Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Hexyl salicylate

EC Number: 228-408-6
CAS Number: 6259-76-3

CLH-O-0000007103-85-01/F

Adopted

18 March 2022

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18 March 2022

CLH-O-0000007103-85-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Hexyl salicylate**

EC Number: **228-408-6**

CAS Number: **6259-76-3**

The proposal was submitted by **France** and received by RAC on **7 December 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **8 February 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **9 April 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2022** by **consensus.**

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Hexyl salicylate	228-408-6	6259-76-3	Skin Sens. 1 Repr. 2	H317 H361d	GHS07 GHS08 Wng	H317 H361d			
RAC opinion	TBD	Hexyl salicylate	228-408-6	6259-76-3	Skin Sens. 1 Repr. 2	H317 H361d	GHS07 GHS08 Wng	H317 H361d			
Resulting Annex VI entry if agreed by COM	TBD	Hexyl salicylate	228-408-6	6259-76-3	Skin Sens. 1 Repr. 2	H317 H361d	GHS07 GHS08 Wng	H317 H361d			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Hexyl salicylate is used as a fragrance ingredient in a wide range of products, including household cleaners, cosmetics, and personal care products. It does not have an entry in Annex VI to the CLP Regulation. It has been assessed in the framework of CoRAP by the Dutch competent authority that identified a need to classify hexyl salicylate as reproductive toxicant and skin sensitiser.

The Dossier Submitter (DS) initially proposed a read-across approach for the reproductive toxicity endpoint using salicylic acid (SA), sodium salicylate (NaS), and methyl salicylate (MeS) as source substances since no studies with hexyl salicylate are available for this endpoint. According to the DS, this read-across approach is adequate based on the assumption that hexyl salicylate like NaS and MeS are likewise metabolised to SA. The DS based this on one *in vitro* study dealing with the absorption and metabolism after application of hexyl salicylate on human skin explants.

During consultation on the dossier, one MSCA questioned the proposed read-across since no experimental data were available from other tissues than skin and no conclusion could be drawn on the metabolism of hexyl salicylate in other organs, e.g. in the liver. They also pointed out that hexyl salicylate and MeS differ considerably in their physico-chemical properties. Additionally, an industry comment on behalf of the registrants for hexyl salicylate requested clarification why data for two other possible read-across candidates, i.e. benzyl salicylate (BzS) and cyclohexyl salicylate (CHS), were not considered in the CLH report.

A targeted consultation was launched to gather additional information.

All additional information provided are included in this document in the Reproductive Toxicity section.

Available ADME data

Dermal absorption of salicylates

Based on physico-chemical properties Watkinson *et al.* (1992, *apud* Belsito *et al.* 2007) calculated dermal bioavailability of about 2.3 % for MeS (MS). Much lower dermal absorption rates were calculated for butyl salicylate (BtS) (0.068 %), pentyl salicylate (PtS) (0.017 %), hexyl salicylate (HS) (0.005 %), and ethyl hexyl salicylate (EHS) (0.0006 %).

Ethyl Hexyl salicylate

Bury *et al.* (2019) calculated that 3.0 % (mean) of a dermally applied EHS dose was absorbed from a sun screen product (based on data for specific metabolites, see below). In an *in vitro* dermal bioavailability study using human excised and skin samples from six donors, 1.82 ± 1.5 % of the topically applied dose of 1 % EHS in body lotion were recovered in the receptor fluid and the epidermis and dermis layers of the skin after 24 hours (data from an unpublished study cited in Bury *et al.* 2019). These data indicate a higher absorption rate than was calculated based on physico-chemical properties. This might be explained by the fact that test substances were applied in form of skin care products (sun screen and body lotion) that could facilitate skin penetration.

Hexyl salicylate

One *in vitro* dermal absorption and metabolism study in human skin explants is available. In the absorption part of the study, radio-labelled hexyl salicylate was applied to breast or abdomen split-thickness skin explants from four female donors at concentrations of 0.1, 20, or 100 %.

Most of the applied radioactivity was washed off after 8 hours (exposure termination). After 24 hours, very small amounts of hexyl salicylate of up to 1 % were detected in the receptor fluid. In a separate metabolism phase, 0.1 % of ^{14}C-radiolabelled hexyl salicylate in dipropylene glycol was applied to breast or abdomen skin membranes (n=3) from two female donors using static diffusion cells and tissue culture medium as receptor fluid. Analysis of the receptor fluid showed an absence of hexyl salicylate, but identified SA as the major component (92.8-97.8 %, similar in both donors), indicating metabolism of hexyl salicylate by dermal esterases. Analysis of skin extracts showed variable amounts of SA and hexyl salicylate between the two donors: While for one donor, SA accounted for 86.6 to 89.3 % and hexyl salicylate for 5.7 to 10.7 % of compounds found, in the other donor percentages where 59.4 to 77.9 % and 20 to 37.4 % for SA and hexyl salicylate, respectively.

Interestingly, the DS noted one important limitation for this study that was performed according to OECD TG 428: The results for relevant reference chemicals were not made available to demonstrate the performance and reliability of the test system in the performing laboratory.

2-ethylhexyl salicylate metabolism in humans

Bury and coworkers aimed to identify specific urinary metabolites of EHS as biomarkers of oral exposure in humans (Bury *et al.* 2019). They did not use a radio-labelled compound but concentrated on characterization and measurement of metabolites that can be unequivocally attributed to EHS exposure (2-ethyl-5-hydroxyhexyl 2-hydroxybenzoate (5OH-EHS), 2-ethyl-5-oxohexyl 2-hydroxybenzoate (5oxo-EHS), and 5-(((2-hydroxybenzoyl)oxy) methyl)heptanoic acid (5cx-EPS)). In the course of their study, they found SA and salicyluric acid (SUA), another metabolite downstream of the SA metabolic pathway, in urine samples of orally exposed individuals. No numeric data are available in the publication. For SUA, concentrations were described as "rather high". However, the authors acknowledged that apart from EHS, other salicylic acid esters, that are frequently used as fragrance ingredients, and acetyl salicylic acid used as analgesic drug can also be expected to be metabolised extensively to SA. Thus, the source of neither SA nor SUA was identified in this study but can be assumed to be partially related to EHS.

Cyclohexyl and benzyl salicylates

No experimental toxicokinetic data for cyclohexyl and benzyl salicylates were provided with the REACH registration dossiers for these substances. Additional data were not provided by industry. Therefore, RAC decided to exclude these substances from the read-across approach.

Esterases

In an extensive review on human esterases, Lockridge and Quinn (2010) reported that in humans, liver carboxylesterase (CES1) and the carboxylesterase in the small intestine (CES2) have different substrate specificities. While CES1 preferentially hydrolyzes esters with a small alcohol group and a large acyl group; CES2 preferentially hydrolyzes esters with a large alcohol group. In that review, they provided cocaine and the hydrolysis of its methyl ester bond as example for the former and cocaine benzoyl ester as example for the latter. Thus, it can be assumed that similarly salicylate esters with various alcohol moieties might be metabolised by one (or both) of these enzymes.

Furthermore, differences in esterase distribution have been reported between humans and rodents with rodents expressing carboxyl esterases in their blood while humans do not (Li *et al.* 2005 *apud* Lockridge and Quinn 2010).

These differences in the site of metabolism for different salicylates together with inter-species differences in carboxyl esterases expression should be considered in the proposed read-across.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification for all physical hazards, based on test results and the results of the screening procedure relevant for each hazard class.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Hexyl salicylate is a liquid, therefore hazard classes for gases and solids do not apply.

Hexyl salicylate does not contain any molecular structures associated with explosive properties, self-reactive properties and no peroxide or acidic moieties. Thus, it does not fulfil screening criteria for explosives, self-reactive substances, organic peroxides, and corrosive to metals.

The substance has a flash point of 151°C at 1013 hPa, therefore it does not fulfil the criteria for classification as flammable liquid.

Based on handling and manufacturing experience, hexyl salicylate is not a pyrophoric liquid, does not emit flammable gases upon contact with water.

Hexyl salicylate contains only oxygen atoms bound to hydrogens or carbons, thus it doesn't have oxidising properties.

Thus, RAC agrees with the assessment of the DS on the physical hazards and proposes **no classification**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitising property of hexyl salicylate was investigated in four animal studies, including two standard test methods, an local lymph node assay (LLNA) and a guinea pig maximisation test (GPMT). The LLNA was the only test of high quality, conducted according to OECD TG 429 which led to clearly positive results. Overall, hexyl salicylate was positive at concentrations above 0.25% in the LLNA, the only animal study of good quality available, with an EC3 = 0.18%, indicating a strong potency of sensitisation.

There is also data available in two human volunteer induction studies, one human repeated insult patch test (HRIPT) and one Human maximisation (HMT) and two diagnostic studies (in selected and unselected patients). However, in humans, hexyl salicylate does not seem to induce skin sensitisation based on the data available.

The DS proposed a classification as Skin Sens. 1.

Comments received during consultation

No comments were received on this hazard class during consultation.

Assessment and comparison with the classification criteria

Animal data

There are four studies available to assess skin sensitisation property of hexyl salicylate in a LLNA, a modified Draize test, a maximisation assay and in a photoallergy study. The studies are listed in the table below.

***Table**: Summary table of animal studies on skin sensitisation*

Method, guideline, deviations	**Species, strain, sex, no./group**	**Concentrations, exposure duration**	**Results**	**Reference**
LLNA equivalent or similar to OECD Guideline 429 GLP compliant	Mouse (CBA), female, 4/group	1, 2.5, 5, 10, 25% w/v (experiment 1) 0.05, 0.25, 0.5, 1, 2.5% w/v (experiment 2) Vehicle used: 1:3 ethanol:diethylphthalate Daily for 3 consecutive days	**Positive** Stimulation index (relative to vehicle control): First experiment: > 3 at all concentrations Second experiment: 0.05%: 1.87 0.25%: 3.56 0.5%: 5.60 1%: 10.83 2.5%: 10.80 EC3 = 0.18%	Unnamed (2006) Cited in Scientific Committee on Consumer Safety (SCCS) Opinion on Fragrance allergens in cosmetic products (2011)
Modified Draize test Induction: 4 intradermal injections (0.1 mL at 0.25%) First challenge: intradermal injection 14 days later (0.1 mL at 0.1%) and topical application (0.1 mL at 5%) Second challenge conducted 7 days later Secondary literature Limitations: vehicle not specified	Inbred Hartley albino guinea pigs 4 or 6 of each sex, 10 total	0.25% for intradermal induction 0.1% and 5% for challenge (vehicle not reported)	**Positive** Sensitisation reactions observed after the second challenge at 5%	Sharp (1978) Cited in Lapczynski *et al.* (2007)

Maximisation assay Intradermal induction: 6 injections (2 x 0.1 mL injections of 1% HS in 0.01% DOBS/saline, 2 x 0.1 mL injections of 1% HS in 50% Complete Freund's Adjuvant and 2 x 0.1 mL injections of 50% Complete Freund's Adjuvant Topical induction 7 days later: 40% HS in acetone (48h occluded patch) Topical challenge 13-14 days later: 10% HS in acetone (24h occluded patch) Similar to OECD 406 Limitation: low number of animals, tested concentrations not justified	Dunkin/ Hartley albino guinea pigs, 10 total	1% in 0.01% DOBS/saline and 1% in 50% Complete Freund's Adjuvant for intradermal induction 40% in acetone for topical induction 10% in acetone for challenge	Negative	cited in Lapczynski *et al.* (2007)
Sensitisation evaluated as part of a photoallergy study Intradermal induction: injection of 0.1 mL of a formulation of sterile water and Freund's complete adjuvant (1:1 v/v) Topical induction: 0.3 mL of 100% HS in 3:1 DEP:ethanol applied to 25 mm Hilltop Chambers® and then to the dorsal skin of animals (occluded patch for 2h) Followed by UVR exposure using a 6.5 kW long-arc xenon water-cooled lamp with a filter used to attenuate mid-range UVB. Delivered dose: 2.25 Minimal Erythema Doses (MED) (~2.25h). Procedure repeated once daily on days 3, 5, 8, 10 and 12 of the induction phase Topical challenge on day 22: 50% HS in 3:1 DEP:EtOH and 100% HS Observations 1, 4h later and 1, 2, 3 days later.	Male albino hairless guinea pigs (5/group)	100% for topical induction 50 and 100% HS in 3:1 DEP:ethanol for topical challenge	Negative	cited in Lapczynski *et al.* (2007)

All four studies used hexyl salicylate as testing substance, whereas only in the LLNA the purity was stated to be 98.5%. Hexyl salicylate was tested diluted in various solvents (1:3 ethanol:diethylphthalate, acetone or petrolatum) and the concentrations tested ranged from 0.05 to 100%. The LLNA is considered the key study since it is in compliance with OECD TG 429. In this test, hexyl salicylate gave a positive result with a clear dose response from the lowest concentration tested (0.05%). The test was performed twice and the stimulation index was >3 in both experiments performed from 0.25% to 25% hexyl salicylate in 1:3 ethanol:diethylphthalate, leading to an EC3 of 0.18%.

In a maximisation test and a photoallergy study evaluating sensitisation hexyl salicylate was negative. However, although the maximisation assay was performed similar to OECD 406 the number of animals tested was too low, with 10 animals used instead of at least 20 animals recommended. In addition, there is no justification for the concentrations used.

Limitations of the low number of animals also apply to the photoallergy study, where 5 animals per group were used.

In a modified Draize test, 5% hexyl salicylate induced sensitising reactions in Hartley albino guinea pigs after a second challenge. The lack of specification of the vehicle used was a limitation in this test.

In a genomic allergen rapid detection (GARD) assay utilising an *in vitro* model of dendritic cells, hexyl salicylate was predicted to be a skin sensitiser (Forreryda *et al.* (2018), cited in the Cosmetic Ingredient Review on salicylic acid and salicylates (2019)).

Human data

Two human volunteer induction studies and two diagnostic studies where available as listed in the table below.

***Table**: Summary table of human data on skin sensitisation*

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Induction studies				
Human repeated insult patch test (HRIPT) with 103 volunteers (29 male and 74 female)	30% hexyl salicylate in 3:1DEP:EtOH	Nine induction applications, 3 per week over a 3-week period After 2 weeks rest period, single application challenge test. Reactions were scored at 24h after challenge.	0/103 positive reactions Induction phase: 3 subjects with equivocal transient reactions After challenge: 2 subjects with equivocal transient responses	RIFM (2004a) Cited in Lapczynski *et al.* (2007)
Human maximization test (HMT) with	3% hexyl salicylate probably	Applications of 3% hexyl salicylate in petrolatum under occlusion for 5 alternate-day 48h	Initial positive "equivocal" reactions after challenge	RIFM (1975b) Cited in Lapczynski *et al.* (2007)

22 selected volunteers	formulated in petrolatum	periods after pretreatment of patch site for 24h with 5% aqueous SLS under occlusion. After 10-14 days rest period, 2% SLS was applied under occlusion for 30 min on the left side of the back prior to challenge patch of hexyl salicylate under occlusion for 48h on the right side.	Subjects are re-tested later. No positive evidence of sensitisation was observed.	
Diagnostic studies				
Patch test in 218 fragrance sensitive patients with contact dermatitis (selected patients)	5% hexyl salicylate in petrolatum	Various fragrance materials including hexyl salicylate	0% positive reactions	Larsen *et al.* (2002) Cited in Lapczynski *et al.* (2007) and in SCCS Opinion on Fragrance allergens in cosmetic products (2011)
Patch test in ~100 patients with dermatitis (unselected patients)	5%, 7.5%, 11.3%, 16.9%, 25.3% hexyl salicylate	Test material suspended in pet. was applied to the upper back in Finn Chambers under occlusion for 2 days. Patch test readings performed on day (D) 2, D3, D4, D5 and D7	0% positive reactions in all test concentrations 5%: 2/100 "doubtful" reactions 16.9%: 1/100 "doubtful" reactions 25.3%: 1/87 "doubtful" reactions	Bennike *et al.* (2019)

In a HRIPT, no signs of sensitisation of hexyl salicylate (30%) were reported on 103 volunteers. Three subjects showed equivocal transient reactions during the induction phase, which might be linked to irritation, as the test substance was repeatedly applied to the same site. Two further subjects had equivocal transient responses after challenge. However, it is not clear if they were the same as during the induction phase. Due to these reactions, a second reading was performed after 48h, followed by a re-challenge 3 weeks later. Benzyl salicylate was used as negative control in this study whereas this substance was recently proposed by RAC for Skin Sens. 1B under the CLP regulation. This information may question the negative result of this study. In addition, the number of tested volunteers was low (103) in comparison with the recommendations of the Scientific Committee on Consumer Safety (SCCS) (150-200 volunteers). Although the equivocal reactions after challenge may be linked to irritation, the reported data do not allow to rule out an allergic reaction. Considering these limitations, this HRIPT is not considered reliable.

A maximisation assay performed on 22 selected volunteers gave negative results with positive equivocal reactions being observed after the challenge phase but not after a re-challenge. The test was performed on 22 volunteers instead of 25, but it was overall in compliance with the

method. However, the use of sodium lauryl sulphate as adjuvant in order to maximise the reaction increases the risk of sensitising reactions.

In a diagnostic study Larsen *et al.* included 218 selected fragrance sensitive patients with contact dermatitis. It aimed at identifying new sensitising substances to screen on patients with suspect fragrance allergy. The 218 patients were exposed to a fragrance mixture (FM) and several individual fragrance materials including hexyl salicylate. The FM did not contain hexyl salicylate. This mixture induced positive reactions in 76% of the subjects. The patch test following the exposure to 5% hexyl salicylate appeared negative.

Bennike *et al.* investigated hexyl salicylate on unselected patients with dermatitis. As the substance is used in consumer products, exposure is commonly occurring. The substance was tested in concentrations from 5 to 25% on approximately 100 patients with dermatitis per concentration group. Some patients showed doubtful reactions at first reading but these reactions were not confirmed at second reading. According to the authors, no positive patch test reaction occurred up to a concentration of 25% and the maximum tolerated concentration for most of the patients was 12.5%.

There are no case reports in the literature for patients with dermatitis after the use of a product containing hexyl salicylate.

Skin sensitising vs skin irritating reactions

Contradictory results were found in both animal and human studies. In animals, positive effects were reported in one LLNA. The results of the LLNA suggested that hexyl salicylate would be a strong sensitiser as the EC3 is clearly below 2%. In addition, hexyl salicylate was predicted to be a skin sensitiser in a GARD assay but data from other studies (maximisation assay and photoallergy study) showed negative results. In humans, studies were all considered negative, despite some methodological deficiencies (in particular in HRIPT). Special caution has to be paid to differentiate if the positive results are linked to irritating or sensitising effects of hexyl salicylate.

Some studies published in the open literature indicated that the positive result of the LLNA was considered false positive because hexyl salicylate was non-sensitising up to 30 % in a human HRIPT (Roberts *et al.* 2015a & b). This argument should be disregarded as the reliability of this HRIPT is questionable and negative human data cannot normally be used to negate positive results from animal studies according to the CLP Regulation. Another study explained the positive result of the LLNA by mentioning that the very low EC3 (0.18%) might be due to irritating properties of hexyl salicylate or potential sensitising impurities (Urbisch *et al.* 2015).

Contradictory results were found in literature regarding irritating properties of hexyl salicylate (Lapczynski *et al.* 2007, Belsito *et al.* 2007). However, it can be noted that irritation was only observed for high concentrations of hexyl salicylate: at least 25% but rather with concentrations above 50%. These concentrations are clearly above the concentrations for which skin sensitisation was observed in the LLNA.

Moderate skin irritation was also reported in an OECD Guideline 404 study available in the registration dossier (Haynes, 1986). In this study, female rabbits were exposed to 50% and 100% hexyl salicylate in DEP for 4 hours under semi-occlusive conditions. At 50% hexyl salicylate, the mean erythema and oedema scores were respectively 2.0 and 1.4. The observed effects were fully reversible within 7 days. For the undiluted substance, the mean scores for erythema and oedema over the 24-72 hour period were respectively 2.0 and 2.16. In this case, it was reported that one rabbit showed remaining erythema and oedema after 7 days. Nevertheless, these effects concerned only one animal and no information was available until 14 days, which is the normal observation period recommended by OECD Guideline 404. Overall, the results of the study could not trigger a classification for skin irritation according to the CLP criteria.

Regarding the argument of potential sensitising impurities, the purity of hexyl salicylate in the LLNA is > 98% and there are no impurities in amount exceeding 1% based on registration data. Besides, no impurities that would impact the classification of hexyl salicylate were identified.

There is no indication of irritating effects of hexyl salicylate in humans.

Overall, there is no sufficient information to discount the effects reported in the LLNA. Thus, the reported positive reactions should be considered as sensitising effects.

Conclusion

With EC3 values $\leqslant$ 2% in the LLNA, hexyl salicylate fulfils criteria for classification Skin Sens. 1A according to the CLP guidance. Regarding human data, the HRIPT cannot be used for the purpose of classification due to its low reliability. Nevertheless, the maximisation assay and both diagnostic studies were negative and were considered reliable.

There are several possible reasons for the absence of sensitising reactions in these studies:

- The patch test for hexyl salicylate is not marketed. In fact, 46 fragrances are marketed by Chemotechnique for patch testing, but hexyl salicylate is not part of the list. Hexyl salicylate was therefore only tested for prospecting purposes. This could explain why only 2 diagnostic studies with different concentrations of this substance have been published.
- Hexyl salicylate is not included in the list of 26 sensitising fragrances for humans that require labelling. Therefore, it would be difficult to determine whether hexyl salicylate is responsible for contact dermatitis following exposure to a fragrance.
- Although this substance is widely used in perfumes, the concentrations used are low. In leave-on products for face and body, the concentrations are between 0.02 and 0.03 % and between 0.08 and 0.12 %, respectively. The highest concentrations are used in rinse-off products, reaching 0.52 % in soaps and cleansers (Cosmetic Ingredient Review on salicylic acid and salicylates (2018)). These concentrations are below the concentration limits recommended by the International Fragrance Association (IFRA).

Therefore, the absence of sensitising reactions observed in humans could be due to primary prevention related to these concentration limits, more than the absence of sensitising properties.

Due to the significant discrepancies between positive animal data and negative human studies, sub-categorisation does not seem appropriate according to the CLP-guidance.

With the positive results of the LLNA of good quality, Category 1A would be justified. However, since data are not sufficient for sub-categorisation, RAC agrees to the DS that hexyl salicylate should be classified Skin Sens. 1 – H317.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's initial proposal

There are no fertility or developmental studies available for hexyl salicylate. Therefore, the assessment of reproductive toxicity has been based on read-across data from animal studies on MeS for fertility as well as SA, NaS and MeS for developmental toxicity (see Annex II of the CLH dossier for rationale). According to the DS, the read-across approach is considered adequate since NaS, MeS and hexyl salicylate metabolise to form SA.

They summarised the following studies for effects on fertility:

Method, guideline, deviations, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
Study of fertility and early embryonic development to implantation Crj:CD(SD)IGS rats male/female Subcutaneous administration GLP and ICH guidelines	MeS (purity: 100.1%) 0, 30, 100, 300 mg/kg bw/d in corn oil From 2 weeks prior to mating until sacrifice (total of 52 days) for males and until gestation day 6 for females (total of 30 days). Sacrifice of females on GD13.	NOAEL for general toxicity: 100 mg/kg bw/d based on one mortality in males, decreased body weight gain and food consumption at 300 mg/kg bw/d. NOAEL for fertility: 300 mg/kg bw/d (no effect). Increased plasmatic SA concentration dependent on the dose ratio but scarcely affected by repeated dosing. No clear sexual difference.	FDA (2006a) Klimisch score: 1 Key study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Two-generation study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 4 (offspring assessment) of the NTP continuous breeding protocol Limited examination NTP protocol, GLP	MeS (purity ≥ 99%) 0, 25, 50 and 100 mg/kg bw/d (nominal conc.) Exposure: 7 days prior to mating, during 98 days of cohabitation (allowing the production of about 4 litters) and then during a separation period of 21 days during which final litters were delivered (task 2). A second generation was then produced only for the highest dose group (task 4): the mothers were dosed through weaning and F1 mice were dosed until mated at about 74 days of age.	NOAEL (reproductive effects): 100 mg/kg bw/d – no adverse effect	NTP (1984a) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Lamb *et al.*, (1997) Klimisch score: 2 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
One generation study + crossover mating study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 3 (crossover mating) of	MeS (purity ≥ 99%) 100, 250 and 500 mg/kg bw/d (nominal conc.) Exposure: 7 days prior to mating, during 98 days of cohabitation (allowing the production of about 4 litters) and then during a separation period of 21 days during which final litters were delivered (task 2). Task 3: high-dose animals of each sex were	500 mg/kg bw/d – no effect on fertility index Task 3: due to fertility problem in the control groups (26% in the first task 3 and 41% in the second task 3) and lack of significant results in the litter analysis, an affected sex cannot be determined.	NTP (1984b) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Klimisch score: 2 Supporting study (See Annex I of the BD for more details on the results)

the NTP continuous breeding protocol Limited examination NTP protocol, GLP	mated to control mice of the opposite sex.		(See Annex II of the BD for justification of read-across)
Three-generation study Rat (Osborne-Mendel); male/female (20/sex/dose) Oral: feed (no vehicle) A supplementary study was performed with adding calcium carbonate to MeS diet with the same examination. Examination very limited Several deficiencies from OECD 416, not GLP	MeS 0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw/d as MeS) (nominal in diet) Exposure: 100 days before the first mating and then throughout the experiment (until weaning of the 3rd generation).	NOAEL (fertility): 250 mg/kg bw/d (male/female) based on no statistically significant effect reported. The results after addition of calcium carbonate did not markedly differ from those obtained after administration of MeS alone.	Collins TFX *et al.* (1971) Gross MA, Fitzhugh OG (1977) Klimisch score: 3 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Two-generation study Rat (Wistar) male/female 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	MeS 0.25% and 0.5% (2500 ppm and 5000 ppm equivalent to 125 and 250 mg/kg bw/d MeS/day) (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (weaning of the F2b litters).	No adequate NOAEL can be set based on the low quality of the reported results. Decreased litter size at all doses. Higher number of unsuccessful matings for the first generation and decreased reproduction index for both generations at the highest dose. Higher number of death between birth and day 5 at 250 mg/kg bw/d.	Anonymous (1978a) Klimisch score: 3 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Two-generation study Mouse male/female (no data on strain); 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	MeS 0.25% and 0.5% (2500 ppm and 5000 ppm, equivalent to 375 and 750 mg/kg bw/d) (nominal in diet) Exposure: 30 days before the first mating and then throughout the experiment (weaning of the pups).	No adequate NOAEL can be set based on the low quality of the reported results. Litter size slightly smaller in test groups only in the first generation.	Anonymous (1978b) Klimisch score: 3 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
One-generation study Rat (Sprague-Dawley); male/female; 24-27 animals/dose	MeS 4000 ppm and 6000 ppm equivalent to 200 and	NOAEL (F1): 300 mg/kg bw/d (male/female) based on no effect	FDA (1966) CIR (2003) Klimisch score: 4

Oral: feed (no vehicle) Guideline and GLP not stated – secondary literature	300 mg/kg bw/d (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (until weaning of offspring on day 20-21)	No abnormalities. Neonate survival at weaning was higher in the test group than in control.	Disregarded study (See Annex II of the BD for justification of read-across)

For developmental toxicity, the DS summarised the following studies:

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Data on salicylic acid			
Prenatal developmental assay (GD8-14) Rat (Wistar) (female) oral: in the diet equivalent or similar to OECD Guideline 414	SA 0.06, 0.1, 0.2, 0.4% (corresponding to 50.7 +/- 0.6, 77.4 +/- 1.0, 165 +/- 2.1, 205.9 +/- 18.9 mg/kg bw/d) Exposure: day 8 to 14 (daily)	NOAEL (maternal toxicity): 165 mg/kg bw/d NOAEL (developmental toxicity): 77.4 mg/kg bw/d	Tanaka S *et al.* (1973a) Klimisch score: 2 (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Prenatal developmental assay (GD 8-14) Rat (Wistar) (female) oral: gavage equivalent or similar to OECD Guideline 414	SA 75, 150, 300 mg/kg bw/d in CMC (carboxymethyl cellulose) Exposure: day 8 to 14 (daily)	NOAEL (maternal toxicity): 150 mg/kg bw/d NOAEL (developmental toxicity): 75 mg/kg bw/d	Tanaka S *et al.* (1973b) Klimisch score: 2 (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Rat (Sprague-Dawley) (17 female) subcutaneous no guideline followed Limitation: not GLP compliant	SA 380 mg/kg (nominal conc.) Vehicle: water Exposure: 2 SA administrations at 2 hr interval, on day 9, followed by mineral isotopes administration on day 9 or 16 of pregnancy Urinary excretion and foetal uptake of the mineral isotopes were measured and the foetuses were removed and inspected noting death, resorption, as well as external congenital malformations (on day 20 of gestation).	No NOAEL identified Marked maternal body weight loss, loss of appetite, complete relaxation, weakness, drowsiness, muscular limpness, inactivity, accelerated respiration rate, and occasionally elevated water intake and urinary excretion High incidence of foetal malformations and resorption, abnormally small foetuses	Koshakji and Schulert (1973) Klimisch score: 3 (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)

