COMPILED COMMENTS ON CLH CONSULTATION

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Last data extracted on 21.08.2023

Substance name: eugenol; 2-methoxy-4-(prop-2-en-1-yl)phenol
CAS number: 97-53-0
EC number: 202-589-1
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	1
Comment received				
Overall, DE CA supports the dossier submitter's proposed classifications of acute (oral) toxicity, skin sensitisation as well as STOT SE and no classification for reproductive toxicity and STOT RE. The classification for skin irritation requires more elaboration, and we do not agree with the current approach of extrapolating the skin irritation classification for the evaluation of eye damage/irritation. Our main comment pertains to the evaluations for carcinogenicity and germ cell mutagenicity. The current toxicity dataset for eugenol is not sufficient to clearly conclude that it does not have genotoxic potential, and there are concerns regarding possible substance-related tumours (e. g., liver) in rats and mice. Considering genotoxicity and carcinogenicity data of structurally similar substances, in particular methyleugenol, critical interpretation of the appropriateness of the standard genotoxicity assays and the carcinogenicity data on eugenol is required.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	2
Comment received				
We currently do not agree with the dossier submitter's conclusion of no classification for carcinogenicity for eugenol. Based on the existing data on carcinogenicity for eugenol and structurally similar substances such as methyleugenol and isoeugenol, we are of the opinion that eugenol might warrant a Category 2 classification for carcinogenicity. From the 2-year NTP study on eugenol in F344/N rats and B6C3F1 mice, tumour formation was observed in both species and sexes. In particular, increases of uterine non-neoplastic lesions (cystic hyperplasia; 11/50 vs. 1/40 in control) and tumours (endometrial stromal polyp/sarcoma; 16/50 vs. 6/40 in control) in female rats at the highest dose group (12500 ppm or default dose of 625 mg/kg bw/d) were observed when compared to the control group. The uterine tumour incidence (32 %) clearly exceeds the HCD from the Southern Research Institute (15 %), and the non-neoplastic uterine lesions might provide some				

indication of progression to malignancy (uterine tumours). We agree with the dossier submitter's rationale to only use the HCD from the Southern Research Institute (1983) for comparison because the other HCD from NTP are either not acceptable (e.g. past the 5-year period of the study) or questionable due to missing information as specified in Regulation (EU) 283/2013.
In mice, there was a dose-related increase of hepatocellular adenoma/carcinoma (combined) in females, and at the higher dose of 6000 ppm, the increase was statistically significant (9/49 vs. 2/50 in control). An increasing trend was also observed with the Cochran-Armitage trend test. On the other hand, even though the increase of hepatocellular adenoma/carcinoma (combined) in male mice was not dose-dependent, the incidence at 6000 ppm (18/49; 37 %) slightly exceeds the HCD from SRI (32 %).
The authors of the NTP 2-year study of eugenol (1983) concluded that there was equivocal evidence of carcinogenicity for mice because of the increased incidence of liver tumours in both male and female mice.
It is also worth mentioning that in the NTP (2000) carcinogenicity study with methyleugenol (TR 491), a similar lack of dose-dependent relationship in the combined hepatocellular adenoma/ carcinoma incidence in male mice (incidence of 63 %, 94 %, 92 % and 80 % at 0, 37, 75 and 150 mg/kg, respectively) was observed, and methyleugenol was identified as a carcinogen. Furthermore, methyleugenol was recently classified by IARC as Group 2A ("probably carcinogenic to humans") based on hepatocellular adenoma and carcinoma in mice and rats as well as other tumours (https://doi.org/10.1016/S1470-2045(23)00341-8).
Considering the structural similarity of the two substances and the same target organ for tumour formation in mice (liver), we are of the opinion that eugenol might also have carcinogenic potential.
We acknowledge the experimental limitations of the NTP study and the uncertainties in drawing a clear conclusion due to lack of statistical significance or dose-response relationship; however, the current weight of evidence on eugenol and its structurally similar substances may be sufficient to classify eugenol as Category 2 for carcinogenicity.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	3

