Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2-phenylpropene; α-methylstyrene

EC Number: 202-705-0
CAS Number: 98-83-9

CLH-O-0000007252-80-01/F

Adopted

16 March 2023

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-PHENYLPROPENE; α - METHYLSTYRENE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during 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 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 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. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 2-phenylpropene; α-methylstyrene
EC number: 202-705-0
CAS number: 98-83-9
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	Belgium	ReachCentrum on behalf of the Phenols & Derivatives Reach Consortium	Company-Manufacturer	1

Comment received

On behalf of INEOS Phenol GmbH as lead registrant of 2-phenylpropene (CAS Number: 98-83-9; EC Number: 202-705-0) and representing the 41 EU manufacturers and suppliers of this substance under REACH we are pleased to have the opportunity to submit comments/information on the harmonised classification and labelling proposal for this substance (Version number 2.0, Dated March 2022).

Note: Summaries and general statements are included in the comment boxes. Detailed comments concerning the CLH Report are provided in the attachment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-phenylpropene_detailed comments on the CLH Report_PandD Consortium_20220623.pdf

Dossier Submitter's Response

The DS appreciates the comments raised by the organisation and acknowledges that the general conclusions on the classification of 2-phenylpropene are supported (see comment no. 4). With respect to some of the detailed comments, the DS would like to respond as follows.

In line with the deletion of the testing guideline for a lack of mechanistic understanding, the biological relevance of a positive finding in an *in vitro* sister chromatid exchange

(SCE) assay for the endpoint germ cell mutagenicity is considered uncertain as stated in the CLH report. The DS, however, supports the initiative announced under comment no. 7 to address the positive SCE results obtained with 2-phenylpropene by generating new data to further clarify the genotoxic potential of 2-phenylpropene.

The DS agrees with the organisation on the unclear relevance of the proposed primary metabolite, 2-phenylpropene oxide, for the endpoint *in vivo* mutagenicity. As stated in the CLH report, the formation of the epoxide has not been experimentally verified. Human information on the pace of the first metabolic step or the tissue specificity is lacking. Similar to what has been described for the close structural analogue, styrene, the metabolic activation of 2-phenylpropene likely depends on the activity of cytochrome P450 (CYP) enzymes, which may be expressed in a species- and/or tissue-specific manner. CYP450-dependent epoxidation yielding 2-phenylpropene oxide is also predicted to occur in humans according to the knowledge-based expert system for metabolism prediction, METEOR (Lhasa Limited).

The CLH report compares (it does not group) 2-phenylpropene with close structural analogues with respect to available genotoxicity data.

The conclusion that *"the existence of some genotoxic potential attributed to 2-phenylpropene, or its metabolite(s) cannot be ruled out"* is based on the outcome of the weight-of-evidence approach and not solely on the *"comparison to the structural analogue styrene where epoxidation of the side chain double bond results in the formation of the reactive styrene-7,8-oxide (SO)"* as stated by the organisation. The DS disagrees with the organisation that *"a genotoxic MoA can reasonably be excluded based on the available data"*. As listed in Table 16 of the CLH report, a number of arguments are in support of some genotoxic potential of 2-phenylpropene.

RAC's response

Thank you for the detailed comments. RAC agrees that 2-phenylpropene is conclusively negative for mutations in bacteria. In vitro mutagenicity assays in mammalian cells are negative but some of them have deviations from the current TGs, potentially reducing their sensitivity (e.g. short harvest time, lower number of scored cells). Thus, the recently submitted guideline-compliant in vitro micronucleus test in human lymphocytes (Gilby, 2023, the study report submitted by industry as informed during the consultation and taken into consideration by RAC) represents a valuable contribution to the dataset. In vitro SCE assays are indeed given lower weight than mutagenicity tests.

As to the MN test by NTP, the protocol is well-established and able to produce positive results. The fact that the concurrent control in the test with 2-phenylpropene is markedly above the historical control introduces some uncertainty, as does the fact that the increase in MN-NCEs in top concentration females was not accompanied by an increase in MN-PCEs. Excessive toxicity in this group further questions the relevance of the positive finding. Given these uncertainties and the negative in vitro mutagenicity database, RAC agrees that available data do not meet the criteria for classification.

Metabolism of 2-phenylpropene via the Ames-positive side-chain epoxide is highly plausible based on the urinary metabolites in the rat ADME study and analogy with styrene. On the other hand, the levels of this metabolite in tissues might be low depending on the kinetics of oxidation and hydrolysis/conjugation (cf. Morgan et al., 1999). The classification criteria are mainly based on experimental evidence for the parent substance.

The available information on styrene, cumene and ethylbenzene has been reviewed by RAC and compared with the toxicological profile of 2-phenylpropene (see the RAC opinion and background document). Besides similarities, there also important differences and therefore the information on structurally related substances has been used by RAC only to a limited extent.

Date	Country	Organisation	Type of Organisation	Comment number
16.06.2022	Netherlands		MemberState	2
Comment received				
We would like to thank the DS for preparing the CLH dossier on 2-phenylpropene. Please find our comments on the proposed classifications in the relevant sections.				
Dossier Submitter's Response				
See below.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	France		MemberState	3
Comment received				
Study in rats: Regarding kidney tumours in males and according to IARC criteria, we note that some elements to support the exclusive role of alpha-2microglobuline are not available for the substance (table 22). Thus, relevance to humans cannot be excluded. Regarding MNCL and testis tumours in males, we would like to highlight that the incidence at 1000 ppm reaches statistical significance and is higher than HCD ranges. Moreover, a dose-response relationship is observed (with the trend test). It is stated in the CLH report that the high background incidence of these tumours reduces the level of evidence. However, despite this high background incidence, the comparison with HCD shows that the incidence at 1000 ppm cannot be considered as incidental. Moreover, the fact that the statistical significance only occur at the highest concentration would not be used to consider the tumour as "negligible for the purpose of classification" since CLP Regulation is hazard-based. Overall, we are of the opinion that these results may justify a conclusion of "sufficient evidence" rather than "some evidence" for male rats. Could you please add further arguments on this point? Study in mice: We agree that the level of evidence for liver tumours is lower in males than in females, in particular in the absence of clear dose-response. However, it may justify a conclusion of "some evidence" rather than "equivocal evidence" considering the higher incidence than HCD (even if not statistical significance is not reached when adenoma and carcinoma are considered individually). Regarding the mode of action, there is no specific investigation to conclude that these effects are due to CAR activation. Thus, these tumours should be considered as relevant to humans. Overall, tumours were reported in two species: - male rats: kidney tumours, MNCL and testis tumours - male and female rats: liver tumours				

In the comparison to CLP criteria (i), you can also mention epoxidation process leading to high reactive metabolites with possible role in carcinogenicity. In particular, adverse effects on liver and kidney can be related to local formation of a reactive metabolite. As noted above, we question if "sufficient evidence" cannot be reached in male rats. In this case, there would be sufficient evidence of carcinogenicity in two species (male rats and female mice) and this could thus warrant a classification as Carc. Cat. 1B. Moreover, data on analogous substance also support Carc. Cat 1B. Indeed, cumene identified as a metabolite and a precursor of 2-phenylpropene is classified as Carc. Cat. 1B according to CLP Regulation. Styrene is currently not classified for its carcinogenic property; however, it seems that this hazard class was not subject to evaluation by the RAC. Styrene is classified by the IARC in the group 2A which is considered equivalent to Carc. Cat. 1B, based on a comparison of IARC and CLP criteria.
Dossier Submitter's Response
The DS appreciates the comments raised by the MS and would like to respond to the main points as follows. The findings in male rats regarding mononuclear cell leukaemia (MNCL) and interstitial cell adenoma in testis may, indeed be seen as additional evidence of carcinogenicity (statistically significant increase, concentration-response relationship based on trend test, increase above the historical control incidence (HCI) range). Albeit, acknowledging that these are borderline cases, the DS is, for reasons explained in the CLH report, of the opinion that both findings are insufficient for classification. Both tumour types are characterised by high background incidences. The human relevance of MNCL has been called into question (spontaneous occurrence in aged F344 rats with variable and high incidence, species-specific characteristics, mechanistic considerations and reproducibility issues). According to the guidance on the application of the CLP criteria, *"appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification"*. As for the increased incidences of interstitial cell adenoma in testis, the authors of the NTP study considered this finding unrelated to the treatment. The DS also notes that (1) the increased incidences at 100 ppm and 1000 ppm only slightly exceed the range of the HCI, (2) there is no statistically significant increase at 300 ppm (mid concentration), the increase, therefore, lacks a clear concentration dependency, and (3) the incidence for the concurrent control is below the range of the HCI. Hence, the biological significance of the findings is questionable. The liver tumours in male mice have been considered of equivocal evidence, as statistically significant effects are only seen when incidences of hepatocellular adenoma or carcinoma were analysed in combination. Furthermore, the increased incidences are not concentration-related and only slightly above the HCI. Altogether, the biological significance of liver tumours in male mice is less clear. As compared to the close structural analogue, cumene, lung tumours in mice and nasal tumours in rats have not been observed with 2-phenylpropene. The DS agrees with France in that the formation of a reactive metabolite may be responsible for liver and/or kidney toxicity.

Based on uncertainties, specifically related to a potential species-specific MoA in the formation of liver tumours in mice and kidney tumours in male rats, it is the opinion of the DS that a Category 1B classification may not be justified.

RAC's response

Thank you for your comments. The RAC response to the individual points raised in comments no. 3 and 5 is provided below.

Testicular tumours in male rats
The rat strain used had a high and variable background incidence and there is no clear dose-response relationship (the incidences at the top and low dose are identical). Thus, the increase may be unrelated to treatment.

Mononuclear cell leukaemia in male rats
The increase in incidence at 1000 ppm may be treatment-related. Tumour latency appears to be slightly reduced. On the other hand, this tumour type has a very high background incidence in F344 rats, and poor reproducibility even in the same strain has been observed for several substances (Scheepmaker et al., 2005).

Kidney tumours in male rats
Not all elements of the IARC criteria have been fulfilled and human relevance can therefore not be excluded. Still, the concern is reduced by partial involvement of α2u-globulin-related MoA and absence of renal neoplasms in females.

Liver tumours in male mice
There was a statistically significant increase in hepatocellular tumours (adenomas + carcinomas) but it was not clearly dose-related and the incidence remained close to the historical control mean.

Liver tumours in female mice
The increase at 600 ppm is clearly treatment-related and there was a biologically plausible sequence of increased eosinophilic foci, adenoma and carcinoma. RAC agrees that there is no robust MoA information. The tumours are relevant for classification but the concern is reduced by the relatively high background incidence and high susceptibility of B6C3F1 mice.

Genotoxic metabolite
Depletion of hepatic glutathione detected in a short-term mouse inhalation study (Morgan *et al.*, 1999) is consistent with formation of a reactive metabolite in the liver. If the metabolic profile in mice is similar to that in rats, this reactive metabolite would most likely be the side-chain epoxide. However, whether this metabolite is responsible for the increase in tumours (e.g. via genotoxicity or cytotoxicity) is curently unknown.

Similarity to cumene and styrene
Cumene has a harmonised classification as Carc. 1B mainly based on malignant lung tumours in both sexes of mice (RAC opinion on cumene, 2020). Nasal tumours (mostly benign) in male and female rats and kidney tumours in male rats were considered to provide limited evidence of carcinogenicity.
Styrene has no harmonised classification for carcinogenicity and has not been evaluated by RAC for this endpoint. The main carcinogenic finding was an increase lung tumours in both sexes of mice.
The carcinogenic profile of 2-phenylpropene is quite different from that of cumene and styrene, particularly with regard to respiratory tract tumours. Thus, it is not considered

justified to use cumene and styrene as an argument for a cat. 1B classification of 2-phenylpropene.

Comparison with CLP criteria
Since malignant tumours were observed in two species, Category 1B has to be considered. To aid in the weight of evidence assessment, the CLP regulation provides a list of factors increasing or decreasing the level of concern for human carcinogenicity (CLP, Annex I, 3.6.2.2.6).
The main factors increasing the concern in this case are:

- Increases in malignant tumours in two species
- Multi-site response in male rats

Main factors decreasing the concern:

- High spontaneous incidence of hepatocellular tumours in B6C3F1 mice and high susceptibility of this strain to induction of liver tumours by chemicals
- High spontaneous incidence of mononuclear cell leukaemia in F344 rats, not seen in other strains and species, and poor reproducibility observed for some substances
- Involvement of α2u-globulin accumulation in the development of renal tumours in male rats (although human relevance cannot be completely excluded)
- The fact that kidney tumours and mononuclear cell leukaemia were limited to a single sex a species

Taking into account all available information, RAC agrees with the DS's proposal to classify 2-phenylpropene as Carc. 2; H351.

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	Belgium	ReachCentrum on behalf of the Phenols & Derivatives Reach Consortium	Company-Manufacturer	4

Comment received

Summary:
In principle, we support the Dossier submitters proposal as stated. In particular, we agree to proposal for classification as Carcinogen category 2 (H351) with no classification for germ cell mutagenicity.

With regard to the classification for carcinogenicity, we have adopted this position on the basis that while we believe that there is strong evidence that an α2u-globulin-mediated nephropathy may be involved in the carcinogenic effects of 2-phenylpropene in the kidney of male rats, we recognise that not all the IARC criteria are met and there are no studies with this substance at present to exclude the possibility of other mechanisms being involved. We also consider the likelihood that PPARα activation, aryl hydrocarbon receptor (AhR) and the constitutive androstane receptor (CAR) mechanisms are involved in the mouse liver tumour development and consequently these are of questionable relevance for human carcinogenicity. We are of the view that the available mutagenicity data on 2-phenylpropene is not only insufficient to justify classification as a germ cell mutagen, but also not sufficiently convincing to justify regarding it as a somatic cell mutagen responsible for the cancers observed in the rodent cancer studies. However, in the absence of a robust negative genotoxicity database and specific cancer MOA data on 2-phenylpropene, we are of the opinion that classification as Carcinogen category 2 is perhaps justified at this time.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-phenylpropene_detailed comments on the CLH Report_PandD Consortium_20220623.pdf
Dossier Submitter's Response
The DS appreciates the comments raised by the organisation and acknowledges that the general conclusions on the classification of 2-phenylpropene are supported. As stated under response to comment no. 1, the DS disagrees with the organisation that *"a genotoxic MoA can reasonably be excluded based on the available data"*. As listed in Table 16 of the CLH report, several arguments are in support of a genotoxic potential of 2-phenylpropene.
RAC's response
Thank you for your comments. RAC agrees with the DS's proposal to classify 2-phenylpropene as Carc. 2.

Date	Country	Organisation	Type of Organisation	Comment number
16.06.2022	Netherlands		MemberState	5
Comment received				
The proposed classification is based on a 2-year inhalation study in both rats and mice where carcinogenic effects were observed including increased incidences in renal tubular adenoma/carcinoma in male rats and increased incidences of liver tumours in male and female mice. The DS proposed to classify 2-phenylpropene as a category 2 carcinogen because the mode of actions (MoA) related to the observed carcinogenic effects may not be relevant for humans. Although the NL-CA agrees with this, the MoA should be regarded as relevant for humans until clear evidence is provided supporting the opposite. Especially the evidence regarding the MoA of liver tumours in mice is insufficient. In Table 24 (p. 50), the DS downgrades the concern for carcinogenicity significantly based on the MoA argumentation, but the NL-CA believes the concern should only be marginally reduced without further evidence supporting non-relevant MoAs. The criteria support classification as a carcinogen in category 1B when clear carcinogenic effects are present in both sexes of one species or in two species. For 2-phenylpropene, both is the case. In addition, there is clear malignancy in liver tumors in mice, multi-site responses and structural similarity to substances with good evidence of carcinogenicity. Even if, hypothetically, the irrelevant modes of actions would have been fully investigated, it seems unlikely that all factors would be disregarded to such extent that a lower classification is warranted. It is also questioned whether the adenomas in the testis of the rats should be regarded as not treatment related as the incidence was increased clearly in all dose levels and exceeded the incidence in historical controls in the low and high dose group. In conclusion, the NL-CA is of the opinion classification as a category 1B carcinogen is warranted based on the current information.				
Dossier Submitter's Response				
The DS appreciates the comments raised by the MS and would like to refer to response to comment no. 3.				
RAC's response				
Thank you for your comments, please see the response to comment no. 3.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	France		MemberState	6

Comment received

The complexity of this case is clearly described and balanced in the CLH report.

We note that there are some arguments in favour to classification as Muta 2:
- Positive result found in female mice from the NTP MN assay by inhalation (2007). The other MN assay (2012) cannot be used to dismiss the positive results since the protocols are not similar (duration of exposure, route of exposure, sex used). Furthermore, it is regrettable that this study which is more recent than the NTP study was only performed in male mice, that seem less sensitive than females based on the results from the NTP study.
- In vitro SCE assays, even of various qualities, are consistently positive
- Toxicokinetics considerations point to the formation of an epoxide, with epoxides being highly reactive substances.

However, we recognize that the evidence of a possible mutagenic effect is reduced (without completely removing the concern) considering that:
- In vivo positive result is found only in one study and in one sex. Is there any evidence from other studies that female would be more sensitive to toxicity of this substance?
- No in vitro mutagenic assay reported positive results.

Dossier Submitter's Response

The DS appreciates the comments raised by the MS. Apart from the carcinogenic response in mice, which was considerably stronger in females when compared to males, there are no other indications that females would be more sensitive.

RAC's response

Thank you for your comments. Female B6C3F1 mice were more sensitive to general toxicity of the substance in the 2-week inhalation study by Morgan et al. (1999) (mortality in females from 600 ppm, no mortality in males up to 1000 ppm) and in the 3-month study itself (early mortality of 2 out of 10 females at 1000 ppm, no mortality in males). Nevertheless, the positive result in the MN test by NTP is associated with uncertainties such as high general toxicity, absence of a concomitant increase in micronucleated immature erythrocytes and abnormally high concurrent control values. Due to these uncertainties, the evidence is not considered sufficient for a Category 2 classification.

RAC agrees that the oral MN assay (2012) should have been performed in females. It also has some deviations from the current OECD TG potentially decreasing its sensitivity (e.g. a single sampling time).

Epoxide formation raises a concern but the levels of the reactive metabolite in tissues may be low depending on the kinetics of oxidation and hydrolysis/conjugation (cf. Morgan et al., 1999). The classification criteria are mainly based on experimental evidence for the parent substance.

In vitro SCE is given lower weight than in vitro mutagenicity tests.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-PHENYLPROPENE; α - METHYLSTYRENE

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	Belgium	ReachCentrum on behalf of the Phenols & Derivatives Reach Consortium	Company-Manufacturer	7

Comment received

Summary:
In principle, we support the Dossier submitters proposal as stated. In particular, we agree to proposal for classification as Carcinogen category 2 (H351) with no classification for germ cell mutagenicity.

We consider that the deficiencies in the available genotoxicity data on 2-phenylpropene are not best addressed by speculative read-across to other "similar" substances and hypothesising as to the formation of reactive metabolites, but are more appropriately addressed by conducting new high-quality toxicity studies on 2-phenylpropene that can definitively resolve any remaining concerns.
Consequently, industry is planning to conduct a GLP guideline OECD 487: In Vitro Mammalian Cell Micronucleus Test in human whole blood both with and without S9. We believe that this study will be sufficient to address the increase in SCE with whole blood observed in two albeit of very limited quality in vitro sister chromatid exchange test in human lymphocytes and possible concern relating to a putative reactive metabolite generated due to erythrocyte-mediated metabolic activation (Norppa and Tursi, 1984; Norppa and Vainio, 1983).
In this regard, we note that as indicated in the CLH review that the SCE endpoint is not actually indicative of genetic damage and that the OECD TG for in vitro SCE was deleted because of this. We also note (more comments below) that the available in vitro mammalian studies for chromosomal endpoints although showing no signs that there is genetic activity, also were not conducted using the current OECD recommendations. Thus, a new study will provide a definitive answer as to whether 2-phenylpropene causes chromosome damage and by using whole blood will address any issues arising out of the in vitro SCE publications indicating that whole blood can generate genotoxic metabolites. We further propose that subject to a negative outcome of this guideline study that a new in vivo study is undertaken to investigate and clarify the finding of a statistically significant trend for the frequency of micronucleated normochromatic erythrocytes (NCEs) observed in peripheral blood samples of female but not in male B6C3F1 mice at the end of the 90-day subchronic repeated dose toxicity study (NTP, 2007). The design of this study will be discussed with the dossier submitter and ECHA prior to contracting.
Regarding the CLH dossier itself, we commend the dossier submitter on their comprehensive review of the available data on 2-phenylpropene, however, we have concerns as to both the interpretation of some of the available genotoxicity data on this substance and the use of analogy/read across to similar substances to infer an increased concern for mutagenicity. Our comments are therefore focused in section A) on the assessment of mutagenicity and in section B) on the grouping of 2-phenylpropene with structurally related styrene, cumene and ethylbenzene.

Note: The detailed comments following the pagenumbering of the CLH Porposal are enclosed in the attachment

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2-phenylpropene_detailed comments on the CLH Report_PandD Consortium_20220623.pdf

Dossier Submitter's Response
The DS appreciates the comments raised by the organisation and would like to refer to response to comment no. 1. The DS particularly appreciates and supports the announcement to conduct an *in vitro* mammalian cell micronucleus test to address the concern raised by the positive SCE findings.
RAC's response
Thank you for your comment, please see the response to comment no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
16.06.2022	Netherlands		MemberState	8
Comment received				
The NL CA agrees that the biological relevance of the effects in female mice at the highest dose in the MN test is questionable given the lethality of two female mice, limited positive effect only in a single sex, the absence of a positive control and the high number of NCE also in the negative control. It is also agreed that standard in vitro studies are all negative. However, the in vitro sister chromatid exchange (SCE) tests were all positive. In particular the SCE test in hamster ovary cells in presence of S9 fraction is clearly positive with a dose-response relationship. This in turn supports the hypothesis of the formation of a genotoxic metabolite. The main metabolite in blood was 2-phenyl-1,2-propanediol and one of the main metabolites in urine was 2-phenyl-1,2-propanediol glucuronide. These metabolites are very similar to styrene glycol and styrene glycol glucuronide and it is thus likely that the biotransformation is similar to that of styrene and that the metabolites are derived from 2-phenylpropene oxide as is already stated on page 8 of the CLH proposal. Perhaps the DS could perform an additional QSAR analysis of the parent and metabolites. This may help identify the likelihood of the parent and/or the metabolites to be mutagenic. This could in turn provide stronger evidence for classification. According to the guidance on the application of CLP criteria, in vitro data may be used as a basis for classification when supported with chemical structure activity relationships. Overall, the body of evidence contains limited positive results with uncertain biological relevance for causing mutagenicity. The hypothesis for metabolic activation being required to cause mutagenicity is plausible but also requires further investigation. With the current information, the NL-CA is supportive for no classification based on inconclusive data. However, mutagenicity in category 2 could be considered with further support from QSARs.				
Dossier Submitter's Response				
The DS appreciates the comments raised by the MS and acknowledges that additional information would help clarifying the role of the proposed epoxide, 2-phenylpropene oxide. As stated in the CLH report, a non-guideline bacterial gene mutation study conducted with 2-phenylpropene oxide was positive while bacterial gene mutation studies with 2-phenylpropene were consistently negative (+/- S9). Hence, 2-phenylpropene oxide formation may be insufficient in this test system under the conditions of the test. The relevance of the alleged metabolite for *in vivo* mutagenicity remains, however, obscure. Using the knowledge-based expert system for metabolism prediction, METEOR (Lhasa Limited), the formation of 2-phenylpropene oxide as the first metabolic step is predicted to occur in humans.				

