Australian Government
Department of Health and Aged Care
Australian Industrial Chemicals Introduction Scheme

Benzoic acid, 2-hydroxy-, methyl ester (methyl salicylate)

Evaluation statement

15 April 2024

Draft

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AICIS evaluation statement

Subject of the evaluation

Benzoic acid, 2-hydroxy-, methyl ester (methyl salicylate)

Chemical in this evaluation

Name	CAS registry number
Benzoic acid, 2-hydroxy-, methyl ester	119-36-8

Reason for the evaluation

Evaluation Selection Analysis indicated a potential human health risk.

Parameters of evaluation

The chemical is listed on the Australian Inventory of Industrial Chemicals (the Inventory).

This evaluation is a human health risk assessment of all identified industrial uses of the chemical in Australia (except for use in e-cigarettes due to absence of relevant hazard data).

The chemical is a major component of some essential oils and plant extracts in Australia. These oils and extracts are subject to a separate evaluation.

Summary of evaluation

Summary of introduction, use and end use

There is currently no specific information about the introduction, use and end use of the chemical in Australia.

Based on international information, the chemical is used as a fragrance ingredient in a variety of cosmetic products. The predominant use appears to be in oral care products at concentrations up to 2.5%. Typical use concentrations are expected to be less than 0.1% in leave-on and rinse-off products (except hand soaps which can be up to 0.6%).

The chemical is used as a fragrance in professional and domestic cleaning products with typical concentrations below 1%. The chemical is used in air freshener products at concentrations up to 13%.

The chemical has non-industrial uses in therapeutics and foods.

Human health

Summary of health hazards

The identified health hazards are based on the available data for the chemical. Conclusions on the systemic endpoints were supported with read-across information from the major metabolite salicylic acid (CAS No. 69-72-7).

Based on the available data, the chemical:

- has low acute dermal and inhalation toxicity
- is at most slightly irritating to skin
- is not considered to have genotoxic potential
- is not expected to be carcinogenic
- is not expected to cause specific adverse effects on fertility.

Based on available data, the chemical is expected to have moderate acute oral toxicity (median lethal dose (LD50) = 887 mg/kg bw in rats).

Based on the results from an in vitro guideline study (OECD TG 491), the chemical is expected to cause serious eye damage.

The chemical is expected to be a weak skin sensitiser based on animal and human data. In local lymph node assay (LLNA) studies, reported concentrations producing a three-fold increase in lymphocyte proliferation (EC3) values ranged from 15–65%. Guinea pig maximisation tests (GPMT) were mostly negative for sensitisation. In human patch testing with the chemical applied to dermatitis patients there was a 1–2% positive reaction rate, indicating that the chemical can cause skin sensitisation.

Based on the available data, the chemical may cause adverse effects on development. The main effects observed were increased incidences of neural tube defects in pups born from rats or hamsters that were exposed to the chemical during gestation. Other adverse effects noted in multiple studies were increased incidences of skeletal variations in the pups and a lower pup body weight when compared to control groups. The lowest no observed adverse effect level (NOAEL) for development is 75 mg/kg bw/day based on a 3 generation study in rats. In addition, the metabolite salicylic acid is reported to cause adverse effects on development in rats and monkeys including increased foetal mortality, increased incidences of neural tube defects and foetal growth retardation. There is extensive human use of acetylsalicylic acid as aspirin which shares a metabolite with the chemical. There is a lack of evidence to support an increased risk of birth defects following exposure to aspirin.

For further details of the health hazard information see **Supporting information**.

Hazard classifications relevant for worker health and safety

This chemical satisfies the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) for hazard classes relevant for work health and safety as follows. This does not consider classification of physical hazards and environmental hazards.

Health hazards	Hazard category	Hazard statement
Acute toxicity – oral	Acute Tox. 4	H302: Harmful if swallowed
Serious eye damage/eye irritation	Eye Damage 1	H318: Causes serious eye damage
Skin sensitisation	Skin Sens. 1B	H317: May cause an allergic skin reaction
Reproductive toxicity	Repr. 2	H361d: Suspected of damaging the unborn child

Summary of health risk

Public

Based on the available use information, the public may be exposed to the chemical in:

- cosmetic products at concentrations up to 2.5% in oral care products, up to 0.1% in leave-on products and up to 0.6% in rinse-off products
- household cleaning and air freshener products at concentrations up to 13%.

Exposure to these products may occur by the:

- oral route, when using oral care products
- dermal route, when using leave-on and rinse-off skin products or cleaning products
- inhalation route, as the chemical is volatile and can be used in sprayed products.

Exposure to the chemical is expected to be predominantly from cosmetic products (particularly oral care products). Exposure from household products is expected to be marginal in comparison to cosmetics products. Australian use patterns for the various cosmetic product categories are assumed to be similar to those in Europe.

The critical health effect for risk characterisation is systemic long term effects (developmental toxicity).

The European Commission's Scientific Committee on Consumer Safety (SCCS) recently reassessed the safety of the chemical in cosmetics and personal care products. A conservative quantitative risk assessment based on European Union (EU) concentration limits resulted in a calculated margin of safety (MoS) of 145. In general, an MoS value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences. The SCCS also undertook a quantitative risk assessment to assess the risks of the chemical in products used by children under 6 years of age. The MoS based on aggregated exposure were all calculated above 100. Based on our exposure estimates, the presence of the chemical in household products such as air fresheners or cleaners is not expected to significantly change existing exposure or risk estimates.

The chemical is also acutely toxic by the oral route, can cause eye damage and is a weak sensitiser. Under the typical conditions of use in cosmetic and household products at low concentrations, the risk of these adverse effects to the public is low.

Overall, when used in cosmetic and household products at low concentrations there are no identified risks that require management.

The public could also be exposed to the chemical at high concentrations in some essential oils and plant extracts in Australia. These oils and extracts are subject to a separate evaluation and any means for managing risk will be determined as part of that evaluation.

Workers

During product formulation and packaging, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing these chemicals. The level and route of exposure will vary depending on the method of application and work practices employed. Good hygiene practices to minimise incidental oral exposure are expected to be in place.

Given the critical systemic long-term and local health effects, the chemical could pose a risk to workers. Control measures to minimise dermal, ocular and inhalation exposure are needed to manage the risk to workers (refer to **Proposed means of managing risk**).

Proposed means for managing risk

Workers

Recommendation to Safe Work Australia

It is recommended that Safe Work Australia (SWA) update the Hazardous Chemical Information System (HCIS) to include classifications relevant to work health and safety.

Information relating to safe introduction and use

The information in this statement including recommended hazard classifications, should be used by a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) to determine the appropriate controls under the relevant jurisdiction Work Health and Safety laws.

Control measures that could be implemented to manage the risk arising from oral, dermal and inhalation exposure to the chemical include, but are not limited to:

- using closed systems or isolating operations
- minimising manual processes and work tasks through automating processes
- adopting work procedures that minimise splashes and spills
- cleaning equipment and work areas regularly
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Measures required to eliminate, or manage risk arising from storing, handling and using this hazardous chemical depend on the physical form and how the chemical is used.

These control measures may need to be supplemented with:

- conducting health monitoring for any worker who is at significant risk of exposure to the chemical if valid techniques are available to monitor the effect on the worker's health.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Model codes of practice, available from the Safe Work Australia website, provide information on how to manage the risks of hazardous chemicals in the workplace, prepare an SDS and label containers of hazardous chemicals. Your Work Health and Safety regulator should be contacted for information on Work Health and Safety laws and relevant Codes of Practice in your jurisdiction.

Conclusions

The Executive Director proposes to be satisfied that the identified risks to human health from the introduction and use of the industrial chemical can be managed.

Note:

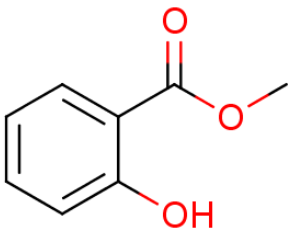
1. Obligations to report additional information about hazards under *Section 100* of the *Industrial Chemicals Act 2019* apply.
2. You should be aware of your obligations under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Supporting information

Chemical identity

Chemical name	Benzoic acid, 2-hydroxy-, methyl ester
CAS No.	119-36-8
Synonyms	methyl salicylate (INCI)
	methyl 2-hydroxybenzoate
Molecular formula	C8H8O3
Molecular weight (g/mol)	152.15
SMILES (canonical)	O=C(OC)C=1C=CC=CC1O
Chemical description	-

Structural formula:



Relevant physical and chemical properties

Measured physical and chemical property data for the chemical were identified from the European Union Registration, Evaluation and Authorisation of Chemicals dossiers (REACH n.d.).

Physical form	Liquid at 25°C
Melting point	-8.6°C
Boiling point	221°C at 101 kPa
Vapour pressure	13 Pa at 20°C
Water solubility	625 mg/L at 30°C
pK_a	9.76
log K_{ow}	2.55

Introduction and use

Australia

There is no specific information about the introduction, use and end use of the chemical in Australia.

The chemical has non-industrial uses including in therapeutics (as an analgesic in topical pain relief ointments and creams at up to 50%).

International

The chemical is manufactured and/or imported in Europe at 1,000–10,000 tonnes per year (ECHA 2018).

The chemical is used in a wide variety of cosmetic and personal care products, predominantly as a fragrance. The chemical has reported functions as a denaturant, and as an oral care, soothing, perfuming and flavouring agent in the CosIng database (EC n.d.). The chemical is listed on the International Fragrance Association (IFRA) Transparency List (IFRA n.d.). The products which can contain the chemical include (SCCS 2021):

- oral care products including toothpastes, mouthwashes and mouth sprays
- hydroalcoholic-based fragrances
- rinse-off skin and hair products including shampoos, hair conditioners and shower gels
- leave-on skin and hair products including body lotions, face creams, hand creams, hair styling products and deodorants
- hand washes
- make-up products including liquid foundations, lipsticks, eye make-ups, mascaras, eyeliners and make-up removers.

According to the Environmental Working Group (EWG) Skin Deep database, most products containing the chemical are oral care products (EWG n.d.). The reported concentrations of the chemical in some of these products were 0.1–1.5% in toothpastes and 0.06–1.0% in mouthwashes (DeLima Associates n.d.). Recent survey data from the United States of America (USA) indicates that the chemical was used in 18 leave-on and 15 rinse-off cosmetic products. Reported concentrations were 0.0000013–1% in leave-on products and 0.00000006–0.23% in rinse-off products (CIR 2019). In 2002, the 97.5 percentile use level of the chemical in cosmetic and personal care products was 0.13% and is consistent with reports from industry indicating use concentrations are less than 0.6% (ECHA 2018).

The chemical has reported domestic use as a fragrance in:

- household cleaning products and disinfectants
- air freshener products
- automotive cleaning products.

The concentration of the chemical in cleaning products is typically between 0.1 and 1%. However, the concentration in some air freshener products can be between 7.0 and 13.0%. The chemical is used in some commercial and professional cleaning and disinfecting products as a fragrance (DeLima Associates n.d.).

The chemical has non-industrial uses including in therapeutic products (as an analgesic in topical pain relief ointments and creams). Reported concentrations of the chemical in therapeutic products are between 15% and 30% (SCCS 2021). The chemical is also used in foods as a fragrance and flavouring ingredient.

Existing Australian regulatory controls

Public

The chemical is listed in the *Poisons Standard* (SUSMP) as follows (TGA 2024).

Schedule 4:

“METHYL SALICYLATE in preparations for internal therapeutic use.”

Schedule 5:

“METHYL SALICYLATE in preparations containing 25% or less of methyl salicylate **except**:

a) in preparations for therapeutic use; or
b) in preparations containing 5% or less of methyl salicylate.”

Schedule 6:

“METHYL SALICYLATE **except**:

a) when included in Schedule 5; or
b) in preparations for therapeutic use; or
c) in preparations containing 5% or less of methyl salicylate.”

Schedule 4 chemicals are labelled with ‘Prescription Only Medicine or Prescription Animal Remedy’ and are described as: “Substances, the use or supply of which should be by or on the order of persons permitted by State or Territory legislation to prescribe and should be available from a pharmacist on prescription.”

Schedule 5 chemicals are labelled with ‘Caution’ and are described as: “Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.”

Schedule 6 chemicals are labelled with 'Poison’ and are described as: “Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label.” (TGA 2024).

Workers

The chemical is listed on the Hazardous Chemical Information (HCIS) with the following hazard categories and statements for human health (SWA n.d.):

Health hazards	Hazard category	Hazard statement
Acute toxicity – oral	Acute Tox. 4	H302: Harmful if swallowed
Skin sensitisation	Skin Sens. 1B	H317: May cause an allergic skin reaction
Reproductive toxicity	Repr. 2	H361d: Suspected of damaging the unborn child

No specific exposure standards are available for the chemical in Australia (SWA n.d.).

International regulatory status

Exposure standards

The following exposure standards were identified (Chemwatch n.d.):

- maximum permissible concentration of hazardous substances in the air of the working area of 1 mg/m^3 – Belarus
- maximum allowed concentration of harmful substances in the air of the workplace zone of 1 mg/m^3 – Russia.

The following temporary emergency exposure limits (TEELs) have been recommended by the United States Department of Energy for the chemical (Chemwatch n.d.):

- 150 ppm (TEEL-3)
- 25 ppm (TEEL-2)
- 2.3 ppm (TEEL-1).

Canada

The chemical is listed on the Health Canada Cosmetic Ingredient Hotlist - List of Ingredients that are Restricted for Use in Cosmetic Products, with a maximum concentration permitted of 1% (Government of Canada 2022).

European Union

The chemical is listed in Annex III of the EU Cosmetic Regulation (EC) No.1223/2009 - List of substances which cosmetic products must not contain except subject to the restrictions laid down. The maximum concentrations permitted in cosmetic products are listed in **Table 1**.

Table 1 – Maximum concentrations of methyl salicylate permitted in cosmetic products in the European Union.

Product type	Maximum concentration in ready for use preparation
Leave-on skin products (except face makeup, spray/aerosol body lotion, spray/aerosol deodorant and hydroalcoholic-based fragrances) and leave on hair products (except spray/aerosol products)	0.06%
Face makeup (except lip products, eye makeup and makeup remover)	0.05%
Eye makeup and makeup remover	0.002%
Leave-on hair products (spray/aerosol)	0.009%
Deodorant spray/aerosol	0.003%
Body lotion spray/aerosol	0.04%
Rinse-off skin products (except hand wash) and rinse-off hair products	0.06%
Hand wash	0.6%
Hydroalcoholic-based fragrances	0.6%
Lip products	0.03%
Toothpaste	2.52%
Mouthwash intended for children aged 6–10 years	0.1%
Mouthwash intended for children above 10 years of age and adults	0.6%
Mouth spray	0.65%

The chemical is listed with an additional restriction: “Not to be used in preparations for children under 6 years of age, with the exception of (k) “Toothpaste”. The presence of the substance shall be indicated in the list of ingredients referred to in Article 19(1), point (g), when its concentration exceeds: 0.001 % in leave-on products; 0.01 % in rinse-off products.” (EC n.d.).

New Zealand

The chemical is listed in the New Zealand Cosmetic Products Group Standard — Schedule 5 Components Cosmetic Products ‘Must Not Contain Except Subject to the Restrictions and Conditions Laid Down’. The maximum authorised concentrations in finished cosmetic products are the same as that in the EU Cosmetic Regulation (see **European Union**) (NZ EPA 2024).

United States of America

The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the chemical is “safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating and non-sensitizing” (CIR 2019).

Under the US Food and Drug Administration (FDA) Federal Food, Drug, and Cosmetic Act Code of Federal Regulations (21CFR201.303) “any drug containing more than 5 percent methyl salicylate (wintergreen oil)” must be labelled “to warn that use otherwise than as directed therein may be dangerous and that the article should be kept out of reach of children to prevent accidental poisoning” (FDA n.d.).

Asia

The chemical is restricted under a group entry in the Japan Ministry of Health and Welfare’s Standards for Cosmetics (Ministry of Health and Welfare Notification No.331 of 2000). The entry in “Appendix 3: The ingredients restricted in all types of cosmetics” states that total concentration of all salicylates in cosmetics has a concentration limit of 1.0% (Ministry of Health and Welfare Japan 2000).

Human exposure

Public

As the chemical is used in a wide range of cosmetic and household products (see **Introduction and use**), there is expected to be significant public exposure to the chemical. Australian use patterns for the various product categories are assumed to be similar to those in Europe and the USA. Therefore, existing international exposure estimates are suitable for estimating Australian public exposure to the chemical.

Cosmetic products

Depending on the type of product, dermal contact with cosmetic products can be limited to specific areas on the body such as the eye region, face, hands, nails, or feet, or it can be more extensive, covering large areas of the trunk as well as the face. The duration of exposure for various products may differ substantially. For rinse-off products such as soaps or shampoos, exposure might only be for a few minutes, although some residual product can remain. Whereas for leave-on products, exposure could last for several hours.

The SCCS has conducted exposure assessments to determine the aggregate exposure to the chemical from a range of cosmetic and personal care products at the current EU concentration limits (see **International restrictions**). The calculated aggregate daily systemic exposure to the chemical from oral, dermal and inhalation exposure to all product types was 0.52 mg/kg bw/day (SCCS 2021).

The exposure assessment was considered conservative as it included all product types which contain the chemical and used maximum concentrations permitted in the EU. Exposure to the chemical was calculated as an internal dose which is proportional to the use volumes, product retention factors (reflecting proportions of product remaining on the skin during normal use) and the dermal absorption of the chemical. Default absorption values of 100% were used for oral and inhalation exposure. A default value of 50% dermal absorption was chosen, based on the data reported in human in vivo studies and on the physical and

chemical properties of the chemical (see **Toxicokinetics**). The SCCS also accounted for the inhalation exposure of the chemical from products that are expected to be inhaled as well as residual vapours from leave-on skin products.

The SCCS also estimated the aggregate exposure to the chemical in children under 6 years of age. The estimate differed from the above as it:

- only included products that are likely to be used by children
- used a concentration of 0.02% for dermal products for children aged 0–3 years
- used toothpaste as the only oral care product at the EU concentration limit of 2.52% for children over 1 year of age
- did not include inhalation exposure as it was expected to be incidental.

The aggregate daily systemic exposure doses in children aged 0.5–1, 1–3 and 3–6 years were 0.035, 0.463 and 0.454 mg/kg bw/day respectively (SCCS 2023). For children over 1 year of age, the predominant source of exposure was from toothpaste products, resulting in the significant discrepancy in values between the age groups.

Although there is some reported use in the USA in leave-on products at concentrations higher than current EU limits (see **Introduction and use**), the SCCS exposure estimates are considered relevant for risk characterisation given that:

- oral exposure (from rinse-off oral care products) is a significant contributor to the overall exposure (SCCS 2021)
- the reported number of leave-on products in the USA was relatively low (CIR 2019); therefore, use of products with concentrations higher than the EU limits is not expected to be widespread.

In 2002, the dermal daily systemic exposure to the chemical was determined to be 0.0034 mg/kg bw/day by the IFRA (Lapczynski et al. 2007). This exposure assessment only considered products applied to the skin and excluded exposure from oral care products. A concentration of 0.13% was chosen for all products based on the 97.5 percentile level of use in 2002, resulting in a lower aggregate daily systemic exposure estimate than other exposure assessments.

Household products

The public may also be exposed to the chemical due to its presence in household products. In general, exposure from cleaning products with low concentrations of the chemical (typically less than 1%) would be incidental. International exposure estimates listed above did not account for exposure from domestic products.

Based on product information in the USA, the chemical has been identified in a drop air freshener product at concentrations up to 13% (see **Introduction and use**). As this product has concentrations of the chemical that are order(s) of magnitude higher than other reported household products, the daily systemic exposure by inhalation from this product was estimated. The exposure to vapours of the chemical from use in this air freshener product was estimated using the ConsExpo web tool to be 0.0053 mg/kg bw/day (RIVM n.d.).

For this type of air freshener, one drop (assumed to be 0.05 g) is added to a room with frequency of use of once per day. Despite limited data on absorption of the chemical by inhalation (see **Toxicokinetics**), the default inhalation absorption value is assumed to be 100% (SCCS 2021). The default inhalation rate of 0.629 m^3/hour was set using the default

Canadian population aged 19+ years in ConsExpo (RIVM n.d.). The remaining parameters were based on a previous exposure estimate of a different salicylate chemical used in an air freshener product (Government of Canada 2020):

- Exposure model: exposure to vapour – constant rate
- Exposure duration and emission duration – 24 hours
- Room volume – 20 m^3
- Ventilation rate – 0.6 per hour.

Health hazard information

Toxicokinetics

The oral absorption of the chemical is expected to be 100%. Human data on the chemical indicates typical dermal absorption ranges from 2 to 43%. Absorption is highly dependent on the vehicle. A dermal absorption value of 93% was reported in a single study when the chemical was administered in acetone under occluded conditions (ECHA 2018; ECHA 2021). Despite this, a default dermal absorption value of 50% is often considered as a conservative estimate (SCCS 2021). No data is available on the absorption of the chemical via inhalation, but its physiochemical properties suggest that it can be absorbed by the inhalation route.

After administration, the chemical is expected to be widely distributed. Studies of the radiolabelled chemical administered orally in mice showed peak blood radioactivity after 30 minutes, and only traces of radioactivity after 48 hours. The levels of radioactivity were highest in the kidneys, liver and adrenals. Lower levels of radioactivity were detected in the uterus, ovaries, lungs, heart, spleen and pancreas, with the lowest levels in the brain (ECHA 2018; ECHA 2021).

The chemical hydrolyses into the metabolites, salicylic acid (CAS No. 69-72-7) and methanol in vivo. The rate of hydrolysis is species dependent, as it is completely hydrolysed to the salicylate anion (“free salicylate”) within 20 minutes in rats, 95% hydrolysed within 1 hour in dogs, and 80% hydrolysed in humans after 90 minutes (ECHA 2018; ECHA 2021). Free salicylate is detected in the plasma 1–2 hours after oral or dermal administration, in a dose dependent manner. The free salicylate is usually bound to proteins in plasma. Oral exposure typically results in higher plasma salicylate levels than topical applications (ECHA 2018; ECHA 2021; Overman and White 1983). Studies conducted with hamsters have indicated that free salicylate can reach the developing foetus within the first 2.5 hours after oral administration of the chemical. The concentrations of free salicylates in the foetus were 50–90% of the concentrations in the dam (Overman and White 1983).

Free salicylate is later conjugated with either glycine or glucuronide and excreted in the urine, with total recovery levels near 100%, indicating a low potential for bioaccumulation (ECHA 2018; ECHA 2021).

Acute toxicity

Oral

Based on the available data, the chemical has low to moderate acute oral toxicity.

The chemical is classified as hazardous in the HCIS with hazard category “Acute Toxicity – Category 4” and hazard statement “H302: Harmful if swallowed” (SWA n.d.). There is limited

information on the studies performed and none of the studies are good laboratory practice (GLP) compliant or conducted according to guidelines. Most of the available studies report an LD50 between 300 and 2000 mg/kg in a range of animal species. The available data support the existing classification.

In an acute oral toxicity study conducted similarly to the Organisation for Economic Cooperation and Development Test Guideline (OECD TG) 401, Osborne-Mendel rats (5/sex/dose) were treated with a single dose of the chemical. The LD50 was 887 mg/kg. Depression was observed soon after treatment (Lapczynski et al. 2007; REACH n.d.).

In an acute oral toxicity study conducted similarly to OECD TG 401, guinea pigs (5/sex/dose) were treated with a single dose of the chemical. The LD50 was 1060 mg/kg. Clinical signs of toxicity included convulsions and gastro-intestinal irritation (Lapczynski et al. 2007; REACH n.d.).

Other reported oral LD50 values for the chemical with limited study details were (ECHA 2018; Lapczynski et al. 2007; REACH n.d.):

- 1060–2820 mg/kg in rats
- 580–1440 mg/kg in mice
- 1300–2800 mg/kg in rabbits
- 700 mg/kg in guinea pigs
- 2100 mg/kg in dogs.

The reported clinical signs of toxicity in rats included piloerection, shaggy coat, hunched posture, lethargy, oscillated movements, difficulty breathing, convulsions and mydriasis (dilated pupil) (ECHA 2018; Lapczynski et al. 2007; REACH n.d.).

Dermal

Based on the available data, the chemical is not expected to be acutely toxic via the dermal route.

In an acute dermal toxicity study conducted in accordance with OECD TG 402, rabbits (10/dose, sex not specified) were treated with a single dose of the chemical. The dermal LD50 was determined to be greater than 5000 mg/kg. One mortality was recorded during the study, but no other clinical signs of toxicity were reported (REACH n.d.).

Other reported values from studies with limited details include an LD50 greater than 2500 mg/kg in rats and LD50 of 700 mg/kg in guinea pigs (REACH n.d.).

Inhalation

Based on the information from a subchronic inhalation toxicity study (see **Repeat dose toxicity**), the chemical is not expected to be acutely toxic by the inhalation route. Median lethal concentrations (LC50) from studies with limited details were reported to be greater than 114 and 400 mg/m^3 in rats and greater than 400 mg/m^3 in mice (REACH n.d.).

Observation in humans

In 2004, 12,005 cases of poisoning from exposure to this chemical were reported in the USA. The reported signs and symptoms included haematemesis, tachypnoea, hyperpnoea, dyspnoea, tinnitus, deafness, lethargy, seizures or confusion (ECHA 2018).

Corrosion/Irritation

Skin irritation

Based on the available data, the chemical is at most, slightly irritating to skin.

In a GLP compliant skin irritation study conducted similarly to OECD TG 404, 4 female Albino Mol:Russian rabbits were treated with the chemical at concentrations of 1, 5, 10, 25 or 100% in 1:1 ethanol/diethyl phthalate for 4 hours under semi-occlusive conditions. Observations were recorded at 24, 48, 72 hours after patch removal. No signs of irritation were observed in the rabbits treated with 1, 5 or 10% of the chemical. For the 25% application, the mean score was 0.2 for erythema and 0 for oedema. At this dose, erythema was fully reversible within 72 hours. For the 100% application of the chemical, the mean score was 1.33 for erythema and 0.67 oedema. At this dose, erythema was fully reversible within 14 days and the oedema was fully reversible within 7 days (REACH n.d.).

In a primary skin irritation study, 3 rabbits were administered the chemical at concentrations of 1, 3 or 6% in different vehicles (water, polyethylene glycol (PEG) 400, 70% ethanol or 70% ethanol with emollients) by occluded patch for 24 or 72 hours. Moderate irritation was observed at all concentrations in the vehicles containing ethanol. Mild irritation was observed from the chemical in all other conditions, except for 1% of the chemical in water, for which there was no irritation (Lapczynski et al. 2007).

There are several other incidental reports of irritation in animals, primarily from screening studies to determine maximum non-irritant concentrations for maximisation tests. These studies have limited details and used conditions different to the OECD TG including the use of guinea pigs or mice and long administration times (24 hours) for the chemical.

Eye irritation

Based on the available in vivo and in vitro data, the chemical is considered to cause serious eye damage, warranting hazard classification.

In a GLP compliant in vitro eye corrosion study conducted according to OECD TG 491, the chemical was dissolved or suspended in physiological saline and applied to a single layer of rabbit corneal cells at 0.05 % and 5%. After a 5 minute exposure, the percentage cell viability was 25.5–31.0% and 3.9–19.9% for 0.05% and 5% concentrations, respectively. Based on the decision criteria for this test (cell viability at 5 and 0.05 % <70%), the chemical is predicted to cause serious eye damage (REACH n.d.).

In an eye irritation test, the chemical was instilled into 1 eye of 5 albino rabbits. The eyes were observed between 18–24 hours and then stained with fluorescein. Necrosis was observed across 13–37% of the cornea after staining and assigned a score of 3 (Lapczynski et al. 2007).

There are numerous other studies reporting some ocular irritation in animals after exposure to the chemical. However, these studies have limited details (ECHA 2021).

In a non-GLP compliant eye irritation study conducted similarly to OECD TG 405, the chemical was instilled into 1 eye each of a New Zealand white (NZW) rabbit (sex not specified). The eyes were observed at 1, 24, 48 and 72 hours. The following mean scores were reported at 24, 48 and 72 hours: corneal opacity 0/4, iritis 0/2, conjunctival redness 0/3,

chemosis 0/4 (REACH n.d.). In this study the chemical was not irritating to eyes, but only a single animal was tested.

Observation in humans

The chemical is not considered to be irritating at typical use concentrations. The available data in humans for the chemical is limited, and not in controlled studies. As a class of chemicals, salicylate esters are not considered irritating to skin (Belsito et al. 2007).

In a screening study for a human maximisation test, no irritation was reported in 27 healthy male volunteers after exposure to a 48 hour occluded patch containing 8% of the chemical in petrolatum. In another study, 9 volunteers were exposed to the chemical at various concentrations in 4:1 ethanol:water under occlusive conditions. There were reports of irritation in this study at 30% and 60% concentrations of the chemical, but the study was not performed using standard guidelines (Lapczynski et al. 2007).

The SCCS considers the chemical not to be irritating to skin up to a concentration of 12% (SCCS 2021).

Sensitisation

Skin sensitisation

Based on the available data from animal and human studies, the chemical is considered to be a weak sensitiser.

The chemical is classified as hazardous in the HCIS with hazard category “Skin sensitisation – Category 1B” and hazard statement “H317: May cause an allergic skin reaction” (SWA n.d.). Based on the weight of evidence showing a low to moderate frequency of reactions in humans and a low to moderate sensitisation potency in animals, the data supports the classification of the chemical as a Category 1B skin sensitiser.

Local lymph node assays (LLNA)

The results from numerous local lymph node assays (LLNA) gave positive and negative indications of sensitisation. Positive results were generally observed in guideline studies where the tested concentration was 25% or above. The reported concentrations producing a three-fold increase in lymphocyte proliferation (EC3) ranged from 15–65%, depending on the animal and vehicle used. The majority of negative results were obtained when the concentration tested was below 20% (ECHA 2018; Lapczynski et al. 2007; REACH n.d.). The results are summarised in **Table 2**.

Many of the reports below are from screening studies that were used to validate the original LLNA protocol. The chemical was included in these screens as a known skin irritant, although it is not currently classified as irritating (REACH n.d.).

Table 2 – Summary of results from local lymph node assays (LLNAs) with methyl salicylate

OECD TG	Animals	Concentration range	Vehicle	Result	EC3 (%)
429	4 female CBA/Ca mice	10–100%	DMF	Positive	65
429	4 female CBA/Ca mice	10–50%	MEK	Positive	15
429	4 female CBA/Ca mice	12.5–100%	DMF	Positive	33
429	4 female CBA/Ca mice	12.5–100%	MEK	Positive	28
429	4 female CBA/Ca mice	1–25%	DMF	Positive (SI = 3 at 25%)	-
429	4 female CBA/Ca mice	5–25%	MEK	Positive (SI = 7.5 at 25%)	-
429	9 female BALB/c mice	20–80%	Acetone:olive oil (4:1)	Positive	48.15
429	4 CBA mice	25–100%	Not specified	Negative	-
429	mice	5–25%	Acetone:olive oil (4:1)	Negative	-
429	female CBA/Ca or CBA/JHsd mice	1–20%	Acetone:olive oil (4:1)	Negative	-
429	4 CBA mice	5–25%	Acetone:olive oil (4:1)	Negative	-
429	4 CBA/Ca mice	1–5%	Acetone:olive oil (4:1)	Negative	-
429	4–6 female BALB/c mice	25%	Acetone	Negative	-
442B	4 BALB/c mice	50%	Acetone:olive oil (4:1)	Negative	-
None	5 female CBA mice	5%	Acetone	Negative	-
None	4–6 female BALB/c mice	25%	Acetone	Positive (SI = 3)	-
None	female Wistar rats or Brown Norway rats	5–25%	Acetone:olive oil (4:1)	Negative	-
None	female Hartley albino guinea pigs	10%	DMSO	Negative	-

DMF = *N,N*-dimethylformamide; DMSO = dimethyl sulfoxide; EC3 = concentration producing a three-fold increase in lymphocyte proliferation; MEK = methyl ethyl ketone; SI = stimulation index.

Maximisation tests

The results from guinea pig maximisation tests (GPMT) were reported to be negative. These studies were conducted similarly to OECD TG 406. However, fewer animals than recommended in the guideline were used, which may have limited the sensitivity of these studies. Other maximisation tests conducted with guinea pigs that were performed differently from OECD guidelines gave conflicting sensitisation results (ECHA 2018; Lapczynski et al. 2007; REACH n.d.). All maximisation tests are summarised in **Table 3**.