Comment received

We do not agree with the dossier submitter's conclusion of no classification of genotoxicity/germ cell mutagenicity for eugenol on the basis of "conclusive data but not sufficient for classification". The available dataset on eugenol is not robust enough to draw a conclusion on genotoxicity/mutagenicity due to a very high degree of uncertainty in the studies. Below is a brief overview of our assessment on genotoxicity/mutagenicity.
1. Assessment of gene mutation in vitro cannot be completed due to lack of the fifth strain (e.g. TA102 or E. coli WP2 strain) in the Ames test to identify cross-linking mutagens and lack of metabolic activation investigation in the mouse lymphoma assay.
2. We agree with the dossier submitter that the data demonstrate that eugenol can induce chromosomal aberrations, sister chromatid exchange (SCE) and DNA damage in vitro.
3. The in vivo genotoxicity data are mostly, if not entirely, supplementary. The only study considered acceptable by dossier submitter (Chen et al., 2021) has major limitations, e.g. unknown purity of the substance and limited reporting of the study. The in vivo dataset is not conclusive enough to resolve the incomplete investigation on gene mutation in vitro or negate the positive findings with chromosomal aberrations/SCE and DNA damage.
Nearly all of the in vivo genotoxicity studies were generated using male animals, but it should be pointed out that tumour formations potentially from long-term eugenol exposure were observed more prominently in female mice and rats. Data from short-term toxicity

(and repeated dose toxicity, to some extent) appear to show some sex-specific differences in toxicity.
Of note, early in vitro mutagenicity studies with the structurally related substance methyleugenol performed under standard conditions were negative (e.g., NTP Toxicology and Carcinogenesis Studies of Methyleugenol (CAS NO. 93-15-2) in F344/N Rats and B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser. 2000; 491:1-412. PMID: 12563349.), while it was later discovered that through bioactivation by sulfotransferases, DNA adducts are formed and respective modification of the test system is thus required (Herrmann, K. et al., Mutagenesis 27(4): 453–462, https://doi.org/10.1093/mutage/ges004). Relevance of these observations to eugenol should be discussed.
Overall, due to the high uncertainties in the dataset (e. g. nearly all of the studies are considered supportive) and unresolved concerns regarding positive in vitro findings, no classification on genotoxicity / germ cell mutagenicity can be proposed at the moment for eugenol but rather on the basis of inconclusive and insufficient data.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	4
Comment received				
For assessment of sexual function and fertility, there are no suitable reproductive toxicity studies on eugenol available for evaluation. On the other hand, there is a GLP-compliant two-generation reproductive toxicity study on isoeugenol available, which shows no notable adverse effects on sexual function and fertility. The dossier submitter does not accept the use of isoeugenol as a read-across substance for eugenol with the rationales of the difference in ADME between isoeugenol and eugenol due to the difference in the position of the double bond in the alkene chain and difference in physicochemical properties (such as water solubility) between the two substances. We do not entirely agree with their justifications. In the RIFM safety assessment of eugenol (http://dx.doi.org/10.1016/j.fct.2015.12.013), the authors presented plausible rationales for using isoeugenol as a read-across substance for eugenol. Eugenol and isoeugenol are structural isomers with a single difference of the position of the double bond. While there might be some kinetic differences in the metabolism, QSAR analyses (e. g., using OECD QSAR Toolbox) showed similar alerts for the two substances regarding protein binding and metabolites. Therefore, it is expected that both eugenol and isoeugenol would undergo similar metabolism, and we question the dossier submitter’s rationale that the position of the double bond would truly result in different metabolism of the two substances and different toxicity profiles. Nevertheless, in either case, there is no sufficient evidence to classify eugenol for effects regarding sexual function and fertility as well as lactation. