituitary gland lesions at 250 mg/kg bw/day and an increase of relative weights of testis (males), heart and kidneys (females) in the 500 mg/kg bw/day group. In a supplemental study, it is reported that chondroclastic and especially osteoclastic activity was virtually completely blocked and that chondroblastic and osteoblastic activity was somewhat diminished. A set of six mechanistic studies of 6 to 12 week duration examined the effects of methyl salicylate on bone metabolism and growth in rats when administered in the diet at doses between 0.2 and 2% (equivalent to 100 to 1000 mg/kg bw/day) (Study Report #1, 1978). Bone lesions associated with growth retardation were reported in all studies at doses equal or higher than 1.13% (equivalent to 560 mg/kg bw/day). These effects were not reported in dogs exposed for 2 years to methyl salicylate administered in capsule at doses up to 350 mg/kg bw/day.

Secondly, developmental effects consisting in skeletal malformations, delay of the cleavage of the balanopreputial gland and delay of eye opening were observed in rats. **These effects could be a sign of a disruption of oestrogenic and/or androgenic pathways.** Pregnant female rats were exposed subcutaneously to methyl salicylate at doses up to 200 mg/kg/day from gestation day 6 to lactation day 21. There was a significantly lower mean body weight and body weight gain during gestation and a decrease in food consumption during gestation and lactation at 200 mg/kg/day. In offspring, the balanopreputial separation was delayed in males and the eyelid separation was delayed in females at the highest dose. At this same dose, an increase of skeletal anomalies (such as fusion of cervical vertebra and misshapen sternebra) was noted leading to the conclusion that methyl salicylate is teratogen in rats. Other effects in offspring included a significant decrease in the birth index, a lower body weight gain that was more pronounced in males and a delay in incisor eruption slightly more pronounced in females in the 200 mg/kg/day group (FDA, 2006d). Delayed tooth eruption may be associated with decreased osteoclastic activity.

In a prenatal developmental toxicity study where rats were exposed subcutaneously to methyl salicylate, skeletal variations were also observed at the highest tested dose of 200 mg/kg bw/day. Other developmental effects included a decreased fetal body weight and external anomalies. At this dose, dams presented a decrease in body weight, body weight gain and food consumption. Similar effects were not reported in rabbit (FDA, 2006c).

Overall, the effects described above can represent a sign of an endocrine disruption. In this context, a link between these effects with an endocrine disruption mechanism was assessed.

Since several publications report effects of various endocrine disruptors on bone, the type of bone lesions reported with methyl salicylate were compared with those observed with known endocrine disruptors in order to identify if they can be related to an alteration of an endocrine pathway.

As methyl salicylate, the phytoestrogens, such as psoralidin and coumestrol to a lesser extent, are able to reduce osteoclastic activity and increase bone matrix and bone mineral density (Zhai *et al.,* 2017). Moreover, experimental and clinical data attest phytoestrogens beneficial effects on bone since two decades (Whitten and Patisaul, 2001). It must be noted that although phytoestrogen effects on bone involve ERβ pathway, such type of interaction has never been investigated until today with methyl salicylate (only interaction with ERα investigated).

Methyl salicylate effects on bone present also some similarities with those of tributyltin which reduces ossification in the fetuses by disrupting thyroid axis (Adeeko *et al*., 2003), suppresses osteoclast differentiation *in vitro* (Yonezawa *et al*., 2007) and alters tooth development (Salmela *et al.,* 2008). In contrast, several studies show that tributyltin has

obesogenic effects, in addition to the deleterious bone effects (Chamorro-Garcia *et al*., 2013; Watt & Schlezinger, 2015), which was not evidenced for methyl salicylate.

Finally, the effects on bone reported with methyl salicylate present some similarities to those of BPA (bisphenol A) which is also able to delay ossification of rat fetuses at high doses (Kim *et al.,* 2001) and to increase bone matrix at low-dose. Nevertheless, these effects occurred in female rats only, whereas opposite effects were observed in males (Lejonklou *et al.,* 2016). This sex dimorphic effect on bone was also observed with the known endocrine disruptor, diethylstilbestrol (Rowas *et al.,* 2012). Other endocrine disrupting chemicals such as PCB126 and benzo[α]pyrene, acting through AhR-binding, also suppress osteoclast differentiation (Izawa *et al.,* 2016) and impair bone mineralization leading to decreased bone mineral density, which is not the case with methyl salicylate which increases bone density.

Altogether, available data on deleterious effects of methyl salicylate on bone do not clearly correspond to the effects of other endocrine disruptors. Indeed, the effects on bone may be due in fact to the main biological activity of salicylates, i.e., a COX (cyclo-oxygenase) inhibition, leading to a decrease of prostaglandins reflecting by a decreased inflammation associated to a reduced osteoclast activity.

After these theoretical assumptions, QSAR models and experimental studies available with methyl salicylate have been reviewed.

As a first step, the eMSCA estimated endocrine properties of methyl salicylate using the Danish QSAR Database. The only alert reported is related to androgen receptor antagonism using Leadscope model.

Oestrogenic properties of methyl salicylate were investigated *in vitro* and *in vivo*.

The activity of 73 phenolic additives was assessed using a recombinant yeast estrogen assay (Miller *et al.,* 2001). Methyl salicylate, hexyl salicylate, salicylic acid and triethanolamine salicylate had no activity on ERα. In contrast, phenyl salicylate and benzyl salicylate showed estrogenic activity. The authors concluded that the major criteria for activity appear to be the presence of an unhindered phenolic OH group in a para position and a molecular weight of 200-250 Da. Nevertheless, it should be noted that this conclusion is only based on potential interaction with ERα only; interactions of methyl salicylate with other sex receptors (oestrogenic or not) are not investigated in this study.

Zhang *et al*. (2012) assessed estrogenic potential of salicylate esters (methyl salicylate (MeS); ethyl salicylate (ES); phenyl salicylate (PhS); phenethyl salicylate (PES) and benzyl salicylate (BzS)). Using an automated molecular docking, it was found that PhS, BzS and PES have high estrogenic potentials whereas methyl salicylate and ES have much lower estrogenic activity. *In vitro*, PhS, BzS and PES showed positive results in a ligand-dependent coactivator recruiting assay for hERα. ES and methyl salicylate showed low or no oestrogenicity. These results were confirmed *in vivo* by using the uterotrophic assay in mice and rats: the uterine weight was statistically increased by BzS, PES and PhS exposure, but a similar effect was not observed after MeS and ES exposure. Thus, MeS appears unable to activate ERα but activation of other subtypes of ER was not investigated.

Zhang *et al.* (2013) developed an *in vitro* nuclear receptor coactivator recruiting assay to evaluate the binding activities of parabens, salicylates and benzoates via antagonist competitive binding on the human oestrogen-related receptor ɣ (ERRɣ). The results showed that all tested parabens possessed clear inverse antagonist activities on ERRɣ, whereas salicylates (MeS and ES) possessed much lower activities and the benzoates showed no obvious activity.

From these studies, methyl salicylate does not possess oestrogenic activity *in vitro* on ERα genomic activity and *in vivo* in an uterotrophic assay. However, it should be noted that this type of assay is only based on an assessment of uterus weight which is only related

to ERα alteration. Thus, this type of test cannot allow a definitive conclusion on all possible oestrogenic modes of action. Only a low activity was found on ERRγ *in vitro*. Methyl salicylate was not tested for its activity on ERβ. In addition, no data are currently available for endocrine disruption pathways other than oestrogenic.

Literature search with other salicylates was performed in order to identify possible ED alerts with this chemical family. From the literature, there are recent publications suggesting endocrine activities with the metabolite of methyl salicylate (salicylic acid) or with acetyl salicylic acid (aspirin). These publications were analysed in order to know if endocrine disruption potential is raised for methyl salicylate from other substances within salicylate family. Most of the found publications were related to aspirin.

An anti-androgenic activity was reported with aspirin in humans, in rats and in *ex-vivo* or *in vitro* models. 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. This publication also assessed the effect of aspirin in rat and in *ex-vivo* model. After intra-uterine exposure in rats from gestation days 13 to 21, aspirin from 150 to 350 mg/kg bw/day resulted in a shorter anogenital distance (AGD) in all males associated with intra-uterine foetal growth retardation to such a degree that the difference in AGD was undetectable after adjusting for body weight. Examination of testosterone production by the testes exposed *in utero* showed a decrease of testosterone (either significant or non-significant). A dose-dependent reduction in testosterone was also seen in the *ex-vivo* testis rat model. In a further *ex-vivo* rat testis assay performed by Kristensen *et al.* (2012), a decrease in testosterone was also reported without any modification in testis morphology. Albert et *al.* (2013) evaluated the endocrine effects of mild analgesic, including aspirin, in adult human testis explants or NCI-H295R adrenocortical human cells. In human testis, aspirin had no effect on morphology and did not induce a significant decrease in testosterone whereas a significant decrease in INSL3 (insulin like factor 3) level and inhibin production was found following a 24h exposure. In NCI-H295R adrenocortical human cells, testosterone production was inhibited after 24 and 48 hour exposure to 10^{-4} and 10^{-5} M of aspirin.

Acetyl salicylic acid also induced a decrease of thyroid hormones in humans and dogs. A reduction of total T4 and T3 was observed after administration of aspirin to 18 dogs at 25 mg/kg bw twice a day for 7 days (Daminet *et al*., 2003). A decrease in total and free T4, total and free T3 and TSH was found in a single dose study (1000 mg) or in a 1-week study (1000 mg four times a day; one week after the single study) with 25 healthy subjects (Samuels *et al*., 2003). The hypothesized mechanism of action consists in a displacement of thyroid hormones from serum protein binding site.

Finally, some effects on the response to stress, in particular on cortisol production, were found with acetyl salicylic acid in human (Di Luigi *et al*., 2001). Aspirin ingestion (800 mg two times daily for 3 days before and 800 mg in the morning or each physical-stress exercice) by male volunteers significantly blunted the increased serum ACTH, β-endorphin, cortisol, and growth hormone levels before exercise (anticipatory response) and was associated with reduced cortisol concentrations after exercise (Di Luigi *et al*., 2001). Further data are reported in fishes: with acetyl salicylic acid in tilapia (van Anholt *et al*., 2003) and with salicylic acid in rainbow trout (Gravel & Vijayan, 2006). In tilapia, aspirin treatment decreased plasma PGE2 (prostaglandin E2), basal PGE2 and cortisol levels. Basal plasma T3 was also reduced whereas prolactin (PRL)188 increased. After a mild stress (5 minutes of net confinement), aspirin depressed the cortisol response (van Anholt *et al*., 2003). In rainbow trout, ingestion of salicylic acid for 3 days depressed the acute ACTH-stimulated cortisol production in the interrenal tissue. This disruption of steroidogenesis corresponded to a significant drop in the gene expression of StAR (steroidogenic acute regulatory protein) and PBR (peripheral-type benzodiazepine receptor), but no P450scc or 11β-hydroxylase, compared to the sham-treated fish. Also brain glucocorticoid receptor (GR) protein content, but not GR mRNA level, was

significantly reduced. The authors concluded that salicylic acid is a corticosteroid disruptor in trout and the targets include the key rate-limiting step in interrenal steroidogenesis and brain glucocorticoid signalling.

Overall, methyl salicylate was not deeply assessed for its possible activities on endocrine pathways. Some concerns are raised from other salicylates. However, uncertainties remain to what extent data with other salicylates can be extrapolated to methyl salicylate (see also remarks above on the read-across with aspirin). In particular, oestrogenic activity varied depending on the salicylate tested. Anti-androgenic effects and changes in thyroid hormones are reported with aspirin. Aspirin and salicylic acid were also associated with some effects on the response to stress. Based on these results and considering the wide endocrine pathways, a conclusion on the validity of a read-across for endocrine disrupting properties with other salicylates is difficult to reach. **In summary, it can only be concluded that methyl salicylate has no effect on ERα genomic activity and only a low activity on ERRɣ *in vitro*.**

7.10.3. Conclusion on endocrine disrupting properties

Environnement

Taken together, even if the validity of a read-across is questionable for endocrine disruption endpoint (See HH section), these results raised some endocrine concerns in fish in the absence of adequate data with methyl salicylate. However, no clear ED mode of action can be defined considering the only two available studies, and the a priori level of concern can not be determined to propose a robust testing strategy for the environmental species.

Human health

Methyl salicylate induces bone effects in rats after exposure during adulthood (decrease in bone resorption and increase in metaphysal cancelous bone density) and also after *in utero* and peri-post-natal exposures (skeletal anomalies). Similar effects are not reported in other species tested such as rabbits (prenatal developmental toxicity study and repeated-dose toxicity study), dogs (repeated-dose toxicity studies) and mice (two generation fertility studies), even if in most cases, the level of details does not allow to assess if bones were specifically examined to identify such type of lesions.

Since bone growth and modelling are regulated by several hormones, it has been questioned if the bone effects observed with methyl salicylate are mediated by an endocrine mode of action. The investigation of this hypothesized action cannot be deeply conducted since methyl salicylate was only tested for a part of oestrogenic pathways (ERα and ERRɣ). Therefore, in order to clarify this concern and considering the numerous hormones involved in bone growth and modelling, the profiles of bone lesions reported with methyl salicylate and with different known endocrine disruptors were compared but differed on some points. In particular, endocrine disruptors generally provoke effects at low doses and eventually, with a sex-specificity, which is not the case with methyl salicylate inducing effects only at high doses (from 200 mg/kg bw/day) with a general toxicity and without a clear sex specificity. In summary, the different profiles of bone lesions between methyl salicylate and known endocrine disruptors do not allow to evidence an endocrine mode of action, even if no firm conclusion can be made since endocrine pathways involved in bone growth and modelling are not investigated with methyl salicylate.

Other effects reported with methyl salicylate could be a sign of an alteration of endocrine pathways. In particular, the developmental effects such as delayed eyelid separation and cleavage of the balanopreputial gland found after *in utero* and peri/post-natal exposures may be related to an anti-androgenic activity. Nevertheless, the biological relevance of these effects on male fertility cannot be assessed since no specific examination was made on the fertility of the offspring (ex. no sperm examination) (FDA, 2006d). In addition,

methyl salicylate was not tested in regard to androgenic activity. Some anti-androgenic effects were reported with aspirin, but the extrapolation of these results to methyl salicylate is questionable. Thus, investigation of the hypothesized underlying mode of action cannot be conducted. However, considering the unknown impact of the delayed eyelid separation and cleavage of the balanopreputial gland on adulthood and the absence of fertility effects in the parental generation, there is no major concern related to endocrine disruption, if any.

In conclusion, in regard to these data, there is no strong concern of an endocrine disruption induced by methyl salicylate exposure. However, it should be noted that the available dataset does not allow covering all endocrine pathways that can be impacted since only a part of the oestrogenic pathways (especially ERα and ERRɣ) was investigated.

One alternative hypothesis is the inhibition of the cyclooxygenases (COX) leading to a decrease in prostaglandins which play a role in many biological systems, including bones and reproduction. Inhibition of COX pathway is known as the biological activity of several salicylates, including salicylic acid and aspirin. Even if there is no data investigating specifically the effects of methyl salicylate on COX and/or prostaglandins, there is some indirect evidence allowing concluding on this activity since methyl salicylate is expected to be rapidly and extensively metabolized into salicylic acid.

Therefore, considering that:

- **The available dataset corresponds to the level 4 set in the OECD conceptual framework for testing and assessment of endocrine disrupters, with several reproductive toxicity studies;**
- **Fertility defects are not reported in reliable studies (FDA, 2006a);**
- **Knowing that following a proposal by the evaluating MSCA to classify the substance as Repro Cat. 1B, discussion took place at the RAC leading to an agreement to classify the substance as Repro Cat. 2 ;**
- **The available data allow to perform a risk characterization;**

No further data related to reproductive toxicity and endocrine disruption properties are requested in the framework of Substance Evaluation under Reach regulation at this time. However, it is recommended to pay attention to new publications on this topic or to promote toxicovigilance for this substance to ensure that no further concern will rise.

Impact of assessment of salicylic acid under Biocidal Product Regulation

Salicylic acid was evaluated as biocidal active substance by NL under Biocidal Product Regulation (BPR) for product types 2, 3 and 4. From June 2018, a guidance document setting scientific criteria to identify endocrine disruptors in pesticides and biocides is available. According to the 24th meeting of the Biocidal Products Committee (March 2018), the evaluating member state (e.g. NL for salicylic acid under BPR) has to revise its assessment on the endocrine properties of salicylic acid in the light of the new ED criteria. Considering that methyl salicylate is rapidly and mostly metabolized to salicylic acid, this assessment will also impact the present evaluation of methyl salicylate in this SEv framework. Depending on the outcome of the assessment of endocrine properties of salicylic acid by NL, further investigation on endocrine properties of methyl salicylate could be required. The possible options will be investigated in the RMOA to come.

7.11. PBT and VPVB assessment

7.11.1 Persistence

Evidence of non-P / non-vP properties

Screening criteria

- *Not P / vP based on ready biodegradability*: Methyl Salicylate was found to be readily biodegradablebased on a weight of evidence approach. Therefore, it is not considered to be persistent in water, sediment and soil.

Conclusion on P / vP properties: not P/vP

7.11.2 Bioaccumulation

Evidence of non-B / non-vB properties

Screening criteria

- *Not B / vB based on Log Kow <= 4.5*: available logKow values between 1.45 and 2.98

Conclusion on B / vB properties: not B/vB

7.11.3 Toxicity

Evidence of non-T properties

Criteria based on Annex XIII of REACH

- *Not T based on criteria laid down in Annex XIII of REACH*:

- *EC10 / NOEC >= 0.01 mg/L for marine / freshwater organisms (long-term toxicity)*: Lowest NOEC > 0.01 mg/L
- *Substance is not classified as carcinogenic (category 1 or 2), mutagenic (category 1 or 2), or toxic for reproduction (category 1, 2 or 3) according to Directive 67/548/EEC (or the DSD) or carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to Regulation EC No 1272/2008 (or CLP Regulation) (see also section "3. Classification and labelling")*: Not classified
- *No other evidence of chronic toxicity, as identified by the classifications: T, R48, or Xn, R48 according to Directive 67/548/EEC or specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Regulation EC No 1272/2008*: Not classified

Conclusion on T properties: not T

Overall conclusion:

Based on the assessment described in the subsections above the substance is not a PBT/ vPvB substance.

7.12. Exposure assessment

After a first round of evaluation, Decisions[4] were sent to the existing registrants on 19 December 2018, requesting them to update their CSR. The CSR have been updated accordingly. When the evaluation resumed in 2019, new registrants had arrived and 3 also provided CSR.

[4] https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e9072

7.12.1. Overview of the uses

This information has been moved to a confidential Annex

7.12.1.1. Overview of the exposure scenarios

Based on the available information, methyl salicylate is used as an intermediate to manufacture other substances, as an odour agent in various products (air care products, biocidal products, polishes and wax blends, washing and cleaning products, cosmetics and personal care products), as a solvent for laboratory chemicals and as a fuel additive.

Table 23. Brief description of the uses declared by the 5 Registrants

This table has been moved to a confidential annex.

7.12.2. Human health

For human health, the 5 registrants describe a total of 842 exposure scenarios. There are 19 exposure scenarios for manufacture (2 registrants), 183 for formulation (all registrants), 316 for industrial uses (all registrants), 300 for professional uses (all registrants) and 60 for consumer uses (4 registrants). Some other exposure scenarios are declared by other registrants but they are not assessed in separate CSR (no CSR is required due to lower tonnages).

When taken individually, the information is different from one CSR to the other, in terms of approach (targeted or generic), exposure scenarios, conditions of use, and models used for the estimation of exposure.

The current evaluation covers the exposure scenarios of Registrant 1 and 2, which were concerned by the listing to the initial CoRAP in 2015 and were addressees of the requests in formal Decisions. However all registrants can be taken into account during subsequent work on methyl salicylate.

As detailed in Table 24, based on the available information in the registration dossiers, industrial workers can be exposed by inhalation and dermal routes during the manufacture of methyl salicylate, the formulation of compounds and of end-products, the use of methyl salicylate as intermediate to manufacture other substances, and the use of formulated products.

Professional workers can be exposed by inhalation and dermal routes during the use of formulated products.

Consumers can be exposed by inhalation, dermal and oral routes during the use of formulated products.

In addition, the general public can be exposed to methyl salicylate as a result of its use, for example after it has been applied on a surface (eg as component of a cleaning product) or sprayed to the air (eg as component of an air care product).

Human exposure via the environment is also expected to its hydrolysis product salicylic acid.

Table 24: Uses assessed in the available CSR (the uses of Registrant 3, 4 and 5 are given for information purpose)

This table has been moved to a confidential Annex

7.12.2.1. Worker

When considering Registrant 1 and 2, there are 142 exposure scenarios for workers. The chemical safety assessments were carried out with Chesar[5] and take into account the requests expressed by the eMSCA in the Decisions that were addressed to the registrants. All the exposure estimates were calculated with ECETOC TRA Workers 3.0, except for some exposure scenarios for which either Stoffenmanager 8 (Registrant 1) or ART 1.5 (Registrant 2) were used for the inhalation route.

The eMSCA observes that:

- Similar uses (based on use titles) are assessed by registrants based on different combinations of contributing scenarios process categories and conditions of use. It

[5] https://chesar.echa.europa.eu/

is unclear if this is the result of intentional different approaches (more or less targeted or generic) or if the declared scenarios/PROCs are really specifically relevant for each use.
- The eMSCA does not have the possibility to verify to which extent the condition of use described in the exposure scenario are adequately implemented.
- No measured data is given by registrants to support the modelled exposure estimates.

7.12.2.2. Consumer

When considering Registrant 1 and 2, there are 22 exposure scenarios for consumers. The chemical safety assessments were carried out with Chesar and take into account the requests expressed by the eMSCA in the Decisions that were addressed to the registrants. All the exposure estimats were calculated with ECETOC TRA Consumers 3.1, except for some exposure scenarios for which either AISE REACT 2010 or ConsExpo 1.0.6 (Registrant 2) were used.

Remarks on washing and cleaning products:

Overall, very different results are obtained by Registrant 1 and 2 for similar uses.

Laundry products: A Registrant did not assess the inhalation route, and justified this choice by the vapour pressure of methyl salicylate (relevant > 10 Pa) and by considering that inhalation exposure is negligible compared to dermal exposure. However, methyl salicylate has a vapour pressure of 10 Pa, and exposure via dermal route is quite high based on his assessment (RCR very close to 1 for dermal route alone). In addition, based on the ConsExpo Fact Sheet on cleaning products[6], the inhalation route is relevant for laundry products (inhalation of dust, aerosols or inhalation of volatile compounds depending of the physical form of the product). The eMSCA assumes that all physical forms of laundry products should be assessed. Moreover, secondary exposure to residues left in washed textile should also be assessed (see 7.13.1.3).

Dishwashing products: A Registrant did not assess the inhalation route, and justified this choice by considering that inhalation exposure is negligible compared to dermal exposure. However, exposure via dermal route is quite high based on his assessment (RCR very close to 1 for dermal route alone). In addition, exposure via oral route has not been assessed. Based on the ConsExpo Fact Sheet on cleaning products, both inhalation and oral route are relevant when using dishwashing products (the oral exposure being due to residues on washed table ware).

Cleaning products (surface cleaners, liquid and trigger sprays all purpose cleaners, liquid and trigger sprays sanitary products, liquid floor cleaners, liquid and trigger sprays glass cleaners, liquid carpet cleaners, liquid metal cleaners): the oral route has not been assessed. However, cleaning of surfaces used for food preparation can be relevant. Some use phases are missing: the mixing and loading and post-application phases should be taken into account (for example rubbing off of the substance by sensitive population such as children).

Consequently, the exposure assessment should be revised (also taking into account the other considerations in this report).

7.12.3. Environment

Table 25: Environmental exposure scenario

[6] Cleaning Products Fact Sheet – Default parameters for estimating consumer exposure - Updated version 2018 (RIVM Report 2016-0179).

The table has been moved to the confidential annex

Additional relevant details for exposure scenario

Input parameters for calculating the fate and distribution in the local environment		
Input	**Value**	**Unit**
Molecular weight	152.147	g/mol
Vapour pressure	12.3 (at 25°C)	Pa
Water solubility (at 20 °C)	670	mg/L
Organic carbon/water partition coefficient (Koc)	222	L/kg
Biodegradability	Readily biodegradable	--
Total rate constant for removal from agricultural top soil	2.56E-02	d-1 (12°C)
Total rate constant for removal from grassland top soil	2.80E-02	d-1 (12°C)

Calculated fate and distribution in the STP – Simple Treat model	
Compartment	**Percentage [%]**
Air	0.417
Water	12.3
Sludge	2.03
Degraded in STP	85.3

7.13. Risk characterisation

7.13.1. Human health

Methyl salicylate is a skin sensitiser, an eye irritant, and is also classified as reprotoxic (category 2). Uncertainties remains regarding the endocrine disrupting effects.

7.13.1.1. Workers

- Local effect (skin sensitisation and severe eye irritation):

No local DNEL has been proposed by the lead registrant and there is no quantitative risk assessment. However, based on a qualitative approach, it is necessary to implement appropriate risk management measures to protect workers from skin sensitisation and eye irritation, taking into account the hierarchy of protective measures set out in the occupational health and safety legislation. Where exposure cannot be prevented by other means, personal protective equipments, such as gloves and goggles, should be worn to avoid skin and eye contacts. These would also reduce systemic exposure to methyl salicylate in occupational setting. **Update of the CSR by registrants is strongly recommended** to account for these hazards in the chemical risk assessment and communicate adequate risk management measures to downstream users.

- Systemic effects

DNELs have been established for long term systemic effects by inhalation and dermal route. However they are considered as not adequate by the eMSCA (refer to section 7.9.9).

For inhalation, the DNEL could be 2.5 to 25 times lower by taking into account an interspecies assessment factor of 2.5, and an additional assessment factor of 10 to reflect the low quality of the dataset.

For dermal route, the DNEL could be 2.5 to 62.5 times lower, for the same reasons and additionally considering a higher dermal absorption.

The evaluating MSCA highlights that these are only estimations and that the adequate DNEL values have to be calculated and justified properly. The evaluating MSCA intends to write a RMOA to establish the DNELs.

The impact on the workers exposure scenarios is given in Table 26 below. In the table, the "RCR « cut off » value" is the value of RCR as calculated by registrants which should actually be considered as above 1 (risks not controlled). It means that all RCR calculated by a registrant in its CSR which are above the "cut-off" value should be considered as if above 1 instead. For example, for long term systemic effects via dermal route, if the DNEL were 2.5 times lower (new DNEL = DNEL / 2.5), the "cut-off" RCR would be 1 / 2.5 = 0.4, meaning that all calculated RCR > 0.4 mean that the risk are not controlled.

Table 26: Impact of lower DNEL on workers RCR

This table has been moved to a confidential annex

The eMSCA has not verified the calculations for each individual scenario but consider that, based on the information provided by the registrants, as the DNELs are not appropriate, many scenarios become unacceptable (RCR become > 1).

For workers, the eMSCA concludes that le DNELs need to be recalculated, which will impact many exposure scenarios depending on the values that will be determined. Even with the more favourable DNEL (as presented above), risks are identified for several exposure scenarios.

The evaluating MSCA intends to launch a RMOA to establish the DNELs and investigate risk management options to address the risks due to local and systemic effects. Registrants may need to reconsider whether they wish to still support the uses, and if so, adapt the conditions of use in order to demonstrate safe use.

If new information about endocrine disruption becomes available, they will be taken into account as well.

7.13.1.2. Consumers

- Local effect (skin sensitisation and severe eye irritation):

No local DNEL has been proposed by the lead registrant and there is no quantitative risk assessment. However, based on a qualitative approach, it is necessary to implement appropriate risk management measures to protect consumers from skin sensitization and eye irritation. For consumers, the use of personal protective equipments is not an adequate risk management tool, and therefore other risk management measures have to be implemented. The consumers uses described in the registration dossiers reveal that methyl salicylate is intentionally applied on skin (cosmetics, repellents), and can also be in contact with the skin when applying or handling products manually. Dermal exposure can also occur via contact with surfaces where product has been applied or where aerosol/droplets are deposited, or with residues eg on washed laundry. Exposure of the eyes can occur due to spraying or splashing. **Update of the CSR by registrants is strongly recommended** to account for these hazards in the chemical risk assessment and communicate adequate risk management measures to formulators of consumer products.

- Systemic effects

DNELs have been established for long term systemic effects by inhalation and dermal route. However they are considered as not adequate by the eMSCA (refer to section 7.9.9).

For inhalation, the DNEL could be 2.5 to 25 times lower by taking into account an interspecies assessment factor of 2.5, and an additional assessment factor of 10 to reflect the low quality of the dataset.

For dermal route, the DNEL could be 5 to 50 times lower, for the same reasons.

The eMSCA highlights that these are only estimations and that the adequate DNEL values have to be calculated and justified properly. The eMSCA intends to launch a RMOA for this purpose.

The impact on the consumer exposure scenarios is given in Table 27 below.

Table 27: Impact of lower DNEL on consumer RCR

This table has been moved to a confidential Annex

The eMSCA has not verified the calculations for each individual scenario but consider that, based on the information provided by the registrants, as the DNELs are not appropriate, many scenarios become unacceptable (RCR become > 1).

For consumers, the eMSCA concludes that the DNELs need to be recalculated, which will impact many exposure scenarios depending on the values that will be determined. Even with the more favourable DNEL (as presented above), risks are identified for several exposure scenarios.

The evaluating MSCA intends to write a RMOA to establish the DNELs and investigate risk management options to address the risks due to local and systemic effects. Registrants may need to reconsider whether they wish to still support the uses, and if so, adapt the conditions of use in order to demonstrate safe use.
If new information about endocrine disruption becomes available, they will be taken into account as well.

7.13.1.3. General public

For all uses, the risks related to secondary exposure of the general public (indirect exposure as a result of use) have not been assessed. This is relevant for consumer uses but also for industrial and professional uses, depending on the product types and on the places where the products are used. However, the places where the products are used are not described, and therefore an uncertainty remains. Exposure of bystanders, i.e. persons that are present during the use of end-products (even if they are not using the products themselves), and exposure of persons after use of the products, in particular if products are used in public/private areas where vulnerable populations are (for example: floor cleaning in a kindergarten where yound children crawl), are not addressed. Persons indirectly exposed are not expected to wear any personal protective equipment, can have different behaviours, can be exposed for longer period of time and via additional routes (e.g. oral), and can be particularly vulnerable (e.g. children and pregnant women). Therefore as a worst case, based on the information provided in the exposure scenarios and considering the health effects, the eMSCA consider that risks for the general public and especially vulnerable population cannot be excluded.

Additionally, considering that methyl salicylate is hydrolysed into salicylic acid (half-life in water: 14 days), the general public would likely be exposed to salicylic acid as well. Therefore, for the purpose of risk management, salicylic acid should be taken into account. There are many other sources of salicylic acid (from direct use and from the degradation of other substances) and as of now, salicylic acid is under evaluation for endocrine disruption concerns.

The conclusions and proposal for a RMOA presented above (on workers and consumer uses) will likely impact the uses and the conditions of use, and subsequently will impact the exposure of the general public (for example, if a use is not supported anymore, or if a use is narrowed down with more stringent risk management measures). Consequently, the eMSCA will assess the need to address secondary exposure depending on the consequence that the RMOA may have on the supply chain.

7.13.2. Environment

Table 28: Conclusion of the environmental risk assessment for each exposure scenario of Methyl salicylate

Exposure Scenario n°		Exposure Scenario name	compartment with risk	Conclusion
Life Cycle Stage (LCS) M: Manufacture				

1		Manufacture of methyl salicylate	Aquatic Agricultural soil Groundwater	Unacceptable. RMM site specific (no sludge application)
Life Cycle Stage (LCS) F: Formulation or re-packing				
2		Formulation of fragrances	Aquatic Agricultural soil Groundwater	Unacceptable
3		Formulation & (re)packing of substances and mixtures; Cosmetics, personal care products; Washing and cleaning products	**Cosmetics, personal care products** Aquatic Agricultural soil Groundwater **Washing and cleaning products** Aquatic Agricultural soil Groundwater	Unacceptable Unacceptable
4		Formulation of fuel	**No onsite WWTP** Aquatic Agricultural soil Groundwater **Onsite WWTP** Aquatic Agricultural soil Groundwater	Unacceptable Unacceptable
5		Formulation of fragrances	**No onsite WWTP** Agricultural soil Groundwater **Onsite WWTP** Aquatic Agricultural soil Groundwater	Unacceptable Unacceptable
6		Formulation or (re)packing -Formulation of end-products - Odour agent in cosmetics,	**AISE 2.1a.v2** : **AISE 2.1b.v2** :	Acceptable

		cleaning and maintenance products	Aquatic Agricultural soil: Groundwater > **AISE 2.1c.v2 : AISE 2.1k.v2:** Aquatic: Agricultural soil: Groundwater **AISE 2.1j.v2** : Aquatic Agricultural soil Groundwater **AISE 2.1l.v2:** Aquatic Agricultural soil Groundwater **Cosmetics Europe 2.1g.v2**: Aquatic Agricultural soil Groundwater	Unacceptable Unacceptable Unacceptable Unacceptable Unacceptable
Life Cycle Stage (LCS) IS: Use at industrial sites				
7		Use as an intermediate for industrial manufacturing	**No onsite WWTP** Aquatic Agricultural soil Groundwater **Onsite WWTP** Aquatic Agricultural soil Groundwater	Unacceptable Unacceptable
8		Industrial use of fuels		Acceptable
9		Industrial end-use of washing and cleaning products	Aquatic Agricultural soil Groundwater	Unacceptable
10		Intermediate Use resulting in manufacture of another substance		Acceptable
11		Industrial end-use of washing and cleaning		Acceptable

		products		
Life Cycle Stage (LCS) PW: Widespread use by professional workers				
12		Professional use of fuels		Acceptable
13		Professional end-use of washing and cleaning products products		Acceptable
14		Professional end-use of washing and cleaning products		Acceptable
15		Professional end-use of polishes and wax blends		Acceptable
Life Cycle Stage (LCS) C: Consumer use				
16		Private use of cosmetics, personal care products, and fragranced products	**Cosmetics, personal care products** **Washing and cleaning products**	Acceptable
17		Consumer end-use of washing and cleaning products		Acceptable
18		Consumer end-use of air products		Acceptable
19		Consumer end-use of biocides		Acceptable
20		Consumer end-use of polishes and wax blends		Acceptable
21		Consumer end-use of cosmetics		Acceptable

Conclusion

To conclude, the eMSCA has verified the calculations for each individual scenario and considers that, based on the relevant information provided by the registrants, many scenarios are unacceptable (RCR > 1), mainly for the following life cycle stages: manufacture, formulation and some uses at industrial sites.

7.14. References

David R Lide, CRC Handbook of chemistry and physics 86th edition, 2005-2006.

Merck Index 14th edition, 2006

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. British Journal of Dermatology; 107: 261–274.

Adeeko A, Li D, Forsyth DS, Casey V, Cooke GM, Barthelemy J, Cyr DG, Trasler JM, Robaire B, Hales BF. 2003. Effects of in utero tributyltin chloride exposure in the rat on pregnancy outcome. Toxicol Sci.;74(2):407-15.

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.

Albert O, Desdoits-Lethimonier C, Lesné L, Legrand A, Guillé F, Bensalah K, Dejucq-Rainsford N, Jégou B. 2013. Paracetamol, aspirin and indomethacin display endocrine disrupting properties in the adult human testis in vitro. Human Reproduction; 28(7): 1890–8

Arts JHE, de Jong WH, van Triel JJ, Schijf MA, de Klerk A, van Lovereen H, Kuper CF. 2008. The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological sciences: 106(2),423-434.

Bi J.L., Murphy J. B. & Felton G. W. 1997: Does salicylic acid act as a signal in cotton for induced resistance to Helicoverpa zea? Journal of Chemical Ecology, 23 (7), 1805-1818

Bisesi MS. 1994. Esters of Aromatic Monocarboxylic Acids and Monoalcohols. In: Patty's Industrial Hygiene and Toxicology. 4th Ed., Clayton DG, Clayton FE eds., John Wiley & Sons, NY, Vol II part D, p3013-3019.

Burdette WJ, Strong LC. 1941. Comparison of methyl salicylate and benzene as solvents for methylcolanthrene. Cancer Research 1:939-941.

Carpenter, C.P., Smyth, H.F., 1946. Chemical burns of the rabbit cornea. American Journal of Ophthalmology 29, 1363–1372

Castagnou R, Larceban S, Quement A. 1952. Toxicology of methyl salicylate. In: Opdyke DLJ, Monographs on fragrance raw materials. Methyl salicylate. 16S1:821-825 (1978).

Chamorro-García R, Sahu M, Abbey RJ, Laude J, Pham N, Blumberg B. 2013. Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. Environ Health Perspect.;121(3):359-66. doi: 10.1289/ehp.1205701.

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.

Collins TFX, Hansen WH, Keeler HV. 1971. Effect of methyl salicylate on rat reproduction. Toxicology and applied pharmacology;18:755-765.

Coffing S, Engel M, Dickinson D, Thiffeault C, Spellman R, Shutsky T, Schuler M. 2011. The rat gut micronucleus assay: a good choice for alternative in vivo genetic toxicology testing strategies. Environmental and molecular mutagenesis; 52: 269-79.

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.

Cross SE, Megwa SA, Benson HAE, Roberts MS. 1999. Self promotion of deep tissue penetration and distribution of methyl salicylate after topical application. Pharmaceutical Research. Vol. 16, No 3.

Daminet S, Croubels S, Duchateau L, Debunne A, van Geffen C, Hoybergs Y, van Bree H, de Rick A. 2003. Influence of acetylsalicylic acid and ketoprofen on canine thyroid function tests. The Veterinary Journal.; 166: 224–32.

Daston, G.P., Rehnberg, B.F., Carver, B., Rogers, E.H., Kavlock, R.J., 1988. Functional teratogens of the rat kidney. I. Colchicine, dinoseb, and methyl salicylate. Fundamental and Applied Toxicology 11, 381–400.

Daubert, T.E., R.P. Danner. 1989. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis

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; 42:184–185.

Di Luigi L, Guidetti L, Romanelli F, Baldari C, Conte D. 2001. Acetylsalicylic acid inhibits the pituitary response to exercise-related stress in humans. Med Sci Sports Exerc.; 33(12):2029-35.

ECHA. Registration dossier salicylic acid. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14544

ECHA. Registration dossier sodium salicylate. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13593

ECHA. Registration dossier acetylsalicylic acid. https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/10841

Eisenreich SJ et al. 1981. Environ Sci Technol 15: 30-8

EMEA. 1999. Committee for veterinary medicinal products. Acetylsalicylic acid, sodium acetylsalicylate, acetylsalicylic acid DL-lysine and carbasalate calcium – summary report. EMEA/MRL/695/99-final.

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.

Hansch, C., Leo, A., D. Hoekman. 1995a. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995., p. 29

Hansch, C., Leo, A., D. Hoekman. 1995b. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995., p. 54

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. Epub 2011 Dec 1.

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

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): 394–396. doi: 10.1093/ndtplus/sfr104

Fassett DW (1963). Esters. In: Industrial Hygiene and toxicology. 2nd Ed Edited by FA Patty. Vol. 2 p 1897, Interscience, NY.

Ferguson J, Sharma S. 1984. Cinnamic aldehyde test concentrations. [Letter to the Editor]. Contact Dermatitis; 10:191–192.

Gage JC. 1970. Subacute inhalation toxicity of 109 industrial chemicals. Br J Ind Med 27:1-18.

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.,U.S. EPA Environmental Research

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., 19:438-445.

Giri AK, Adhikari N, Khan KA. 1996. Comparative genotoxicity of six salicylic acid derivatives in bone marrow cells of mice. Mutation Research, 370, 1-9.

Gravel A, Vijayan MM. 2006. Salicylate Disrupts Interrenal Steroidogenesis and Brain Glucocorticoid Receptor Expression in Rainbow Trout. Toxicological Sciences; 93(1), 41–9.

HSDB, 1996. Hazardous Substances Data Bank.

IARC. 1997. Non-steroidal anti-inflammatory drugs. IARC handbooks of cancer prevention. Volume 1.

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.

INRS. Fiche toxicologique n°5. Methanol. 2018.

INRS. Fiche toxicologique n°24. Acide acétique. 2011.

Ishidate M Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A. 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22:623-636.

Izawa T, Arakaki R, Mori H, Tsunematsu T, Kudo Y, Tanaka E, Ishimaru N. 2016. The Nuclear Receptor AhR Controls Bone Homeostasis by Regulating Osteoclast Differentiation via the RANK/c-Fos Signaling Axis. J Immunol.;197(12):4639-4650.

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. Vol 2, pp. 327-343.

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

Kavlock, R.J., Chernoff, N., Rogers, E., Whitehouse, D., Carver, B., Gray, J., Robinson, K., 1982. An analysis of fetotoxicity using biochemical endpoints of organ differentiation. Teratology 26, 183– 194.

Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M. & Sugiyama, T. 1980a. Cooperative programme on short-term assays for carcinogenicity in Japan. In: Montesano, R., Bartsch, H. & Tomatis, L., eds, Molecular and Cellular Aspects of Carcinogen Screening Tests (IARC Scientific Publications No. 27), Lyon: IARC Press, pp. 323–330.

Kawachi, T., Komatsu, T., Kada, T., Ishidate, M., Sasaki, M., Sugiyama, T. & Tazima, Y. 1980b. Results of recent studies on the relevance of various short-term screening tests in Japan. In: Williams, G.M., Kroes, R., Waajiers, H.W. & van de Poll, K.W., eds, The Predictive Value of Short-term Screening Tests in Carcinogenicity Evaluation (Applied Methods in Oncology Vol. 3) Amsterdam: Elsevier/North Holland Biomedical Press, pp. 253–267.

Kim JC, Shin HC, Cha SW, Koh WS, Chung MK, Han SS. 2001. Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol Aduring pregnancy. Life Sci.;69(22):2611-25.

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.

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. Toxicologist; 42:268.

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

Koshakji RP and Schulert AR. 1973. Biological mechanisms of salicylate teratogenicity in the rat. Biochemical Pharmacology, vol22, pp407-416.

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.

Kristensen DM, Lesné L, Le Fol V, Desdoits-Lethimonier C, Dejucq-Rainsford N, Leffers H, Jégou B. 2012. Paracetamol (acetaminophen), aspirin (acetylsalicylic acid) and indomethacin are anti-androgenic in the rat foetal testis. Int J Androl.; 35(3):377-84.

Kuboyama N , Fujii A. 1992. Mutagenicity of analgesics, their derivatives, and anti-inflammatory drugs with S-9 mix of several animal species. J Nihon Univ Sch Dent.;34(3):183-95.

Lamb J, Gulati D, Choudhury H, Chambers R, Sabharwal. 1997. Methyl salicylate. Environmental Health 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–S452

Lejonklou MH, Christiansen S, Örberg J, Shen L, Larsson S, Boberg J, Hass U, Lind PM. 2013. Low-dose developmental exposure to bisphenol A alters the femoral bone geometry in wistar rats. Chemosphere;164:339-346. doi: 10.1016/j.chemosphere.2016.08.114.

Lewis RB, Schulman JD. 1973. Influence of acetylsalicylic acid an inhibitor of prostaglandin synthesis on the duration of human gestation and labour. The Lancet; 2(7839):1159-61.

Miller D, Wheals BB, Beresford N, Sumpter JP. 2001. Estrogenic Activity of Phenolic Additives Determined By an In Vitro Yeast Bioassay. Environ Health Perspect.; 109(2):133-8.

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: 336–337.

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 Dermato-Veneriologica; 78:433-437.

Montelius J, Wahlkvist H, Boman A. 1994. Experience with the murine local lymph node assay: Inability to discriminate between allergens and irritants. Acta Dermato-Veneriologica; 74:22-27.

Moreno OM. 1973. Acute toxicity studies on rats and rabbits. Report to RIFM 12th November. In: Opdyke DLJ, Monographs on fragrance raw materials. Methyl salicylate. Food Cosmet Toxicol, 16S1:821-825 (1978).

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.

Muller L., Kasper P., Madle S. Further investigations on the clastogenicity of paracetamol and acetylsalicylic acid in vitro. Mutation Research. 1991; 263: 83-92.

National Toxicology Program (NTP), 1984a. Methyl salicylate: Reproduction and fertility assessment in CD-1 mice when administered by gavage. August. NTP-84-156; PB84-241140.

National Toxicology Program (NTP), 1984b. Methyl salicylate: Reproduction and fertility assessment in CD-1 mice when administered by gavage. Final Report, November. NTP-85-022; PB85-164283.

Nethercott JR, Nield G, Holness DL. 1989. A review of 79 cases of eyelid dermatitis. Journal of the American Academy of Dermatology; 21:223–230.

Oda Y, Hamono Y, Inoue K, Yamamoto H, Nihara T, Kunita N. 1978. Mutagenicity of food flavors in bacteria. Shokuhin Eisei Hen 9, 1977-181.

OECD. 2004. SIDS Initial assessment profile. Methanol. US/ICCA. SIAM, 19-22 October 2004.

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

Overman DO, White JA. 1983. Comparative teratogenic effects of methyl salicylate applied orally or topically to hamsters. Teratology. 28(3):421-6.

Rai R. Backos M, Baxter N, Chilcott I, Regan L. 2000. Recurrent miscarriage – an aspirin a day? Human reproduction; 15 (10): 2220-2223.

Rainsford KD, Schweitzer A, Green P, Whitehouse MW, Brune K. 1980. Bio-distribution in rats of some salicylates with low gastric ulcerogenicity. Agents Actions. 1980 Nov;10(5):457-64.

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. Epub 2007 Sep 14.

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

Rowas SA, Haddad R, Gawri R, Al Ma'awi AA, Chalifour LE, Antoniou J, Mwale F. 2012. Effect of in utero exposure to diethylstilbestrol on lumbar and femoral bone, articular cartilage, and the intervertebral disc in male and female adult mice progeny with and without swimming exercise. Arthritis Res Ther.;14(1):R17. doi: 10.1186/ar3696.

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

Sax NI, Lewis RJ. 1992. Dangerous properties of Industrial Materials. Van Nostrand Reinhold, 8th Ed. NY

SCCNFP. 2002. Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning salicylic acid. SCCNFP/05322/01, final.

SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 2018, https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_224.pdf

Stich HF, Rosin MP, Wu CH, Powrie WD. 1981. The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. Cancer letters, 14, 251-260.

Stingeni L, Lapomarda V, Lisi P. 1995. Occupational hand dermatitis in hospital environments. Contact Dermatitis; 33: 172–176.

Stoner GD, Shimkin MB, Kniazeff AJ, Weisburger JH, Weisburger EK, Gori GB. 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Research 33:3069-3085.

Salmela E, Sahlberg C, Alaluusua S, Lukinmaa PL. 2008. Tributyltin impairs dentin mineralization and enamel formation in cultured mouse embryonic molar teeth. Toxicol Sci.;106(1):214-22. doi: 10.1093/toxsci/kfn156.

Samuels MH, Pillot K, Asher D, Nelson JC. 2003. Variable Effects of Nonsteroidal Antiinflammatory Agents on Thyroid Test Results. J. Clin Endocrinol Metab.; 88(12):5710-6.

Unpublished study Report#1, Methyl salicylate: studies of osseous changes in the rat, reproduction in the rat and mouse and liver and kidney effects in the dog, 1978.

Unpublished study Report#2, Reproductive and teratogenic risks of low salicylic acid doses in humans, 2012.

Unpublished study Report#3, Methyl salicylate: Safety Assessment Screen Comprising: Acute eye irritation and delayed dermal sensitisation, 2001.

Unpublished study Report#4, Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals, 1986.

Unpublished study Report#5, Primary skin Irritation in the Rabbit, 1999.

Unpublished study Report#6, Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with salicylic acid pharmaceutical grade-CAS# 69-72-7, 2008.

Unpublished study Report#7, Metabolic fate of methyl salicylate. I. Absorption, distribution and excretion in hairless mice, 1976.

Study Report#8, YMS PHARMACEUTICAL METHYL SALICYLATE:Short Time Exposure in vitro Test Method for Identifying Eye Damage, 2019.

Unpublished tudy Report#9, The biodegradability of Perfume Ingredients in the Sealed Vessel Test, 1993.

Unpublished tudy Report#10, Ethyl Salicylate: Acute Immobilisation Test (48h) to Daphnia magna, 2001.

Unpublished tudy Report#11, MethylSalicylate (CAS: 119-36-8): algal growth inhibition test, -2010.

Unpublished tudy Report #12, Salicylate de Methyle, détermination de l'effet inhibiteur du développement de Pseudomonas putida, selon le projet de norme ISO/CD 10712 du 10.10.1990. 1992.

Unpublished study Report #13, Toxicity of modified HL simulant and methyl salicylate in soil on cucumbers and earthworms, 1993.

