Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Methyl salicylate

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

CLH-O-0000006716-67-01/F

Adopted

20 September 2019

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: methyl salicylate
EC number: 204-317-7
CAS number: 119-36-8
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Belgium	<confidential>	Company-Downstream user	1
Comment received				
N/A				
Dossier Submitter's Response				
France: N/A				
RAC's response				
N/A				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	France	<confidential>	Company-Downstream user	2
Comment received				
Our comments relate to exposure scenarios to methyl salicylate in the French CLH dossier which are not relevant for the purpose of deriving conclusions under the EU Regulation (EC) No 1272/2008 (CLP) ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Methyl Salicylate - CLH public consultation comments.pdf				
Dossier Submitter's Response				
France: - Comment related to use of exposure data: see response to comment 8. - Comment related to reproductive / developmental toxicity: see responses to comment 8 and 16. - Comment related to salicylic acid classification: see response to comment 10. - Comment related to epidemiological data on aspirin: see response to comment 10.				

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Belgium	Procter & Gamble	Company-Downstream user	3
Comment received				
COMMENTS to the CLH REPORT: Methyl Salicylate: EC# 204-317-7; CAS# 119-36-8 1. Skin Sensitisation: Based on an overall weight of evidence using animal, human and alternative data, we conclude that Methyl Salicylate should not be classified as a Skin Sensitiser. See details and justification in relevant section below 2. Developmental Toxicity: We conclude that methyl salicylate should not be classified as Repro. Cat 1B. See details and justification in relevant section below. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments to the CLH report_PG FINAL Version_25 Jan 2019.zip				
Dossier Submitter's Response				
France: see responses to comments 13 & 25.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Germany		MemberState	4
Comment received				
Regarding the hydrolysis of salicylates, ECHA noted explicitly in the decision (CCH-D-21 14387555-36-0L/F) to 2-ethylhexyl salicylate (CAS Number 118-60-5) that information have to be given on all hydrolysis products. The toxicity of all potential metabolites has to be taken into account and discussed in the dossier. This information is required to justify the read-across. Regarding the subcutaneous route of administration in the FDA studies, the following statement can be found in the Guidance on the Application of the CLP Criteria, Version 5.0, July 2017 on page 55: "As CLP is hazard-based, it does not take exposure into consideration arriving at a classification. As it is already noted in the UN GHS, the classification according CLP is only based on the intrinsic hazards. "				
Dossier Submitter's Response				
France: We agree that all hydrolysis products should be taken into account when a read-across is considered. In the case of methyl salicylate, the substance is hydrolized into salicylic acid and methanol. Methyl salicylate, salicylic acid and sodium salicylate present a similar pattern of developmental effects, suggesting that these effects are likely related to an effect of methyl salicylate and/or its metabolite, salicylic acid. Methanol also presents developmental effect at high exposure levels, that may not be reached after exposure to methyl salicylate. However, a specific toxicity of methanol cannot be neither excluded if it				

is present in the target organs (in this case we agree, as you noted in comment 14, that the potential of the alcohol can be higher than by oral administration). In the present CLH report, the proposed classifications are actually based on methyl salicylate data. Human data with aspirin are presented in the absence of such data with methyl salicylate. However, uncertainties linked to differences in kinetics and biological effects can be raised between these two substances (see also response to comments 10 and 16 related to data on aspirin). Therefore, the relevance of an extrapolation between aspirin and methyl salicylate remains doubtful. The human data with aspirin was judged inadequate, even if it should be noted that some publications reported developmental effects that seem consistent with those reported in experimental studies with methyl salicylate. Thus, these data cannot be used to disregard the effect reported in experimental animals with methyl salicylate. Thank you for the statement that supports the relevance of the subcutaneous studies. See also response to comment 8 on this topic.
RAC's response
Thanks for your comment.

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2018	United Kingdom	<confidential>	Company-Downstream user	5
Comment received				
Comments refer to the reproductive toxicity endpoint ECHA note – An attachment was submitted with the comment above. Refer to public attachment clh_opinion_salicylic_acid_6425_en.pdf				
Dossier Submitter's Response				
France: see response to comment 15. The attachment refers to CLH opinion on salicylic acid.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	France	NOVACYL	Company-Manufacturer	6
Comment received				
The comments to the following endpoints have been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company. We agree in the general statement provided by IFRA. In Summary: - H412: A classification on the basis of the available OECD 201 study is premature due to deficiencies in the study (outlined below) and the final assessment should be postponed until a valid and scientifically robust study is available. - A classification as Repr. Cat 1B for developmental effects for Methyl Salicylate (MeS) is neither warranted nor justified in the light of the overall data set. - Based on an overall weight of evidence using animal, human and alternative data, Methyl Salicylate should not be classified as a Skin Sensitiser. Please find a more precise discussion in the specific fields. ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip				

Dossier Submitter's Response
France: see responses to comments 8, 16,28 and 31
RAC's response
H412: RAC paid attention to the EC_{10} value calculated in the PC comments. Please see the opinion for further details.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	United States	Colgate-Palmolive Company	Company-Downstream user	7
Comment received				
The purpose of this document is to support the safety of methyl salicylate (MeS) for use in personal and oral care products, regardless of the classification as cosmetic, medical device or medicinal product, as a direct addition and also when used as a fragrance/ flavor ingredient. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Reproductive Toxicity Public.docx ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Reproductive Toxicity CP.docx				
Dossier Submitter's Response				
France: According to the attachments, the comments are similar to those submitted by Novacyl and Symrise: see responses to comment 16. Specific comment related to doses of methyl salicylate in personal and oral care product: your statement is not based on any references and cannot be checked. Anyway, it should be reminded that exposure considerations should not be part of classification decision in CLP regulation which is only based on intrinsic properties of a substance. In regards to the ADI of 0.5 mg/kg/day set by the WHO (reference 1 of the attachment), this value was derived in 1967. According to the summary, it seems that developmental data were not taken into account (or not exist yet). http://www.inchem.org/documents/jecfa/jecmono/v44aje28.htm. It should be noted that in 2005, WHO identified that methyl salicylate has margin of safety of less than 100. They suggest that this substance might form a basis for prioritization for future investigation. http://www.fao.org/3/a-at876e.pdf				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	Belgium	INTERNATIONAL FRAGRANCE ASSOCIATION	Industry or trade association	8
Comment received				
IFRA's comments are related to the use of exposure data of Methyl Salicylate in cosmetics and medicinal products as a basis and justification for deriving conclusions under the EU Regulation (EC) No 1272/2008 (CLP), with which IFRA strongly disagrees. Only end-use applications of fragrance substances covered by the scope of CLP should be considered				

and taken into account. See attached document for the rationale. Additionally, we also include comments related to the use of two rat studies with sub-cutaneous injection (FDA 2006c and 2006d) which are used to justify a proposal to classify Methyl Salicylate as a developmental toxic substance Repr. Cat 1B. The rationale is also included under the Reproductive toxicity end-point.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment IFRA comments to Methyl salicilate consultation final.pdf

Dossier Submitter's Response

France:

The numerous uses of methyl salicylate (including cosmetic but not only) strongly support the need of action in the CLP framework. We agree that CLP shall not apply to cosmetic products, in the finished state (article 1 of CLP regulation). However, there are interconnections between CLP and cosmetic regulations, in particular for CMR 1 and 2 (see (32) of Regulation (EC) No. 1223/2009). In addition, cosmetic ingredients are covered by CLP regulation in order to protect workers.

Skin sensitisation:
Regarding the level of exposure to take into account for subcategorization decision, there is no reason to disregard data from cosmetic and medicinal products (in particular, CLP guidance does not mention exclusion of such exposure data). According to the CLH report (page 31), it is clearly stated that methyl salicylate is used at concentration ≤ 0.6% in cosmetics, that is consistent with your comment. Concerning the concentration/dose score of 2 set in the CLH report, it is considered justified based on the concentration found in topical medication.
It has to be noted that your proposed score of 0 instead of 2 would not significantly change the conclusion. Indeed, if a score of 0 was retained, it would lead to a total score for exposure data of 4 (instead of 6 proposed in the CLP report) and thus to a classification as Skin Sens. Cat. 1 (table 3.4 of the CLP guidance) instead of Skin. Sens. Cat. 1B (proposed in the CLH report). Same risk management measures apply for substances classified as Skin Sens. Cat. 1 or Cat. 1B.

Reprotoxicity:

- Regarding your comment on subcutaneous injection in FDA (2006) studies: According to the authors of the study reports: "*Percutaneous route was planned but is difficult in a reproductive and developmental toxicity study. The subcutaneous route was chosen as a substitute route because higher plasma levels of the test article are expected with this route than with the percutaneous route*." The dermal route was preferred than the oral route by the laboratory because the studies were performed to characterize the safety of methyl salicylate, as a ingredient of a topical patch. Even if subcutaneous injection is expected to maximise systemic exposure, the results obtained in these studies are consistent with those obtained after other routes of administration (oral, dermal, intra-peritoneal). Indeed, whatever the route of exposure, developmental toxicity was reported after methyl salicylate administration. The proposed classification is thus based on the developmental effects reported with methyl salicylate in the FDA (2006) studies performed by subcutaneous injection (considered as the most robust studies) but also on the fertility studies performed by oral route (NTP, 1984 b; Collins, 1971; Anonymous, 1978 a&b), supported by the other prenatal developmental toxicity studies of rather low quality by oral, dermal and intraperitoneal route (Infurna, 1990; Overamn & White, 1983; Who, 1972; Daston, 1988; Kavlock, 1982). No well-designed prenatal developmental toxicity study is available by oral route. In the absence of an oral

study and considering an oral absorption of 100% for methyl salicylate, the studies performed by subcutaneous route should be considered as predictive for the potential of developmental toxicity, with potential differences in metabolism due to first pass effect. Methyl salicylate is metabolized in salicylic acid and methanol. A similar pattern of developmental toxicity is reported with other salicylates, such as sodium salicylate or salicylic acid. Thus, based on consistency of effects between methyl salicylate and its metabolite, salicylic acid, remaining differences in metabolism due to first pass effect are not considered as a major issue.

Moreover, according to the CLP regulation, the objectif of hazard classification is to identify the intrinsic hazard of a substance. The route of exposure is not indicated in the CLP guidance as a major limitation in the decision making for classification as Reprotoxic. All the elements above support that the developmental effects reported with methyl salicylate are clearly an intrinsic hazard of this substance.

- Regarding your comment on maternal toxicity at 200 mg/kg bw/day in FDA (2006d): as stated in the CLH report (page 53), "*Although some of the developmental effects (such as skeletal variation, decreased body weight, delay in post-natal differentiation indices) may be secondary to maternal toxicity, it is not possible to explain the other effects such as offspring lethality and external/skeletal anomalies by the observed maternal toxicity. Indeed, the maternal toxicity is considered rather slight in view of the severity of the developmental effects as lethality and anomalies"*. In this study, methyl salicylate was administered subcutaneously from gestation day 6 to lactation day 21. There were no clinical signs in dams. Decreased body weight was < 5% during gestation. The decreased body weight gain reported during gestation recovered during lactation, with higher body weight gain at 200 mg/kg bw/day (*versus* control) from post-natal day 10. Two dams died on gestation day 22 (number 970 and 971). Individual data of dam n°970 and n°971 do not report any decrease of body weight or body weight gain during gestation. Necropsy findings of the first dead dam (n° 970) consist on slight macule, dark red stomack and slight retention fluid oily of the subcutis (treated site). In contrast, for the other dead dam (n°971), only slight retention fluid oily of the subcutis (treated site) was noted at necropsy. Although 14 dead foetuses (no information if live foetuses were obtained) were observed in the uterus in the dam (n° 970), with 2 craniorachischisis among the dead foetuses; in contrast, for the other dead dam, no specific findings were reported in the study report. These dams were not further considered in the calculation of parameters assessed (such as external examination of live newborn showing craniorachischisis, terminal delivery parameters showing a decreased birth index, skeletal examination on PND22 showing skeletal anomalies). Anyway, it should be noted that lethality of offspring and malformations are consistently found in different studies, in particular in the well-performed prenatal developmental toxicity FDA (2006c) study. The classification as Repr. 1B – H360D is thus considered justified.

- Regarding your comment on the occurrence of skeletal variations reported at 60 mg/kg bw/day in the FDA (2006d): indeed similar observations were not reported at 100 mg/kg bw/day in the FDA (2006c) study. However, the design of both studies is not similar: administration from gestation day 6 to post-natal day 21 in the FDA (2006d) study and from gestation day 6 to 17 in the FDA (2006c) study; skeletal examination was performed on PND22 in the FDA (2006d) study and on gestation day 20 in the FDA (2006c). As written in the CLH report (page 45-46), in the absence of historical control data and considering that skeletal anomalies are consistently reported in other studies, it cannot be excluded that skeletal variations observed at 60 mg/kg bw/day are treatment-related. This is in line with FDA (2006) conclusions. Skeletal anomalies occurred in these 2 different studies (clearly treatment-related at

200 mg/kg bw/day in both studies and probably related to treatment at 60 mg/kg bw/day in FDA (2006d) study) and thus should be considered for classification. • Regarding your comment on the level of details available in the FDA (2006) report, we would like to highlight that the assessment made in this CLH report is based on the original study reports. Therefore, the Klimisch scores of 1 (well-performed studies) have been established based on these original study reports (and not only on the detailed summaries provided in FDA report).
RAC's response
Thanks for your comment.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	Germany	Symrise AG	Company-Importer	9
Comment received				
The comments to the following endpoints have been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company. We agree in the general statement provided by IFRA. In Summary: - H412: A classification on the basis of the available OECD 201 study is premature due to deficiencies in the study (outlined below) and the final assessment should be postponed until a valid and scientifically robust study is available. - A classification as Repr. Cat 1B for developmental effects for Methyl Salicylate (MeS) is neither warranted nor justified in the light of the overall data set. - Based on an overall weight of evidence using animal, human and alternative data, Methyl Salicylate should not be classified as a Skin Sensitiser. Please find a more precise discussion in the specific fields ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip				
Dossier Submitter's Response				
See response to comment 6 and 31.				
RAC's response				
H412: RAC paid attention to the EC_{10} value calculated in the PC comments. Please see the ODD for details.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Belgium	<confidential>	Company-Downstream user	10
Comment received				
Hazard Assessment of Reproductive Toxicity Potential of Methyl Salicylate The available data do not provide sufficiently convincing evidence that methyl salicylate should be placed in Category 1b in the light of the overall data set: •The rat reproductive EFD (FDA2006c) and PND (FDA 2006d) studies were conducted by the subcutaneous route, and therefore it is not appropriate to use them to draw definitive conclusions about reproductive toxicity following oral and dermal exposure in humans. •The data support a NOAEL of 100 mg/kg for reproductive toxicity and not < 60 mg/kg.				

There is no effect on skeletal abnormalities up to 100 mg/kg in the study specifically designed to evaluate this i.e. the EFD study (FDA2006c). The effect on skeletal abnormalities in the PND study (FDA 2006d) at 60 mg/kg are not consistent with the EFD study where they dosed during organogenesis at 100 mg/kg. Since the formation of ribs occurs and is completed during organogenesis, while the dosing duration is longer in the PND there is no plausibility for further developmental skeletal abnormalities of the ribs to occur in the PND study vs. the EFD. Given that there is a lack of any statistical significance or any clear dose response relationships for these skeletal findings in the PND study or the EFD study, it is highly unlikely that the skeletal abnormalities are test article related at 60 mg/kg and the appropriate NOAEL to use for reproductive toxicity is 100 mg/kg.

•The reproductive toxicity findings at 200 mg/kg in FDA 2006c occur at clearly maternally toxic doses based on the significant reduction in body weight gain and food consumption and are therefore not relevant for humans at and above the ADI. This is observed by the significant decrease in body weight gain vs. control during entire treatment period in that group (314%, 155.2%, 89%, 68%, 48%, 39%, 36%, 24%, 21% 17%, 12%, 10% and 12% on Days 7-20, respectively) and even body weight loss on Days 7 and 8. In addition, the reduction in body weight gain returns to below 20% of the control once the dosing period is completed, which is consistent with the test article causing maternal toxicity.

•There are limitations of the data that confound interpretation of the results:
oData are reported on a fetus basis and not a litter basis for the skeletal abnormalities in the PND and EFD study; it is not possible to establish whether these are spread out across litters or in a single litter, which might explain the data.
oBody weight gain data do not fully capture the potential maternal toxicity because the individual maternal carcass and fetal weights have not been provided and therefore the body weight gain differences relative to the control may be understated

•The rabbit study is a valid study that shows no potential for reprotoxic effects. These animals were exposed throughout the entire period of organogenesis (days 6 to 18) and further exposure would only have served to demonstrate subsequent effects on the growth of the fetus and not on the occurrence of developmental abnormalities. This dosing period is internationally accepted as covering the period of organogenesis as stated in both the ICH S5 and the OECD 414 guidelines. Therefore, this study was performed to international standards and would qualify for a Study Quality Score of 1 according to the approaches recommended by Klimisch et al. (1997).

•The hamster study is not relevant to humans as the dose tested is well above the human toxic level. Methyl salicylate was administered topically at 3500 and 5250 mg/kg bw to pregnant LVG hamsters on day 7 and teratogenic results were compared with those obtained following oral treatment at 1750 mg/kg bw. Both treatments produced neural tube defects, especially in the area of the developing brain. Percentage of neural tube defect was 72% at 1750 mg/kg bw/day after oral exposure versus 11% in control. This was a poorly conducted study designed to produce an adverse effect as methyl salicylate was administered at an excessively high dose at GD7, which is a very sensitive window for neural tube development. This is evidenced by the high plasma salicylate levels in the animals that reached a peak of 125 mg/100 ml at about 2 hours after oral treatment and is well above the toxic human level. As a result, this result is not relevant to humans and should not be used as part of the weight of evidence for developmental toxicity classification of methyl salicylate.

•No human data has been found with methyl salicylate. The CLH reports that there are some indications of developmental effects from human data with acetyl salicylic acid,

which can support the relevance of the observed effects in experimental studies to humans. However, epidemiological evidence from ASA clearly indicate that there are no effects in humans. The use of ASA also during pregnancy up to the maximum recommended therapeutic dose of 4000 mg/day or 66.7 mg/kg using 60 kg body weight (bw) have demonstrated no evidence of an increased risk of adverse pregnancy outcomes in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay (Slone et al., 1976; Shapiro, 1976; Kozer et al., 2002). A further meta-analysis of studies on the use of therapeutic low-dose ASA at 50–150 mg/day (Kozer et al., 2003) has demonstrated that this dose range is not associated with any adverse pregnancy outcome, in terms of perinatal mortality, birth complications, congenital malformations or adverse effect on subsequent development. For pregnancies with a moderate or high risk of pre-eclampsia and/or premature delivery, the adverse pregnancy outcome rate was reduced with therapeutic low dose ASA (50–150 mg/day) and there was no increased risk of early miscarriage with this dose regime (Roberge et al., 2016). These studies strongly support that neither ASA nor its principal metabolite salicylic acid induce adverse developmental effects up to the maximum recommended therapeutic dose in humans, i.e. 4000 mg/day (66.7 mg/kg for a 60 kg person, equivalent to 51 mg/kg SA).

•Once absorbed, the majority of methyl salicylate is converted to salicylic acid (Opdyke 1978). Davison et al. (1961) conducted a study to compare the toxicity and metabolism of methyl salicylate and salicylic acid. Davison et al. (1961) concluded that the results indicated the toxicity and metabolism of both methyl salicylate and salicylic acid are comparable across multiple species (rats, dogs, and monkeys).

Conclusion:

Based on the data and applying weight of evidence in interpreting them, it can be concluded that:
•The developmental effects in rats were only observed in the presence of excessive signs of maternal toxicity including severe decrements in maternal body weight.

•Methyl salicylate did not result in developmental toxic effects in a well-designed rabbit study

•Epidemiological studies in ASA indicate that there are no effects in humans.

•Once absorbed the majority of methyl salicylate is converted to salicylic acid which has RAC opinion of Repr. 2

Based on data presented, a Category 1B for methyl salicylate is not justified.

References:

•Davidson C et al. (1961): On the metabolism and toxicity of Methyl Salicylate, J Pharm Exp Ther 132, 3, 207

•Kozer, E., Nikfar, S., Costei, A., Boskovic, R., Nulman, I., Koren, G., 2002. Aspirin consumption during the first trimester of pregnancies and congenital anomalies: a metaanalysis. Am. J. Obstet. Gynecol. 187, 1623–1630.

•Kozer, E., Costei, A.M., Boskovic, R., Nulman, I., Nikfar, S., Koren, G., 2003. Effects of aspirin consumption during pregnancy on pregnancy outcomes: a meta-analysis Birth Defects Res. (Part B) 68, 70–84.

•Opdyke DLJ (1978): Monographs on fragrance raw materials. Canadian snakeroot oil. Food and Cosmetics Toxicology 16: 821.

•Overman DO, White JA (1983). Comparative teratogenic effects of methyl salicylate applied orally or topically to hamsters. Teratology 28(3): 421–426

•Roberge, S., Odibo, A.O., Bujold, E., 2016. Aspirin for the prevention of preeclampsia and intrauterine growth restriction. Clin. Lab. Med. 36 (2), 319–329.

•Slone, D., Siskind, V., Heinonen, O.P., Monson, R.R., Kaufman, D.W., Shapiro, S., 1976.Aspirin and congenital malformations. Lancet 1 (7974), 1373–1375.

•Shapiro, S., 1976. Perinatal mortality and birth-weight in relation to aspirin taken during pregnancy. Lancet 1, 1375–137

Dossier Submitter's Response

France:

- Comment related to subcutaneous route: see response to comment 8.

- Comment related to skeletal variations noted at 60 mg/kg bw/day in the FDA (2006d) study: see response to comment 8.

- Comment related to maternal toxicity at 200 mg/kg bw/day in the FDA (2006c) study: no clinical sign was noted at this dose. Indeed, the body weight gain was particularly affected during gestation at 200 mg/kg bw/day. However, as reported in your comment, the reduced body weight gain was more severe on GD7 (start of the treatment) compared to GD17 (end of the treatment): -314% versus -17%. The relationship of the severity of this effect is clearly inverse to the duration of treatment, which can question the role of methyl salicylate in this effect. In addition, the reduced body weight gain has only small impact on the actual body weight. Statistically significant decreases in body weight were noted on GD7 (-3.5%), 8 (-4.2%), 9 (-3.4%), 10 (-3.9%), 12 (-3.4%), 13 (-3.6%). While the treatment stopped on GD17, the lower body weight was not statistically significant from GD14. Therefore, the developmental effects reported at 200 mg/kg bw/day cannot be considered as secondary to maternal toxicity, especially when considering the severity of the developmental effects (external malformation, visceral anomalies).

- Comment related to the limitations of the data that confound interpretation of the results (FDA (2006c):
    - we acknowledge that the data are only reported on a foetus basis in the summary table. However, even if the data are not reported in Annex I of the CLH report, individual observations on caesarean section of F0 dams are available in the study report. In particular, among the effects observed, it can be noted that craniorachischisis occurred in 8 foetuses from 3 different litters, dead foetuses were found in 11/20 litters, dilatation of the ureter (unilateral) were observed in 2 foetuses from 2 different litters and thymic remnant in the neck in 8 foetuses among 6 litters. In addition, external/visceral/skeletal anomalies are also reported in other studies confirming the role of methyl salicylate in these effects.