RAC's response
Thank you for your comments. RAC agrees that metabolism of 2-phenylpropene via the Ames-positive side-chain epoxide is very likely. The positive reliable in vitro SCE by NTP (2007) is also noted. Nevertheless, the most relevant evidence for classification comes from vivo mutagenicity/genotoxicity assays and in vitro mutagenicity assays with the parent substance. As to structural similarity to styrene, styrene has as yet no harmonised classification for mutagenicity, so the criterion of 'chemical structure activity relationship to known germ cell mutagens' does not seem applicable here. In addition, the rate of side-chain epoxide formation and detoxification (by hydrolysis or conjugation) is likely to differ between the two substances, which may be one of the reasons for the differences in their toxicity profiles (a brief overview of the toxicity profiles can be found in the RAC opinion and background document).

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
16.06.2022	Netherlands		MemberState	9
Comment received				
LLNA in CBA/CA mice is clearly positive. NL CA agrees with classification as Skin Sens 1B.				
Dossier Submitter's Response				
The DS appreciates the comment raised by the MS and acknowledges that the conclusion on the classification is supported.				
RAC's response				
Thank you, RAC agrees with classification as Skin Sens. 1B.				

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	France		MemberState	10
Comment received				
FR agrees with the proposed classification as Skin Sens. 1B based on the EC3 > 2% from the well-conducted LLNA.				
Dossier Submitter's Response				
The DS appreciates the comment raised by the MS and acknowledges that the conclusion on the classification is supported.				
RAC's response				
Thank you, RAC agrees with classification as Skin Sens. 1B.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
23.06.2022	France		MemberState	11
Comment received				
Based on the multiple nasal lesions reported in the subchronic toxicity study in mice, a classification as STOT RE 2 can be justified (0.2 <GV ≤1 mg/L). Nasal lesions consist on atrophy and hyperplasia/metaplasia and are reported at all tested concentrations from 75 ppm (0.36 mg/L). Therefore, it cannot be excluded that the effective dose is lower.				

Atrophy of the Bowman's glands and of the olfactory epithelium is reported in nearly all males and females. Metaplasia of the olfactory epithelium is observed in about 5/10 animals. Even if the severities were graded as minimal, such types of lesions (atrophy and metaplasia) should be considered as significant toxic effects according to CLP criteria.
Dossier Submitter's Response
The DS appreciates the comments raised by the MS and acknowledges that the nasal lesions observed at concentrations applicable for classification (75/150 ppm) might be a borderline case. While the lesions (atrophy, metaplasia, hyperplasia) can be considered adverse, the DS is of the opinion that the findings at 75/150 ppm do not support classification given their minimal severity. The effects may be the consequence of chronic treatment-related cytotoxicity and are consistent with an existing harmonised STOT SE 3 (respiratory irritant) classification. While STOT SE 3 covers acute effects, the lesions observed in the subchronic NTP study indicate that similar effects also manifest following repeated insult of the epithelium with low concentrations of 2-phenylpropene.
RAC's response
Thank you for your comment. Atrophy and metaplasia of olfactory epithelium is generally a significant toxic effect that may warrant classification, but in this case the severity at relevant concentrations is low (minimal to mild; cf. the criteria of "significant organ damage" or "marked organ dysfunction" according to CLP, Annex I, 3.9.2.7.3) and no substantial increase in severity was observed at higher levels. RAC agrees with the DS that a STOT RE classification for respiratory tract effects is not justified.

PUBLIC ATTACHMENTS
1. 2-phenylpropene_detailed comments on the CLH Report_PandD Consortium_20220623.pdf [Please refer to comment No. 1, 4, 7]