Unpublished Report #14. Report on Eye irritation study of methyl salicylate in rabbits. 1963.

Technical guidance document on risk assessment: Part III, European Chemicals Bureau, 2003

Van Anholt RD, Spanings T, Koven W, Wendelaar Bonga SE. 2003. Effects of acetylsalicylic acid treatment on thyroid hormones, prolactins, and the stress response of tilapia (Oreochromis mossambicus). Am J Physiol Regul Integr Comp Physiol.; 285(5): R1098-106.

Watt J, Schlezinger JJ. 2015. Structurally-diverse, PPARγ-activating environmental toxicants induce adipogenesis and suppress osteogenesis in bone marrow mesenchymal stromal cells. Toxicology;331:66-77. doi: 10.1016/j.tox.2015.03.006

Webb WK, Hansen WH. 1963. Chronic and subacute toxicology and pathology of methyl salicylate in dogs, rats and rabbits. Toxicol Appl Pharmacol 5: 576-687.

Whitten PL, Patisaul HB.2001. Cross-species and interassay comparisons of phytoestrogen action. Environ Health Perspect.;109 Suppl 1:5-20.

Woo, D.C., Hoar, R.M., 1972. "Apparent hydronephrosis" as a normal aspect of renal development in late gestation of rats: the effect of methyl salicylate. Teratology 6, 191–196.

Yonezawa T, Hasegawa S, Ahn JY, Cha BY, Teruya T, Hagiwara H, Nagai K, Woo JT. 2007. Tributyltin and triphenyltin inhibit osteoclast differentiation through a retinoic acid receptor-dependent signaling pathway. Biochem Biophys Res Commun.;355(1):10-5.

Zhai Y, Li Y, Wang Y, Cui J, Feng K, Kong X, Chen L. 2017. Psoralidin, a prenylated coumestan, as a novel anti-osteoporosis candidate to enhance bone formation of osteoblasts and decrease bone resorption of osteoclasts. Eur J Pharmacol.;801:62-71. doi: 10.1016/j.ejphar.2017.03.001.

Zhang Z, Jia C, Hua Y, Sun L, Jiao J, Zhao L, Zhu D, Li J, Tian Y, Bai H, Li R, Hu J. 2012. The estrogenic potential of salicylate esters and their possible risks in foods and cosmetics. Toxicology Letters; 209: 146– 53.

Zhang Z, Sun L, Hu Y, Jiao J, Hu J. 2013. Inverse antagonist activities of parabens on human oestrogen-related receptor γ (ERRγ): In vitro and in silico studies. Toxicology and Applied Pharmacology; 270: 16–22.

7.15. Annex I

Rationale for read-across:

The registrant proposed a read-across from data on salicylic acid (SA), acetylsalicylic acid (ASA) or sodium salicylate (NaS) to methyl salicylate (MeS), in particular for genotoxicity and reproductive/developmental toxicity.

The justification provided by the registrant consists on a similar metabolism pathway. Indeed, MeS, NaS, SA and ASA were all metabolized initially to free salicylate (by hydrolysis for MeS and deacetylation for ASA), then mainly to salicyluric acid and conjugated salicylic acid compounds, with a small proportion of oxidative metabolites. This scenario is consistent with the scenario 1 (analogue approach for which the read-across hypothesis is based on (bio)transformation to common compound) of the Read-across Assessment Framework.

Figure 2 Biotransformation of MeS, NaS and ASA into SA

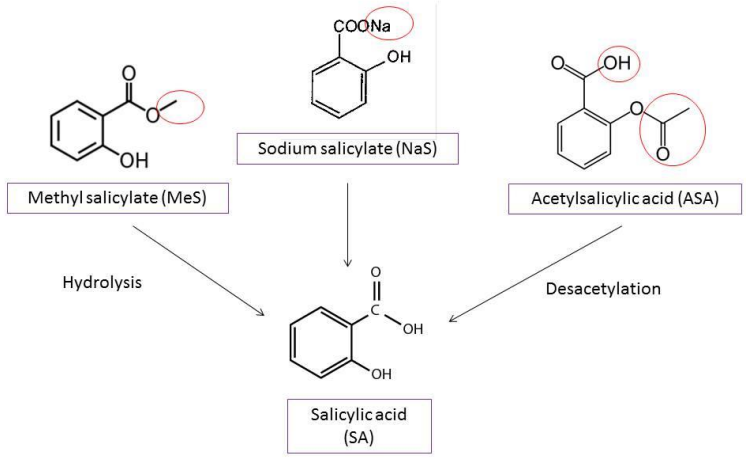
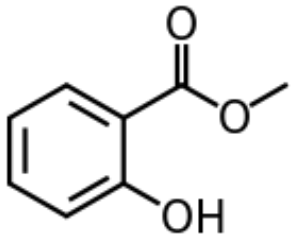
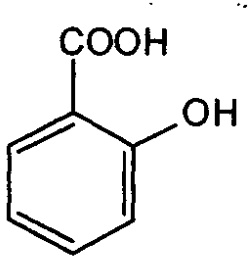
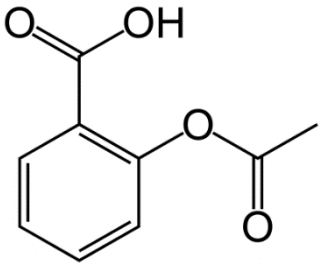
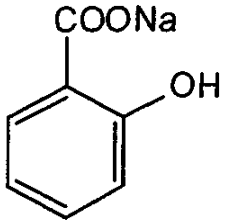


Table 29 Read-across scenario

	Parent substances	(Bio)transformation	Common compound	Non-common compound
Target	Methyl salicylate (MeS)	MeS → SA + methanol	Salicylic acid (SA)	Methanol
Source	Acetylsalicylic acid (ASA)	ASA → SA + acetic acid	Salicylic acid (SA)	Acetic acid
Source	Sodium salicylate (NaS)	NaS → SA + sodium	Salicylic acid (SA)	Sodium

Source	Salicylic acid (SA)	-	Salicylic acid (SA)	-

In order to assess the relevance of this read-across, the evaluating MSCA has compared physico-chemical and toxicological data of MeS, SA, ASA and NaS. Data on MeS were based on the CSR and on a literature search performed by the evaluating MSCA. Data on SA, ASA and NaS were issued from a literature research and on registration data published on ECHA website. It should be noted that most of the reported data are of low quality (studies not following guideline, only small amount of parameters examined…). This was summarized in the table below.

	Methyl salicylate	Salicylic acid	Acetylsalicylic acid	Sodium salicylate
CAS number	119-36-8	69-72-7	50-78-2	54-21-7
Structure				
Classification	Classification agreed by the RAC in 2019: - Acute Tox 4 – H302; - Skin Sens. 1B – H317 - Repr. Cat. 2 – H361 Not yet implemented in an ATP	Harmonized classification (ATP13): - Acute Tox 4 – H302; - Eye Dam. 1 – H318 - Repr. Cat. 2 – H361	No harmonized classification	No harmonized classification
Solubility	0.67g/L in water at ambient T, miscible in chloroform, ether, anhydrous methanol, ethanol, benzene, CCl4, oils	Slightly soluble in water (2.17 mg/ml at 20°C); soluble in organic compounds 0.262% at 25°C in CCl_4, 0.775% at 25°C in benzene, 27.36% at 21°C in propanol, 34.87% at 21°C in ethanol, 396% at 23°C in acetone (Merck 2006)	Soluble in water, ether, chlorophorm, very soluble in ethanol, soluble in benzene 3.3g/L at 25°C and 10g/L at 37°C in water, 200g/L in alcohol, 58.8g/L in chloroform, 66.7-100g/L in ether (Merck 2006)	Soluble in water 125g/100ml in water, 17g/100ml in methanol (Merck 2006)
Log P_{ow}	2.55	2.26 (Hansch, Leo 1995a)	1.19 (Hansch, Leo 1995b)	No data
Vapour pressure	10 Pa at 22°C 100 Pa at 51°C	$8.2.10^{-5}$ mmHg at 25°C (Daubert & Danner 1989)	$2.52.10^{-5}$ mmHg at 25°C (Eisenreich, 1981)	No data