    - Decreased maternal body weight noted at 200 mg/kg bw/day may be not due to a direct general toxic effect of methyl salicylate to dams but may be due to the lower body weight of foetuses and/or to the increased

embryolethality. No calculation of corrected body weight for the dams is provided in the study report to clarify this point. It should be noted that even if the data are not reported in Annex I of the CLH report, individual body weights for dams and mean live foetus weight/dam are available in the study report.

- Comment on the rabbit study: please note that a Klimisch score of 1 for this study is already noted in the CLH report. Therefore, this study has been judged valid and not disregarded. From the available database, the rabbit seems clearly less sensitive to methyl salicylate toxicity than rodents. Some explanations have been searched to explain the difference between rabbit and rodent's response. First, it should be noted that it is recommended in the guideline OECD 414 (version updated in 2018) to dose "*from implantation to one day prior to the day of scheduled humane killing, which should be as close as possible to the normal day of delivery*"; however, this is not the case in the available study (stop of treatment on gestation day 18 compared to a total duration of gestation for rabbits of about 30 days). This short exposure duration may explain, at least partially, the difference of sensitivity between these two species (in particular regarding foetal lethality). In addition, plasma salicylic acid concentration was measured in the rats (FDA (2006a): study of fertility and early development to implantation) and in rabbits (FDA (2006b): prenatal developmental toxicity study). When comparing the plasma salicylic acid concentrations just after the first administration, higher concentrations were found in the plasma of rats compared to rabbits. This can also, at least partially, explain the difference of sensitivity between these two species.

- Comment on hamster study: as reported in the CLH report, the hamster study was judged as a low quality study: high doses (from 3500 mg/kg bw by dermal route and at 1750 mg/kg bw/day by oral route) administered on a single day (GD7). However, it should be noted that the incidence of neural tube defects was very high in particular when considering the single oral dose of 1750 mg/kg bw: 72% of neural tube defects. Therefore, this effect could already have been detected at lower doses if tested, in particular if the substance had been administered for several days of gestation (instead of administration on GD7 only).

  Furthermore, plasma salicylate levels were also analysed in the rats in the FDA (2006a) study and in the rabbits in the FDA (2006b) study. In rats exposed to 300 mg/kg bw/day by subcutaneous route, the plasma salicylic acid concentration was 239 µg/ml in males and 277 µg/ml in females, 4 hours after the first administration. In rabbits exposed to 300 mg/kg bw/day by subcutaneous route, the concentration of plasma salicylic acid concentration was 142 µg/ml, 4 hours after the first administration. Therefore, even if very high doses were administrated to hamsters, plasmatic concentrations reported in this species (125 mg/100 ml) are below those found in rats and rabbits exposed to 300 mg/kg bw. In comparison, it is noted that clinical toxicity (salicylism) in humans occurs at salicylic acid plasma levels above 350 µg/mL (Labib, 2018), that is higher than the concentrations found in rats exposed to 300 mg/kg bw/day. Therefore, developmental effects occurring in rats exposed to administrated doses between 60 and 200 mg/kg bw/day are considered relevant to humans.

- Comment on human data with aspirin: human data on aspirin have been included in the CLH report, in the absence of such data with methyl salicylate, in order to identify possible effects of salicylates (especially, salicylic acid) in humans, but not as a direct extrapolation for methyl salicylate. We agree that most of the epidemiological studies do not report an increased risk of adverse effect on

development. However, there are also some publications reporting developmental effects, consistent with those identified from animal studies, in particular gastroschisis, craniorachischisis, decreased body weight and mortality. Furthermore, these data refer to aspirin at therapeutic dosage. In particular, data showing no effects refer to low-dose aspirin treatment. However, it is important to note that the biological effect of aspirin is highly dependent of its dosage. For example, the effect of aspirin on COX-dependent prostaglandin synthesis is dose dependent, with irreversible acetylation of COX-1 at low doses and inhibition of both COX-1 and COX-2 at higher doses. Therefore, data on low-dose aspirin cannot be used to conclude on intrinsic toxicity of aspirin in general. Finally, no comparison can be made with methyl salicylate since there is no information on the level of exposure of workers and/or general population to this substance in its intended uses. Thus, it cannot be excluded that exposure to methyl salicylate occur at higher level. Anyway, it should be reminded that exposure considerations should not be part of classification decision in the CLP regulation. Finally, as reported in the CLH report, the epidemiological data on aspirin are judged inadequate to conclude on developmental toxicity of salicylates. Therefore, these data should not be used to conclude to a lack of effect (especially since there are some publications reporting developmental effects consistent with experimental data) or to disregard the developmental effects reported in animals with methyl salicylate. Overall, the proposed classification is based on animal data performed with methyl salicylate.

- Comment on metabolisation of methyl salicylate to salicylic acid which is classified as Repr. 2.: Salicylic acid has been classified as Repr. 2 by the RAC based on animal data with different salicylates (salicylic acid, aspirin and sodium salicylate) and human data with aspirin. The data on methyl salicylate present in this CLH report was not included in the CLH report on salicylic acid for classifying this substance as reprotoxic for the development. Even if it is acknowledged that salicylates induce similar pattern of developmental effects, classifying methyl salicylate on the basis of the classification of salicylic acid raises uncertainties (linked to kinetics and potency), in particular when considering that adequate data are available with methyl salicylate. For example, a specific toxicity of the parent molecule, methyl salicylate, on development cannot be excluded as 21% remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans according to Davison (1961). In addition, a potential toxicity of methanol (the other metabolite) cannot be neither excluded (see also response to comment 4). Therefore, in the present case, it is considered more robust to conclude on classification category based on the animal data performed with methyl salicylate, itself. All these studies show consistent effects on foetal development.

- Concerning the references listed in this comment and not cited in the CLH report, see response to comment 16.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	France	<confidential>	Company-Downstream user	11

Comment received

We consider that the classification of Methyl Salicylate as Repr. Cat 1B for developmental effects is not justified in the light of the data set available:
- Salicylic Acid (SA) as the biologically relevant metabolite of Methyl Salicylate was

recently evaluated by the RAC (2016) and classified as CMR Repr 2.
- developmental toxicity findings reported are not consistent and derived from subcutaneous route studies which are not relevant, as it circumvents the first path metabolism

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Methyl Salicylate - CLH public consultation comments.pdf

Dossier Submitter's Response

France: Same attachment as that submitted in comment 2. See response to comment 2.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Finland		MemberState	12

Comment received

Human data
Questions regarding causality and potential confounding factors e.g. general maternal health status do not allow for the drawing of conclusions from the data. FI CA notes that the salicylates have a long history of use. Considering the similarity of the compounds and their common hydrolysis product, salicylic acid, it may be of value in terms of robustness to add also the data from the other salicylates to the weight of evidence assessment for developmental effects.

Animal data
Developmental effects are observed in various studies with rats but not with rabbits. Although the two well conducted studies with rats and the well conducted study with rabbits employed a non-physiological route of exposure (namely subcutaneous injection), high plasma concentrations can be expected to be achieved also via the oral route.
With regard to the animal data, the severity and consistency of the observed effects on development substantiate the proposed classification.

An additional publication for your information, in which craniorachischisis and gastroschisis are observed amongst other effects at maternally toxic doses:
Warknay J; and Takacas E. Experimental production of congenital malformations in rats by salicylate poisoning. Amer. J. Path. 1959, 35: 315-331.

FI CA considers the classification of methyl salicylate as toxic to reproduction category 1B (H360D) based on the animal data suitable.

Dossier Submitter's Response

France:

Thank you for your support.

We acknowledge that salicylates (in particular salicylic acid and sodium salicylate) induce similar pattern of developmental effects:

- Salicylic acid induce high fetal mortality, high frequency of complex anomalies (cranioschisis, myeloschisis, pes varus, oligodactyly etc.) and dose-related foetal growth retardation in rats (Tanaka et al., 1973 a&b).

- Similar effects (including decreased viability and body weight, malformations - such as craniorachischisis - and skeletal anomalies) are also reported with sodium salicylate in rats (Fritz et al., 1990; Janice et al., 1986; Keplinger et al., 1974 ; Beyer et al., 1986) and in mice (Beyer et al. 1986)

All these studies are quite old and present limitations. In addition, a specific toxicity of the parent molecule, methyl salicylate, on development cannot be excluded as 21% remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans according to Davison (1961). In addition, a potential toxicity of methanol (the other metabolite) cannot be neither excluded (see also response to comment 4). Overall, in the present case, it is considered more robust to conclude on classification category based on the animal data performed with methyl salicylate, itself. All these studies show consistent effects on foetal development.

Warkany (1959) administered subcutaneously methyl salicylate on the 9th, 10th or 11th day of pregnancy female rats to synthetic methyl salicylate at doses from 0.1 to 0.5 cc. Several methodological limitations can be identified in the study. Thus, it should be considered of low reliability. However, it can also be noted that developmental effects consistent with those already reported in the CLH report with more robust studies were observed, in particular resorption, craniorachischisis and gastroschisis.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Belgium	Procter & Gamble	Company-Downstream user	13

Comment received

DEVELOPMENTAL TOXICITY
(text is also included as attachment)

We conclude that methyl salicylate should not be classified as Repro. Cat 1B, (H360D) for the following reasons:
• Adverse developmental effects of salicylate in rodents are only observed at maternally toxic dose levels
• There are substantial species differences in sensitivity, with rodents being much more sensitive
• Acetylsalicylic acid is being increasingly used as a therapy for pre-eclampsia, and epidemiology indicates no adverse outcomes (in fact, it has beneficial effects) in humans
Accordingly, there is no basis for a Cat 1B classification. Using virtually the same data set, RAC came to the same conclusion for salicylic acid (1).
As noted in other public commentary, methyl salicylate is rapidly metabolized to salicylic acid, as is acetylsalicylic acid (aspirin, ASA); therefore, it is reasonable to consider the data for salicylic acid and aspirin when developing classification decisions for methyl salicylate. The Dossier Submitter considers this to be a reasonable approach as well, in that the methyl salicylate animal data conclusions are supported by ASA data in the CLH dossier, as does the RAC, which used the epidemiology data on ASA in its Opinion for classification of salicylic acid (1).
Animal data
The CLH dossier cites two unpublished studies in rats using methyl salicylate administered via subcutaneous injection. The protocol used in the studies was compliant with

regulatory guidelines in place at the time they were conducted. Although intended to circumvent the possibility of dermal irritation from topical administration, this route of administration almost certainly produces a higher peak concentration than would be possible from topical administration. This difference makes quantitative comparisons between routes difficult. The effects observed included fetal abnormalities and decreased fetal weight at dose levels that were also maternally toxic. Unfortunately, the prenatal developmental toxicity study design does not provide enough information for us to distinguish between maternally-mediated and primary developmental toxicity. In any case, the kinds of adverse effects observed in fetuses and dams were similar to those observed in the rat after administration of salicylic acid or ASA.
The CLH dossier also cites a rabbit study on methyl salicylate but discounts it because the dosing period was shorter than prescribed under current guidelines. This study should be considered, as it followed earlier guidelines and included dosing over the period of organ formation, gestation days 6-18 in the rabbit. This dosing period was appropriate for identifying the potential for methyl salicylate to cause malformations in the rabbit; it did not. Nor did studies on the developmental toxicity of ASA cited in the RAC Opinion on salicylic acid (1). The lack of effect in rabbits is important, as it suggests species differences, with rodents being more sensitive. The relative species sensitivity can be explained by the extent of protein binding of salicylate (2).
On the subject of species differences, there is one non-human primate study on ASA (Wilson et al 1977, TAP 41: 67-78), but the study is too flawed to provide any interpretation about teratogenicity. The biggest flaw is that there was no control group, only treatment groups (two different dosages). With no control, or even any data on historical background rate of malformations in this lab, there is no basis for drawing any conclusions about teratogenicity.

Human Data

ASA has been in clinical use for more than a century, and there is growing use of ASA therapy during pregnancy to prevent pre-eclampsia. The ongoing EAGeR study is investigating the effects of pre- and peri-conceptional aspirin. This is a randomized clinical trial to test whether aspirin treatment, begun prior to conception and continued through pregnancy, improved live birth rate in women with prior pregnancy loss. The trial included more than 1000 women. There were improvements in pregnancy outcome, with no adverse effects noted (3). Randomized clinical trials are the best evidence of effects of therapies, and the lack of any adverse effect in this trial is strong evidence that salicylate is not developmentally toxic in humans.

Aside from the EAGeR study, there are several epidemiology studies of varying power that have been conducted on aspirin use in pregnancy. The Kozer et al. meta-analyses (4 and 5) summarize the vast majority of the literature on aspirin and pregnancy outcome. The CLH dossier cites a meta-analysis by Bard. Although we do not have access to this study, it would have relied on the same data set as Kozer, and the Kozer studies have the advantage of being peer-reviewed. The peer-reviewed meta-analysis confirms a lack of adverse effect of aspirin on human reproduction and development.

The only significant finding in the Kozer (4) meta-analysis was an odds ratio of 2 for gastroschisis. This is not a very compelling odds ratio, especially in a study that makes multiple comparisons, but because it is statistically significant it needs to be addressed. Gastroschisis has been the focus of a lot of epidemiology studies because of an increasing incidence over the past 20-30 years. A number of risk factors have been identified. The most important of these is younger maternal age (6), which mirrors an increase in adolescent pregnancies (7). Multiple partners with shorter co-habitation between partners is also a risk factor for both gastroschisis (8) and pre-eclampsia (9). Thus, there

may be a link between pre-eclampsia and gastroschisis. Since pre-eclampsia (or the risk of pre-eclampsia) is a principal reason for aspirin therapy during pregnancy, this appears to be a case where the effects of the treatment (aspirin) may be confused by the effects of the disease (pre-eclampsia). This is precisely the problem that the EMA representative quoted by the Dossier Submitter warned about in interpreting the epidemiology data. At any rate, this casts even further doubt on aspirin as having any adverse developmental effects in humans.

Consistency with the RAC Opinion on salicylic acid
The RAC Opinion on salicylic acid was based on animal and human data for salicylic acid and ASA and relied heavily on the latter. The Opinion for the hazard classification of salicylic acid is Repro. 2, H361d. A possible classification of Repro 1B was rejected by RAC based on a consideration of the entire data set for ASA and salicylic acid and concluding that the data were not strong enough to support a presumption of human developmental toxicity. The same data are applicable for methyl salicylate, and the same conclusion should be reached.

REFERENCES
1) ECHA (2016). Opinion proposing harmonised classification and labelling at EU level of SA. CLH-O-0000001412-86-110/F, Committee for Risk Assessment (RAC), 10 March 2016
2) Sturman JA, Smith MJ. The binding of salicylate to plasma proteins in different species. J Pharm Pharmacol. 1967 Sep;19 (9):621-3
3) Connell et al., Semin Reprod Med 2017; 35(04): 344-352 DOI: 10.1055/s-0037 - 1606384
4) Kozer, E., Nikfar, S., Costei, A., Boskovic, R., Nulman, I., Koren, G., 2002. Aspirin consumption during the first trimester of pregnancies and congenital anomalies: a metaanalysis. Am. J. Obstet. Gynecol. 187, 1623–1630.
5) Kozer, E., Costei, A.M., Boskovic, R., Nulman, I., Nikfar, S., Koren, G., 2003. Effects of aspirin consumption during pregnancy on pregnancy outcomes: a meta-analysis Birth Defects Res. (Part B) 68, 70–84.
6) Kazaura et al, Increasing Risk of Gastroschisis in Norway: An Age-Period-Cohort Analysis. 2004 Am J Epidemiol 159:358-363
7) Skinner and Hickey Current priorities for adolescent sexual and reproductive health in Australia. 2003 Med J Australia 179:158-161
8) Rittler et al Risk for gastroschisis in primigravidity, length of sexual cohabitation, and change in paternity. 2007 BDRA 79: 483-7
9) Li and Wi Changing Paternity and the Risk of Preeclampsia/Eclampsia in the Subsequent Pregnancy. 2000 Am J Epidemiol 151: 57-62

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments to the CLH report_PG FINAL Version_25 Jan 2019.zip

Dossier Submitter's Response

France:

- Comment related to subcutaneous injection: see response to comment 8. In addition, referring to your comment: "*Unfortunately, the prenatal developmental toxicity study design does not provide enough information for us to distinguish between maternally-mediated and primary developmental toxicity*"; as you highlighted in your comment, the subcutaneous study was performed according recognised guideline; therefore the study design is judged adequate to provide enough information for concluding on developmental toxicity of methyl salicylate.

- Comment related to the rabbit study: see response to comment 10.

- Comment related to the non-human primate study on aspirin (Wilson et al., 1977): please note that this study was described in the RAC opinion (2016) on salicylic acid. Animal data on ASA or other salicylates were not included in the CLH report since more robust data are available with methyl salicylate. In addition, a specific toxicity of the parent molecule, methyl salicylate, on development cannot be excluded as 21% remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans according to Davison (1961). In addition, a potential toxicity of methanol (the other metabolite) cannot be neither excluded (see also response to comment 4). This data raise some uncertainties in particular when using aspirin data. Overall, in the present case, it is considered more robust to conclude on classification category based on the animal data performed with methyl salicylate, itself.

- Comment related to human data with aspirin: see response to comment 10. Please also note that Kozer (2002) is already cited in the CLH report (page 51). This author reported an increase risk of gastroschisis, which is consistent with findings reported with methyl salicylate in rats.

  Reference 6 assessed the link between young paternal and maternal age with occurrence of gastroschisis. Thus, this reference did not assess the link between aspirin use and gastroschisis. This publication can suggest that it would be interesting to take into account parental age as potential confounding factor when assessing the link between aspirin use and risk of gastroschisis. However, it should be emphasized that gastroschisis is clearly reported after methyl salicylate exposure in experimental animals. Therefore, this publication is not relevant to conclude on classification of methyl salicylate.

  Reference 7 is related to current priorities for adolescent sexual and reproductive health in Australia, and thus not related to any salicylate use during pregnancy. Therefore, this publication is not relevant for classification of methyl salicylate.

  Reference 8 cited in your comment evaluate the risk of gastroschisis as a function of primigravidity and / of length of sexual cohabitation and changing partners in multigravid mothers. They identified short cohabitation time (≤ 1 year between the beginning of cohabitation with the current partner and the last menstrual period before pregnancy) at significant risk for gastroschisis. In addition to others biais identified in the publication, differences between group characteristics were noted that can affect the conclusion. Furthermore, it can be noted that women using cyclooxygenase inhibitors (such as aspirin) were excluded from this study since this medication has been identified as risk factors. This publication can suggest that it would be interesting to take into account cohabitation time as potential confounding factor when assessing the link between aspirin use and risk of gastroschisis. However, it should be emphasized that gastroschisis is clearly reported after methyl salicylate exposure in experimental animals. Therefore, this publication is not relevant to conclude on classification of methyl salicylate.

  Reference 9 cited in your comment assessed the link between changing paternity and the risk of pre-eclampsia/eclampsia in the subsequent pregnancy. Therefore, it is not relevant to conclude on classification of methyl salicylate.

- Comment related to consistency with RAC opinion on salicylic acid: Salicylic acid has been classified as Repr. 2 - H361d by the RAC based on animal data with different salicylates (salicylic acid, aspirin and sodium salicylate) and human data with aspirin. Developmental data with methyl salicylate were not assessed by the

RAC to conclude on the classification of salicylic acid. Even if it is acknowledged that salicylates induce similar pattern of developmental effects, classifying methyl salicylate on the basis of the classification of salicylic acid raises uncertainties (linked to kinetics and potency), in particular when considering that adequate data are available with methyl salicylate. For example, a specific toxicity of the parent molecule, methyl salicylate, on development cannot be excluded as 21% remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans according to Davison (1961). In addition, a potential toxicity of methanol (the other metabolite) cannot be neither excluded (see also response to comment 4). Therefore, in the present case, it is considered more robust to conclude on classification category based on the animal data performed with methyl salicylate, itself. All these studies show consistent effects on foetal development. Epidemiological data on aspirin are judged inadequate to conclude on developmental toxicity of salicylates. Thus, these data should not be used to conclude to a lack of effect (especially since there are some publications reporting developmental effects consistent with experimental data) or to disregard the developmental effects reported in animals with methyl salicylate. Overall, the proposed classification is based on animal data performed with methyl salicylate.

- Other references listed in the comment and not cited in the CLH report: see response to comment 16.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Germany		MemberState	14

Comment received

The classification Repro 1B, H360D for effects on development was proposed for Methyl Salicylate (MeS). This proposal is mainly based on developmental effects (skeletal and visceral anomalies and lower foetal bw) observed in rats after exposure to MeS by subcutaneous administration (FDA, 2006 c, d). In the FDA-study (2006 c) administering dose levels of 0, 50, 100 and 200 mg/kg bw/d on GD 6 – 17, cranio(rachi)schisis, the most relevant effect for classification purposes, was observed in 8 fetuses from 3 litters and gastroschisis in 1 fetus in the high dose group. We agree with the interpretation of the dossier submitter (DS) that lower bw gain (GD 7-20) and lower body weight (< 5 %, on GD 7-13) in the dams is unlikely to have caused the anomalies. The body weight loss in dams on GD 7 and 8 was associated with lower food consumption (-25.7 % and -18.2 %). The reduction in food consumption, however, does not fully explain the strong effect on body weight (body weight gain remained > 30 % lower during the first half of gestation and > 10 % in the remaining days until GD 20). While some uncertainties remain whether the marked effect on body weight gain and even loss of body weight in dams during the first half of gestation are related to the anomalies, the conclusion is supported by a rather low extent (≤ 4.2 %) of the overall (significant) reduction in body weight on GD 7-13. If available, data on corrected dam weights are helpful to clarify the contribution of maternal toxicity and those of the reduced foetal weight. Lower foetal body weight may be secondary to the lower body weight gain in dams.
Cranio(rachi)schisis was consistently observed in a second FDA study on rats (FDA, 2006 d), 4 out of 7 stillborns in the highest dose group (200 mg/kg bw/d, GD6-Lact Day21) showed cranio(rachi)schisis. (Data on the number of affected litters and historical control data may be useful, if available.) The mentioned malformations could not solely be explained by the lower maternal body weight (-4.6 % on GD 20) and body weight gain (-15.7 % on GD20) in comparison to controls.

The effects indicating fetal toxicity are considered as supporting (adverse) effects of less clear nature. The lower foetal body weight in male newborns may be, at least partly, a consequence of lower body weight gain and lower food consumption in dams.
In addition to the acquisition time for 100 % of pups for the differentiation parameters (which indicated a dose-related effect only for the balanopreputial separation) mean values could be more informative. The rate of stillborns was elevated at 200 mg/kg bw/d. This has to be interpreted in the context of 2 mortalities in dams at this dose.

The subcutaneous application is generally considered of minor relevance as this route is not relevant for humans exposed. In the particular case, the subcutaneous route of administration is considered as relevant as the oral. For MeS data following subcutaneous application should be considered as predictive for the potential of developmental toxicity since the absorption rate for the oral route was 100 % (however, remaining differences in metabolism due to first pass effects for the oral route have to be kept in mind).

In terms of a common metabolite read-across to data obtained from Salicylic acid (SA) is considered justified. MeS is hydrolysed to SA in rats and humans (Davison et al., 1961). Cranioschisis is also a malformation observed in rats orally treated with SA (see RAC Opinion on SA, 2016). Also, gastroschisis and ventricular septal defects were observed in rat studies on MeS (subcutaneous) as well as in studies performed with SA (oral) at comparable doses. These observations may point towards SA as a common toxic agent acting irrespective of the administration route.

However, for MeS further aspects have to be taken into consideration:
(1) Upon cleavage/hydrolysis of MeS, methyl alcohol (methanol) is also produced in addition to SA. It is well known that methyl alcohol causes adverse effects (e.g. lethality, neurotoxicity/blindness) in humans. Methyl alcohol is classified as Flam Liq 2, H225; Acute Tox 3*, H301; Acute Tox 3*, H311; Acute Tox 3*, H331 and STOT SE 1, H370**. Its contribution to the MeS toxicity is unknown.
(2) Differences in toxicokinetics between MeS and o-acetyl salicylic acid (ASA) have to be considered. It might be possible that intact (i.e. non-hydrolysed) MeS could be distributed (at least to some extent) in its parent form to possible target organs (e.g. ovaries, liver or kidneys) to act there either in its parent form or to liberate Methanol and SA in the target organ(s). If MeS is hydrolysed in target organs to SA and methyl alcohol, the hazard potential of the alcohol will be higher than by oral administration. We kindly ask the dossier submitter to deliver information on all potential metabolites in future dossiers. Davison et al. (1961) showed that after oral ASA administration the total plasma salicylate concentration of human volunteers clearly exceeded those obtained with MeS. Furthermore, the hydrolysis of MeS to free salicylate was slower and less complete after MeS administration. While MeS hydrolyses within 60 minutes to free salicylate in rats and was distributed to several organs (RAC Opinion on SA, 2016), in man 21 % of MeS remained unhydrolysed after 90 minutes.
(3) We consider the effects caused by SA are pretty similar to those observed by administration of MeS, especially the external anomalies (e.g. cranioschisis). SA is classified as Repr. 2 based on animal data and taking into account the human data obtained from read-across to SA from ASA. Based on read across to SA alone MeS should at least be classified as Repr. 2. In its opinion on SA RAC argued that human data were inconclusive (no clear evidence of malformations in humans in few reported cases at higher doses). No robust human data are available for MeS.
The animal data on MeS alone on the other hand could justify classification as Repr. 1B. The fact that SA is a common hydrolysis product of ASA and MeS and the observation of similar malformations (mainly cranioschisis) supports the classification as reprotoxicant. In addition it cannot be excluded that in humans methanol, especially produced by hydrolysis in target organs, may act as neurodevelopmental toxicant and/or that parent

MeS itself might also exert toxic effects. Due to the differences in metabolism, developmental studies on MeS in animals are unlikely to cover this concern from methanol.
Taken together the available animal and toxicokinetic data deliver clear evidence of developmental effects independent of the route of administration. The similarity of teratogenic effects of MeS and SA, the lack of human data on MeS, the generation of methanol as an additional hydrolysis product and the toxicokinetic differences (possibility of distribution of intact parent MeS into target tissues and toxic action of either parent and/or hydrolysis products SA and Methanol in the target tissue) point to different and possibly additional effects of MeS in humans when compared to SA.
In case the same classification strategy is applied as for SA, MeS would need to be classified as Repr. 2, H361d. In case not, a harmonised classification as Repr. 1B, H 360D has to be discussed.
It is recommended to consider potency and SCL setting.

Dossier Submitter's Response

France:

Thank your for you support for considering the anomalies not secondary to maternal toxicity.

FDA (2006c) study: Unfortunately, corrected dam weights are not available in the report to clarify the contribution of maternal toxicity and those of the reduced foetal weight. See also response to comment 10.

FDA (2006d) study:

- Regarding craniorachischisis reported in FDA (2006d), as written in the CLH report: "*craniorachischisis was noted in 4 stillborns (among 6 stillborns reported in 4 females; there is no indication in how many litters craniorachischisis occurred) in the 200 mg/kg/day group*". In addition, during necropsy of one dead dam on GD22, craniorachischisis was noted in 2/14 of the dead fetuses. No further information is available. No historical control data is provided in the study report. However, according to historical control data for development and reproductive toxicity studies using the Crl:CD®BR rat compiled by MARTA (1993), craniorachischisis are rarely observed in rats: fetal incidence of 7 among a total of 88270 foetuses considered.

- Further information on differentiation parameters:
  - cleavage of the balanopreputial gland:
    - on PND 46: 20/20 in control; 19/20 at 20 mg/kg bw/day; 16/20 at 60 mg/kg bw/day and 12/18 at 200 mg/kg bw/day.
    - 100% reached on day 47 at 20 mg/kg bw/day; on day 61 at 60 mg/kg bw/day and on day 72 at 200 mg/kg bw/day.
  - Incisor eruption:
    - on PND 12: 81/81 in control; 78/79 at 20 mg/kg bw/day; 80/81 at 60 mg/kg bw/day and 35/62 at 200 mg/kg bw/day.
    - 100% reached on day 13 at 20 and 60 mg/kg bw/day and on day 16 at 200 mg/kg bw/day.
  - Eyelid separation:
    - on PND 15: 81/81 in control; 73/79 at 20 mg/kg bw/day; 81/81 at 60 mg/kg bw/day and 53/62 at 200 mg/kg bw/day.
    - 100% reached on day 16 at 20 mg/kg bw/day and on day 17 at 200 mg/kg bw/day.

- The 2 died dams were not included in the calculation of stillborns.

See also response to comments 8 and 10.

Thank you for your support for considering the subcutaneous route of administration as relevant as oral route. See also response to comment 8.

Regarding read-across to data obtained from salicylic acid and further metabolism consideration: please note that the proposed classification is mainly based on animal data with methyl salicylate and not on read-across with other salicylates.

Methyl salicylate is hydrolyzed into salicylic acid and methanol. Other salicylates, such as salicylic acid and sodium salicylate, present the same pattern of developmental toxicity, suggesting that these effects are likely related to an effect of methyl salicylate and/or its metabolite, salicylic acid. Methanol also presents developmental effect at high exposure levels, that may be not reached after exposure to methyl salicylate. However, a specific toxicity of methanol cannot be excluded neither. As stated in your comment, it is possible that methyl salicylate, as parent molecule, reaches target organ (based on Davison (1961) study showing that in man 21% of methyl salicylate remains unhydrolized after 90 minutes). This suggest a possible additional effect of methyl salicylate when compared to salicylic acid. See also response to comment 4.

We agree that some differences in toxicokinetics between methyl salicylate and aspirin should be considered. In addition, aspirin and methyl salicylate present some differences in prostaglandin inhibition. Biological differences can also be suspected since their commercial uses are quite different (even if methyl salicylate is used topically for its anti-inflammatory properties, its major uses are in fragrance compounds, that is not the case for aspirin). These elements suggest that direct extrapolation from aspirin to methyl salicylate without other consideration is hazardous since a role of parental substance in developmental effect cannot be excluded. Overall, we would like to highlight that the proposed classifications are actually based on methyl salicylate data, which are sufficiently robust to conclude to a clear evidence of teratogenic effect. Human data with aspirin are presented in the absence of such data with methyl salicylate and in order to provide some information on the developmental toxicity of salicylates (in particular salicylic acid) in humans. However, the human data with aspirin have been judged as inadequate to conclude on teratogenic effects of salicylates in humans. Thus, these data should not be used to conclude to a lack of effect (especially since there are some publications reporting developmental effects consistent with experimental data) or to disregard the developmental effects reported in animals with methyl salicylate.

In conclusion, it is considered more robust to conclude on classification category based on actual experimental data with methyl salicylate. These data provide clear evidence of developmental toxicity.

See also responses to comments 4, 12 and 16.

SCL setting: ED10 has been calculated

- from FDA (2006c) study for % external anomalies, craniorachischisis and % visceral anomalies
- from FDA (2006d) study for % birth index and skeletal anomalies
- from NTP (1984b) study for litter/pair, average live pups per litter and proportion of pups born alive

Effect	ED10
Study FDA 2006c	

% external anomalies	422.74
% craniorachischisis	449.65
% visceral anomalies	274.45
Study FDA 2006d	
% birth index	170.56
% skeletal anomalies	143.09
Study NTP 1984b	
litter per pair	564.29
average live pups per litter	169.31
proportion of pups born alive	685.00

The ED10 were between 143 and 685 mg/kg bw/day. The lowest ED10 was estimated for % skeletal anomalies from FDA (2006d) study. It should also be noted that craniorachischisis and gastroschisis are both rare malformations. Thus derivation of an ED10 is maybe not appropriate, with possible underestimation of the hazard. However, since different ED10 were between 4-400 mg/kg bw/day, methyl salicylate is considered of medium potency and thus GCL applies.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2018	United Kingdom	<confidential>	Company-Downstream user	15

Comment received

For the endpoint of reproductive toxicity, classification of methyl salicylate (MeS) as a; Repr. 2; H361d (Suspected of damaging the unborn child) is justified. Therefore it follows that the proposed classification by the French MSCA as a; Repr. 1B, H360d, (Suspected of damaging the unborn child) is not justified.
The justification for a classification of Repr. 2; H361d (Suspected of damaging the unborn child) is based on the RAC opinion on Salicylic acid (RAC, 2016). Details in that opinion state that for toxicokinetics and read-across that the class of salicylic compounds has been widely studied. To overcome the information gaps, the Dossier Submitters (DS) performed read across to and from MeS and salicylic acid from; sodium salicylate (NaS), and o-acetyl salicylic acid (ASA).
The toxicokinetic data in the opinion states that upon oral administration in rats, salicylic acid, MeS, NaS and ASA are all rapidly absorbed even at high concentrations. A publication by Davison (1961) has compared the oral absorption and metabolism of MeS and NaS in rats and humans with that of ASA. Several publications demonstrated that salicylic acid is the initial metabolite (hydrolysis product) for the related salicylates: ASA, NaS, MeS. Salicylic acid is found in blood both bound to plasma albumin and as the unbound (free) moiety. Plasma analyses in rats showed rapid hydrolysis to free salicylate for MeS, NaS and ASA, resulting in comparable plasma concentrations of salicylate at 60 minutes post dosing. However, in humans hydrolysis of MeS to salicylic acid was slower and less complete. The publications of Rainsford et al. (1980) and Tjalve et al. (1973) revealed that salicylic acid was found in the stomach, liver, kidney, lungs, bone marrow, intestine, inflamed paws and spleen of rats; the in vivo distribution of ASA and the methyl ester of ASA (AME) were very similarly to that observed with salicylic acid. Tjalve et al. (1973) confirmed that there was no difference between the distribution of salicylic acid compared to ASA in mice after injection. In mice, rats, monkeys and humans, salicylic acid was found in the placenta and readily passed into the foetus. Studies reported by Emudianughe (1988) and McMahon et al. (1989), both performed on rats, demonstrated that salicylic acid is metabolised to two major urinary metabolites, salicyluric acid and

salicyl-glucuronic acid and oxidative metabolites (2,3- and 2,5 -dihydroxybenzoic acid) and other conjugated salicylic acid compounds (salicyl ester glucuronide or salicyl ether glucuronide). All these metabolites as well as unchanged salicylic acid are eliminated almost entirely via the urine.
The opinion summarises that, 'the toxicokinetics of salicylic acid and the selected salicylates indicate that following absorption, the initial metabolic step for MeS, NaS and ASA is hydrolysis to free salicylate. Since salicylate is the principal species circulating in plasma at comparable concentrations, it follows that data from the selected salicylates are acceptable for read across to salicylic acid.'
Following that conclusion, it is therefore reasonable to conclude that read across to MeS from; salicylic acid, sodium salicylate (NaS), and o-acetyl salicylic acid (ASA) is also scientifically justified

The RAC assessment concludes on reproductive toxicity for salicylic acid.
Effects on sexual function and fertility
'RAC concludes that there is insufficient evidence that salicylic acid exhibits adverse effects on sexual function and fertility. Consequently, for this endpoint RAC supports the proposal of the DS and concludes that no classification for salicylic acid for adverse effects on sexual function and fertility is justified
Effects on development
In the assessment and comparison with the criteria for the development of the offspring endpoint, RAC took into consideration the following:
• There is robust evidence of developmental effects in animals which justifies classification. In animals, the developmental toxicity was clearly shown in two out of three species. The pattern and magnitude of the effects shown in rats but also in monkeys are sufficient to presume that salicylic acid is a developmental toxicant and to justify classification in Category 1B;
• According to experts in the field of pharmaceuticals, ASA is not considered as being a major teratogen, but may have some potential for teratogenic effects, and it should be noted that prostaglandin inhibitors in general, including ASA, could have other adverse effects on foetuses, especially on their renal development and during the third trimester on the development of the circulatory system;
• However, neither ASA nor salicylic acid are proven human developmental toxicants. There is a lack of evidence to support an increased risk of birth defects following exposure to ASA. Also, the evidence for other developmental effects has uncertainties. Taking that into account, classification in Category 1A is not justified.
• Although the information on effects of ASA on development in humans at "high doses" is marginal, it should be acknowledged and cannot be discarded when discussing classification in Category 1B versus Category 2.
• It is noted that the available human epidemiological data on ASA was rather contradictory and with only a few reported exposures at higher doses, nevertheless demonstrated no clear evidence of malformations in humans. Hence, the RAC concluded that Category 1B may not be justified.
'Taking into account the available data, including pharmacokinetics, in vitro tests with ASA and salicylic acid, developmental studies in animals (positive findings in rat and monkey studies and a negative rabbit study), human epidemiology and medical experience, the RAC considered classification of salicylic acid as Repr. 2; H361d (Suspected of damaging the unborn child) to be justified'
As MeS and salicylic acid have been used extensively as read across to each other, and given the toxicokinetic data has shown the metabolic pathways are identical for the substances, classification of MeS as a Repr. 2; H361d (Suspected of damaging the unborn child) is also justified.
References
Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and

labelling at EU level of Salicylic acid, EC Number: 200-712-3, CAS Number: 69-72-7, CLH-O-0000001412-86-110/F, Adopted 10 March 2016

ECHA note – An attachment was submitted with the comment above. Refer to public attachment clh_opinion_salicylic_acid_6425_en.pdf

Dossier Submitter's Response

France:

Comment related to consistency with RAC opinion on salicylic acid: Salicylic acid has been classified as Repr. 2 - H361d by the RAC based on animal data with different salicylates (salicylic acid, aspirin and sodium salicylate) and human data with aspirin. Developmental data with methyl salicylate were not assessed by the RAC to conclude on the classification of salicylic acid. Even if it is acknowledged that salicylates induce similar pattern of developmental effects, classifying methyl salicylate on the basis of the classification of salicylic acid or aspirin raises uncertainties (linked to kinetics and potency), in particular when considering that adequate data are available with methyl salicylate. For example, a specific toxicity of the parent molecule, methyl salicylate, on development cannot be excluded as 21% remained unhydrolyzed at 90 minutes (no information after 90 minutes) in humans according to Davison (1961). In addition, a potential toxicity of methanol (the other metabolite) cannot be neither excluded (see also response to comment 4). Therefore, in the present case, it is considered more robust to conclude on classification category based on the animal data performed with methyl salicylate, itself. All these studies show consistent effects on foetal development. Epidemiological data on aspirin are judged inadequate to conclude on developmental toxicity of salicylates. Thus, these data should not be used to conclude to a lack of effect (especially since there are some publications reporting developmental effects consistent with experimental data) or to disregard the developmental effects reported in animals with methyl salicylate. Overall, the proposed classification is based on animal data performed with methyl salicylate.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	France	NOVACYL	Company-Manufacturer	16

Comment received

Reproductive/developmental toxicity
The comments to the following endpoint have been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company.
A classification as Repr. Cat 1B for developmental effects for Methyl Salicylate (MeS) is neither warranted nor justified in the light of the overall data set.
• In rats after subcutaneous application of MeS developmental effects were observed only in the presence of excessive signs of maternal toxicity both in the Embryo-Fetal Development (EFD) and in a Post-Natal Development (PND) study (FDA 2006c, FDA 2006d) including lethality and severe body weight reduction (instead of gain) during the sensitive gestation period in dams. At less toxic doses no clear evidence for developmental toxicity was noted.
• In a hamster study of poor scientific quality and documentation, adverse effects were only reported at exaggerated doses of MeS well beyond any limit dose recommended for OECD studies that caused massive toxicity.
• Salicylic Acid (SA) as the biologically relevant metabolite of MeS was recently evaluated by the RAC (2016) and classified as CMR Repr 2.

• Epidemiological data on Acetyl Salicylic Acid (ASA) which is used for read across for SA and MeS demonstrate a lack of evidence of an increased risk of birth defects following exposure to ASA.

General comments:
Before commenting in detail on this point, we would like to mention a few key findings also stated in the CLH report with regard to kinetics and metabolism important for the overall assessment.
Based on kinetic data, MeS is rapidly absorbed when administered orally in humans and in rats and very quickly hydrolyzed to SA.
However, there is a marked species-dependence in the binding of salicylate to serum proteins, with high binding in man, rhesus monkey, rabbit and guinea pig, while several other species, including the rat, mouse and dog, have much lower binding (1,2).
For skin penetration also very high absorption of up to 100% is reported for rodents whereas controversial findings are reported from low percentages up to around 50% for human skin. It is well known that rat skin is more permeable to substances than human skin (mean difference 10.9 fold) (3). The value of 93 % for human considered and mentioned several times in the CLH report appears not to be realistic as most of the substance was absorbed into the epidermis and did therefore not cross the skin, thereby significantly reducing the overall bioavailability. Therefore and based on the generally lower skin absorption of human skin compared to rodent skin, the RIFM range of 2-43% also cited in the CLH report seems to better reflect the reality.
As noted for oral administration the substance is quickly hydrolyzed and converted into salicylic acid (SA) and methanol (4). Thus, the biologically relevant and systemically available structure is SA. Davison et al. (5) conducted a study to compare the toxicity and the metabolism of MeS and SA and concluded that the results on the toxicity and metabolism of both MeS and SA are comparable across multiple species (rats, dogs, and monkeys). We therefore strongly consider the most recent assessment of SA by the RAC (6) as most relevant also for MeS.

Effect on sexual function and fertility
We agree with the argumentation in the CLH report, that the data available allow a scientific valid assessment and does not warrant a classification with regard to effects on sexual function and fertility. This assessment is also in line with a recent evaluation by RAC for the relevant metabolite SA. For this compound the RAC concluded in 2016: "there is insufficient evidence that SA exhibits adverse effects on sexual function and fertility. Consequently, for this endpoint RAC supports the proposal of the DS and concludes that no classification for SA for adverse effects on sexual function and fertility is justified".

Developmental toxicity Proposal Repr.1B (H360D):
We do not agree with the conclusion drawn in the CLH report, that MeS should be classified as a developmental toxic substance Repr. Cat 1B.
In the CLH report, two argumentation lines are used to justify this proposal for MeS. One are the results obtained in two rat studies using subcutaneous administration, which are only available via secondary source (FDA 2006c and mainly FDA 2006d) together with evidence from studies of low quality and a read across to the findings with ASA) which is used as a drug and for which human data are at hand. The read across to ASA is justified by the fast hydrolysis of both substances to SA. It should be mentioned here, that the biologically relevant substance (SA) was recently evaluated by the RAC and a classification as Repr. Cat 2 (for developmental/reproductive toxic effects) was deduced after a comprehensive and detailed assessment on both the animal findings and the human data for ASA.
In the following we would like to comment on the two arguments provided in the CLH

report separately.
Animal data
Studies in rats (subcutaneous application)
Before addressing details of the studies being considered key for the conclusion that Repr. 1B is warranted we would like to emphasize that we do not have full access to these studies. We assume that also ECHA does not have access to these studies as FDA as second source is cited in the CLH report. Therefore, we do not agree to consider these studies as Klimisch 1, since many details which are relevant for the final assessment are missing (see below).

Relevance of the route of administration
The results of these two studies are not an appropriate basis for the hazard classification under the CLP regulation due to the use of subcutaneous injection as route of exposure. In case of a classification of chemicals, the oral exposure is the most relevant route as stated in the OECD guideline 414 and data via this route is available for the biologically relevant hydrolysis product SA. The direct "systemic" application via the subcutaneous (s.c.) route circumvents the enterohepatic pathway/circulation, which has been shown to eliminate the major amount of the mother-compound via metabolism (7). Consequently, after s.c. administration the blood concentration is significantly higher than following oral application. Also after dermal application (the most relevant exposure route after oral exposure), the mechanical as well as the metabolic "barrier" of the skin (fast hydrolysis) will significantly reduce the bioavailability. Thus, the external doses between s.c. and oral studies cannot be compared due to the kinetic and metabolic differences. Consequently, the results derived from the two rat studies using s.c. application can be considered of limited relevance due to an increased systemic exposure compared to oral (7) and topical (8) application routes and are not an appropriate basis for the hazard classification under the CLP regulation.
In addition, substances given subcutaneously should be non-irritating. However, in these two s.c. studies MeS was used at 100% concentration which is clearly an irritating concentration (9, 10). Thus, the results were clearly distorted and are not considered appropriate for the hazard classification under the CLP regulation.
Our interpretation of the findings of those studies differs from the CLH report. Assessing both studies, the data supports a NOAEL of 100 mg/kg for reproductive/developmental toxicity and not < 60 mg/kg. There is no evidence of treatment-related skeletal abnormalities up to 100 mg/kg in the Embryo-Fetal Development (EFD) study which is a study type specifically designed to evaluate this (FDA 2006c). The occurrence of skeletal abnormalities at 60 mg/kg in the Post-Natal Development (PND) study (FDA 2006d) is not consistent with the absence of those findings in the EFD study with dosing up to 100 mg/kg during the complete period of organogenesis (see also below). The formation of ribs is completed during organogenesis. Thus, it is not plausible why additional abnormalities of the ribs should occur at a lower dose in the PND study compared to the EFD study. It is thus highly unlikely that the skeletal abnormalities observed at 60 mg/kg in the PND study are test article related based on the lack of any statistical significance or a clear dose response relationship for these skeletal findings. Therefore, the NOAEL for reproductive toxicity should be established at 100 mg/kg.
The developmental toxicity findings at 200 mg/kg bw noted in the FDA2006d study are considered to be secondary to maternal toxicity since they occurred at clearly maternally toxic doses which induced not only a significant reduction in body weight gain and food consumption but also lethality in 2 dams. We clearly disagree with the interpretation regarding this study in the CLH report: "Although some of the developmental effects (such as skeletal variation, decreased body weight, delay in post-natal differentiation indices) may be secondary to maternal toxicity, it is not possible to explain the other effects such as offspring lethality and external/skeletal anomalies by the observed maternal toxicity (FDA, 2006d)". From our point of view the observations noted in the

dams classify the effects noted in the foetuses as highly likely to be secondary due to massive maternal toxicity. In such cases according to the CLP (Annex 1: 3.7.2.4.3 and 3.7.2.4.4) either a classification as Cat. 2 or even no classification should be considered. Especially point 3.7.2.4.4 states, that in case of lethality for more than 10 % of the dams in a given dose group effects in the offspring should normally not be taken into account for C&L purposes.
A similar picture for the EFD study was noted at 200 mg/kg bw/day. In the CLH report it is cited "All these effects occurred at concentrations including slight toxicity in dams. However, considering the severity of the observed external malformations and visceral anomalies, these effects cannot be secondary to the slight maternal toxicity".
However, in this study significant decrease in body weight gain vs. control was noted during the entire treatment period in that group (314%, 155.2%, 89%, 68%, 48%, 39%, 36%, 24%, 21% 17%, 12%, 10% and 12% on Days 7-20, respectively) and even body weight loss on Days 7 and 8 causing significant malnutrition to the offspring. In addition, the reduction in body weight gain returned only to below 20% of the control once the dosing period was completed, which clearly demonstrates that the test article caused massive maternal toxicity. Thus the effects noted in the offspring are considered to be a result of this malnutrition and the bad health status of the dams.
In addition, there are also some limitations of the reports (as far as accessible) that confound interpretation of the results especially for the group of 200 mg/kg bw/day for which massive maternal toxicity was noted
• Data are reported on a fetus basis and not a litter basis for the skeletal abnormalities in the PND and EFD study. Therefore it is not possible to establish whether the findings are spread out across litters or in a single litter, which might be explained by a bad health status of a single dam. This is of key importance as severe toxicity up to lethality was noted already for two dams.
• Body weight gain data do not fully capture the potential maternal toxicity because the individual maternal carcass and fetal weights have not been provided and therefore the body weight gain differences relative to the control may be underestimated.
It should also be noted that for the same time windows of exposure, significant difference with regard to maternal toxicity was observed in the FDA 2006d compared to the FDA 2006c study.
Indeed, in the EFD study (FDA 2006c) in the same rat strain specifically designed to investigate the effects of concern, different and significantly less pronounced effects indicative for developmental toxicity (no statistical differences noted between control and treated group for any external anomaly) were noted although the same dose was applied during the critical time window (see also above).
Nevertheless, effects/variations noted in this study at 200 mg/kg bw/day can be explained by maternal toxicity and consequently malnutrition of the offspring (see above). This can result in a delayed ossification too and might so explain some of the skeletal variations seen.
No final evaluation of these two s.c. studies is possible without details about the potential local effects and the resulting stress to the animals. Substances injected subcutaneously should be non-irritating. However, in these two s.c. studies MeS was used at clearly irritating concentration.

Study in rabbits:
We do not agree with the conclusion that the rabbit study (FDA 2006b) is not valid and does not allow a robust and scientifically sound assessment for this species. The validity and the usefulness of the study was not challenged by FDA according to our knowledge. The CLH report considered the exposure period from day 6-18 as of limited relevance, because the gestation period in the rabbit is longer (30-32 days (11). Therefore, it was concluded, that the negative results (no teratogenic effects detected) were questionable due to a too short exposure time to MeS. We disagree and emphasize that the exposure

period in rabbits was sufficiently relevant; according to the OECD GL 414, the test substance was administered during the sensitive period of organogenesis (GD 6-18 in rabbits as it was done in the rat study (GD 6-17).
In addition, the described malformations in the CLH report, such as craniorachischisis, gastrochisis, or ventricular septal defects are clearly originating from the organogenesis phase, which is completely covered in the rabbit between gestation days 6-18 (12). A prolonged exposure period may only have demonstrated potential effects on the growth of the fetus but not any additional developmental abnormalities. Again, the dosing period of GD 6-18 in rabbits is internationally accepted as covering the period of organogenesis as stated in both the ICH S5 and the OECD 414 guidelines.
The lack of malformations in the rabbit rather indicates a difference in species sensitivity between the rabbit and rat, most probably due to species differences in plasma levels/kinetics. Similar species differences have been observed for the relevant metabolite SA (see above).

Study in Hamsters and other evidence
In the CLH report a hamster study is used as additional evidence for developmental toxicity supporting Repr. Cat 1B, although considered of low quality already in the CLH report.
MeS was administered to pregnant LVG hamsters on day 7 (topically) or day 9 (orally) at exaggerated doses far beyond any limit dose recommend for OECD studies, i.e. topically at 3500 and 5250 mg/kg bw, orally at 1750 mg/kg bw (well above the reported oral LD50 in guinea pigs).
We consider the hamster study of poor scientific quality and not relevant for classification and labelling as the doses tested very likely cause massive toxicity such as high pup lethality, also confirmed by the high plasma SA levels in the animals that reached a peak of 125 mg/100 ml at about 2 hours after oral treatment and were well above the toxic level in humans or rats. Moreover, important information is lacking such as clinical signs in the dams as well as historical controls for the effects seen in the pups.
The study is therefore not suitable for any conclusion with regard to classification and labelling.
Moreover, in the CLH report on page 46 the studies Collins et al. (1971) and Anonymous (1978a and 1978b) assessed as Klimisch 3 ("not reliable") were used to support the Repr. Cat 1B classification. Whereas a one generation feeding study in rats evaluated Klimisch 4, which did not show any abnormalities at dose levels of 200 and 300 mg/kg bw day, was disregarded. Klimisch score 3 and even more score 4 data can in fact be used as supporting evidence, but then all studies have to be used as part of an appropriate weight of evidence approach including studies with negative results.

2.3. Human data
Pharmacovigilance data provides no evidence of adverse effects from MeS in humans, although MeS is used since decades as a pharmaceutical substance for local treatment.
The CLH report states that there are some indications of developmental effects from human data with ASA, which can support the relevance of the observed effects in experimental studies to humans. The CLH report for MeS refers to "well-known" malformations caused by ASA as the second major argument for classification of MeS as Repr.1B.
Before addressing in detail the conclusions and findings, it should be mentioned that both MeS and ASA undergo extensive hydrolysis to SA but behave differently with regard to prostaglandin inhibition (more precisely inhibition of COX-1 and COX-2). This mechanism of action of ASA requires the transfer and covalent binding of the acetyl group of ASA to the amino acid Serine in the catalytic center of the enzyme. This mechanism of action is however not possible for MeS. Thus data/findings in humans linked to this mechanism of action cannot be transferred 1:1 to the assessment of MeS.

The data from ASA can however be used to assess the relevant biological metabolite for both substances, SA.
In addition, the data set available for ASA has been recently assessed by the RAC in the context of the CLH report for SA (13) emphasizing that data from ASA and MeS were acceptable for read across to SA. Taking into account the available human epidemio¬logical data on ASA which show a lack of evidence to support an increased risk of birth defects following exposure to ASA, the RAC considered classification of SA as Repr. 2 to be justified based on the findings noted in animals.
The current report does not contain any new information/data suggesting any change for the conclusion (see below).
We would like to first summarize the conclusion by RAC for which the Bard publications (2012, 2015) are key:

"The assessment of "Low doses" in pregnancy
The "low doses" in pregnancy are those referred to in the table above as being indicated for "prevention of multiple miscarriage, pregnancy-induced hypertension and other complications of pregnancy". The (retrospective) cohort study performed by Bard (2012) was provided for this dose range. To further extend the analysis, the DS submitted an additional critical review by the same author (Bard 2015).
The aim of the study and the analysis was to address the effects of ASA within this dose range on the following endpoints: maternal bleeding, neonatal haemostatic abnormalities, pregnancy duration and labour, prevention of pre-eclampsia and intra-uterine foetal growth retardation, stillbirths and infant mortality, birth weight, birth defects and early childhood development. Particular aspects that raised concern were also analysed; the premature closure of Ductus arterious, the occurrence of gastroschisis and congenital cryptorchidism.
As a final conclusion of the study it was stated that: "no adverse effect of aspirin treatment can be considered as established, either at low (<150 mg daily) or higher, usual dose". To further illustrate the overall conclusion with respect to dosages higher than that mentioned above, three epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) were cited; the conclusion was that the use of aspirin at up to the maximum recommended therapeutic dose of 4000 mg/d (equivalent to 66.7 mg/kg bw/d as ASA, or 51 mg/kg bw/d as SA) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay."
This conclusion is well in line with our interpretation of the data and is supported by new publications (see below). ASA is used broadly for the treatment and prevention of gestational complications as e.g. pre-eclampsia. Furthermore, there are numerous studies available that demonstrate the safe use of low-dose ASA also during the first trimester of pregnancy, that is the phase of blastogenesis and organogenesis (in humans: blastogenesis: day 1-16; embryogenesis (organogenesis): day 16-60; fetogenesis: day 61 - onwards) (14-18). This is supported by obstetricians' and gynecologists societies from several countries recommending the use of ASA in women with e.g. pre-eclampsia. The use of ASA during pregnancy up to the maximum recommended therapeutic dose of 4000 mg/day or 66.7 mg/kg using 60 kg body weight (bw) has demonstrated no evidence of an increased risk of adverse pregnancy outcomes in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay (19, 20, 21). A further meta-analysis of studies on the use of therapeutic low-dose ASA at 50–150 mg/day (22) has demonstrated that this dose range is not associated with any adverse pregnancy outcome, in terms of perinatal mortality, birth complications, congenital malformations or adverse effect on subsequent development. For pregnancies with a moderate or high risk of pre-eclampsia and/or premature delivery, the adverse pregnancy outcome rate was reduced with therapeutic low dose ASA (50–150 mg/day) and there was no increased risk of early miscarriage with this dose regime (23). These studies strongly support that

neither ASA nor its principal metabolite SA induce adverse developmental effects up to the maximum recommended therapeutic dose in humans, i.e. 4000 mg/day (66.7 mg/kg for a 60 kg person, equivalent to 51 mg/kg SA).

In addition the results of the ongoing EAGeR study, which is investigating the effects of pre- and peri-conceptional aspirin also comes to this conclusion. This randomized clinical trial is performed to test whether aspirin treatment, begun prior to conception and continued through pregnancy, improved live birth rate in women with prior pregnancy loss. The trial included more than 1000 women. There were improvements in pregnancy outcome, with no adverse effects noted (24).

In the CLH report on page 50 regarding the Collins & Turner study, 1975 the following interpretation is noted "Findings of a survey performed in 144 regular takers of salicylates (including ASA) reported that salicylate consumption was associated with perinatal mortality, decreased intra-uterine growth and birth weight".
The evaluation of the RAC for SA states: "The studies of Turner and Collins have received criticism over time; although the publications are widely cited, the conclusions are mainly presented as having limited reliability due to the relatively small database and due to lack of consistent support from further studies. In addition, the authors themselves underscored a series of confounding factors such as the concurrent maternal exposure to Phenacetin or the low reliability of the serum levels of SA."
In the CLH report, Li et al. (2003) (cited in the Bard review (2012), which is part of RAC evaluation for SA) is cited, in which a significant increase of miscarriage in women using aspirin from conception is reported. This is consistent with the hypothesis that prostaglandin inhibition by aspirin interferes with implantation.
A re-evaluation of this study was performed by Nielsen et al. (2004) (cited in the Bard (2012) review) who showed a positive association between NSAIDS (non-steroidal anti-inflammatory drugs; not further specified) use and miscarriage. However, it was not statistically significant when gestation age was included in the calculation." However, based on the above mentioned differences, we consider this finding not relevant for MeS.
Also the Farid et al, 2011 study included in the CLH report on MeS, but not in the CLH report on SA change the overall assessment of ASA and SA. The case reported by Farid is a single case of an 19-year-old, gravida 1, para 0 woman at 39 weeks gestation with a past medical history notable for mild mental retardation, bipolar disorder and hypothyroidism presented to an outside hospital with bloody emesis and tinnitus after a self-reported ingestion of 100 tablets (32.5 g) of aspirin several hours before. Effects at this very high dose that may be even fatal to the mother does not allow any conclusion relevant for classification and labelling.
Regarding the study of Nelson & Forfar (1971) cited in the CLH report Bard states that the evidence of several confounders possibly impacting the results: "No detailed information is provided on confounders, e.g., study population lifestyle and other risk factors. Furthermore, the average number of drugs taken during the 1st trimester of pregnancy by study and control group was 2.1 and 1.8, respectively. The analysis did not allow to delineating the effect of a single drug, e.g. from multiple regression techniques. In addition, no information appears on the malformation types, except above for the first 28 days of gestation exposure to aspirin, where the organs hit are extremely diverse, which is not in favour of a single ethiopathological mechanism."
Similarly regarding CLH report page 51 (Lynberg et al, 1994) the Bard review states "it should be stressed that the mothers had flu with fever".
The CLH report on page 54 states: "Finally, even if not conclusive, there are some indications of developmental effects from human data with ASA, which can support the relevance of the observed effects in experimental studies to humans. In particular, it can be noted that mortality, craniorachischisis and gastroschisis are both reported in rats after MeS exposure and from human data with ASA".

We disagree with this statement and want to point out to the SA risk assessment where the RAC 2016 concluded the following:
"Summary of medical concerns regarding the usage of ASA during pregnancy
According to a literature search performed and results from a written consultation with representatives from the European Medicines Agency (EMA), ASA doses up to 100 mg/d are generally considered safe during pregnancy (FASS.se; 25 September 2015). A dose of 100 mg/d corresponds to 1.6 mg/kg bw/d of ASA for a 60 kg woman. For the dose range of 100-500 mg (equiv. to 1.6-8.3 mg/kg bw/d) it seems that "there is not enough clinical experience" for specific recommendations to be given, so a precautionary approach has been taken, giving the same warnings as for higher doses (above 500 mg/d). For doses exceeding 500 mg/d the concern is related to effects caused by prostaglandin synthesis inhibition having a negative impact on pregnancy and/or foetal development."
In this context we emphasise again, that ASA and MeS are not identical with regard to the mechanism of action (see above).
As SA is the biologically relevant metabolite of MeS and ASA read across between these substances taken the specific mechanism of action of ASA into account is scientifically justified.
For SA the RAC (2016) justified the classification with CMR Repr 2 as follows:
• According to experts in the field of pharmaceuticals, ASA is not considered as being a major teratogen, but may have some potential for teratogenic effects, and it should be noted that prostaglandin inhibitors in general, including ASA, could have other adverse effects on foetuses, especially on their renal development and during the third trimester on the development of the circulatory system;
• However, neither ASA nor SA are proven human developmental toxicants. There is a lack of evidence to support an increased risk of birth defects following exposure to ASA. Also, the evidence for other developmental effects has uncertainties. Taking that into account, classification in Category 1A is not justified.
It is noted that the available human epidemiological data on ASA was rather contradictory and with only a few reported exposures at higher doses, nevertheless demonstrated no clear evidence of malformations in humans.
Hence, the RAC concluded that Category 1B may not be justified.
Taking into account the available data, including pharmacokinetics, in vitro tests with ASA and SA, developmental studies in animals (positive findings in rat and monkey studies and a negative rabbit study), human epidemiology and medical experience, the RAC considered classification of SA as Repr. 2; H361d (Suspected of damaging the unborn child) to be justified".

For MeS the picture is very similar, with some indication of developmental effects (noted at severely maternal toxicity) and no clear evidence for either MeS nor its biologically relevant metabolite SA to cause malformations in humans. Therefore, Category 1 B is not justified.

References

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ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip

Dossier Submitter's Response

France:

- Comment related to kinetics and metabolism:

  Oral exposure: Some information on toxicokinetics are already provided in the CLH report. Data from mice and rats indicate an oral absorption of 100% (Yamagata et al., 1976; Davison et al., 1961). Comparison of plasma analysis between rats, dogs and humans indicate a high level of hydrolysis of methyl salicylate to free salicylate, with some differences: 100% hydrolysis in 20 minutes in rats; 95% in 1 hour in dogs and 79% in 90 minutes in humans (Davison et al., 1961). These results suggest that humans can be more than rats systemically exposed to the parent molecule, methyl salicylate,.

  Reference 1 (Sturman, 1967: *in vitro* assay: sodium salicylate added to blood from different species) suggests that binding of salicylate to plasma protein is different between species, with rather low percentage of salicylate bound to plasma protein (between 0 to 25% depending on concentration of sodium salicylate used) in rat, baboon, horse, dog, mouse, turkey and toad from one side and rather high percentage of salicylate bound to plasma protein (between 40 to 100% depending on concentration of sodium salicylate used) in monkey, man, guinea pigs and rabbits. The authors concluded that "*the species which show a low capacity to bind salicylate to their plasma proteins may be at higher risk, i.e. the LD50 values for salicylate would be lower, and may show lower values for the circulating half-life of salicylate than the species which possess a much greater affinity for binding salicylate to the plasma proteins*". However, based on the table 12 of the CLH report, some studies with guinea pigs and rabbits (Jenner (1964); Rumyantsev (1992); Castagnou (1952)) reported similar LD_{50} as in rats, that is in contradiction with the hypothesis of Sturman (1967) who suggested a higher sensitivity of species with low binding to plasma protein. In addition, longer systemic exposure could be expected in species with high binding to plasma protein, since elimination half-time would be slower than in species with low binding to plasma protein.

  Reference 2 (Labib, 2018) focuses on risk assessment of salicylic acid in topical cosmetic products. Please note that the SCCS published an opinion on salicylic acid in December 2018. Regarding to toxicokinetics, they concluded that "*Regarding salicylic acid kinetics for rats and humans, no robust data have been provided to enable comparison of the kinetic parameters of the test substance between species (rats and human)*". Regarding developmental toxicity, "*SCCS agrees that salicylic acid can be considered as a developmental toxicant*". Labib (2018) and SCCS (2018) publications are not applicable to methyl salicylate in its intended uses. Anyway, exposure and risk considerations are not part of the CLP regulation. In addition, conclusion of Labib (2018) on human data is only based on Slone et al. (1976), Shapiro (1976), Kozer (2002) and Roberge et al. (2016), not on an exhaustive review of the literature. Please note that these references were already discussed in the CLH report or below in the responses to this comment.

Dermal absorption: we agree that in general it is recognized that rat skin is more permeable than human skin. For methyl salicylate, data by dermal route are available in both animals and humans, however, none are fully adequate. From these studies both in animals and in humans, large ranges of dermal absorption were noted, which do not allow concluding on a specific dermal absorption value for methyl salicylate. Considering physico-chemical properties of methyl salicylate and the high dermal absorption of methyl salicylate reported in some human data (i.e. Yano et al., 1986), a conservative approach is to retain a dermal absorption value of 100% in the absence of more adequate studies.

Reference 8 assessed the penetration of salicylate from commercial formulations, including one formulation containing 20% methyl salicylate (no information on the other ingredients present in the formulation). The authors concluded to direct tissue penetration of methyl salicylate formulation. No conclusion can be made from this publication on a dermal absorption value.

- Comment on effect on sexual function and fertility: thank you for your support.

- Comment on developmental toxicity proposal Repr. 1B (H360D): please note that the assessment of the FDA (2006) studies was made based on the original study report and not *via* secondary source. No read-across with aspirin was proposed in the CLH report (in particular, animal data with aspirin were not included in the CLH report). Instead human data with aspirin were included in the CLH report in order to identify possible effects of salicylates (especially, salicylic acid) in humans, in the absence of such data with methyl salicylate, but not as a direct extrapolation for methyl salicylate. Anyway, the epidemiological data on aspirin are judged inadequate to conclude on developmental toxicity of salicylates. Therefore, these data should not be used to conclude to a lack of effect (especially since there are some publications reporting developmental effects consistent with experimental data) or to disregard the developmental effects reported in animals with methyl salicylate. Overall, the proposed classification is based on animal data performed with methyl salicylate. See also response to comment 10 related to human data and comment 15 related to consistency with RAC opinion on salicylic acid.

- Comment on Klimisch score for FDA (2006) studies: see response to comment 8.

- Comment on subcutaneous administration: see response to comment 8. In addition, you state in your comment that the "*s.c studies MeS was used at 100% concentration which is clearly an irritating concentration*". However, necropsy of the dams on gestation day 20 (for FDA (2006c)) or on lactation day 22 (for FDA (2006d) did not report any findings that can be related to a local irritation. Only retention of oily fluid in the subcutis of the treated site was observed. In this context, we do not agree with your statement that the "*results were clearly distorted*".

- Comment on the NOAEL of 100 mg/kg bw/day instead of 60 mg/kg bw/day based on skeletal variations: see response to 8.

- Comment on maternal toxicity in FDA (2006d) study: see response to comment 8.

- Comment on maternal toxicity in FDA (2006c) study: see response to comment 10.

- Comment on limitations associated to FDA (2006c) study: see response to comment 10. Furthermore, we would like to remind that consistent effects are

reported between FDA (2006c) and FDA (2006d) studies, including craniorachischisis and skeletal anomalies.

- Comment on rabbit study: see response to comment 10.

  In addition, reference 11 (Meredith, 2001) was used in this comment to support the duration of gestation in rabbit. We have no access to this book, nevertheless, the duration of gestation cited in your comment is in accordance with that noted in the CLH report. The reference 12 (Symeon et al., 2015) is not related to methyl salicylate. The aim of this study is to assess the effects of gestational maternal undernutrion on growth, carcass composition and meat quality of rabbit offspring. This reference was only used in your comment to support duration of organogenesis in rabbits (GD6-18). We agree with this information; however, even if rabbits were exposed during this period, it cannot be excluded that further effects can be identified with longer exposure, in particular, lethality in late gestation.

- Comment on hamster comment: see response to comment 10.

- Comment on human data : see response to comment 10. In contrast to your comment stating that "*The CLH report for MeS refers to "well-known" malformations caused by ASA as the second major argument for classification of MeS as Repr.1B.*", it is noted in the CLH report that "*human data are considered inadequate to firmly conclude on the developmental toxicity of salicylates*" (page 52). In addition, we agree that a direct extrapolation from aspirin to methyl salicylate is doubtful since a role of parental substance (and also maybe of methanol) in developmental effect cannot be excluded. In addition, as you mention, aspirin and methyl salicylate present some differences in prostaglandin inhibition. Biological differences can also be suspected since their commercial uses are quite different (even if methyl salicylate is used topically for its inflammatory properties, its major uses are in fragrance compounds, that is not the case for aspirin).

  In addition, the reference 14 (Atallah et al., 2017) is not relevant for classification purpose. The aim of this study was to assess the efficacy of aspirin used at low doses for prevention of preeclampsia and not its potential teratogenic effects. It is however noted in this publication that side effects (including teratogenicity and inhibition of fetal platelet aggregation) can be associated with aspirin used at higher doses. Considering the topic of this publication, it appears not to be relevant for the classification of methyl salicylate.