Data on Sodium Salicylate			
Prenatal developmental assay (GD 6-15) Rat (Sprague-Dawley) (17-19 female/dose) oral: gavage equivalent or similar to OECD Guideline 414	NaS 30, 90 or 180 mg/kg bw/d (nominal conc.) Vehicle: water Exposure: day 6 to 15 (daily)	NOAEL (embryotoxicity/ foetotoxicity): 90 mg/kg bw/d NOAEL (teratogenicity): 30 mg/kg bw/d	Fritz and Giese (1990) Klimisch score: 2 (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Rabbit (New Zealand White) (4 female) (GD 4-7) oral: gavage Limitation: few number of animals, only one concentration tested	NaS 100 mg/kg bw/d (actual ingested) Vehicle: water Exposure: day 4 to 7 (daily)	No effect on the number of implantations or on foetal development	Fabro S *et al.* (1984) Klimisch score: 3 (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Data on methyl salicylate			
Prenatal developmental assay (GD 6-18) Rabbit New Zealand White (18-20 females/group) Subcutaneous administration Study performed according to ICH guidelines and GLP	MeS (purity: 100.1%) 0, 30, 100, 300 mg/kg bw/d in corn oil Exposure: day 6 to 18 (daily)	NOAEL (development): 300 mg/kg bw/d based on no effect. NOAEL (maternal): 100 mg/kg bw/d based on abortion in one dam and on decreased body weight gain at 300 mg/kg bw/d. Increase of the plasma SA concentration nearly dependent of increases in the dose ratio and scarcely affected by repeated dosing.	FDA (2006b) Klimisch score: 1 Key study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Prenatal developmental assay (GD 6-17) Rat Crj:CD(SD)IGS (20 females/group) Subcutaneous administration Study performed according to ICH guidelines and GLP	MeS (purity: 100.1%) 0, 50, 100, 200 mg/kg bw/d in corn oil Exposure: day 6 to 17 (daily)	NOAEL (development): 100 mg/kg bw/d based on decreased body weight, external and skeletal anomalies at 200 mg/kg bw/d. NOAEL (maternal): 100 mg/kg bw/d based on depression of the body weight gain and decrease in food consumption at 200 mg/kg bw/d.	FDA (2006c) Klimisch score: 1 Key study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Study for effects on pre and postnatal development including maternal function Crj:CD(SD)IGS pregnant female rats (20/group) Subcutaneous administration. Groups of offspring sacrificed on lactation day 22 for organ weight and	MeS (purity: 100.1%) 0, 20, 60, 200 mg/kg bw/d in corn oil Exposure: from gestation day 6 to lactation day 21	NOAEL maternal: 60 mg/kg bw/d based on decreased body weight, food consumption and mortality at 200 mg/kg bw/d. NOAEL development < 60 mg/kg bw/d based on skeletal variations at 60 mg/kg bw/d. Decreased birth index, delayed balanopreputial	FDA (2006d) Klimisch score: 1 Key study (See Annex I of the BD for more details on the results) (See Annex II of the BD for

skeletal examination. Remaining males and females were mated to assess reproductive performance. Females sacrificed on gestation day 13. GLP and ICH guidelines		separation, delayed incisor eruption and skeletal anomalies and variations at 200 mg/kg bw/d.	justification of read-across)
Two-generation study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 4 (offspring assessment) of the NTP continuous breeding protocol NTP protocol, GLP	MeS (purity ≥ 99%) 0, 25, 50 and 100 mg/kg bw/d. (nominal conc.) Exposure: 7 days prior to mating, during 98 days of cohabitation (allowing the production of about 4 litters) and then during a separation period of 21 days during which final litters were delivered (task 2). A second generation was then produced only for the highest dose group (task 4): the mothers were dosed through weaning and F1 mice were dosed until mated at about 74 days of age.	NOAEL (reproductive effects): 100 mg/kg bw/d – no adverse effect NOAEL (developmental effects): 100 mg/kg bw/d – no adverse effect	NTP (1984a) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Lamb *et al.*, (1997) Klimisch score: 2 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
One generation study + crossover mating study Mouse (CD-1) male/female 20/sex/dose for MeS groups and 40/sex for vehicle group. Oral: gavage in corn oil Task 2 (continuous breeding phase) & 3 (crossover mating) of the NTP continuous breeding protocol NTP protocol, GLP	MeS (purity ≥ 99%) 100, 250 and 500 mg/kg bw/d. (nominal conc.) Exposure: 7 days prior to mating, during 98 days of cohabitation (allowing the production of about 4 litters) and then during a separation period of 21 days during which final litters were delivered (task 2). Task 3: high-dose animals of each sex were mated to control mice of the opposite sex.	500 mg/kg bw/d – no effect on fertility index NOAEL (developmental effect): 100 mg/kg bw/d based on a reduction in pup weight from 250 mg/kg bw/d. At 500 mg/kg bw/d, a significant decrease in the mean number of litter and in the average of pups per litter, the proportion of pups born alive was observed. Task 3: due to fertility problem in the control groups (26% in the first task 3 and 41% in the second task 3) and lack of significant results in the litter analysis, an affected sex cannot be determined.	NTP (1984b) Chapin & Sloane (1997) Morrissey *et al.*, (1989) Klimisch score: 2 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Three-generation study Rat (Osborne-Mendel); male/female (20/sex/dose) Oral: feed (no vehicle) A supplementary study was performed with adding calcium carbonate to MeS diet with the same examination. Examination very limited Several deficiencies from OECD 416, not GLP	MeS 0, 500, 1500, 3000 and 5000 ppm (equivalent to 25, 75, 150, 250 mg/kg bw/d as MeS) (nominal in diet) Exposure: 100 days before the first mating and then throughout the experiment (until weaning of the 3rd generation).	NOAEL (fertility): 250 mg/kg bw/d (male/female) based on no statistically significant effect reported. NOAEL (development): 75 mg/kg bw/d 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/d. The addition of calcium carbonate did not markedly differ from those obtained	Collins TFX *et al.* (1971) Gross MA, Fitzhugh OG (1977) Klimisch score: 3 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for

		after administration of MeS alone.	justification of read-across)
Two-generation study Rat (Wistar) male/female 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	MeS 0.25% and 0.5% (2500 ppm and 5000 ppm equivalent to 125 and 250 mg/kg bw/d) (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (weaning of the F2b litters).	No adequate NOAEL can be set based on the low quality of the reported results. Decreased litter size at all doses. Higher number of unsuccessful matings for the first generation and decreased reproduction index for both generations at the highest dose. Higher number of death between birth and day 5 day at 250 mg/kg bw/d.	Anonymous (1978a) Klimisch score: 3 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
Two-generation study Mouse male/female (no data on strain); 25/sex/dose (F0); 30/sex/dose (F1) Oral: feed (no vehicle) Examination very limited Several deficiencies from OECD 416, not GLP	MeS 0.25% and 0.5% (2500 ppm and 5000 ppm, equivalent to 375 and 750 mg/kg bw/d) (nominal in diet) Exposure: 30 days before the first mating and then through the experiment (weaning of the pups).	No adequate NOAEL can be set based on the low quality of the reported results. Litter size slightly smaller in test groups only in the first generation.	Anonymous (1978b) Klimisch score: 3 Supporting study (See Annex I of the BD for more details on the results) (See Annex II of the BD for justification of read-across)
One-generation study Rat (Sprague-Dawley); male/female; 24-27 animals/dose Oral: feed (no vehicle) Guideline and GLP not stated – secondary literature	MeS 4000 ppm and 6000 ppm equivalent to 200 and 300 mg/kg bw/d (nominal in diet) Exposure: 60 days before the first mating and then throughout the experiment (until weaning of offspring on day 20-21)	NOAEL (F1): 300 mg/kg bw/d (male/female) based on no effect. No abnormalities. Neonate survival at weaning was greater in the test group than in control.	FDA (1966) CIR (2003) Klimisch score: 4 Disregarded study (See Annex II of the BD for justification of read-across)

Based on a read-across approach with SA and MeS the DS concluded that hexyl salicylate is likely to induce similar developmental effects in animals but no effects on fertility induced by hexyl salicylate are expected. Therefore, considering the RAC opinions for the read-across substances as Repr. 2 for development, the DS proposed that hexyl salicylate should also be classified as **Repr. 2 – H361d.**

Comments received during consultation

One MS questions the read-across approach to MeS as the data do not provide any experimental evidence of the hydrolysis of hexyl salicylate in other tissues than the skin, e.g. in the liver. According to the MS, it is not possible to conclude that hydrolysis of hexyl salicylate in the body would occur as extensively as for MeS which means that it is not possible to decide if the same level of toxic effects would occur taking into account the differences in solubility and logPow between hexyl and MeS.

One industry consortium claimed that relevant data available on BzS and CHS were not considered in the CLH proposal, which do not show developmental effects in rats. Furthermore,

they announced that registrants of hexyl salicylate have submitted testing proposals for an OECD TG 421/OECD TG 408 combined study and OECD TG 414 studies in two species to ECHA and that an assessment should be postponed until the new data are available.

Updated proposal by the Dossier Submitter for targeted consultation

In preparation of the targeted consultation, the DS proposed to use EHS as another source substance for read-across to SA and provided the following rationale.

Table: *comparative data on physico-chemical parameters and human health endpoints (modified from table 3 of AIR)*

Salicylic acid	**Sodium salicylate**	**Methyl salicylate**	**Hexyl salicylate**	**Ethylhexyl salicylate**
Classification				
Acute Tox 4 – H302 Eye Dam. 1 – H318 Repr. 2 – H361d (ATP13)	No harmonized classification	Acute Tox 4 – H302 Repr. 2 – H361d Skin Sens. 1B – H317	No harmonized classification	No harmonized classification
Water solubility				
2.17 x 10^3 mg/L at 20°C (Merck 2006)	1.25 x 10^6 mg/L in water (Merck 2006)	0.67 x 10^3 mg/L in water at ambient T (FR Sev 2021)	2 mg/L at 23°C (NL Sev 2018)	0.074 mg/L at 20°C (registration dossier)
Log P_{ow}				
2.26 (Hansch, Leo 1995)	No data	2.55 (FR Sev 2021)	5.5 (NL Sev 2018)	5.94 (registration dossier)
Vapour pressure				
8.2.10^{-5} mmHg at 25°C (Daubert, Danner 1989) 1.1 x 10^{-2} Pa at 25°C	No data	10 Pa at 22°C 100 Pa at 51°C (FR Sev 2021)	0.077 Pa at 23°C (NL Sev 2018)	0.018 Pa at 20°C
ADME				
- Absorption: rapid by oral route - Distribution: distributed to several organs - Metabolism: 2 major urinary metabolites, SUA and salicyl-glucuronic acid found in rats; also metabolism in a small proportion to oxidative metabolites (2,3- and 2,5-dihydroxybenzoic acid) found in rats. - Elimination: these metabolites and free	- Absorption: rapid by oral route in rats. - Distribution: data from structurally-related salicylates (MeS) indicate wide distribution via blood and no bioaccumulation is expected after oral and dermal exposure.	- Absorption: well absorbed by oral route; oral bioavailability of 100% is assumed; very different values from 1 to 93% for dermal route; no data for inhalation exposure. - Distribution: widely distributed via blood and no bioaccumulation	- Absorption: no data for oral and inhalation route; expected to be poorly absorbed by inhalation route based on Log P and water solubility; data are contradictory for oral route; absorption varied from 0.8% to 7.8% for dermal route for concentrations	- Absorption: well absorbed via the oral route (100% absorption assumed), low absorption via the dermal route in an *in vitro* study (3%); inhalation exposure is not relevant due to low vapour pressure. (registration dossier)

unchanged SA are almost exclusively excreted in the urine. (CLH report on SA 2014)	- Metabolism: rapid hydrolysis to free salicylate in rats. - Elimination: data from structurally-related salicylates (MeS) indicate main and rapid excretion in the urine. (CLP report on SA 2014)	expected after oral and dermal administrations. - Metabolism: rapid and extensive hydrolysis to SA and methanol. After oral administration, 80% of MeS were hydrolysed in 90 minutes in humans; in dogs, hydrolysis is 95% complete in 1h and in rats, MeS is completely hydrolysed to free salicylate within 20 min. After dermal administration, free salicylate rapidly appears in blood and level of unhydrolysed MeS is low. SA obtained is then conjugated with either glycine or glucuronide and excreted inthe urine as SUA and acyl and phenolic glucuronides. Methanol is metabolized to corresponding aldehyde and acid and ultimately to CO_2. (CLH report on MeS 2018) QSAR modelling with Meteor and TIMES predicted hydrolysis of MeS (50% in vitro) to SA and methanol (ECHA 2021) - Elimination: mainly and rapidly in the urine after oral and dermal administration; low level in the faeces. (CLH	between 100 and 0.1% HS. - Distribution: data from structurally-related salicylates (MeS) indicate wide distribution via blood and no bioaccumulation is expected after oral and dermal exposure. - Metabolism: metabolism to SA by human skin esterases in an *in vitro* dermal absorption test; the QSAR Toolbox predicted the metabolites SA, hexanol, hexanal and hexanoic acid. (CLH report on hexyl salicylate 2020) QSAR modelling with Meteor and TIMES predicted hydrolysis of HS (50% in vitro) to SA and hexanol, hydroxylation of the alkyl chain at different sites leading to different metabolites that may be further biotransformed to SA and the corresponding alcohol (ECHA 2021). - Elimination: data from structurally-related salicylates (MeS) indicate main and rapid excretion in the urine.	- Distribution: data from structurally-related salicylates (MeS) indicate wide distribution via blood and no bioaccumulation is expected after oral and dermal exposure. - Metabolism: unchanged EHS in traces (t_R = 16.6 min) and metabolism to hydroxyl-EHS (5OH-EHS) (t_R = 12.5 min), 5oxo-EHS (t_R = 12.9 min), carboxylheptyl salicylate (cx-EHS) (t_R = 12.1 min), dinor EHS carboxylic acid metabolite, SA (t_R = 9.6 min), SUA (t_R = 8.4 min) in humans after oral exposure (Bury *et al.* 2019); also metabolism to 2-ethylhexanol (registration dossier). QSAR modelling with Meteor and TIMES predicted hydrolysis of EHS (50% in vitro) to SA and 2-ethyl-1-hexanol, hydroxylation of the alkyl chain at different sites leading to different metabolites that may be further biotransformed to SA and the corresponding alcohol (ECHA 2021). - Elimination: fast excretion in the urine (peak urinary concentrations of 5OH-EHS, 5oxo-EHS and cx-EHS

		report on MeS 2018)	(CLH report on hexyl salicylate 2020)	were found 1.6-2.6h after dose and >95% of the total amounts were excreted within 24h); it is expected that the major share of EHS dose was eliminated via urine as SA and SUA. (Bury *et al.* 2019)
Acute toxicity				
Classified as Acute Tox 4 - H302 LD_{50} oral = 400-3700 mg/kg LD_{50} dermal > 2000 mg/kg bw	LD_{50} oral = 930-1200 mg/kg LD_{50} dermal > 2000 mg/kg bw	Classified as Acute Tox 4 – H302 ATE = 580 mg/kg bw LD50 dermal > 2000 mg/kg bw	LD_{50} oral and dermal > 5000 mg/kg bw	LD_{50} oral and dermal (rat) > 5000 mg/kg bw
Acute oral toxicity of salicylates is moderate, with toxicity generally decreasing with increasing size of the alcohol moiety. Likely related to the relative proportion of SA followed hydrolysis. Methanol is of higher toxicity than the other alcohol metabolites and this is likely to explain the higher acute toxicity of MeS compared to the other salicylates.				
Repeated-dose toxicity				
No target organ reported (registration data), bones (Abbott, 1978)	Target organs: kidney and liver (registration data); bones (Abbott, 1978)	Target organs: bone and liver NOAELs of 50 mg/kg bw/d based on 2-year studies in rats and dogs (FR SeV 2021)	No data available for oral route	No particular target organ reported in a OECD 421 study at doses up to 250 mg/kg bw/d (registration data)
Fertility				
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. Increased duration of gestation (CIR, 2003)	No effect on fertility (FDA, 2006; FR SeV 2021)	No data available	No effect on fertility (registration data)
Development				
Foetal death, growth retardation and malformations (kidney and skeletal) in rats. Classified as Repr. 2 – H361 based on experimental studies with SA, MeS, NaS and	Foetal death, growth retardation and malformations (mainly skeletal) in prenatal toxicity study in rats.	Lethality, external malformations, visceral/skeletal anomalies and growth retardation in rats	No data available	Increased post-implantation loss, reduction in gestation index and lower litter size in an OECD 421 study

acetylsalicylic acid and on human data with acetylsalicylic acid.		(registration data; FDA (2006); FR SeV 2021). The lowest NOAEL for developmental toxicity can be set at < 60 mg/kg bw/d (but > 20 mg/kg bw/d) based on skeletal variations. Classified as Repr. 2 based on findings in studies in rats (malformations) and on a read-across with SA.		(registration data). LOAEL set by the registrants: 80 mg/kg bw/d and NOAEL: 25 mg/kg bw/d.

Studies with Ethyl Hexyl salicylate

EHS was administered once daily by gavage in corn oil as vehicle at dosages of 25, 80, and 250 mg/kg bw/d in male and female rats. Control animals received the vehicle only. Male rats were exposed for 28 days and female rats for approximately 7 weeks, i.e. 14 days prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum.

At the high dose level, one female was found dead on day 23 of the gestation period which was considered to be a result of birth complications. Slight but non-significant changes on body weight gain in female rats were also observed at this dose.

Reduction in gestation index (number of females with living pups as a percentage of females pregnant), increase in incidence of post-implantation loss resulting in a lower litter size and prolonged gestation period were observed at 80 and 250 mg/kg bw/d. Reduction in gestation index and increase in incidence of post-implantation loss were statistically significant and dose dependent effects, so these findings were considered to be test item-related adverse effects. Based on the individual data, increased post-implantation loss occurred predominantly in females with prolonged gestation. Reduction in absolute body weights of pups was observed at 250 mg/kg bw/d and was considered to be test item-related adverse effect.

Based on the observation of increased post-implantation loss, reduction in gestation index and lower litter size, the LOAEL for developmental toxicity is 80 mg/kg bw/d and the NOAEL is 25 mg/kg bw/d. The LOAEL for maternal toxicity is 250 mg/kg bw/d.

The DS concluded that developmental toxicity of EHS, and the effects reported are similar to those found with other salicylates (as MeS, NaS or SA).

QSAR studies

Furthermore, ECHA provided a QSAR analysis of the putative metabolism of salicylates using Meteor Nexus (Lhasa Ltd.) and TIMES. Meteor Nexus calculates scores for the likelihood of occurrence of metabolic reactions. The higher the yielded score, the larger the relative probability for a specific pathway to occur within the realm of predicted metabolic transformations. Based

on these calculations, the DS argued that hydrolysis to SA is as probable for hexyl salicylate and ethylhexyl salicylate as it is for MeS (see table).

***Table**: Probability scores for SA formation calculated with Meteor Nexus*

Biotransformation	**Phase (enzyme)**	**MeS (MeS)**	**Hexyl Salicylate (HS)**	**Ethylhexyl salicylate (EHS)**
144: Hydrolysis of acyclic carboxylic Esters	Phase I (hydrolase)	831	887, 541, 297, 463, 359, 284, 393, 300, 345	904

The DS considered the (extended) read-across plausible and proposed classification of hexyl salicylate as Repr. 2, H361d based on developmental effects seen in studies using the source substances SA, MeS, and EHS.

Comments received during targeted consultation

One MSCA considered it highly likely that the formation of SA after oral exposure would be sufficient to cause developmental toxicity *in vivo* at relevant oral dose levels.

Another MSCA accepted the read-across approach but asked for an explanation why data from ethylhexyl salicylate were considered in addition, but those from benzyl salicylate and cyclohexyl salicylate were not.

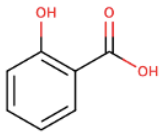
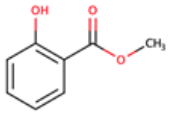
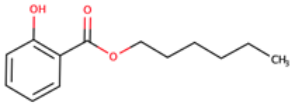
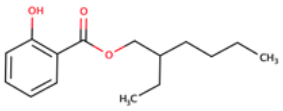
Two registrants clarified that they have submitted testing proposals for hexyl salicylate (OECD TG 421/OECD TG 408 combined study and OECD TG 414 studies in two species) to fill data gaps in the registration dossier. This would also include data on toxicokinetic analysis to determine SA exposure levels to use them as part of the reproductive toxicity risk assessment for hexyl salicylate. They also reiterated former requests to include data on cyclohexyl and benzyl salicylates in the assessment.