Table 3 – Summary of maximisation tests on guinea pigs for sensitisation with methyl salicylate

Test	Number/Sex/Strain	Induction concentration(s)	Challenge concentration	Reactions
GPMT	9–10 albino Dunkin-Hartley	2.5% in 0.01% DOBS in saline (intradermal); 100% (topical)	10% in acetone/ polyethylene glycol	None
GPMT	6–8 male and female outbred Himalayan white-spotted	5% in FCA (intradermal); 25% in petrolatum (topical)	Sub-irritant concentration	None
GPMT	5 Dunkin-Hartley	5% in water (intradermal); 60% in water (topical)	20% in water	None
GPMT	10 albino Dunkin-Hartley	1% in FCA (intradermal); 40% in acetone (topical)	10% in acetone	None
OET	6–8 male and female outbred Himalayan white-spotted	0.03–100% in various vehicles (topical)	3% (vehicle not specified)	Yes, >2/8 animals at 30%
Optimisation test	10 male and 10 female Pirbright White	0.1% in saline (intradermal, 10 times over 3 weeks)	0.1% in saline (intradermal)	Yes, 2/20 animals
Optimisation test	10 male and 10 female Pirbright White	0.1% in saline (intradermal, 10 times over 3 weeks)	10% in petrolatum (topical)	None
CET	5 (strain not specified)	30% (topical, 3 times per week for 2 weeks)	1% (vehicle not specified)	None
Draize test	6–8 male and female outbred Himalayan white-spotted	0.1% in saline (intradermal, 10 times on alternate days)	0.1% (vehicle not specified)	None
FCAT	6–8 male and female outbred Himalayan white-spotted	50% in FCA (intradermal)	Sub-irritant concentration	None

CET = closed epicutaneous test; DOBS = dodecyl benzene sulfonate; FCA = Freund's complete adjuvant; FCAT = Freund's complete adjuvant test; GPMT = guinea pig maximisation test; OET = open epicutaneous test.

Respiratory sensitisation

The chemical is not expected to be a respiratory sensitiser based on the limited data available. There are no reports of respiratory sensitisation from the use or manufacture of the chemical in the EU (REACH n.d.).

In a respiratory sensitisation LLNA, BALB/c mice (6/dose) were exposed to the chemical at 30.4 mg/m^3 for 45, 90, 180, or 360 min/day. There was no increased proliferation in either the mandibular or auricular lymph nodes. The chemical was considered to be non-sensitising but was reported to be slightly toxic to nasal tissues (Arts et al. 2008; ECHA 2021).

Observation in humans

The results of human patch testing with the chemical in human diagnostic and workplace studies are summarised in **Table 4**. While most studies were conducted in the 1970s and 1980s with limited details, the results indicate a low to moderate frequency of reactions in both selected and unselected subjects (ECHA 2018; Lapczynski et al. 2007).

Table 4 – Summary of human patch testing with methyl salicylate

Number of subjects	Subject details	Concentration and vehicle	Positive reactions (%)
4600	Selected (3108 with dermatitis; 1491 healthy subjects)	2% in petrolatum	0.13 (0.19 excluding healthy subjects)
183	Selected from North American Contact Dermatitis Group	2% (vehicle not specified)	1.6
241	Selected (61 males; 180 females)	2% in yellow soft paraffin	1.2
585	Selected subjects with eczema (301 subjects in 1978–1979 and 284 subjects in 1979–1980)	2% in petrolatum	1 (1978–1979 group) 2 (1979–1980 group)
89	Selected (19 with eyelid dermatitis; 70 with dermatitis at other sites)	1% in petrolatum	0 (eyelid dermatitis) 1.4 (other subjects)
1825	Unselected subjects in multicentre study	2% in petrolatum	0.4
539	Selected – 50 with photosensitivity dermatitis with actinic reticuloid syndrome; 32 with polymorphic eruption; 457 with contact dermatitis	2% in yellow soft paraffin	2 (photosensitivity dermatitis with actinic reticuloid syndrome) 0 (other groups)
197	Not specified	0.05-0.5% in base cream or 99% ethanol	2.0
267	Selected health care employees with contact dermatitis (82 males; 194 females)	2% in petrolatum	0

No reactions were reported in subjects exposed to the chemical at 1.25% concentration in 9 applications over 3 weeks in a human repeat insult patch test. No reactions were reported in 27 subjects exposed to the chemical at 8% concentration in petrolatum under occlusive conditions on 5 alternate day 48 hour periods in a maximisation study (ECHA 2018; Lapczynski et al. 2007). No further study details are available.

There are 2 isolated case reports of allergy to the chemical. A 79 year old female had a positive patch test to the chemical at 2% in olive oil after using a compress containing the chemical In another case, a 63 year old male had a positive patch test to the chemical at 2% in arachis oil after developing contact dermatitis following exposure to an analgesic ointment (ECHA 2018; Lapczynski et al. 2007).

Repeat dose toxicity

Based on the weight of evidence of the available data, the chemical is expected to cause adverse effects on foetal development if pregnant dams are exposed to the chemical (see **Reproductive and development toxicity**). Other adverse effects reported in repeat dose studies only occurred at very high doses.

Oral

Subchronic

In a 17 week study in Osborne-Mendel rats (10/sex/dose), the chemical was administered in diet at 0, 50 or 500 mg/kg bw/day. Reduced body weight gain was observed in the 500 mg/kg bw/day group. There were no changes in organ weights (liver, kidneys, spleen and testes). No haematological or biochemical examinations were performed. There were no histopathological changes in the liver, kidneys, spleen and testes (males) or liver, kidneys, spleen, thyroid and adrenals from rats receiving the highest dose. The NOAEL was 50 mg/kg bw/day based on reduced bodyweight at 500 mg/kg bw/day (ECHA 2021; REACH n.d.; Webb and Hansen 1963).

In 5 11–12 week oral toxicity studies, specifically designed to detect bone lesions in Sprague Dawley (SD) rats, the chemical induced bone lesions at doses greater than or equal to 560 mg/kg bw/day (Lapczynski et al. 2007; REACH n.d.). In one of the studies, supplementation with 0.3% calcium carbonate prevented bone lesions in rats (5/sex) receiving 600 mg/kg bw/day.

In a 59 day study in dogs (1/sex/dose), the chemical was administered by oral capsule at doses of 50, 100, 250, 500, 800 or 1200 mg/kg bw/day for 6 days/week. A high rate of mortality or severe adverse effects were observed in all dogs receiving the chemical at 500 mg/kg bw/day or higher. No adverse effects were observed at doses below 500 mg/kg bw/day. The NOAEL was 250 mg/kg bw/day (REACH n.d.).

In a 6/7 month study in Beagle dogs (3/sex/dose), the chemical was administered by oral capsule at 150, 300, 500 or 800 mg/kg bw/day. Survival was low in the 2 highest dose groups (0% at 800 mg/kg bw and 44% at 500 mg/kg bw). Increased kidney and liver weights were reported in the 150 and 300 mg/kg bw/day dose groups, but with no correlation to other histopathological parameters. No other effects on clinical chemistry were noted in the two lowest dose groups. The NOAEL was 300 mg/kg bw/day. In a follow up 6 month study in Beagle dogs (4–6/sex/dose), the chemical was administered by oral capsule at doses of 50, 100 or 167 mg/kg bw/day for 6 days/week. No significant adverse effects were reported at any dose. The NOAEL was 167 mg/kg bw/day (REACH n.d.).

Chronic

In a 2 year study in Osborne-Mendel rats (25/sex/dose), the chemical was administered in diet at 50, 250, 500 or 1000 mg/kg bw/day. In the 500 and 1000 mg/kg bw/day groups, there were significant reductions in body weight gain, development of rough hair coats, increased amounts of cancellous bone in the metaphyses. No animals survived in the 1000 mg/kg bw/day group. In the 500 mg/kg bw/day group, the relative weight of the testis was increased in males and the relative weights of the heart and kidney were increased in females. Gross pituitary gland lesions were increased in the 250 mg/kg bw/day group but no other group. No effects were reported in the lowest dose group, giving an NOAEL of 50 mg/kg bw/day. However, observations were made on random selections of the animals from each group and may not reflect statistically significant changes (REACH n.d.).

In a 2 year study in Beagle dogs (2/sex/dose), the chemical was administered by oral capsule at doses of 0, 50, 150 or 350 mg/kg bw/day. In the 150 and 250 mg/kg bw/day groups, there were significant reductions in body weight gain, enlarged livers (larger hepatic cells) and retarded growth. No effects were reported in the lowest dose group, giving an NOAEL of 50 mg/kg bw/day. However, observations were made on random selections of the animals from each group and may not reflect statistically significant changes (REACH n.d.).

In a 2 year study in albino rats (25/sex/dose), the chemical was administered in diet at 35 or 100 mg/kg bw/day. No adverse effects on growth, survival, food consumption, blood and urine parameters, necropsy or histology were observed (Lapczynski et al. 2007).

Dermal

In a 96 day study in rabbits (3/dose), the chemical was topically administered at 590, 1180, 2360 or 4720 mg/kg bw/day on 5 days/week. No animals in the 4720 mg/kg bw/day group survived the study. Clinical signs of toxicity included anorexia, weight loss and depression. In the 2360 mg/kg bw/day group, slight sloughing of the epidermis was observed. No dermal effects were observed in rabbits in the 590 or 1180 mg/kg bw/day groups, giving an NOAEL of 1180 mg/kg bw/day (REACH n.d.).

In a 16 day study, dogs (3/dose) received topical applications of the chemical at 2000 mg/kg twice per day for 16 days. Clinical signs of toxicity included significantly decreased diuresis, albumin in urine, excess blood nitrogen and decreased alkaline reserve (Lapczynski et al. 2007).

Inhalation

In a 28 day study conducted similarly to OECD TG 412, 4 female Alderley Park rats were exposed to the chemical at a near saturated concentration of 700 mg/m^3 for 7 hours/day for 5 days/week for 4 weeks. No adverse effects were reported (REACH n.d.).

Observation in humans

Retrospective studies of children receiving oral salicylate therapy for management of rheumatoid arthritis revealed no changes in bone pathology. It should be noted that the children received various forms of salicylate and varying doses (100–3240 mg daily for months to intermittent daily dosage). In a related retrospective study, there was no association between incidences of hepatomegaly in children and the dosages of salicylate they received (ECHA 2021).

Genotoxicity

Based on the available data on the chemical and its metabolites, the chemical is not expected to have genotoxic potential.

Negative results were reported in the following in vitro genotoxicity studies (Lapczynski et al. 2007; REACH n.d.):

- a bacterial reverse mutation (Ames) assay (OECD TG 471) in *Salmonella typhimurium* TA 92, 94, 98, 100, 1535 and 1537 with and without metabolic activation at concentrations up to 10,000 µg/plate
- a bacterial reverse mutation (Ames) assay in *Salmonella typhimurium* TA 1535, 1537, 98 and 100 with and without metabolic activation at concentrations up to 333 µg/plate

- an in vitro mammalian chromosome aberration assay (OECD TG 473) in Chinese hamster lung fibroblasts without metabolic activation at concentrations up to 0.25 mg/mL
- in a Rec-assay using *Bacillus subtilis* H 17 (rec+) and M 45 (rec-), dosed up to 23 µg
- in a Rec-assay using *Bacillus subtilis* H 17 (rec+) and M 45 (rec-), dosed up to 5000 µg/plate.

No in vivo genotoxicity studies are available for the chemical. Other chemically similar esters of salicylic acid were not mutagenic in various Ames assays (Belsito et al. 2007). The metabolite salicylic acid is not expected to have genotoxic potential based on in vitro and in vivo data (NICNAS 2013).

Carcinogenicity

Based in the limited available data, the chemical is not expected to have carcinogenic potential. The metabolite salicylic acid is not expected to be carcinogenic (NICNAS 2013). Chronic studies of wintergreen oil (of which the chemical is a major component) shows no evidence of carcinogenicity (SCCS 2021).

In a 2 year study in rats (see **Repeat dose toxicity**) with limited information available (no biochemical, urinalysis and ophthalmological examination and limited histopathological examination), similar type and numbers of tumours occurred in rats on all diets, except in the highest dose group (1000 mg/kg bw/day) where no animals survived. Benign pituitary tumours occurred in similar numbers of surviving rats on all diets. Malignant pituitary tumours occurred in 1 male and 2 female rats receiving 250 mg/kg bw/day. The benign pituitary tumours were more common in females (ECHA 2021; Webb and Hansen 1963).

Reproductive and development toxicity

The chemical is classified as hazardous in the HCIS with hazard category “Reproductive Toxicity – Category 2” and hazard statement “H361d: Suspected of damaging the unborn child” (SWA n.d.). The available data support the existing classification.

In animal studies, the chemical was able to reach the developing foetus and caused adverse effects which included reduced litter sizes, decreased pup survival and increased incidences of neural tube defects and skeletal abnormalities. The effects were observed when the chemical was administered to the animals orally, dermally or by subcutaneous injection. The same adverse effects were seen in multiple studies in rats and monkeys, using the metabolite salicylic acid, providing further evidence of developmental toxicity (AICIS 2024).

There is extensive human use of acetylsalicylic acid as aspirin which shares the metabolite salicylic acid with the chemical. There is a lack of evidence to support an increased risk of birth defects following exposure to aspirin.

Based on the weight of evidence available for the chemical and its metabolite salicylic acid, the chemical is not expected to cause adverse effects on fertility.

Reproductive toxicity studies with methyl salicylate

3 generation study in rats

In a non-guideline 3 generation study, Osborne-Mendel rats (20/sex/group) were administered the chemical in diet at doses equivalent to 0, 25, 75, 150 or 250 mg/kg bw/day

for 100 days prior to mating. The diet was continued through two further mating, gestation and lactation periods until weaning of the third generation. Each generation was mated twice (ECHA 2018).

There were no significant changes in the fertility index at any dose level in any generation. Significant decreases in pup body weight at weaning was noted in the 150 and 250 mg/kg bw/day dose groups. In the pups, there were decreases in average litter size, number of pups per female, pup viability survival to day 4 and survival to weaning noted in the higher dose groups. These decreases were only significant in the second generation and were dose dependent between 75 and 250 mg/kg bw/day. No gross abnormalities were reported in any pup from any generation. No histopathological effects on the kidneys or liver were noted in the pups from the third generation (other tissues or generations were not investigated).

Only a limited set of reproductive parameters were evaluated in this study (general parental toxicity was not reported). The NOAEL for fertility is 250 mg/kg bw/day and the NOAEL for development was 75 mg/kg bw/day based on decreased pup survival and decreased pup body weight in the higher dose groups.

Reproductive toxicity study in rats

In a GLP compliant reproductive toxicity study performed according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for the detection of reproduction and developmental toxicities for medicinal products, Crj:CD(SD)IGS rats (20/sex/dose) were administered the chemical by subcutaneous injection once daily at 0, 30, 100 or 300 mg/kg bw/day. Exposure started from 2 weeks prior to mating until sacrifice for males, or until gestation day (GD) 6 for females. Dams were sacrificed on GD 13 and foetuses were examined (ECHA 2018).

Animals in the 300 mg/kg bw/day group had a significantly lower bodyweight, reduced bodyweight gain and decreased food consumption compared to the control groups. Clinical signs of toxicity in the 300 mg/kg bw/day group included hypoactivity, bradypnoea, hypothermia and blanching in one male and crust at the treatment site and/or hair loss in 2 females. The NOAEL for parental toxicity was 100 mg/kg bw/day.

With respect to fertility parameters, there were no significant differences between the control groups and dosed groups, in:

- sperm count, motility or morphology in males
- the weights or testes or epididymides in males
- the length of oestrus cycle or oestrus count in females
- fertility or copulation indices
- the number of days required for copulation
- numbers of implants, viable embryos or dead embryos
- pre-implantation loss index.

A significant decrease in corporea lutea was noted in the 100 mg/kg bw/day group, but not in the other dose groups. The NOAEL for fertility was 300 mg/kg bw/day.

2 generation study in mice

In a GLP compliant 2 generation reproduction study conducted according to National Toxicology Program (NTP) guidelines, Swiss CD-1 mice (20/sex/dose) were administered

the chemical in corn oil by oral gavage once daily at 0, 25, 50 or 100 mg/kg bw/day. Exposure started 7 days prior to mating, through 98 days of cohabitation and then for a further 21 day separation period. The last litter from the dams in the 0 and 100 mg/kg bw/day groups were reared until weaning on postnatal day 21. These pups were dosed until mating on postnatal day 74 and produced a second generation (ECHA 2018).

In the F0 generation there were no significant changes between control and dosed groups in body weight, mortality or clinical signs of toxicity. In the F1 generation there were no significant clinical signs of toxicity or mortality. Pup growth to weaning, adult body weights and liver weights were not significantly different between control and dosed groups.

For F0 generation, the number of litters per pair, number of live pups per litter, average pup weight per litter and number of days to litter were not significantly different between control and dosed groups. In the F1 generation, there were no significant differences between control and dosed groups with regards to:

- absolute testes, epididymis, prostate and seminal vesicles weights
- sperm parameters (count, motility, morphology)
- fertility indices
- number of live pups per litter
- average pup weight per litter.

The NOAEL for fertility was 100 mg/kg bw/day. However, only the limited set of reproductive parameters indicated above were evaluated in this study.

1 generation study in mice

In a GLP compliant 1 generation reproductive toxicity study conducted according to NTP guidelines, CD-1 (ICR)BR outbred albino mice (20/sex/dose) were administered the chemical in corn oil by oral gavage once daily at 0, 100, 250 or 500 mg/kg bw/day. The animals were dosed 7 days prior to mating, through 100 days of cohabitation and then a further 21 day separation period (ECHA 2018).

No significant clinical signs of toxicity were observed in the parents. There were 11 animals across all dose groups that did not survive the study, but it did not appear to be treatment related. There was no significant effect on the fertility index for any dose groups. Between the control and 500 mg/kg bw/day groups, there were significant decreases in:

- the average number of litters
- the average number of pups per litter
- the proportion of pups born alive
- mean live pup body weights.

The NOAEL for reproduction was 500 mg/kg bw/day, and the NOAEL for development was 100 mg/kg bw/day based on decreased pup body weight.

Prenatal and postnatal reproductive study in rats

In a GLP compliant prenatal and postnatal reproductive toxicity study conducted according to ICH guidelines for the detection of reproductive and developmental toxicities for medicinal products, female Crj:CD(SD)IGS rats (20/dose) were administered the chemical in corn oil by subcutaneous injection once daily at 0, 20, 60 or 200 mg/kg bw/day from GD6 to lactation

day 21. The pups from the F1 generation were raised until 12–13 weeks of age, when they were mated for 2 weeks and then sacrificed after successful mating (ECHA 2018).

A significantly lower mean body weight and decreased body weight gain was noted in the 200 mg/kg bw/day group during gestation, but not during lactation. Reduced food consumption was observed in the 200 mg/kg bw/day group during both gestation and lactation. Two dams from the high dose group did not survive the study. The NOAEL was 60 mg/kg bw/day for general toxicity.

In all dose groups, there were no significant differences in the:

- number of implants, litters, gestation index, live pups and stillborns
- sex ratio of live pups
- number of live pups with external anomalies
- weaning index
- differentiation indices for pinna detachment, piliation, gait, descendus testes or vaginal opening
- copulation time, fertility or copulation indices in the F1 generation
- number of corporea lutea, implantation, pre-implantation loss index, live or dead embryos in females from the F1 generation.

Compared to the control group, the 200 mg/kg bw/day group had decreases in the number of implantations (278 to 251; 10% decrease), number of litters (270 to 215; 20% decrease) and number of live pups (268 to 208; 22% decrease). While not statistically significant, these effects were considered treatment related. A significant decrease in the birth index (number of live newborns divided by number of implantation sites, as a percentage) of 6% was observed in male pups from the 200 mg/kg bw group compared to the control. A significant prolongation of gestational days was observed in the 60 mg/kg bw/day group only and was not treatment related.

Pups born from the 200 mg/kg bw/day group had significantly lower mean body weights (9% decrease) when compared to the control group. In the male pups at weaning, there was a significant decrease in the absolute and relative weights of the liver and kidneys and the absolute weights of the brain, adrenals and testes in the 200 mg/kg bw/day group compared to the control. In the female pups at weaning, there were significant decreases in the absolute weights of the brain, heart, lungs, liver, kidneys, adrenals and ovaries in the 200 mg/kg bw/day group compared to the control.

There were significant delays in developmental landmarks including incisor eruption in both sexes, eyelid separation in females and cleavage of the balanopreputial gland in males from the 200 mg/kg bw/day group when compared to the control group. There were incidences of excessive elongation of the maxillary incisors and abnormal pupils in pups from the 200 mg/kg bw/day group.

Neural tube defects (craniorachischisis) were observed in 4 stillborn pups in the 200 mg/kg bw/day group. Skeletal anomalies were observed in 3 (4%) and 20 (32%) pups from the control and 200 mg/kg groups, respectively. When considered by type, there was a significant increase in misshapen sternebrae and fusions of the cervical vertebrae in the 200 mg/kg group compared to the control. Skeletal variations were observed in 20 (26%), 30 (40%) and 58 (94%) pups from the control, 60 mg/kg bw/day and 200 mg/kg bw/day groups, respectively. The incidences of skeletal variations appear to be dose related and are significantly increased in the 200 mg/kg bw/day group compared to the control. In the high dose group, there were significant increases in skeletal variations in the: full supernumerary rib (73% of pups), accessory sternebra (71% of pups), lumbarisation (6% of pups), 7 lumbar

vertebrae (63% or pups), and incomplete ossification of the cervical (39% of pups), thoracic (69% of pups) and caudal (35% of pups) vertebrae. The types of skeletal variations observed in the 60 mg/kg bw/day were in the cervical rib, accessory sternebra and incomplete ossification of the cervical, thoracic and caudal vertebrae. Considering the similarity in the types of variations between the 60 and 200 mg/kg bw/day groups, these incidences are considered treatment related. The NOAEL was below 60 mg/kg bw/day for developmental toxicity, based on the skeletal variations observed in the 60 mg/kg bw/day group.

Developmental studies with methyl salicylate

Prenatal developmental study in rats

In a GLP compliant prenatal developmental toxicity study conducted according to ICH guidelines for the detection of reproductive and developmental toxicities for medicinal products, female Crj:CD(SD)IGS rats (20–22/dose) were administered the chemical in corn oil by subcutaneous injection once daily at 0, 50, 100 or 200 mg/kg bw/day from GD6–17. Dams were sacrificed on GD 20 and foetuses examined (ECHA 2018).

Significantly lower body weights, reduced body weight gain and reduced food consumption were observed in the 200 mg/kg bw/day group compared to the control. The NOAEL was 100 mg/kg bw/day for general toxicity based on these observations.

In all dose groups, there were no significant differences in the:

- numbers of corporea lutea, implants, pre-implant loss, early or late resorptions, live or dead foetuses
- sex ratio of live foetuses
- number of placental abnormalities in live foetuses.
- numbers and type of skeletal and visceral abnormalities in live foetuses.

Foetuses from the 200 mg/kg bw/day group had significantly lower mean body weights (22% decrease) when compared to the control groups. There were external abnormalities in 1 (0.4%) and 9 (3.2%) foetuses in the control and 200 mg/kg bw/day groups respectively, but the difference was not statistically significant. The abnormalities most frequently presented as neural tube defects (craniorachischisis), which were observed in 8 (3%) foetuses in the high dose group. These defects were not observed in any other dose group and typically occur in only 0.01% of foetuses from SD rats, based on historical controls. Therefore, it is likely that these abnormalities are treatment related, even if not statistically significant.

There were 14 and 97 skeletal variations reported in the control and 200 mg/kg bw/day groups respectively, which was a statistically significant increase. The skeletal variations that had significantly increased incidences in the high dose group compared to the control were in the: short supernumerary rib (33% of foetuses), full supernumerary rib (46% of foetuses), splitting of the thoracic (33% of foetuses) and lumbar (12% of foetuses) vertebral bodies, 7 lumbar vertebrae (33% of foetuses) and incomplete ossification of the thoracic centrum (8% of foetuses). A significantly delayed progress of ossification of the cervical, thoracic, lumbar and sacrocaudal vertebrae, the sternebrae, metacarpus, metatarsus and phalanges were observed in the high dose group compared to the control. The NOAEL was 100 mg/kg bw/day for developmental toxicity.

Prenatal developmental study in rabbits

In a GLP compliant prenatal developmental study conducted according to ICH guidelines, pregnant NZW rabbits (20–22/dose) were administered the chemical in corn oil by

subcutaneous injection once daily at doses of 0, 30, 100 or 300 mg/kg bw/day from GD 6–18. Dams were sacrificed on GD 29 and foetuses examined (ECHA 2018).

Clinical observations of toxicity included crust at the administration site and hair loss in one dam from the 100 mg/kg bw/day and vaginal haemorrhaging, blanching, pale eyes, decreased body weight and low food consumption in one dam from the 300 mg/kg bw/day group. There was a significant increase in food consumption in the 30 and 100 mg/kg bw/day groups but was not dose related. There was no significant difference in body weight between the control and dose groups but a slight depression (not statistically significant) in body weight in the 300 mg/kg bw/day group when compared to the control. The maternal NOAEL was 100 mg/kg bw/day for general toxicity based on the slight depression in body weight in the high dose group.

In all dose groups, there were no significant differences in the:

- numbers of corporea lutea, implants, early or late resorptions, live or dead foetuses
- average foetal body weights
- number of external or placental abnormalities in live foetuses
- numbers or types of skeletal variations or abnormalities in live foetuses.

A significant decrease in the pre-implantation loss index was observed in the 30 mg/kg bw/day group but was not dose related. A significant change in the sex ratio (44% increase in males compared to control) of live foetuses was noted in the 300 mg/kg bw/day group. However, sex determination is typically determined before GD 6 and therefore, is not treatment related. Incidences of skeletal abnormalities were observed in all dose groups but were not considered to be treatment related. The NOAEL for development was 300 mg/kg bw/day.

Prenatal developmental study in hamsters

In a non-guideline developmental toxicity study, pregnant LVG hamsters received a single dose of the chemical on GD 7 either by oral gavage at 1750 mg/kg or topical application for 2 hours at 3500 and 5200 mg/kg. Most embryos were recovered and examined on GD 9. The remaining embryos continued to develop but did not typically survive past GD 12 (Overman and White 1983).

In GD 9 embryos from hamsters treated orally with the chemical, incidences of neural tube defects were 11% and 72% in the control and dosed groups, respectively. For those exposed topically, incidences were 0%, 6% and 53% for the control, 3500 mg/kg and 5200 mg/kg groups, respectively. Defects commonly involved both the cranium and spine, exhibited by the failure of the closure of the midbrain region of the skull. Characteristics of the defects from oral and topical groups were independent of treatment type. These neural tube defects are similar to those reported in rat studies with oral or subcutaneous injection of the chemical or the metabolite salicylic acid.

Analysis of blood samples post-treatment indicated that plasma salicylate levels were consistently higher from oral treatment. Blood samples from foetuses (survived past GD 9) demonstrated that a fraction of the oral dose of the chemical reaches the foetus within the first 2.5 hours of treatment (see **Toxicokinetics**). Taken together, it is likely the neural tube defects in this study are treatment related.

Other studies

There are numerous other studies using the chemical of lower reliability. Results of these studies are generally conflicting, with some studies demonstrating similar adverse effects to those above, and some showing no specific effects on development.
(CIR 2019; ECHA 2018; Lapczynski et al. 2007).

Studies with salicylic acid and analogues

The adverse effects on development caused by the chemical are supported by the experimental evidence of similar effects caused by the metabolite salicylic acid. The key supporting evidence from this report is summarised below. For full study details, see the related AICIS Evaluation Statement on salicylic acid and its salts (AICIS 2024).

In 2 prenatal developmental toxicity studies, pregnant Wistar rats were orally exposed to salicylic acid daily at doses between 50 and 300 mg/kg bw/day on GD 8–14. The NOAELs for maternal toxicity were 150 and 165 mg/kg bw/day based on salivation and piloerection. The reported adverse effects on development were high foetal mortality, high frequency of anomalies including neural tube defects (craniorachischisis) and foetal growth retardation. The NOAELs for development were 75 and 77.4 mg/kg bw/day. Studies with the structurally similar chemical, sodium salicylate resulted in similar effects with a NOAEL of 90 mg/kg bw/day.

Acetylsalicylic acid also metabolises to salicylic acid in vivo. Increased incidences of abnormalities and neutral tube defects have been reported in guideline and non-guideline developmental toxicity studies with acetylsalicylic acid in rats and monkeys. For full study details, see the related AICIS Evaluation Statement on salicylic acid (AICIS 2024).

Observation in humans

No human data are available for this chemical. Extensive data are available for acetylsalicylic acid as aspirin which shares a metabolite with the chemical. Aspirin is a widely used medicine and has been used for a long time. Most data indicate that low doses of aspirin do not increase risk of adverse effects on pregnancy. Although some adverse effects such as maternal bleeding and changes in pregnancy duration and labour have been reported no malformations were identified at any dose. The difference in the dose range between the animal studies and the human epidemiology studies is very high (ECHA 2018).

Endocrine effects

The only identified studies for the chemical relate to the estrogenic pathway. No information on the androgen, thyroid and steroidogenesis (EATS) pathways has been identified.

In an in vitro ligand-dependent coactivator recruiting assay with glutathione-S-transferase-tagged hER-alpha-LBD assay, the chemical showed no oestrogenicity. The activity was negligible, so a maximal acceptable daily exposure value was not calculated (SCCS 2021).

In an in vivo mouse uterotrophic assay conducted according to OECD TG 440, female CD-1 mice exposed to up to 300 mg/kg bw/day of the chemical did not have any significant differences in uterine weights. No mortality was noted and a slight reduction in body weight compared to the controls in higher dose groups (SCCS 2021).

There are indications from the literature that salicylates may have endocrine modulating properties. The current available data does not provide sufficient evidence of an adverse

effect of the metabolite salicylic acid from an endocrine mode of action. For further study details, see the related AICIS Evaluation Statement on salicylic acid and its salts (AICIS 2024).

Human health risk characterisation

Critical health effects

The critical health effects for risk characterisation are systemic effects (developmental toxicity). While the NOAEL for developmental toxicity varies significantly in the experimental data, an NOAEL of 75 mg/kg bw/day is selected for risk characterisation based on both the 3 generation study with the chemical and the prenatal studies with salicylic acid (Government of Canada 2020, SCCS 2021).

Public risk

The MoS (also referred to as margin of exposure or MOE) methodology is commonly used to characterise risks to human health associated with exposure to chemicals (ECB 2003).

The MoS risk estimate provides a measure of the likelihood that a particular adverse health effect will occur under the conditions of exposure. As the MoS increases, the risk of potential adverse effects decreases. To decide whether the MoS is of sufficient magnitude, expert judgment is required. Such judgments are usually made on a case-by-case basis and should consider uncertainties arising in the risk assessment process such as the completeness and quality of available data, the nature and severity of effect(s) and intra/inter species variability. In general, an MoS value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences.

The critical health effect for the chemical is developmental toxicity. The SCCS recently reassessed the safety of the chemical in cosmetic products in adults and children. The SCCS used the aggregate daily systemic exposure of 0.52 mg/kg bw/day in MoS calculations (see **Human exposure - Public**). The calculated MoS was 145. The SCCS concluded that based on their safety assessment of all available information, the use of the chemical is safe under the existing EU restrictions on concentrations. The SCCS noted the potential additional exposure to the chemical through use of wintergreen oil in cosmetic products (SCCS 2021).

In children, the aggregate daily systemic exposures used were 0.0347, 0.463 and 0.454 mg/kg bw/day for children aged 0.5–1 years, 1–3 years and 3–6 years, respectively. This gave calculated MoS of 2161, 162 and 165 for children aged 0.5–1 years, 1–3 years and 3–6 years respectively. Based on their safety assessment of all available information, the SCCS concluded that the chemical is safe for use in children under 6 years of age at these levels of use (SCCS 2023).

Australian use patterns for the various product categories are assumed to be similar to those in Europe. Therefore, the calculated MoS indicating that the chemical is safe for use at international exposure levels indicates that current use levels in Australia are unlikely to pose a risk to the public.

The presence of the chemical in household products is not expected to significantly change exposure (see **Human exposure – Public**) or risk estimates. The MoS for a drop air freshener product with the highest reported concentrations of the chemical was 14,000. Other incidental exposures to household products are expected to have much larger MoS and therefore, do not pose a significant risk to the public.

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