  In reference 15 cited in this comment, it is noted that "*Epidemiologic studies suggest increased risk of miscarriage, cardiac malformations, and gastroschisis when used early in pregnancy; the absolute risk of cardiovascular malformations increased from less than 1% to up to approximately 1.5%. The risk is believed to increase with dose and duration of therapy. During the third trimester of pregnancy, administration of nonsteroidal anti-inflammatory drugs (NSAIDs) pregnancy may cause premature closure of the fetal ductus arteriosus, oligohydramnios, fetal renal impairment, pulmonary hypertension, and prolongation of bleeding time*." In addition to this reference, reference to the French Vidal dictionary (including summaries of medicine characteristics) can be made. Based on this dictionary, use of aspirin during pregnancy is not recommended (in particular for doses > 100 mg/day). It refers to epidemiological data suggesting an increase of miscarriage, cardiac malformations and gastroschisis when aspirin was used during the first trimester. After, it is noted that

inhibitors of prostaglandin synthesis can induce cardiopulmonary toxicity and renal toxicity. This is consistent with information from the reference 15.

Reference 16 refers to recommendation of the American College of Ostetricians and Gynecologist to use low-dose aspirin prophylaxis in women at high risk of preeclampsia. It is important to note that biological effect of aspirin is highly dependent of aspirin dosage. For example, the effect of aspirin on COX-dependent prostaglandin synthesis is dose dependent, with irreversible acetylation of COX-1 at low doses and inhibition of both COX-1 and COX-2 at higher doses. Therefore, data on low-dose aspirin cannot be used to conclude on intrinsic toxicity of aspirin in general. At the time being, it seems that there is no international consensus to recommend the use of low-dose aspirin in the prevention of preeclampsia. Considering the subject of this reference, it appears not to be relevant for classification purpose.

Reference 17 is written in German and cannot be adequately checked.

Reference 18: we have no access to the full document. However, based on the abstract, it seems that it refers to recommendations for the prevention of ischemic stroke recurrence. Therefore, it is not relevant in the present case related to classification of methyl salicylate as reprotoxic.

Reference 19: this publication evaluated aspirin use in relation to birth-weight and perinatal mortality. The authors concluded that "*the data suggest that aspirin is not teratogenic*". As other available human studies with aspirin, different biais were described in this publication. This publication is not in contradiction with the CLH report stating that there are contradictory results from epidemiological studies with aspirin. However, it should be noted that this article is rather old and that more recent data is available.

Reference 20: this publication evaluated aspirin use in relation to reduced birth-weight and perinatal death. The authors concluded that they "*found no evidence that aspirin as used by pregnant women in the United Stated is related to perinatal mortality or low birth-weight*". This publication is not in contradiction with the CLH report stating that there are contradictory results from epidemiological studies with aspirin. However, it should be noted that this article is rather old and that more recent data is available.

Reference 21: this reference is already cited in the CLH report. The authors of this meta-analysis did not identified an overall increase in the risk of congenital malformations that could be associated with aspirin. However, they did not excluded the possibililty of an increased risk of rare malformations, as suggested from some of the case control studies. In addition, they also found a statistically significant increase of gastroschisis, that is one of the malformations also identified from experimental studies with methyl salicylate.

Reference 22: this publication is a follow-up of the meta-analysis cited in reference 20. The aim of this meta-analysis is to analyse the effect of aspirin use (up to 150 mg/d) in women with moderate and high-risk pregnancies on preterm deliveries and perinatal death. Since this meta-analysis include studies with consumption of aspirin during the second and/or the third trimester, association between aspirin use and malformations cannot be assessed.

Reference 23: this publication assessed the efficacy of low dose aspirin (up to 160 mg) in pre-eclampsia and intrauterine growth restriction. Considering the topic of this publication, it is considered not relevant for the classification of methyl salicylate.

Reference 24 refers to effects of low-dose aspirin in gestation and reproduction (EAGeR) trial. In particular, the effects of preconception aspirin use and its safety during early pregnancy was assessed. In addition it was noted in the publication that "*This study had insufficient sample size to detect a difference in birth defects*". Considering the topic of this publication, it appears not to be relevant for the classification of methyl salicylate.

- Comment on consistency with RAC opinion on salicylic acid: see response to comment 15. You state that "*The current report does not contain any new information/data suggesting any change for the conclusion".* It should be emphasized that developmental data with methyl salicylate was not assessed by the RAC for concluding on the classification of salicylic acid. In turn, animal data with aspirin were not included in the CLH report of methyl salicylate. Regarding human data with aspirin, the RAC also conclude that they are inconclusive.

- Comment on limitations associated with some epidemiological studies: Collins & Tuerner (1975), Li et al (2003), Farid et al (2011), Nelson & Forfar (1971), Lynberg et al (1994): as stated in the CLH report (page 52), we agree that epidemiological data are inadequate due to limitations including misclassification of exposure, confounding factors, lack of quantitative data etc. Nevertheless, it can be noted that the most important studies raising malformations (gastroschisis and craniochischisis) in humans, which seems consistent with animal data, are the following: Werler et al. 2002; Kozer et al. 2002 and Hernandez et al., 2012.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	United States	Colgate-Palmolive Company	Company-Downstream user	17

Comment received

A classification as Repr. Cat 1B for developmental effects for Methyl Salicylate (MeS) is neither warranted nor justified in the light of the overall data set.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Reproductive Toxicity Public.docx
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Reproductive Toxicity CP.docx

Dossier Submitter's Response

France: same attachments as in comment 7. See response to comment 7.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	United States	Givaudan	Company-Manufacturer	18
Comment received				

Background:

Methyl salicylate (MeS; EC Number: 204-317-7, CAS Number: 119-36-8) has been evaluated by ANSES (French Agency for Food, Environmental and Occupational Health & Safety) on behalf of the French Member State Competent Authority (MSCA) for classification and labeling. ANSES has proposed classification of Cate-gory 1B for Reproductive and Developmental (R) toxicity of the molecule (CLH Report, 2018). ECHA has published its opinion on the proposed CLH for MeS and seeking for public comments.

MeS is a key fragrance ingredient for the fragrance industry. It is used in combination with other ingredients in fragrance applications in cosmetics, fine fragrances, soaps and other toiletries. It is also used in oral care prod-ucts, household cleaners and detergents (Lapczynski et al. 2007). The substance is manufactured and/or import-ed in the European Economic Area at 1000 - 10000 tonnes per year (CLH Report 2018). Due to wide range of applications this molecule has high economic value for the industry as well as to the society in large. Therefore, an unjust toxicity classification will have significant impact to the industry and to the public.

Givaudan, a Switzerland based Fragrance Company with significant business in Europe, would like to take this opportunity, during public commenting period, to review and provide comments to the proposed classification. We believe that the proposed classification for reproduction and developmental toxicity is not warranted based on the weight of evidence analysis. In this 'public comment' document, we are providing a rationale that classi-fication of Category 1B is not justifiable for reproduction and developmental toxicity.

Data availability, quality and interpretation in CLH report

From the CLH report (CLH Report 2018) it is implicit that ANSES did not have access to the primary data. Instead, they have used the summary information that was available from an US FDA report (cited as FDA 2006a,b,c,d). As the information extracted from FDA report has been used in making an important conclusion that this compound is a Presumed human reproductive toxicant (Cat 1B), it is important to fully evaluate the quality and integrity of the studies before making the conclusion. Without evaluating the full report, ANSES has given a Klimisch Score of 1 (K1) which indicates that the data is deemed to be best in class without any major deviation and studies are most reliable. How the agency determined that the reliability of the study is best in its class without reviewing the full report?

In our opinion the information from all available sources may be used in Weight of Evidence analysis. However, in a major CLP assessment the data from secondary source or from poor quality study should be used with caution. We believe a K score of 1 is not appropriate for the studies (FDA 2006) cited in this evaluation as ANSES has not evaluated the data from primary source. We also did not get access to the main study report to evaluate the quality and integrity of the study.

Inappropriateness of the route of exposure for classification

In the Guidance on the Application of the CLP Criteria, 2017, (CLP Version 5.0 – July 2017) the significance of 'Reasonable expected use' to the CLP classification has been

mentioned. The article 8 and 9 (as mentioned below) indicates that test and evaluation should be done in the 'form' and 'physical state' 'which it can be rea-sonably expected to be used'. Although this does not specify the route of exposure in hazard classification but suggested of consideration of 'Form or physical state' and 'reasonably expected use' in CLP evaluation.

The route of exposure used in the FDA cited studies are subcutaneous exposure. This route of exposure may be appropriate for the pharmaceutical applications. However this is not appropriate route for hazard classification for MeS. The subcutaneous route of exposure definitely does not fall under the category of 'reasonably ex-pected use' as defined in the CLP guidance document

Although, the relevance of appropriate route of exposure in repro-developmental toxicity testing section has not been discussed in CLP guidance document; however, this has been mentioned in carcinogenicity section as below.

"Most standard carcinogenicity studies use physiological routes of exposure for humans, namely inhalation, oral or dermal exposure. The findings from such routes are usually considered directly relevant for humans. Studies using these routes will generally take precedence over similar studies using other routes of exposure.
Sometimes other non-physiological routes are used, such as intra-muscular, sub-cutaneous, intra-peritoneal and intra-tracheal injections or instillations. Findings from studies using these routes may provide useful information but should be considered with caution. Usually dosing via these routes provides a high bolus dose which gives different toxicokinetics to normal routes and can lead to atypical indication of carcinogenicity."

These above lines of evidence in CLP guidance document imply the common routes of exposer for classification are oral, dermal and inhalation. The subcutaneous route of exposure should be considered as 'non-physiological' which may leads to 'high bolus dose' leading to 'different toxicokinetics to normal routes'. Therefore, as mentioned in CLP guidance document, the findings from these routes 'should be considered with caution'.

Subcutaneous route of exposure used in the current FDA cited studies does not even qualify for 'reasonably foreseeable accidental exposure'. As mentioned in the background section the major uses of MeS are in cosmetic and household product as a fragrance ingredient. The primary route of exposure to consumers and workers from these applications is topical dermal and secondary route could be oral, in worst case. None of the intended or unintended accidental exposure would justify a subcutaneous exposure. Therefore the subcutaneous studies cited by FDA and used by ANSES should not be considered as key studies. Again, the results should be used with caution.

Exaggerated Exposure and unknown toxicokinetics through Subcutaneous Exposure

From toxicokinetics prospective a subcutaneous exposure will give a different exposure profile than the oral or dermal exposure. Subcutaneous route of exposure is highly exaggerated exposure scenario because of two rea-sons; a) Chemical is 100% bioavailable, b) it bypasses the 'first-pass-effect'. Dermal route of exposure, and oral route of exposure would lead to a different exposure profile due to first pass effect.

Several ADME studies are available for MeS in different species. However these studies are for either dermal or oral route of exposure and there are no studies addressing the toxicokinetic or ADME properties through subcu-taneous exposure. As presented in the CLH document, there are conflicting results for dermal absorption from various studies.

"From all these studies, various dermal absorption values were obtained and varied from 1% (human in vivo study with undiluted MeS; open application 6h to the chest and back) to 93% (human in vivo study with MeS applied to the forearm; 4h occlusion) (CLH report 2018)". There is no sound explanation for the wide range of dermal absorption in human volunteers. However, based on a RIFM review (RIFM review 2007), human in vivo data support a dermal absorption in the range of 2 to 43%.

Yamagata et al., 1976 observed close to 100% absorption of MeS through oral route of exposure in mice within 30 minutes to 48h of exposure. Davison et al., 1961 also reported that MeS was rapidly absorbed and complete-ly hydrolyzed to free salicylate in as little as 20 minutes. Further the authors concluded that the major site of hydrolysis is the liver. The oral route of exposure would lead to first-pass-effect and the molecule would go through metabolism in liver and converting to salicylic acid and other non-toxic metabolites. Although hydroly-sis can occur outside of liver converting MeS to Salicylic acid (SA), but the rate of metabolism, duration of stay in circulation and elimination rate would be different. A subcutaneous exposure will bypass the first-pass-effect make the MeS to be 100% bioavailable leading to a very highly exaggerated exposure situation. Considering the applications of MeS, a dermal route of exposure and in worst-case scenario an oral route would be appropriate. The subcutaneous study cited in this report should be considered inappropriate.

Inconsistent approach in classifying MeS compared to SA

All salicylates have the potential to release SA, but how fast this occurs and whether it is similar in rats and hu-mans is not known. Also there could be other metabolic fates (hydroxylation, conjugation) which could compete with SA formation. MeS is rapidly and extensively hydrolyzed to SA and corresponding alcohol. SA further is conjugated with either glycine or glucuronide and excreted in the urine as salicyluric acid and acyl and phenolic glucuronides. The alcohol is metabolized to corresponding aldehyde and acid and ultimately to CO2 (RIFM, 2007).

SA has been reviewed by the Risk Assessment Committee (RAC) for its classification and has been reviewed by Scientific Committee on Consumer Safety (SCCS) for its safety in cosmetic applications.

It is important to mention here that RAC has used read-across information from MeS and Acetylsalicylic acid (ASA) in evaluating the fertility and reproductive toxicity of SA. The justification provided is the metabolism pathway leading to formation of SA from these salicylates. Based on this read-across analysis RAC concluded that SA does not pose any fertility hazard. RAC has considered epidemiological data (from ASA use as NSAID drug) in concluding the developmental toxicity of SA. RAC in the risk evaluation document states that "Over-all, it can be concluded that salicylic acid does not adversely affect fertility and that the developmental toxicity reported in the rat is of very questionable significance/ relevance for humans" (RAC opinion 2016). Further RAC concluded that "Taking into account the available data, including pharmacokinetics, in vitro tests with ASA and salicylic acid, developmental studies in animals (positive findings in rat and monkey studies and a negative rabbit study), human epidemiology and medical experience, the RAC considered classification of sali-cylic acid as Repro. 2". RAC has considered the human epidemiological data in making the conclusion for R2 and not 1B as mentioned "It is noted that the available human epidemiological data on ASA was rather contra-dictory and with only a few reported exposures at higher doses, nevertheless demonstrated no clear evidence of malformations in humans. Hence, the RAC concluded that Category 1B may not be justified."

RAC has considered MeS, ASA and epidemiological data for the classification of SA. However, in current evaluation ANSES has a different opinion on the human epidemiological information, as they consider the epi-demiological data to be inadequate as stated "In conclusion, even if most of the epidemiological studies with ASA do not report an increased risk of adverse effect on development at therapeutic dosage, there are some indications of fetal lethality and malformations with this compound. These effects seem consistent with those reported in experimental studies with methyl salicylate. However, due to some limitations (such as misclassifica-tion of exposure, confounding factors and lack of quantitative data), human data are considered inadequate to firmly conclude on the developmental toxicity of salicylates." (CLH Report, 2018).

RAC's and ANSES's interpretation of epidemiological data in risk evaluation and classification is contradicto-ry. In principle, all available data including human epidemiological data should be considered in CLP evalua-tion.

Maternal toxicity associated with Developmental toxicity in the subcutaneous study

The developmental toxicity was assessed in a prenatal Developmenal study following ICH guideline. In this study pregnant Crj:CD(SD)IGS rats (20/group)were exposed subcutaneously to methyl salicylate at 0, 20, 60 or 200 mg/kg/day from gestation day 6 to lactation day 21. Dams were sacrificed on day 22 after delivery. The highest tested dose was selected based on a preliminary study showing mortality in almost all dams at 500 mg/kg/day, no live delivery at 300 mg/kg/day and slight effect on birth index and body weight at 80 and 200 mg/kg/day.

As stated in the CLH report, there was severe maternal toxicity in the highest dose of 200 mg/kg/day. Even two dams at 200 mg/kg/day died on gestation day 23 which is attributable to methyl salicylate exposure. There was a significantly lower mean body weight (-3.7% on GD12 and -4.6% on GD20) and body weight gain (between -4.08% on GD9 and -15.7% on GD20) during gestation at 200 mg/kg/day. The food consumption was signifi-cantly decreased on day 9 of gestation (-10.2%) and during lactation (-42.9% on day 1 and -21.9% on day 21) at this same dose. A significant prolongation of gestational days was observed in the 60 mg/kg/day group (with no dose-response relationship and within background data of the institution). A significant lower mean body weight with decreased food consumption was noted during lactation and maturation in the 200 mg/kg/day group.

Skeletal anomalies, especially fusion of the cervical vertebra and misshapen sternebra, were significantly in-creased at 200 mg/kg/day (32.26% versus 3.90% in the control). Skeletal variations slightly increased at 60 mg/kg bw/day and was significantly increased at 200 mg/kg/day (93.55% versus 25.97% in the control). How-ever, no historical control data was presented.

The developmental toxicity findings at 200 mg/kg bw noted in the FDA2006d study occur at clearly maternally toxic doses based on the significant reduction in body weight gain and food consumption as well as lethality. CLH reports states that "Although some of the developmental effects (such as skeletal variation, decreased body weight, delay in post-natal differentiation indices) may be secondary to maternal toxicity, it is not possible to explain the other effects such as offspring lethality and external/skeletal anomalies by the observed maternal toxicity (FDA, 2006d)". It is well known that maternal toxicity can cause physiological disturbance which may cause developmental delays and defects which is secondary to maternal effect. In presence of maternal toxicity the developmental effects should be interpreted with caution. We strongly believe the effects observed at 200 mg/kg/bw are related to maternal toxicity. The effects observed at 60 mg/kg/bw are

not statistical significant and in absence of historical control data the 'slight' skeletal variation may not be considered biological significant. Overall, in our opinion, the teratogenic effects observed in highest dose group is masked by maternal toxicity and therefore this data does not justify for a classification of category 1B.

Developmental data from different species does not support Category 1B classification

It is important to mention that RAC in its evaluation of SA (RAC Opinion 2016) has indicated species differ-ence in developmental toxicity for ASA. Some developmental effects were noted in rats but not in rabbit or in humans. In FDA 2006b study rabbits when exposed between gestation 6-18 did not show any developmental toxicity. The CHL report considered the exposure period from day 6-18 as of limited relevance, because the gestation period in the rabbit is longer (30-32 days). Therefore, it was concluded, that the negative results (no teratogenic effects detected) were questionable due to a too short exposure time to methyl-salicylate.

We do not agree with this conclusion that the malformations mentioned in the CLH report, such as craniorachis-chisis, gastrochisis, or ventricular septal defect are clearly originating from the organogenesis phase, which is completely covered in the rabbit between G 6-18. This dosing period is internationally accepted as covering the period of organogenesis as stated in both the ICH S5 and the OECD 414 guidelines. Therefore, to conclude that the exposure to MeS between days 6-18 is not appropriate to determine the teratogenicity is not logical.

There is a developmental toxicity study in hamster cited in CLH report. This study has been considered as 'ad-ditional evidence' in support of category 1B. However, the hamster study should not be considered as a valid study due to massive dose used which is beyond the limit dose as recommended by OECD guideline. MeS was administered topically at 3500 and 5250 mg/kg bw to pregnant LVG hamsters on day 7. Both treatments pro-duced neural tube defects, especially in the area of the developing brain. Percentage of neural tube defect was 72% at 1750 mg/kg bw/day after oral exposure versus 11% in control.

This is a poorly conducted study designed to produce an adverse effect as MeS was administered at an exces-sively high dose at gestation day 7, is not suitable for any conclusion with regard to classification and labelling. The high toxicity is also evident by the high plasma salicylate levels in the animals that reached a peak of 125 mg/100 ml at about 2 hours after oral treatment and is well above the toxic level in humans or rats. As a result, this finding is not relevant to humans and should not be used as part of the weight of evidence approach for developmental toxicity classification of MeS.

Overall Conclusion:

Based on the weight of Evidence analysis we do not believe a classification 1B for reproduction and develop-mental toxicity of MeS is warranted. The studies conducted for a pharmaceutical product with MeS are subcu-taneous exposure. Subcutaneous exposure is not appropriate for CLP evaluation as this route of exposure does not justify for 'reasonably expected use' scenario. The developmental effects in rats co-occur with severe ma-ternal toxicity. Therefore the results should be used with caution. Methyl salicylate did not result in develop-mental toxic effects in a well-designed rabbit study, indicating species sensitivity. Epidemiological studies on ASA as a read across substance indicate that there are no effects in humans. Similar to RAC evaluation for SA, a classification as Cat 1B for MeS is not warranted.

References:

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

FDA 2006 (a,b,c,d). Center for Drug Evaluation and Research. Pharmacology / Toxicology review and evalua-tion. FS-67 Patch (10% Methyl salicylate & 3% l-menthol Topical patch). NDA number 22-029.

CLH Report. 2018. Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 , International Chemical Identification: Methyl salicylate

RAC Opinion 2016. proposing harmonized classification and labeling at EU level of Salicylic acid.
CLH-O-0000001412-86-110/F

Guidance on the Application of the CLP Criteria. 2017. Guidance to Regulation (EC) No 1272/2008 on classifi-cation, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public Response to Methyl Salicylate CLH .docx

Dossier Submitter's Response

France:

- Comment related to economic considerations: Economic consideration is not part of the CLP Regulation.

- Comment related to "availability, quality and interpretation in CLH report": Please note that ANSES has access to the original studies cited in the FDA report (references FDA (2006a, b, c, d). Therefore, assessment of these studies, including Klimisch score was based on the full reports. Sufficient level of details was provided in the Annex I of the CLH report to make an objective assessment of the studies.

- Comment related to "inappropriateness of the route of exposure for classification": See response to comment 8.

- Comment related to "exaggerated exposure and unknown toxicokinetics through subcutaneous exposure": see responses to comments 8 and 16.

- Comment related to 'inconsistent approach in classifying MeS compared to SA": see response to comment 13. Please note that methyl salicylate data were not used in the RAC opinion to conclude on developmental toxicity of salicylic acid. Regarding human data with aspirin, the RAC conclude that they was rather contradictory. Based on this observation and identified limitations associated with the studies, we conclude that the epidemiological data are inadequate. In addition, there are remaining uncertainties linked to biological and kinetics differences between methyl salicylate and aspirin. Therefore, these data should not be used to conclude to a lack of effect or to disregard the developmental effects reported in animals with methyl salicylate. See also responses to comments 10 and 16.

- Comment related to "maternal toxicity associated with developmental toxicity in the subcutaneous study": see responses to comments 8 (for maternotoxicity in FDA

(2006d) and 10 (for maternotoxicity in FDA (2006c), for rabbit study and for hamster study).
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	Belgium	INTERNATIONAL FRAGRANCE ASSOCIATION	Industry or trade association	19

Comment received

IFRA's comments are related to the use of two rat studies with sub-cutaneous injection (FDA 2006c and 2006d) which are used to justify a proposal to classify Methyl Salicylate as a developmental toxic substance Repr. Cat 1B. Subcutaneous injection as route of administration is not a usual administration route and should be evaluated with caution under the CLP Regulation given the avoidance of typical 'first pass' metabolism. The developmental toxicity findings reported in rat studies using this route of administration are a consequence of maternal toxicity and are therefore not considered relevant for hazard classification purposes. Finally, the assigned reliability of Klimisch score 1 is not considered pertinent given the secondary source availability of the studies in question.

Rationale:
The proposal to classify Methyl Salicylate as a developmental toxic substance Repr. Cat 1B is in large part based on the results obtained in two rat studies using subcutaneous administration (FDA 2006c and 2006d). Developing conclusions based on such a route of administration needs caution since this route circumvents the opportunity for metabolism via the relevant exposure routes which have been shown to hydrolyse methyl salicylate into salicylic acid i.e. (i) the oral route and the 'first past' effect in the liver and (ii) the dermal route and skin metabolism. Consequently, the peak plasma levels of Methyl Salicylate achieved by the subcutaneous route of administration will be significantly higher than through oral and dermal exposure which do not avoid this metabolism. Therefore, from the metabolism and kinetic perspective, the results obtained for Methyl Salicylate by the subcutaneous route should not be compared to oral and dermal exposures. In addition, substances administered subcutaneously should be non-irritating and high percentage concentrations of Methyl Salicylate as applied in those studies are known to be irritating. Overall, the results obtained in these two studies are distorted and are not considered appropriate for hazard classification under the CLP regulation (see CLP Regulation, Annex 1, 1.1.1.5 "For the purpose of classification for health hazards (Part 3) route of exposure, mechanistic information and metabolism studies are pertinent to determining the relevance of an effect in humans."

The oral exposure route is normally the preferred administration route for the hazard classification of chemicals. Since relevant and valid data for Methyl Salicylate via the oral route are available, they should be the basis for the hazard classification.

As per the specific developmental toxicity findings reported at 200mg/kg/day (FDA 2006d), these should be regarded as secondary to the maternal toxicity clearly observed at that dose and not test article related, and therefore, should not be considered for hazard classification purposes, in line with CLP Regulation Annex 1, 3.7.2.4. Furthermore, the occurrence of skeletal variations reported at 60mg/kg/day (FDA 2006d) is inconsistent with the lack of those observations at 100mg/kg/day in a study designed to detect these effects (FDA 2006c).

Moreover, the proposed CLH assessment of the two studies with subcutaneous injection appears to be based on the results summarised in an NDA review report by the FDA (2006). IFRA considers that in order to make a robust evaluation for the purposes of harmonised classification under CLP, far more study details than those publicly available are needed, irrespective of the questionable relevance of the subcutaneous route of exposure of the studies as outlined above.

Unless a direct data source, such as the study report is used in the assessment, the reliability of the reference cannot be considered as Klimisch 1 and therefore, it is not appropriate to use such data to support a key argument for classification.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment IFRA comments to Methyl salicilate consultation final.pdf

Dossier Submitter's Response
See response to comment 8 (reprotoxicity).
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	Germany	Symrise AG	Company-Importer	20

Comment received

Reproductive/developmental toxicity
The comments to the following endpoint has been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company.
A classification as Repr. Cat 1B for developmental effects for Methyl Salicylate (MeS) is neither warranted nor justified in the light of the overall data set.
• In rats after subcutaneous application of MeS developmental effects were observed only in the presence of excessive signs of maternal toxicity both in the Embryo-Fetal Development (EFD) and in a Post-Natal Development (PND) study (FDA 2006c, FDA 2006d) including lethality and severe body weight reduction (instead of gain) during the sensitive gestation period in dams. At less toxic doses no clear evidence for developmental toxicity was noted.
• In a hamster study of poor scientific quality and documentation, adverse effects were only reported at exaggerated doses of MeS well beyond any limit dose recommended for OECD studies that caused massive toxicity.
• Salicylic Acid (SA) as the biologically relevant metabolite of MeS was recently evaluated by the RAC (2016) and classified as CMR Repr 2.
• Epidemiological data on Acetyl Salicylic Acid (ASA) which is used for read across for SA and MeS demonstrate a lack of evidence of an increased risk of birth defects following exposure to ASA.

General comments:
Before commenting in detail on this point, we would like to mention a few key findings also stated in the CLH report with regard to kinetics and metabolism important for the overall assessment.
Based on kinetic data, MeS is rapidly absorbed when administered orally in humans and in rats and very quickly hydrolyzed to SA.
However, there is a marked species-dependence in the binding of salicylate to serum proteins, with high binding in man, rhesus monkey, rabbit and guinea pig, while several

other species, including the rat, mouse and dog, have much lower binding (1,2).
For skin penetration also very high absorption of up to 100% is reported for rodents whereas controversial findings are reported from low percentages up to around 50% for human skin. It is well known that rat skin is more permeable to substances than human skin (mean difference 10.9 fold) (3). The value of 93 % for human considered and mentioned several times in the CLH report appears not to be realistic as most of the substance was absorbed into the epidermis and did therefore not penetrate the skin, thereby significantly reducing the overall bioavailability. Therefore and based on the generally lower skin absorption of human skin compared to rodent skin, the RIFM range of 2-43% also cited in the CLH report seems to better reflect the reality.
As noted for oral administration the substance is quickly hydrolyzed and converted into salicylic acid (SA) and methanol (4). Thus, the biologically relevant and systemically available structure is SA. Davison et al. (5) conducted a study to compare the toxicity and the metabolism of MeS and SA and concluded that the results on the toxicity and metabolism of both MeS and SA are comparable across multiple species (rats, dogs, and monkeys). We therefore strongly consider the most recent assessment of SA by the RAC (6) as most relevant also for MeS.

Effect on sexual function and fertility
We agree with the argumentation in the CLH report, that the data available allow a scientific valid assessment and does not warrant a classification with regard to effects on sexual function and fertility. This assessment is also in line with a recent evaluation by RAC for the relevant metabolite SA. For this compound the RAC concluded in 2016: "there is insufficient evidence that SA exhibits adverse effects on sexual function and fertility. Consequently, for this endpoint RAC supports the proposal of the DS and concludes that no classification for SA for adverse effects on sexual function and fertility is justified".

Developmental toxicity Proposal Repr.1B (H360D):
We do not agree with the conclusion drawn in the CLH report, that MeS should be classified as a developmental toxic substance Repr. Cat 1B.
In the CLH report, two argumentation lines are used to justify this proposal for MeS. One are the results obtained in two rat studies using subcutaneous administration, which are only available via secondary source (FDA 2006c and mainly FDA 2006d) together with evidence from studies of low quality and a read across to the findings with ASA) which is used as a drug and for which human data are at hand. The read across to ASA is justified by the fast hydrolysis of both substances to SA. It should be mentioned here, that the biologically relevant substance (SA) was recently evaluated by the RAC and a classification as Repr. Cat 2 (for developmental/reproductive toxic effects) was deduced after a comprehensive and detailed assessment on both the animal findings and the human data for ASA.
In the following we would like to comment on the two arguments provided in the CLH report separately.
Animal data
Studies in rats (subcutaneous application)
Before addressing details of the studies being considered key for the conclusion that Repr. 1B is warranted we would like to emphasize that we do not have full access to these studies. We assume that also ECHA does not have access to these studies as FDA as second source is cited in the CLH report. Therefore, we do not agree to consider these studies as Klimisch 1, since many details which are relevant for the final assessment are missing (see below).

Relevance of the route of administration
The results of these two studies are not an appropriate basis for the hazard classification

under the CLP regulation due to the use of subcutaneous injection as route of exposure. In case of a classification of chemicals, the oral exposure is the most relevant route as stated in the OECD guideline 414 and data via this route is available for the biologically relevant hydrolysis product SA. The direct "systemic" application via the subcutaneous (s.c.) route circumvents the enterohepatic pathway/circulation, which has been shown to eliminate the major amount of the mother-compound via metabolism (7). Consequently, after s.c. administration the blood concentration is significantly higher than following oral application. Also after dermal application (the most relevant exposure route after oral exposure), the mechanical as well as the metabolic "barrier" of the skin (fast hydrolysis) will significantly reduce the bioavailability. Thus, the external doses between s.c. and oral studies cannot be compared due to the kinetic and metabolic differences. Consequently, the results derived from the two rat studies using s.c. application can be considered of limited relevance due to an increased systemic exposure compared to oral (7) and topical (8) application routes and are not an appropriate basis for the hazard classification under the CLP regulation.

In addition, substances given subcutaneously should be non-irritating. However, in these two s.c. studies MeS was used at 100% concentration which is clearly an irritating concentration (9, 10). Thus, the results were clearly distorted and are not considered appropriate for the hazard classification under the CLP regulation.

Our interpretation of the findings of those studies differs from the CLH report. Assessing both studies, the data supports a NOAEL of 100 mg/kg for reproductive/developmental toxicity and not < 60 mg/kg. There is no evidence of treatment-related skeletal abnormalities up to 100 mg/kg in the Embryo-Fetal Development (EFD) study which is a study type specifically designed to evaluate this (FDA 2006c). The occurrence of skeletal abnormalities at 60 mg/kg in the Post-Natal Development (PND) study (FDA 2006d) is not consistent with the absence of those findings in the EFD study with dosing up to 100 mg/kg during the complete period of organogenesis (see also below). The formation of ribs is completed during organogenesis. Thus, it is not plausible why additional abnormalities of the ribs should occur at a lower dose in the PND study compared to the EFD study. It is thus highly unlikely that the skeletal abnormalities observed at 60 mg/kg in the PND study are test article related based on the lack of any statistical significance or a clear dose response relationship for these skeletal findings. Therefore, the NOAEL for reproductive toxicity should be established at 100 mg/kg.

The developmental toxicity findings at 200 mg/kg bw noted in the FDA2006d study are considered to be secondary to maternal toxicity since they occurred at clearly maternally toxic doses which induced not only a significant reduction in body weight gain and food consumption but also lethality in 2 dams. We clearly disagree with the interpretation regarding this study in the CLH report: "Although some of the developmental effects (such as skeletal variation, decreased body weight, delay in post-natal differentiation indices) may be secondary to maternal toxicity, it is not possible to explain the other effects such as offspring lethality and external/skeletal anomalies by the observed maternal toxicity (FDA, 2006d)". From our point of view the observations noted in the dams classify the effects noted in the foetuses as highly likely to be secondary due to massive maternal toxicity. In such cases according to the CLP (Annex 1: 3.7.2.4.3 and 3.7.2.4.4) either a classification as Cat. 2 or even no classification should be considered. Especially point 3.7.2.4.4 states, that in case of lethality for more than 10 % of the dams in a given dose group effects in the offspring should normally not be taken into account for C&L purposes.

A similar picture for the EFD study was noted at 200 mg/kg bw/day. In the CLH report it is cited "All these effects occurred at concentrations including slight toxicity in dams. However, considering the severity of the observed external malformations and visceral anomalies, these effects cannot be secondary to the slight maternal toxicity".

However, in this study significant decrease in body weight gain vs. control was noted during the entire treatment period in that group (314%, 155.2%, 89%, 68%, 48%, 39%,

36%, 24%, 21% 17%, 12%, 10% and 12% on Days 7-20, respectively) and even body weight loss on Days 7 and 8 causing significant malnutrition to the offspring. In addition, the reduction in body weight gain returned only to below 20% of the control once the dosing period was completed, which clearly demonstrates that the test article caused massive maternal toxicity. Thus the effects noted in the offspring are considered to be a result of this malnutrition and the bad health status of the dams.
In addition, there are also some limitations of the reports (as far as accessible) that confound interpretation of the results especially for the group of 200 mg/kg bw/day for which massive maternal toxicity was noted
• Data are reported on a fetus basis and not a litter basis for the skeletal abnormalities in the PND and EFD study. Therefore it is not possible to establish whether the findings are spread out across litters or in a single litter, which might be explained by a bad health status of a single dam. This is of key importance as severe toxicity up to lethality was noted already for two dams.
• Body weight gain data do not fully capture the potential maternal toxicity because the individual maternal carcass and fetal weights have not been provided and therefore the body weight gain differences relative to the control may be underestimated.
It should also be noted that for the same time windows of exposure, significant difference with regard to maternal toxicity was observed in the FDA 2006d compared to the FDA 2006c study.
Indeed, in the EFD study (FDA 2006c) in the same rat strain specifically designed to investigate the effects of concern, different and significantly less pronounced effects indicative for developmental toxicity (no statistical differences noted between control and treated group for any external anomaly) were noted although the same dose was applied during the critical time window (see also above).
Nevertheless, effects/variations noted in this study at 200 mg/kg bw/day can be explained by maternal toxicity and consequently malnutrition of the offspring (see above). This can result in a delayed ossification too and might so explain some of the skeletal variations seen.
No final evaluation of these two s.c. studies is possible without details about the potential local effects and the resulting stress to the animals. Substances injected subcutaneously should be non-irritating. However, in these two s.c. studies MeS was used at clearly irritating concentration.

Study in rabbits:
We do not agree with the conclusion that the rabbit study (FDA 2006b) is not valid and does not allow a robust and scientifically sound assessment for this species. The validity and the usefulness of the study was not challenged by FDA according to our knowledge.
The CLH report considered the exposure period from day 6-18 as of limited relevance, because the gestation period in the rabbit is longer (30-32 days (11). Therefore, it was concluded, that the negative results (no teratogenic effects detected) were questionable due to a too short exposure time to MeS. We disagree and emphasize that the exposure period in rabbits was sufficiently relevant; according to the OECD GL 414, the test substance was administered during the sensitive period of organogenesis (GD 6-18 in rabbits as it was done in the rat study (GD 6-17).
In addition, the described malformations in the CLH report, such as craniorachischisis, gastrochisis, or ventricular septal defects are clearly originating from the organogenesis phase, which is completely covered in the rabbit between gestation days 6-18 (12). A prolonged exposure period may only have demonstrated potential effects on the growth of the fetus but not any additional developmental abnormalities. Again, the dosing period of GD 6-18 in rabbits is internationally accepted as covering the period of organogenesis as stated in both the ICH S5 and the OECD 414 guidelines.
The lack of malformations in the rabbit rather indicates a difference in species sensitivity between the rabbit and rat, most probably due to species differences in plasma

levels/kinetics. Similar species differences have been observed for the relevant metabolite SA (see above).

Study in Hamsters and other evidence
In the CLH report a hamster study is used as additional evidence for developmental toxicity supporting Repr. Cat 1B, although considered of low quality already in the CLH report.
MeS was administered to pregnant LVG hamsters on day 7 (topically) or day 9 (orally) at exaggerated doses far beyond any limit dose recommend for OECD studies, i.e. topically at 3500 and 5250 mg/kg bw, orally at 1750 mg/kg bw (well above the reported oral LD50 in guinea pigs).
We consider the hamster study of poor scientific quality and not relevant for classification and labelling as the doses tested very likely cause massive toxicity such as high pup lethality, also confirmed by the high plasma SA levels in the animals that reached a peak of 125 mg/100 ml at about 2 hours after oral treatment and were well above the toxic level in humans or rats. Moreover, important information is lacking such as clinical signs in the dams as well as historical controls for the effects seen in the pups.
The study is therefore not suitable for any conclusion with regard to classification and labelling.
Moreover, in the CLH report on page 46 the studies Collins et al. (1971) and Anonymous (1978a and 1978b) assessed as Klimisch 3 ("not reliable") were used to support the Repr. Cat 1B classification. Whereas a one generation feeding study in rats evaluated Klimisch 4, which did not show any abnormalities at dose levels of 200 and 300 mg/kg bw day, was disregarded. Klimisch score 3 and even more score 4 data can in fact be used as supporting evidence, but then all studies have to be used as part of an appropriate weight of evidence approach including studies with negative results.

2.3. Human data
Pharmacovigilance data provides no evidence of adverse effects from MeS in humans, although MeS is used since decades as a pharmaceutical substance for local treatment.
The CLH report states that there are some indications of developmental effects from human data with ASA, which can support the relevance of the observed effects in experimental studies to humans. The CLH report for MeS refers to "well-known" malformations caused by ASA as the second major argument for classification of MeS as Repr.1B.
Before addressing in detail the conclusions and findings, it should be mentioned that both MeS and ASA undergo extensive hydrolysis to SA but behave differently with regard to prostaglandin inhibition (more precisely inhibition of COX-1 and COX-2). This mechanism of action of ASA requires the transfer and covalent binding of the acetyl group of ASA to the amino acid Serine in the catalytic center of the enzyme. This mechanism of action is however not possible for MeS. Thus data/findings in humans linked to this mechanism of action cannot be transferred 1:1 to the assessment of MeS.
The data from ASA can however be used to assess the relevant biological metabolite for both substances, SA.
In addition, the data set available for ASA has been recently assessed by the RAC in the context of the CLH report for SA (13) emphasizing that data from ASA and MeS were acceptable for read across to SA. Taking into account the available human epidemio¬logical data on ASA which show a lack of evidence to support an increased risk of birth defects following exposure to ASA, the RAC considered classification of SA as Repr. 2 to be justified based on the findings noted in animals.
The current report does not contain any new information/data suggesting any change for the conclusion (see below).
We would like to first summarize the conclusion by RAC for which the Bard publications (2012, 2015) are key:

"The assessment of "Low doses" in pregnancy
The "low doses" in pregnancy are those referred to in the table above as being indicated for "prevention of multiple miscarriage, pregnancy-induced hypertension and other complications of pregnancy". The (retrospective) cohort study performed by Bard (2012) was provided for this dose range. To further extend the analysis, the DS submitted an additional critical review by the same author (Bard 2015).
The aim of the study and the analysis was to address the effects of ASA within this dose range on the following endpoints: maternal bleeding, neonatal haemostatic abnormalities, pregnancy duration and labour, prevention of pre-eclampsia and intra-uterine foetal growth retardation, stillbirths and infant mortality, birth weight, birth defects and early childhood development. Particular aspects that raised concern were also analysed; the premature closure of Ductus arterious, the occurrence of gastroschisis and congenital cryptorchidism.
As a final conclusion of the study it was stated that: "no adverse effect of aspirin treatment can be considered as established, either at low (<150 mg daily) or higher, usual dose". To further illustrate the overall conclusion with respect to dosages higher than that mentioned above, three epidemiological studies (Slone, 1976; Shapiro, 1976; Kozer, 2002) were cited; the conclusion was that the use of aspirin at up to the maximum recommended therapeutic dose of 4000 mg/d (equivalent to 66.7 mg/kg bw/d as ASA, or 51 mg/kg bw/d as SA) have largely demonstrated an absence of increased risk of adverse pregnancy outcome in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay."
This conclusion is well in line with our interpretation of the data and is supported by new publications (see below). ASA is used broadly for the treatment and prevention of gestational complications as e.g. pre-eclampsia. Furthermore, there are numerous studies available that demonstrate the safe use of low-dose ASA also during the first trimester of pregnancy, that is the phase of blastogenesis and organogenesis (in humans: blastogenesis: day 1-16; embryogenesis (organogenesis): day 16-60; fetogenesis: day 61 - onwards) (14-18). This is supported by obstetricians' and gynecologists societies from several countries recommending the use of ASA in women with e.g. pre-eclampsia. The use of ASA during pregnancy up to the maximum recommended therapeutic dose of 4000 mg/day or 66.7 mg/kg using 60 kg body weight (bw) has demonstrated no evidence of an increased risk of adverse pregnancy outcomes in terms of frequency of stillbirth, neonatal mortality, birth defects or developmental delay (19, 20, 21). A further meta-analysis of studies on the use of therapeutic low-dose ASA at 50–150 mg/day (22) has demonstrated that this dose range is not associated with any adverse pregnancy outcome, in terms of perinatal mortality, birth complications, congenital malformations or adverse effect on subsequent development. For pregnancies with a moderate or high risk of pre-eclampsia and/or premature delivery, the adverse pregnancy outcome rate was reduced with therapeutic low dose ASA (50–150 mg/day) and there was no increased risk of early miscarriage with this dose regime (23). These studies strongly support that neither ASA nor its principal metabolite SA induce adverse developmental effects up to the maximum recommended therapeutic dose in humans, i.e. 4000 mg/day (66.7 mg/kg for a 60 kg person, equivalent to 51 mg/kg SA).

In addition the results of the ongoing EAGeR study, which is investigating the effects of pre- and peri-conceptional aspirin also comes to this conclusion. This randomized clinical trial is performed to test whether aspirin treatment, begun prior to conception and continued through pregnancy, improved live birth rate in women with prior pregnancy loss. The trial included more than 1000 women. There were improvements in pregnancy outcome, with no adverse effects noted (24).

In the CLH report on page 50 regarding the Collins & Turner study, 1975 the following

interpretation is noted "Findings of a survey performed in 144 regular takers of salicylates (including ASA) reported that salicylate consumption was associated with perinatal mortality, decreased intra-uterine growth and birth weight".
The evaluation of the RAC for SA states: "The studies of Turner and Collins have received criticism over time; although the publications are widely cited, the conclusions are mainly presented as having limited reliability due to the relatively small database and due to lack of consistent support from further studies. In addition, the authors themselves underscored a series of confounding factors such as the concurrent maternal exposure to Phenacetin or the low reliability of the serum levels of SA."
In the CLH report, Li et al. (2003) (cited in the Bard review (2012), which is part of RAC evaluation for SA) is cited, in which a significant increase of miscarriage in women using aspirin from conception is reported. This is consistent with the hypothesis that prostaglandin inhibition by aspirin interferes with implantation.
A re-evaluation of this study was performed by Nielsen et al. (2004) (cited in the Bard (2012) review) who showed a positive association between NSAIDS (non-steroidal anti-inflammatory drugs; not further specified) use and miscarriage. However, it was not statistically significant when gestation age was included in the calculation." However, based on the above mentioned differences, we consider this finding not relevant for MeS.
Also the Farid et al, 2011 study included in the CLH report on MeS, but not in the CLH report on SA change the overall assessment of ASA and SA. The case reported by Farid is a single case of an 19-year-old, gravida 1, para 0 woman at 39 weeks gestation with a past medical history notable for mild mental retardation, bipolar disorder and hypothyroidism presented to an outside hospital with bloody emesis and tinnitus after a self-reported ingestion of 100 tablets (32.5 g) of aspirin several hours before. Effects at this very high dose that may be even fatal to the mother does not allow any conclusion relevant for classification and labelling.
Regarding the study of Nelson & Forfar (1971) cited in the CLH report Bard states that the evidence of several confounders possibly impacting the results: "No detailed information is provided on confounders, e.g., study population lifestyle and other risk factors. Furthermore, the average number of drugs taken during the 1st trimester of pregnancy by study and control group was 2.1 and 1.8, respectively. The analysis did not allow to delineating the effect of a single drug, e.g. from multiple regression techniques. In addition, no information appears on the malformation types, except above for the first 28 days of gestation exposure to aspirin, where the organs hit are extremely diverse, which is not in favour of a single ethiopathological mechanism."
Similarly regarding CLH report page 51 (Lynberg et al, 1994) the Bard review states "it should be stressed that the mothers had flu with fever".
The CLH report on page 54 states: "Finally, even if not conclusive, there are some indications of developmental effects from human data with ASA, which can support the relevance of the observed effects in experimental studies to humans. In particular, it can be noted that mortality, craniorachischisis and gastroschisis are both reported in rats after MeS exposure and from human data with ASA".
We disagree with this statement and want to point out to the SA risk assessment where the RAC 2016 concluded the following:
"Summary of medical concerns regarding the usage of ASA during pregnancy
According to a literature search performed and results from a written consultation with representatives from the European Medicines Agency (EMA), ASA doses up to 100 mg/d are generally considered safe during pregnancy (FASS.se; 25 September 2015). A dose of 100 mg/d corresponds to 1.6 mg/kg bw/d of ASA for a 60 kg woman. For the dose range of 100-500 mg (equiv. to 1.6-8.3 mg/kg bw/d) it seems that "there is not enough clinical experience" for specific recommendations to be given, so a precautionary approach has been taken, giving the same warnings as for higher doses (above 500 mg/d). For doses exceeding 500 mg/d the concern is related to effects caused by prostaglandin synthesis inhibition having a negative impact on pregnancy and/or foetal development."

In this context we emphasise again, that ASA and MeS are not identical with regard to the mechanism of action (see above).
As SA is the biologically relevant metabolite of MeS and ASA read across between these substances taken the specific mechanism of action of ASA into account is scientifically justified.
For SA the RAC (2016) justified the classification with CMR Repr 2 as follows:
• According to experts in the field of pharmaceuticals, ASA is not considered as being a major teratogen, but may have some potential for teratogenic effects, and it should be noted that prostaglandin inhibitors in general, including ASA, could have other adverse effects on foetuses, especially on their renal development and during the third trimester on the development of the circulatory system;
• However, neither ASA nor SA are proven human developmental toxicants. There is a lack of evidence to support an increased risk of birth defects following exposure to ASA. Also, the evidence for other developmental effects has uncertainties. Taking that into account, classification in Category 1A is not justified.
It is noted that the available human epidemiological data on ASA was rather contradictory and with only a few reported exposures at higher doses, nevertheless demonstrated no clear evidence of malformations in humans.
Hence, the RAC concluded that Category 1B may not be justified.
Taking into account the available data, including pharmacokinetics, in vitro tests with ASA and SA, developmental studies in animals (positive findings in rat and monkey studies and a negative rabbit study), human epidemiology and medical experience, the RAC considered classification of SA as Repr. 2; H361d (Suspected of damaging the unborn child) to be justified".

For MeS the picture is very similar, with some indication of developmental effects (noted at severely maternal toxicity) and no clear evidence for either MeS nor its biologically relevant metabolite SA to cause malformations in humans. Therefore, Category 1 B is not justified.

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ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip

Dossier Submitter's Response
France: see response to comment 16.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Germany		MemberState	21

Comment received

Based on animal data the proposed classification for MeS is Acute Tox 4, H302. We agree that the quality/documentation of the available studies shows weaknesses. But in a weight-of-evidence-approach (considering the ranges of the observed LD50 values with a majority corresponding to category 4), the classification in category Acute Tox 4, H302 is justified. Furthermore, the two metabolites, SA and methanol are also classified as Acute Tox 4, H302 or Acute Tox 3, H302, respectively. After oral administration, MeS is not completely hydrolysed to SA and methyl alcohol in man (Davison et al. 1961, Martin et al., 2004). In the dossier the publication by Chyka et al. (2007) regarding salicylate poisoning in humans is cited, but there is no discussion of the eight cases of methyl salicylate associated deaths predominantly in children and elderly adults. Two cases results in classification as Acute Tox. 3, H301 based on the estimated dose and a standard human body weight of 70 kg. Therefore, a discussion of methyl salicylate associated deaths in humans should be added in the dossier especially regarding the effect of the metabolite methanol. It has to be noted that methanol is classified as Acute Tox. 3, H301 based on human data because the rat is known to be insensitive to the toxicity of methanol.
Regarding the animal data the DS proposed an ATE value of 580 mg/kg bw/d which is the lowest ATE in the most sensitive appropriate species (mice) tested. The DS assessed one study with Klimisch score 3: Davison et al. (1961). This publication leads to an ATE value of 1100 mg/kg bw/d and category Acute Tox 4. As the margins of LD50 values are quite wide (580 - >2000 mg/kg bw/d) we propose to consider the converted Acute Toxicity point Estimates as shown in Table 3.1.2 of CLP Regulation, Annex 1. LD50 values of 300 mg/kg < Category 4 ≤ 2000 mg/kg leads to a converted ATE value of 500 mg/kg bw/d.

Dossier Submitter's Response

France: Thank you for this comment.

Concerning the cases of death after ingestion of methyl salicylate reported by Chyka et al. 2007, in particularly in children and elderly adults, they occur after ingestion of 5-120 mL of methyl salicylate. Using density of methyl salicylate and default values for body weight, the ingested lethal doses are estimated to be between 100 and 2000 mg/kg, corresponding to Acute Tox 3 or Acute Tox. 4. However, evaluation of these data for classification purpose is difficult considering the wide range of lethal estimated doses, the low level of information provided in this publication and uncertainties associated.

In addition, according to CLP guidance, *"It should be acknowledged that human data often do not provide sufficiently robust evidence on their own to support classification. They may, however, contribute to a weight of evidence assessment with other available information such as data from animal studies."* It is considered that human data on its own do not allow to conclude definitively on a category for acute toxicity. In contrast, weight of evidence based on animal data allow to conclude to a category Acute Tox. 4. Human data do not contradict this conclusion.

Concerning the metabolites, we would like to specify that for methanol, the current harmonized classification is Acute Tox. 3*. It means that the classification was set under Directive 67/548/EC and not reevaluated according to CLP criteria. Anyway, it is considered more robust to propose a classification for methyl salicylate based on actual data on methyl salicylate rather than estimated from metabolite classifications.

Concerning the ATE, since there are several data with methyl salicylate, we prefer to set a specific ATE (580 mg/kg in this case), based on the lowest LD50 derived. However, considering the weakness of the database, we can also agree to retain the converted ATE value of 500 mg/kg bw.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2018	United Kingdom	<confidential>	Company-Downstream user	22
Comment received				
No comments on this endpoint ECHA note – An attachment was submitted with the comment above. Refer to public attachment clh_opinion_salicylic_acid_6425_en.pdf				
Dossier Submitter's Response				
France: no comment on this endpoint.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Finland		MemberState	23
Comment received				
Although no fully reliable study for acute toxicity via the oral route is available, the majority of the findings from the reported studies result in an LD50 ranging from 580 to 1390 mg/kg bw and thus within the criterion for classification in category 4. FI CA supports the proposal to classify methyl salicylate as Acute tox. 4, H302. Based on the available database, we agree that an ATE of 580 mg/kg bw reflecting the most conservative of the available LD50 data is justified.				
Dossier Submitter's Response				
France: Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Finland		MemberState	24
Comment received				
Contradictory results have been obtained both in animal and human studies investigating the sensitisation potential of methyl salicylate. Skin sensitisation of methyl salicylate has been tested using various methods over a wide range of concentrations. The available maximisation tests of limited reliability due to methodological limitations show negative results for skin sensitisation. The maximisation test nevertheless appears to be less sensitive for the detection of skin sensitising potential of methyl salicylate than the LLNA. Induction of sensitisation was detected with stimulation indices exceeding 3 in three LLNA studies at concentrations exceeding 25 %. In addition to testing conducted with experimental animals, human data from diagnostic studies, human volunteer studies and case studies exist. A sensitisation incidence of < 2				

% in diagnostic studies and two published cases of skin sensitisation support a low to moderate frequency of occurrence of skin sensitisation. The human data support the findings from LLNA studies for the classification of methyl salicylate as a skin sensitiser. FI CA supports the proposal to classify methyl salicylate as a skin sensitiser in category 1B (H317) based on both animal and human data.
Dossier Submitter's Response
France: Thank you for your support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Belgium	Procter & Gamble	Company-Downstream user	25
Comment received				

SKIN SENSITISATION
(text is also included as attachment)

Based on an overall weight of evidence using animal, human and alternative data, Methyl Salicylate should not be classified as a Skin Sensitiser.

Firstly, the OECD Guideline for the Testing of Chemicals, Skin Sensitisation: LLNA (1), considers Methyl Salicylate as an 'irritant' reference substance in the Guideline's Performance Standards (see Table 1 of OECD 429; July 2010). This is in line with the LLNA studies by Montelius et al., indicating that Methyl Salicylate is not a skin sensitiser but that concentrations above 25% may induce false positive results due to its irritation properties (2, 3).

Secondly, assessment of Methyl Salicylate in guinea pig Maximisation tests using optimal conditions for maximal stimulation of the skin immune system (circumventing the dermal barrier by intradermal administration of up to 5% in adjuvant) did not result in skin sensitisation (4, 5).

Thirdly, there is no indication for a skin sensitisation concern from the use of Methyl Salicylate when used for local pharmaceutical treatment at concentrations up to 10% based on pharmacovigilance data.

With regard to the Montelius LLNA studies, the authors conclude that the proliferation effect is likely due to irritation properties and not skin sensitisation as similar findings were reported for other known irritants e.g. nonanoic acid, sodium dodecyl sulfate, Triton X-100, chloroform/methanol and oxalic acid. Mechanistic studies using the B cell marker B220 also support the irritation concern and the lack of skin sensitisation properties for Methyl Salicylate (6). Further, data from Schreiter (1999), Moreno (1973) and Fassett (1963) which are part of the IULCID dossier indicate some irritation potential of this substance in rabbits, rats and mice even though the substance does not warrant hazard classification as a skin irritant according to GHS criteria. There are also human data indicating irritation potential at high test concentrations (7, 8 and 9).

Consequently, the effects noted at high concentration in the LLNA studies are not an indication of skin sensitisation potential of Methyl Salicylate and they do not warrant a hazard classification as a skin sensitiser even in the category 1B. This point is further supported by the fact that all studies with non-irritating concentrations in the LLNA assays

did not show any positive findings.

Based on the different vehicles used and the good skin penetration of Methyl Salicylate, it can also be excluded that a lack of bioavailability in the skin is responsible the non-sensitising properties in the studies using non-irritant concentrations up to 20%. Furthermore, Maximisation tests in guinea pigs (which circumvent the skin barrier) did not show any positive findings. The CLH report states that the Maximisation test is less sensitive than the LLNA without providing any reference for this conclusion. To our knowledge, no published reference indicates that the guinea pig Maximisation test is of limited sensitivity for low molecular weight chemicals structurally related to Methyl Salicylate.

Based on a review of human data by Basketter et al. (10), Methyl Salicylate has a very low intrinsic ability to cause skin sensitisation. It was grouped into the least potent category (category 5), which means that only exceptionally prolonged exposure in combination with high use levels may lead to skin sensitisation. All substances grouped into category 5 do not have a prevalence > 1% in the dermatology clinics.

Methyl Salicylate was also tested in in vitro skin sensitisation assays and evaluated in defined approaches which predict the skin sensitisation hazard of chemical substances (11, 12). Methyl Salicylate was negative in all alternative methods which have an OECD Test Guideline. Results indicated no sensitisation hazard in all defined approach prediction models.

In summary, based on the findings in animal and mechanistic assays, Methyl Salicylate is not a skin sensitiser and should not be classified as such.

The human data also reveal no clear indication that the substance is a skin sensitiser and the data do not show that skin contact can lead to sensitisation in a substantial number of people. In well-performed maximization and HRIPT tests, no sensitisation effects were observed. In patch tests with eczema patients or patients with dermatitis at other sites (eyelids), no reactions were observed in the eyelid dermatitis patients and only a few positive reactions were reported at very low percentages (< 2% and often triggered by 1 patient only) in the other group. For unselected patients the percentage of reported positive findings was even lower (all well below 1 %).

Also, the CLH dossier states that "It should be noted that the available human data are somewhat old. However, Methyl Salicylate is not currently included either in standard battery (such as Fragrance Mix I or II) or in the perfume battery. Therefore, it is difficult to make a clear and definitive conclusion on actual frequency of skin sensitisation to Methyl Salicylate". Importantly, Methyl Salicylate has been used as a dermally applied pharmaceutical substance at high concentrations for decades as a local treatment and the required pharmacovigilance data do not indicate that skin sensitisation is a concern.

The CLH dossier states that Methyl Salicylate is listed by the SCCS as an "established contact allergen in humans". However, in its report (13), the SCCS also confirms that this is based on less than 10 positive reactions (7 out of 1825 patients or < 0.4%)

Taken together, neither the skin sensitisation data in animals nor the data in humans justify a hazard classification as a skin sensitiser.

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ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments to the CLH report_PG FINAL Version_25 Jan 2019.zip

Dossier Submitter's Response

France:

We acknowledge that methyl salicylate is listed as a recommended reference substance in the OECD 429 guideline. In the table 1 of the guideline, negative results were noted for methyl salicylate in AOO in animals (LLNA and guinea pig studies) and in humans. However, the references / rationale behind these results are not provided. From the available data described in the CLH report, similar conclusion can be reached for guinea pigs studies (negative results). For LLNA, it should be highlighted that the positive results were obtained with methyl salicylate in solvents other than AOO (DMF and MEK), except

one study performed in 2012 (and thus after the publication of the OECD guideline 429). Finally, various publications reported consistent cases of sensitization in humans.

Differentiation between irritation and sensitisation: this point has been already investiguated and is discussed in the CLH report from page 26 to 28. Indeed, even if we cannot exclude a potential of skin irritation, the data available are not clearly consistent and tend toward a rather weak irritancy potential. Indeed, in a well-performed OECD 404 study (Anonymous, 1999), the erythema score was 1.3 and the oedema score 0.6 when the substance was undiluted. The score decreased to 0.2 and 0, respectively, when methyl salicylate was tested at 25%. Considering the 3 references cited in your comment, Schreiter (1999) corresponds to the study above already described in the CLH report (Anonymous, 1999). Furthermore, it should be noted that the available data related to Moreno (1973) and Fassett (1963) studies do not allow a proper evaluation of the results. In addition, when the data were available, the positive response obtained in sensitization assay cannot be linked to an irritation (for example in Adenuga et al., 2012; de Groot et al., 2000; Nethercott et al., 1989).

Concerning the impact of the solvent, we agree that no conclusion can be reached since positive results are obtained in LLNA with methyl salicylate in DMF or MEK or AOO solvents.

In conclusion on animal data, according to CLP guidance, when contradictory results occur, the substance should be classified according to the findings resulting to the stricter classification, unless there is sufficient information to discount the results. In this case, the available data do not allow disregarding the positive responses obtained in different studies. However, it should be highlighted that the proposed classification of methyl salicylate is rather derived from human data, supported by the animal data.

Regarding Basketter et al. (2014), the rationale of the categorisation of human data is not clearly detailed and does not correspond to CLP criteria. In particular, for methyl salicylate, the data used to conclude to category 5 are not specified. Indeed, references to positive diagnostic studies cited in the CLH report are not listed in this publication. Finally, it should be noted that even if it is stated that substances in category 5 should not be classified according to CLP regulation, several substances presented in this list have sensitization potential, for example: benzyl salicylate (CLH report submitted by Germany for Skin. Sens.1B); citronellol and hexylcinnamal (even if there is no harmonized classification for these substances, a large majority of notifiers declare self-classification as Skin Sens.). Therefore, concluding that substances listed in the category 5 proposed by Basketter et al. (2014) should not be classified according to CLP regulation is highly doubtful.

According to your comment, methyl salicylate is negative in *in vitro* skin sensitisation assays based on Hoffmann et al. 2018 and Kleinstreuer et al. 2018 publications. Some indications of sensitisation is nevertheless noted in LuSens assay (Urbisch et al., 2015) and in SENS-IS test (Cottrez et al., 2016; weak classification). At the time being, *in vitro* assays cannot replace the *in vivo* data. Therefore this information cannot be used to disregarded the positive findings reported in both human and animals.

Regarding human data, you state that "*Methyl Salicylate has been used as a dermally applied pharmaceutical substance at high concentrations for decades as a local treatment and the required pharmacovigilance data do not indicate that skin sensitisation is a concern*". We have no access to pharmacovigilance data. However, it can be noted that at least 2 cases were described after exposure to methyl salicylate used as an anti-

inflammatory agent and in an analgesic ointment. These cases are reported in the CLH report (Oiso et al., 2004 and Hindson, 1977). We do not agree that human data reveal no clear indication that the substance is a skin sensitiser. There are several diagnostic studies (a total of 7 publications), all reporting consistent positive patch tests with methyl salicylate. Even if the incidence is rather low, the CLP guidance do not set a threshold below which classification is not relevant (table 3.2 of CLP guidance). The incidence should be considered for subcategorization only. A total number of 29 cases over the 7 publications is reported in the literature with methyl salicylate. When considering individual studies, occurrence of skin sensitisation corresponds to a low/moderate frequency according to CLP guidance.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	Germany		MemberState	26
Comment received				
We agree with the proposed classification "Skin Sens. 1B, H317" based on positive reactions in diagnostic patch test studies in humans and supported by positive results in diverse LLNAs.				
Dossier Submitter's Response				
France: Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2018	United Kingdom	<confidential>	Company-Downstream user	27
Comment received				
No comments on this endpoint ECHA note – An attachment was submitted with the comment above. Refer to public attachment clh_opinion_salicylic_acid_6425_en.pdf				
Dossier Submitter's Response				
France: no comment specific to this endpoint. Attachment refers to RAC opinion on salicylic acid.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	France	NOVACYL	Company-Manufacturer	28
Comment received				
The comments to the following endpoint have been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company. Based on an overall weight of evidence using animal, human and alternative data, Methyl Salicylate should not be classified as a Skin Sensitiser.				

In the OECD Guideline for the Testing of Chemicals, Skin Sensitisation: LLNA, Methyl Salicylate (MeS) is reported as an 'irritant reference substance" and not a 'skin sensitising' agent in the list of recommended reference substances in the Guideline's Performance Standards (see Table 1 of OECD 429; July 2010 (1)). In addition, there is quite some evidence that false positive findings (proliferation in the local lymph node) due to irritation at higher concentrations occur for MeS and other irritating substances (2, 3, 4).

This evidence compares well with the studies cited in the CLH report especially the LLNA studies by Montelius (3, 4). If positive findings were noted at all in these studies, they were seen at concentrations of > 25% or even >= 50% and were independent of the vehicle used (3). Thus, the different findings in the literature can hardly be explained by the use of different vehicles as argued in the CLH report, taking into account that the substance shows good skin absorption, which is the factor potentially most affected by the vehicle used.

With regard to the Montelius LLNA studies, even the authors conclude that the proliferation effect observed is likely to be an effect of the irritation properties and not an indication for skin sensitisation as similar findings were reported for other known irritants e.g. nonanoic acid, sodium doedecyl sulfate, Triton X-100, chloroform/methanol and oxalic acid. Mechanistic studies using the B cell marker B220 also support the irritation hypothesis and the lack of skin sensitisation properties for MeS (5). Further, data from Schreiter (1999), Moreno (1973) and Fassett (1963) which are part of the IUCLID dossier indicates some irritation potential of this substance in rabbits, rats and mice even though the substance does not warrant hazard classification as a skin irritant according to GHS criteria. There are also human data indicating irritation potential at higher test concentrations (6,7,14).

Consequently, the effects noted at high concentrations in the LLNA studies are not an indication of skin sensitisations potential of MeS and they do not warrant a hazard classification as a skin sensitiser even in the category 1B. This point is further supported by the fact that all studies with non-irritating concentrations in LLNA assays did not show any positive findings.

Based on the different vehicles used and the skin penetration properties of MeS, it can also be excluded that a lack of bioavailability in the skin is responsible for the non-sensitising properties in the studies using non-irritant concentrations up to 20%. Furthermore, Maximisation tests in guinea pigs representing worst case test conditions (circumventing the dermal barrier by intradermal administration in adjuvant) and fulfilling Klimisch 2 criteria did not show any positive findings. The CLH report states that the Maximisation test is less sensitive than the LLNA without providing any reference for this conclusion. We doubt that such a general statement is scientifically supported. To our knowledge no published reference indicates that the Maximisation test is of limited sensitivity for low molecular weight chemicals structurally related to methyl salicylate.

Based on a review of human data by Basketter et al. (8), MeS has a very low intrinsic ability to cause skin sensitisation. It was grouped into the least potent category (category 5), which means that only exceptionally prolonged exposure in combination with high use levels may lead to skin sensitisation. All substances grouped into category 5 do not have a prevalence > 1% in the dermatology clinics.

MeS was also tested in available skin sensitisation in vitro alternative methods and evaluated in defined approaches which predict the skin sensitisation hazard of chemical

substances (9, 10). MeS was negative in all alternative methods which have an OECD Test Guideline. The data also indicated no sensitisation hazard in all defined approach prediction models. This conclusion is in line with the data set of the QSAR tool box (see attachment 1) considered as a reliable source for the evaluation and assessment of chemicals under REACH. None of the in vitro mechanistic studies indicate a skin sensitising potential.
In summary, based on the findings in animal and mechanistic assays, MeS is not a skin sensitizer and should not be classified as such.

The human data also reveal no clear indication that the substance is a skin sensitizer and the data do not show that skin contact can lead to sensitisation in a substantial number of people. In well-performed maximization and HRIPT tests, no sensitisation effects were observed. In addition, practically no positive findings were noted at work places where accidental exposure to undiluted substance can be expected. In patch tests with eczema patients or patients with dermatitis at other sites (eyelids), no reactions were observed in the eyelid dermatitis patients and only a few positive reactions were reported at very low percentages (< 2% and often triggered by 1 patient only) in the other group. For unselected patients the percentage of reported positive findings were even lower (all well below 1 %).
The CLH dossier states that MeS is listed by the SCCS as an "established contact allergen in humans". However, in its report, the SCCS also confirms that this is based on less than 10 positive reactions (7 out of 1825 patients or < 0.4%)

Also, the CLH dossier states that "It should be noted that the available human data are somewhat old. However, MeS is not currently included either in standard battery (such as Fragrance Mix I or II) or in the perfume battery. Therefore, it is difficult to make a clear and definitive conclusion on actual frequency of skin sensitisation to MeS".

Importantly, MeS has been used for decades for local treatment as a pharmaceutical substance at high concentrations of up to 10 % and the required pharmacovigilance data does not indicate that skin sensitisation is a concern.
E.g. Salonpas (containing 10% MeS) is used in the United States as a topical pain releaser, and no adverse effects were observed in the clinical trials or as case reports (11). In the US and Canada, there are rare cases of burns reported in the users of topical formulations containing MeS, but no allergic reaction (12, 13). The CIR (2018, page 60) confirms an irritation effect in humans from 12% (with no irritation at 8%).
MeS is a counterirritant [an externally applied substance that causes irritation or mild inflammation of the skin for the purpose of relieving pain in muscles or joints by reducing inflammation in deeper adjacent structures (15)] and thus an irritant effect or a pseudo allergic inflammation reaction can explain the skin effect observed. This mechanism of action is in line with the strength of the reactions observed in animals, vanishing abruptly when the concentration is lowered and clearly indicates a false-positive irritant reaction. For allergic reactions a gradually diminishing with decreasing concentration is noted.

Taken together, neither skin sensitisation data in animals nor data in humans justify a hazard classification as a skin sensitiser. This conclusion is in line with mechanistic investigations in vitro showing no indication for a skin sensitising mechanism of action of MeS.

References
1) Local lymph node assays, OECD 429 test guidance, 23. July 2010
2) ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans, NIH publication 11-7709

3) Montelius et al. Murine Local Lymph node Assay for Predictive Testing of Allergenicity: Two Irritants caused significant proliferation. Acta Derm. Venereol (Stockh) 78:433-437, 1998
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8) Basketter, D. A. Alepee N. Ashikaga T. Barroso J. Gilmour N. Goebel C. Hibatallah J. Hoffmann S. Kern P. Martinozzi-Teissier S. Maxwell G. Reisinger K. Sakaguchi H. Schepky A. Tailhardat M. and Templier M (2014). Categorization of chemicals according to their relative human skin sensitizing potency. Dermatitis 25:11-21
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https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM453825.pdf
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https://www.fda.gov/Drugs/DrugSafety/ucm318858.htm
13) Summary Safety Review - Over-the-Counter Topical Pain Relievers Containing Menthol, MeS or Capsaicin - Assessing the Risk of Serious Skin Burns (February 13, 2017)
https://www.canada.ca/en/health-canada/services/drugs-health-products/medeffect-canada/safety-reviews/summary-safety-review-pain-relievers-containing-menthol-methyl-salicylate-capsaicin-risk-serious-skin-surns.html
14) CIR (2018). Amended Safety Assessment of Salicylic Acid and Salicylates as Used in Cosmetics. Draft Final Amended Report for Panel Review, November 9, 2018
15) Medline 2012; MediLexicon 2012; US FDA 1983.
http://webprod.hc-sc.gc.ca/nhpid-bdipsn/atReq.do?atid=counter&lang=en

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip

Dossier Submitter's Response

France:

See response to comment 25.

Concerning specific comment related to dataset of the QSAR Tool box: estimation from non-testing system cannot be used to disregard the positive findings reported in both humans and animals. It can be noted that prediction by CAESAR (version 2.1.) for methyl salicylate is "*sensitizer, the result appears reliable*".

Some references cited in this comment refer to irritant potency of methyl salicylate (e.g references 12, 13, 14 and 15). We acknowledge that irritation is reported in the literature with methyl salicylate. However, the data available are not clearly consistent and tend toward a rather weak irritancy potential. See also response to comment 25.

Reference 14 reports an irritation effect in humans from 12% (with no irritation at 8%). According to reference 15, methyl salicylate is listed as a counterirritant at doses from 10%. In addition, in the comments it is said that no adverse effects were observed in the clinical trials or as case reports with Salonpas (containing 10% MeS; it should be noted that this product also contains menthol).

In the comments it is also said that no allergic reaction were reported in the users of topical formulations containing MeS (references 12 and 13). However, it should be noted that the aim of the references 12 and 13 is to assess the risk of serious skin burns. Therefore, it is not clear if skin sensitisation cases have been investiguated. In addition, it is reported by Health Canada (reference 13) that the available information has established a link between the use of topical pain relievers containing menthol and the risk of rare but serious skin burns; however, there was not enough information to draw the same conclusions for the products containing methyl salicylate alone. Similarly, FDA (reference 12) concludes that burns have been reported with products containing methol as a single active ingredient and with products containing both menthol and methyl salicylate, at concentrations greater than 3% menthol and 10% methyl salicylate.

Overall, these above data cannot be used to disregard the results of the 7 diagnostic studies showing positive patch tests with methyl salicylate. In particular, in these studies, the concentration of methyl salicylate is 1 or 2%. At these concentrations (clearly below 10-12% considered as irritant by the references you cited), positive patch tests were reported, confirming that the reaction are linked to a sensitisation effect and not an irritation.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	Germany	Symrise AG	Company-Importer	29

Comment received

SKIN SENSITISATION

The comments to the following endpoint has been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company.

Based on an overall weight of evidence using animal, human and alternative data, Methyl Salicylate should not be classified as a Skin Sensitiser.

In the OECD Guideline for the Testing of Chemicals, Skin Sensitisation: LLNA, Methyl Salicylate (MeS) is reported as an 'irritant reference substance" and not a 'skin sensitising' agent in the list of recommended reference substances in the Guideline's Performance Standards (see Table 1 of OECD 429; July 2010 (1)). In addition, there is quite some evidence that false positive findings (proliferation in the local lymph node) due to irritation at higher concentrations occur for MeS and other irritating substances (2, 3, 4).

This evidence compares well with the studies cited in the CLH report especially the LLNA studies by Montelius (3, 4). If positive findings were noted at all in these studies, they were seen at concentrations of > 25% or even >= 50% and were independent of the vehicle used (3). Thus, the different findings in the literature can hardly be explained by the use of different vehicles as argued in the CLH report, taking into account that the substance shows good skin absorption, which is the factor potentially most affected by the vehicle used.

With regard to the Montelius LLNA studies, even the authors conclude that the proliferation effect observed is likely to be an effect of the irritation properties and not an indication for skin sensitisation as similar findings were reported for other known irritants e.g. nonanoic acid, sodium doedecyl sulfate, Triton X-100, chloroform/methanol and oxalic acid. Mechanistic studies using the B cell marker B220 also support the irritation hypothesis and the lack of skin sensitisation properties for MeS (5). Further, data from Schreiter (1999), Moreno (1973) and Fassett (1963) which are part of the IUCLID dossier indicates some irritation potential of this substance in rabbits, rats and mice even though the substance does not warrant hazard classification as a skin irritant according to GHS criteria. There are also human data indicating irritation potential at higher test concentrations (6,7,14).

Consequently, the effects noted at high concentrations in the LLNA studies are not an indication of skin sensitisations potential of MeS and they do not warrant a hazard classification as a skin sensitiser even in the category 1B. This point is further supported by the fact that all studies with non-irritating concentrations in LLNA assays did not show any positive findings.

Based on the different vehicles used and the skin penetration properties of MeS, it can also be excluded that a lack of bioavailability in the skin is responsible for the non-sensitising properties in the studies using non-irritant concentrations up to 20%. Furthermore, Maximisation tests in guinea pigs representing worst case test conditions (circumventing the dermal barrier by intradermal administration in adjuvant) and fulfilling Klimisch 2 criteria did not show any positive findings. The CLH report states that the Maximisation test is less sensitive than the LLNA without providing any reference for this conclusion. We doubt that such a general statement is scientifically supported. To our knowledge no published reference indicates that the Maximisation test is of limited sensitivity for low molecular weight chemicals structurally related to methyl salicylate.

Based on a review of human data by Basketter et al. (8), MeS has a very low intrinsic ability to cause skin sensitisation. It was grouped into the least potent category (category 5), which means that only exceptionally prolonged exposure in combination with high use levels may lead to skin sensitisation. All substances grouped into category 5 do not have a prevalence > 1% in the dermatology clinics.

MeS was also tested in available skin sensitisation in vitro alternative methods and evaluated in defined approaches which predict the skin sensitisation hazard of chemical substances (9, 10). MeS was negative in all alternative methods which have an OECD Test Guideline. The data also indicated no sensitisation hazard in all defined approach prediction models. This conclusion is in line with the data set of the QSAR tool box (see attachment 1) considered as a reliable source for the evaluation and assessment of chemicals under REACH. None of the in vitro mechanistic studies indicate a skin sensitising potential.
In summary, based on the findings in animal and mechanistic assays, MeS is not a skin sensitizer and should not be classified as such.

The human data also reveal no clear indication that the substance is a skin sensitizer and the data do not show that skin contact can lead to sensitisation in a substantial number of people. In well-performed maximization and HRIPT tests, no sensitisation effects were observed. In addition, practically no positive findings were noted at work places where accidental exposure to undiluted substance can be expected. In patch tests with eczema patients or patients with dermatitis at other sites (eyelids), no reactions were observed in the eyelid dermatitis patients and only a few positive reactions were reported at very low percentages (< 2% and often triggered by 1 patient only) in the other group. For unselected patients the percentage of reported positive findings were even lower (all well below 1 %).
The CLH dossier states that MeS is listed by the SCCS as an "established contact allergen in humans". However, in its report, the SCCS also confirms that this is based on less than 10 positive reactions (7 out of 1825 patients or < 0.4%)

Also, the CLH dossier states that "It should be noted that the available human data are somewhat old. However, MeS is not currently included either in standard battery (such as Fragrance Mix I or II) or in the perfume battery. Therefore, it is difficult to make a clear and definitive conclusion on actual frequency of skin sensitisation to MeS".

Importantly, MeS has been used for decades for local treatment as a pharmaceutical substance at high concentrations of up to 10 % and the required pharmacovigilance data does not indicate that skin sensitisation is a concern.
E.g. Salonpas (containing 10% MeS) is used in the United States as a topical pain releaser, and no adverse effects were observed in the clinical trials or as case reports (11). In the US and Canada, there are rare cases of burns reported in the users of topical formulations containing MeS, but no allergic reaction (12, 13). The CIR (2018, page 60) confirms an irritation effect in humans from 12% (with no irritation at 8%).
MeS is a counterirritant [an externally applied substance that causes irritation or mild inflammation of the skin for the purpose of relieving pain in muscles or joints by reducing inflammation in deeper adjacent structures (15)] and thus an irritant effect or a pseudo allergic inflammation reaction can explain the skin effect observed. This mechanism of action is in line with the strength of the reactions observed in animals, vanishing abruptly when the concentration is lowered and clearly indicates a false-positive irritant reaction. For allergic reactions a gradually diminishing with decreasing concentration is noted.

Taken together, neither skin sensitisation data in animals nor data in humans justify a hazard classification as a skin sensitiser. This conclusion is in line with mechanistic investigations in vitro showing no indication for a skin sensitising mechanism of action of MeS.

References
1) Local lymph node assays, OECD 429 test guidance, 23. July 2010
2) ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans, NIH publication 11-7709
3) Montelius et al. Murine Local Lymph node Assay for Predictive Testing of Allergenicity: Two Irritants caused significant proliferation. Acta Derm. Venereol (Stockh) 78:433-437, 1998
4) Montelius, et al. Experience with the Murine Local Lymph Node Assay: Inability to Discriminate between Allergens and Irritants. Acta Derm. Venereol (Stockh) 74: 22-27, 1994
5) Gerberick et al. Use of B Cell Marker (B220) to Discriminate between Allergens and Irritants in the Local Lymph Node Assay. Toxicological Sciences 68, 420-428, 2002
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8) Basketter, D. A. Alepee N. Ashikaga T. Barroso J. Gilmour N. Goebel C. Hibatallah J. Hoffmann S. Kern P. Martinozzi-Teissier S. Maxwell G. Reisinger K. Sakaguchi H. Schepky A. Tailhardat M. and Templier M (2014). Categorization of chemicals according to their relative human skin sensitizing potency. Dermatitis 25:11-21
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15) Medline 2012; MediLexicon 2012; US FDA 1983.
http://webprod.hc-sc.gc.ca/nhpid-bdipsn/atReq.do?atid=counter&lang=en

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip

Dossier Submitter's Response

France: same comment as comment 28. See response to comment 28.

RAC's response

Noted.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2018	United Kingdom	<confidential>	Company-Downstream user	30

Comment received

No comments on this endpoint

ECHA note – An attachment was submitted with the comment above. Refer to public attachment clh_opinion_salicylic_acid_6425_en.pdf

Dossier Submitter's Response

France: no comment on this endpoint.

RAC's response
Noted, no comment on this endpoint.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2019	Germany	Symrise AG	Company-Importer	31

Comment received

Comment to H412
The comments to the following endpoint has been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company.
A classification on the basis of the available OECD 201 study is premature due to deficiencies in the study (outlined below) and the final assessment should be postponed until a valid and scientifically robust study is available.

A new classification for the environment with H412,has been proposed. It is based on an algae (OECD 201) study revealing a NOEC < 1 mg/L (NOEC=0.79 mg/L based on the geometric mean measured test concentrations).
IES (Innovative Environmental Services) was asked to assess the study report specifically with regards to paragraphs 37 and 40 of the OECD 201 guideline and to calculate EC10 values(for details see attachment 1). EC10 values are considered as a more relevant measure than NOECs for C&L as they are based on the entire data set.

In summary, the current OECD 201 study does not allow a final assessment of chronic toxicity in algae due to failures in the methodology (mainly regarding analytical measurement) having a manifest impact on the interpretation and validity of the study. Nevertheless, the missing analytical data points at 24 and 48 h would most likely result in a higher NOEC/EC 10 value. In addition, based on the current data an EC10 (growth rate) > 1 mg/L (1.033 mg/L) was deduced (not given in the original report) indicating that the current study most likely overestimates the effects of Methyl Salicylate.
Taking into account the assessment by IES which clearly highlights the failures in the study's methodology and the subsequent impact on the results and their interpretation, we do not consider the current study as a reliable basis for the assessment of chronic effects in algae or any conclusion drawn from it with regard to C&L. Therefore, we request that any decision regarding this endpoint be postponed until a new state of the art and guideline compliant study is available.

Attachment 1 (IES report)

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip

Dossier Submitter's Response

France: According to the Guide R7b p.75, it is stated that "For static tests, where the concentrations do not remain within 80-120% of nominal, the effect concentrations should be expressed relative to the geometric mean of the measured concentrations at the start and end of the test.", as it is the case in the study. That's why we decided not to include the timepoint at 24 and 48h. We think the classification H412 should be kept, with respect to the aquatic environment according to the Regulation (EC) No 1272/200

RAC's response

RAC agrees with the DS in considering the algae test valid. RAC also agrees that the ECHA Guidance advises to use measured concentrations at the start and end of the test for calculating the geometric mean. Unfortunately, no EC_{10} has been calculated with this

information. The IES report uses also measured concentrations at 49 hours in their calculations getting an EC_{10} of 1.033 mg/L.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	United Kingdom		MemberState	32

Comment received

Methyl salicylate (EC: 204-317-7; CAS: 119-36-8)
The available fate data presented in the CLH include a non-GLP ready biodegradation test with limited reliability due to lack of raw data to assess the validity of study controls. In addition, the reported Henry's Law Constant of 4.76 Pa.m3.mol (ECHA's online database) indicates the test item may be lost from the aquatic phase meaning the test method may not be the most appropriate. Additional supporting fate data involve non-standard methods and/or inoculum and unknown sample composition. While QSAR data are quoted, it is unclear if the test item falls within the model domain and if the QSARs are valid.

Given the above uncertainties, we feel additional information should be presented to support methyl salicylate as rapidly degradable. This could include read-across with reliable analogue data and/or valid QSARs. If this is not available, a substance would normally be considered not rapidly degradable.

We agree that algal mean-measured endpoints are reliable for hazard classification.

We note the read-across Daphnia magna 48-hour EC50 of 28 mg/l for ethyl salicylate is based on initial measured concentrations. It is unclear if analytical verification at study termination is available to verify the use of initial measured concentrations. Please can you clarify if 48 hour analytical concentrations are available? This is important as the endpoint is used for acute hazard classification and relevant for chronic classification using the surrogate approach if methyl salicylate is considered not rapidly degradable.

Based on available data, algal appear to be the most acutely sensitive trophic level. However, it is unclear if algae are the most chronically sensitive trophic. Overall, it would be useful if additional toxicity data for relevant analogues were presented with a clear read-across justification. We note that the substance is currently being considered under Substance Evaluation (SEV-FR-019/2015) and that additional data information may become available.

Dossier Submitter's Response

France:
We used Episuite and Danish QSAR database to add information about the biodegradability of the substance, the results are presented below :

Episuite:
Biowin1 (Linear Model Prediction) : Biodegrades Fast
Biowin2 (Non-Linear Model Prediction): Biodegrades Fast
Biowin3 (Ultimate Biodegradation Timeframe): Weeks
Biowin4 (Primary Biodegradation Timeframe): Days
Biowin5 (MITI Linear Model Prediction) : Readily Degradable
Biowin6 (MITI Non-Linear Model Prediction): Readily Degradable
Biowin7 (Anaerobic Model Prediction): Biodegrades Fast
Ready Biodegradability Prediction: YES

Moreover, the Danish QSAR database indicates :

DK	Exp	Battery	E Ultra	Leadscope	SciQSAR
Not Ready Biodegradability (POS=Not Ready)		NEG_IN	NEG_IN	NEG_IN	NEG_IN

Methyl salicylate is readily biodegradable in all models (while being in the applicability domain of each model). This supports the assumption of rapid biodegradation of the substance.

Thank you for your agreement on algal mean-measured endpoints.

Concerning the read-across with ethyl salicylate on Daphnia magna 48-hour leading to an EC_{50} of 28 mg/L, the study was considered of reliability index 2. The test item was only analytically verified at test begin and not at the end. Nevertheless, the test item unstability during experiment was specifically reported for the algae study with methyl salicylate and not in the other assays. For instance, the acute study on ethyl salicylate with fathead minnow **(**Geiger et al. 1985) was conducted in static conditions with measurements of the test item at several times during the experiment and only slight differences with nominal concentrations were noted. To our point of view, the stability of ethyl salicylate in other experiments confirmed that the EC50 (48h) value of 28 mg/L for daphnia is acceptable and the instability of the test item was specific to the algae study.

No additional toxicity data on the chronic level is available on analogs. Regarding the uncertainty of algae to be the most chronically sensitive trophic, according the ECHA Guidance on the Application of the CLP Criteria (2017), Figure 4.1.1 p.504, if there is at least one toxicity data available for one trophic level (i.e algae in our case), we can classify the substance. A SEv is indeed currently being considered, but no additional data will be provided on invertebrates.

Nevertheless, considering the QSAR data on methyl salicylate reported below (from the Danish QSAR database) which are in the applicability domains, the acute toxicity for the 3 trophic levels are in the same order of magnitude (even if invertebrates toxicity is slightly higher). That can justify the proposed chronic classification considering that invertebrates seem as sensitive as algae in the acute tests.

Aquatic toxicity

DK	Exp	Battery	Leadscope	SciQSAR
Fathead minnow 96h LC50 (mg/L)		27.15901	13.46052	40.85751
Domain		IN	IN	IN
Daphnia magna 48h EC50 (mg/L)		22.07411	18.6111	25.53712
Domain		IN	IN	IN
Pseudokirchneriella s. 72h EC50 (mg/L)		44.66078	45.29934	44.02222
Domain		IN	IN	IN

EPI ECOSAR	Fish 96h	Daphnid 48h	Green Algae 96h

LC50 (Fish) or EC50 (Daphnid and Algae) for Most Toxic Class (mg/L)	9.034	3.62	6.809
Max. Log Kow for Most Toxic Class	5	7	6.4
Most Toxic Class	Esters	Phenols	Esters
Note			

ECOSAR Classes: Esters;Phenols

RAC's response

RAC considers the ready biodegradability valid due to the following:

- The study report by King from 1993 The Biodegradability of Perfume Ingredients in the Sealed Vessel Test refers to study report published in Chemosphere, Vol. 23, No 4, pp 507-524 in 1991 for development and validation of the method used. This publication by Birch, R.R. and Fletcher, R.J is titled The Application of Dissolved Inorganic Carbon Measurements to the Study of Aerobic Biodegradability. The article is about developing a test that is essentially the same as the Sturm CO2 Production tests (OECD TG) but with greater simplicity of the technique and the high precision of the data. It does not include any validity criteria as such. This study has been referenced and used as the basis of the OECD TG 310. The study is especially ment for volatile substances (sealed vessels).

RAC agrees with the DS in considering the algae test valid. RAC also agrees that the ECHA Guidance advises to use measured concentrations at the start and end of the test for calculating the geometric mean. Unfortunately, no EC_{10} has been calculated with this information. The IES report uses also measured concentrations at 49 hours in their calculations getting an EC_{10} of 1.033 mg/L.

Concerning the *Daphnia magna* 48-hour test leading to an EC_{50} of 28 mg/L RAC agrees that only initially measured concentrations are available. RAC does not agree with the DS view that the stability of ethyl salicylate has been confirmed in other experiments. The only information we have comes from the *Pimephales promelas* test which is a flow-through test (CLH Report and it's Annex) and concentration have only been measured in the beginning of the test.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2019	France	NOVACYL	Company-Manufacturer	33

Comment received

The comments to the following endpoint have been prepared jointly by Symrise and Novacyl as leading group for the registration dossier. The same comments are posted by the other company.
A classification on the basis of the available OECD 201 study is premature due to deficiencies in the study (outlined below) and the final assessment should be postponed until a valid and scientifically robust study is available.

A new classification for the environment with H412 ,has been proposed. It is based on an algae (OECD 201) study revealing a NOEC < 1 mg/L (NOEC=0.79 mg/L based on the geometric mean measured test concentrations).
IES (Innovative Environmental Services) was asked to assess the study report specifically with regards to paragraphs 37 and 40 of the OECD 201 guideline and to calculate EC10 values (for details see attachment 1). EC10 values are considered as a more relevant measure than NOECs for C&L as they are based on the entire data set.

In summary, the current OECD 201 study does not allow a final assessment of chronic toxicity in algae due to failures in the methodology (mainly regarding analytical measurement) having a manifest impact on the interpretation and validity of the study. Nevertheless, the missing analytical data points at 24 and 48 h would most likely result in a higher NOEC/EC 10 value. In addition, based on the current data an EC10 (growth rate) > 1 mg/L (1.033 mg/L) was deduced (not given in the original report) indicating that the current study most likely overestimates the effects of Methyl Salicylate. Taking into account the assessment by IES which clearly highlights the failures in the study's methodology and the subsequent impact on the results and their interpretation, we do not consider the current study as a reliable basis for the assessment of chronic effects in algae or any conclusion drawn from it with regard to C&L. Therefore, we request that any decision regarding this endpoint be postponed until a new state of the art and guideline compliant study is available. Attachment 1 (IES report) ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH-proposal attachments CAS119-36-8.zip
Dossier Submitter's Response
France: According to the Guide R7b p.75, it is stated that "For static tests, where the concentrations do not remain within 80-120% of nominal, the effect concentrations should be expressed relative to the geometric mean of the measured concentrations at the start and end of the test", as it is the case in the study. That's why we decided not to include the timepoint at 24 and 48h. We think the classification H412 should be kept, with respect to the aquatic environment according to the Regulation (EC) No 1272/200.
RAC's response
RAC agrees with the DS in considering the algae test valid. RAC also agrees that the ECHA Guidance advises to use measured concentrations at the start and end of the test for calculating the geometric mean. Unfortunately, no EC_{10} has been calculated with this information. The IES report uses also measured concentrations at 49 hours in their calculations getting an EC_{10} of 1.033 mg/L.

PUBLIC ATTACHMENTS
1. Comments to the CLH report_PG FINAL Version_25 Jan 2019.zip [Please refer to comment No. 3, 13, 25]
2. CLH-proposal attachments CAS119-36-8.zip [Please refer to comment No. 6, 16, 28, 33]
3. Reproductive Toxicity Public.docx [Please refer to comment No. 7, 17]
4. Public Response to Methyl Salicylate CLH .docx [Please refer to comment No. 18]
5. IFRA comments to Methyl salicilate consultation final.pdf [Please refer to comment No. 8, 19]
6. CLH-proposal attachments CAS119-36-8.zip [Please refer to comment No. 9, 20, 29, 31]
7. clh_opinion_salicylic_acid_6425_en.pdf [Please refer to comment No. 5, 15, 22, 27, 30]

CONFIDENTIAL ATTACHMENTS
1. Methyl Salicylate - CLH public consultation comments.pdf [Please refer to comment No. 2, 11]
2. Reproductive Toxicity CP.docx [Please refer to comment No. 7, 17]