Assessment and comparison with the classification criteria

Read Across

RAC assessed reproductive toxicity data available for all salicylates proposed as read-across source substances for hexyl salicylate, namely SA (and NaS), MeS, EHS, CHS, and BzS. It is noted that salicylates with longer alkane chain (both linear and cyclic) or aromatic side chains have similar physico-chemical (PC) properties (e.g. solubility and Kow) as the target substance, hexyl salicylate. However, as no data are available on the possible hydrolysis of CHS or BzS (N.B. hydrolysis is the basis for the proposed read-across), RAC considered salicylates with cyclic and aromatic side chains not suitable to be used as source substances and limited the use of read-across to linear salicylates. The chemical structures, PC data and selected toxicological data for substances included in the read-across are compiled in the following table.

Substance	**Physico-chemical data**	**Reproductive toxicity data**	**Harmonised or self-classification (sc)**
Salicylic acid	Solubility: 2.17 g/L at 20°C LogP:2.26 Vapour pressure: 0.011 Pa at 25°C)	Reprotox: Foetal death, growth retardation and **malformations** (kidney and skeletal)	**Repr. 2 – H361d** Acute Tox 4 – H302 Eye Dam. 1 – H318

		in rats. (similar to OECD 414)	
MeS 	Solubility: 0.67 g/L LogP: 2.55 Vapour pressure: 10 Pa at 22°C	Lethality, external **malformations,** visceral/skeletal anomalies and growth retardation in rats (acc to ICH guideline)	**Repr. 2 – H361d** Acute Tox 4 – H302 Skin Sens. 1B – H317
Hexyl salicylate 	Solubility: 0.002 g/L at 23°C LogP: 5.5 Vapour pressure: 0.077 Pa at 23°C	No reproductive toxicity studies available	
Ethylhexyl salicylate 	Solubility: 0.074 mg/L at 20°C LogP: 5.94 Vapour pressure: 0.018 Pa at 20°C	Increased post-implantation loss, reduction in gestation index, lower litter size at 80 and 250 mg/kg bw/d, and statistically significantly lower mean pup body weight in rats at 250 mg/kg bw/d in OECD 421 Screening Test	Skin irrit. 2 (sc) Eye irrit. 2 (sc)

Studies with SA and MeS, are described in the proposal by the DS section.

According to the study summary of the additional reproduction / developmental toxicity screening test according to OECD guideline 421 in the registration dossier on EHS, four groups of 11 male and 11 female rats received 0, 25, 80 or 250 mg EHS per kg bw/d via gavage over a period of approximately 7 weeks, 14 to 28 days prior to mating, throughout mating and gestation periods until F1 reached day 4 postpartum. One high dose female was found dead on GD 23, considered to be a birth complication. No further effects were observed in males and females at any dose group. At 80 and 250 mg/kg bw/d a reduction in gestation index as well as an increase in incidence of post-implantation loss resulting in a lower litter size were noted. Mean number of living pups per dam were 5.3 and 9.2 at high and mid dose, respectively compared to 12 in the ctrl group. Birth index (number of pups born alive as percentage of implantations) was also reduced (42.9 % and 66.2 % at high and mid dose, respectively, vs. 88.2 % in the ctrl). No effects on litter size were noted in the low dose group. Mean number of pups was 13.1 per dam and birth index was 94.2 %. According to the registration report, these effects were statistically significant and dose dependent and therefore considered to be test item related.

During lactation, a total number of 18, 3, 10 and 13 pups (which corresponded to mean number per dam of 1.8, 0.3, 1.0 and 1.4) were lost at the dose levels of 0, 25, 80 and 250 mg/kg bw/d, respectively.

Pups sex ratio was not affected by exposure to the test item at any dose level. At the dose level of 250 mg/kg bw/d, reduced body weights of pups were noted. Mean body weights of pups were

5.0 g compared to 6.0 g in the control group (5.9 g and 6.3 g in low and mid dose group, respectively) on day 1 of the lactation period; this difference was statistically significant. Body weights of pups at the high dose level remained lower than the respective control value also on day 4 of the lactation period. Mean body weights were 7.6 g compared to 9.2 in the control group; this difference was however no longer statistically significant.

No test item related effects on body weights or body weight gain in pups were noted at the dose levels of 25 and 80 mg/kg bw/d.

Body weight gain of pups during the first four days of the lactation period was +44.4%, +42.7%, +48.0% and +44.6% in control, low, mid and high dose group, respectively.

At the mid-dose level, statistically significantly higher body weight gain was noted. In the absence of increased body weight gain at the high dose level, this was considered not to be related to the treatment with the test item.

Fertility

No animal studies nor human data are available to assess adverse effects on fertility for hexyl salicylate. No classifiable effects were noted in any of the studies on SA or MeS. Some effects were noted in the screening study with EHS. Reduced number of living pups per dam and increased post-implantation loss may be considered adverse effects on fertility. However, limited details are provided in the study summary from the registration dossier on ECHA's dissemination website. Thus, no firm conclusion can be drawn.

Thus, RAC proposes not to classify hexyl salicylate for adverse effects on fertility due to inconclusive data.

Development

There are no human data available on developmental effects after exposure to hexyl salicylate.

Since no developmental studies are available for hexyl salicylate, the DS summarised studies on SA, NaS and MeS in their initial proposal. During targeted consultation, they added EHS to the list of substances proposed for read-across. As stated by the DS, the 2016 RAC opinion on SA and the studies listed in the above table on this substance and NaS show robust evidence of developmental effects in rats following exposure to SA. In rats, embryo-/fetotoxic effects were observed with dose dependent growth delays, foetal death and malformations without maternal toxicity.

According to the CLH report on MeS and the RAC opinion dated on September 2019 for this substance, there is clear evidence of developmental effects in two well-conducted studies in rats. Following s.c. exposure to 200 mg/kg bw/d of MeS, several developmental effects were observed. FDA 2006d reported lethality, growth retardation, external malformation, delay in post-natal differentiation indices, skeletal anomalies, skeletal variations and delay of ossification at this concentration. FDA 2006c observed significant lower foetal body weight, external malformations, visceral anomalies and skeletal variations. Although maternal toxicity also occurred at 200 mg/kg bw/d in these two studies, the observed developmental effects were not considered to be secondary to this maternal toxicity. Additionally, developmental effects were reported in fertility studies in both mice and rats (Collins *et al.* 1971, Anonymous 1978a, 1978b, NTP 1984b). It should be noted that in this case metabolic transformation to SA was experimentally shown *and* studies with MeS itself showed reproductive toxicity causing malformations and other effects. RAC used read-across to SA in their opinion on classification and labelling for MeS to justify a Repr. 2; H361d classification proposal despite clear effects in animals that could warrant a classification as Repr. 1B.

In the OECD TG 421 study with ethylhexyl salicylate, some effects were observed concerning post-implantation loss (and related mean number of pups born alive per dam) from mid dose onwards as well as on pup body weight in the highest dose group (250 mg/kg bw/d).

Conclusion on classification

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

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

Following the read-across approach using data on methyl and ethylhexyl salicylates as well as on the common metabolite SA, adopting the precautionary principle,RAC concurs with the DS and proposes classification of hexyl salicylate as **Repr. 2, H361d.**

Additional references

O. Lockridge, D.M. Quinn, Esterases in Comprehensive Toxicology, 2010 (ISBN: 978-0-08-046884-6)

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 – Records of the targeted consultation following the submission of further information to clarify the rate and relevance of hexyl salicylate hydrolysis for the oral route of exposure