ADME	Salicylates are all metabolized into salicylic acid. 21% of methyl salicylate remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans (Davison (1961)). Some publications compared kinetics of different salicylates. It was found that MeS does not produce higher plasma or brain concentration of total salicylates in rats than ASA or NaS. In humans, ASA administration induced plasma salicylates concentration higher than those obtained after MeS administration (Davison, 1961). Rainsford (1980) reported similar distribution with methyl ester of ASA, ASA and SA. During this first step of metabolism, methanol is also formed after hydrolysis of methyl salicylate; acetic acid after desacetylation of acetylsalicylic acid and sodium after hydrolysis of sodium salicylate.			
Acute toxicity	LD_{50} oral = 580-2000 mg/kg bw Acute Tox 4 agreed by the RAC in September 2019 LD_{50} dermal > 2000 mg/kg bw	LD_{50} oral = 400-3700 mg/kg Acute Tox 4 (ATP13) LD_{50} dermal > 2000 mg/kg bw	LD_{50} oral = 1100 - 1900 mg/kg LD_{50} dermal > 7940 mg/kg bw	LD_{50} oral = 930-1200 mg/kg LD_{50} dermal > 2000 mg/kg bw
	Acute oral toxicity of salicylates is moderate, with toxicity generally decreasing with increasing size of the ester R-group. Likely related to the relative proportion of the molecular weight release as SA followed hydrolysis.			
Irritation	No dermal irritation	Moderately to minimally irritant in solutions to animal skin. Mild transient irritant to human skin in formulation. Dermal irritation in repeated-dose toxicity studies (CIR, 2003). No dermal irritation (registration data)	No dermal irritation (registration data)	No dermal irritation (registration data)
	Eye Dam. 1	Eye Dam. 1 (ATP13)	Not irritating for eye (registration data)	Mildly irritation to eye (registration data)
Sensitisation	Maximisation assays negative but positive and negative results in LLNA depending on the concentrations tested. Low incidence of reactions in humans. Skin 1B agreed by the RAC in	One LLNA positive (CIR, 2003). Contradictory results in LLNA, negative in a MEST and QSAR model predict no sensitization potential of SA (registration data)	No sensitization in a Maximisation assay (registration data).	QSAR model predict no sensitization potential of NaS. Only 1 positive reaction among 31 patients (registration data)

	September 2019			
Repeated-dose toxicity	Target organs: bone and liver	No target organ reported (registration data), bones (Study Report #1, 1978)	Target organs: gastrointestinal tract, kidney and liver (IARC); bones (Study Report #1, 1978)	Target organs: kidney and liver (registration data); bones (Study Report #1, 1978)
	Study Report #1 (1978) compared bone effects after administration of different salicylates in the same study: Similar effects on body weight, food consumption and bone was observed with MeS, ASA and NaS with a higher mortality reported with ASA and NaS. These findings suggest that the bone lesions are related to the ortho-hydroxybenzoic acid structure.			
Mutagenicity	Negative in bacteria and mammalian cells (FDA, 2006).	Not mutagenic in bacteria and in mammalian cells. Contradictory data for chromosome aberrations (SCCNFP, 2002).	Not mutagenic in bacteria and in mammalian cells. Contradictory data for chromosome aberrations (Giri, 1996; EMEA, 1999).	Negative in Ames test and DNA cell-binding assay using Ehrlich ascites cells (CIR, 2003)
	Negative in a micronucleus assay (FDA, 2006)	Negative *in vivo* in sister chromatid exchange assay and in chromosome aberration assay (Giri, 1996)	Contradictory data for clastogenic effects *in vivo* (EMEA, 1999; Giri, 1996)	Contradictory data for clastogenic effects *in vivo* (Giri, 1996)
Carcinogenicity	Not carcinogenic in rats by oral route (registration data)	Not carcinogenic in rats by oral route (CIR, 2003).	Not carcinogenic in rats in water (SCCNFP, 2002)	No *in vivo* data (CIR, 2003)
Toxicity on reproduction	No effect on fertility (FDA, 2006)	No adequate study on fertility. Inhibition of human sperm mobility *in vitro* (CIR, 2003). Increased mean gestation period after treatment on GD20 & 21 in rodents (CIR, 2003).	No adequate study on fertility. Longer time to pregnancy and lower pregnancy rate after treatment during gestation in rats (registration data).	No adequate study on fertility. Increased duration of gestation (CIR, 2003)
Toxicity for the development	Fetal death and malformations (neural tube defect, kidney) (registration data; FDA (2006)).	Fetal death, growth retardation and malformations (kidney and skeletal) in rats. Classification Repr. 2 – H361 (ATP13) based on experimental studies with salicylic acid, methyl salicylate, sodium	Fetal death, growth retardation and various malformations (skeletal and visceral) in rats. No teratogenic effect in rabbits and	Fetal death, growth retardation and malformations (mainly skeletal) in rats.

	Classification as Repr. 1B proposed by the eMSCA in a CLH report submitted in 2018 Repr. 2 agreed by the RAC in September 2019, based on a read-across with SA.	salicylate and acetylsalicylic acid and on human data with acetylsalicylic acid.	mice (registration data).	
	When various salicylates are administered to rats twice on GD9 (subcutaneous route) (Koshakji, 1973), different developmental toxicity profiles are observed depending on the salicylate tested When comparing molecular structure and developmental findings, it appears that COOH and OH must be adjacent for inducing teratogenic effects. It also appears that substitution of OH group by SH or NH_2, substitution of COOH for $CONH_2$ or addition of OH groups to SA eliminates teratogenic properties.			

ASA, MeS and NaS are all metabolized into SA and corresponding alcohol. From the available studies, it is suggested that, after oral administration, metabolism of ASA is more rapid and more complete than MeS and thus ASA led to higher salicylate concentration in plasma compared to MeS. Considering the chemical structure of NaS, its hydrolysis to SA is also expected to be complete and rapid. In humans, 21% of methyl salicylate remained unhydrolyzed at 90 minutes (no information after 90 minutes) (Davison (1961)). Thus, a possible specific effect of the parent molecule (MeS) can be expected.

Although most of the above data are not fully reliable, it can be shown that MeS, ASA, SA and NaS present a similar toxicological profile. Thus, it can be hypothesized that salicylic acid is responsible of the effects observed.

Methyl salicylate (MeS) also produced methanol during its hydrolysis. The impact of this metabolite in the toxicity of MeS also needs to be taken into account. This substance is currently classified as Flam. Liq. 2 (H225), Acute Tox 3 (H301/311/331) and STOT SE 1 (H370). Furthermore, a CLH report was submitted by Italy focusing on developmental toxicity. In its opinion dated in September 2014, the RAC concluded that there is not sufficient evidence for classifying methanol for developmental toxicity based on inter-species toxicokinetic differences and since effects observed in rodents were observed at very high doses. The main effects reported after methanol exposure consist in central nervous system depression, liver and eye toxicity (INRS, 2018). Concerning mutagenicity, most of the *in vitro* and *in vivo* tests were negative. However, *in vitro*, there were a positive result in a mouse lymphoma test, an ambiguous result in an Ames assay for strain TA102, and an ambiguous result in the DNA damage and repair assay. *In vivo*, methanol was positive for aneuploidy, sister chromatid exchange, and micronuclei. Only limited information was available for the positive studies (OECD SIAP, 2004). In conclusion, it seems that the concerns raised for methyl salicylate regarding genotoxicity and reproductive toxicity are mainly related to the salicylate structure (from the parent molecule or from salicylic acid as a metabolite), even if it cannot be excluded that methanol can be involved in the toxicity of methyl salicylate.

Concerning the acetic acid formed during the metabolism of ASA, the substance is currently classified as Flam. Liq 3 (H226) and Skin Corr 1A (H314). After prolonged exposure by oral route, acetic acid induced inflammatory lesions. It is not mutagenic except when the pH is less than 6 and not teratogenic (INRS, 2011). Therefore the toxicity of ASA is mainly related to the salicylate structure.

However, some uncertainties remains in particular for a read-across between ASA and methyl salicylate. First, biological differences can be suspected since their commercial uses are quite different (even if methyl salicylate is used topically for its anti-inflammatory properties, its major uses are in fragrance compounds, that is not the case for ASA). Secondly, ASA and methyl salicylate present some differences in prostaglandin inhibition. A possible specific effect of the parent molecule can also be highlighted by the fact that 21% of methyl salicylate remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans (Davison (1961)). In addition, a potential toxicity of methanol (the other metabolite) cannot be neither totally excluded. In conclusion, without other robust considerations, it is not known to what extent data with ASA can be extrapolated to methyl salicylate.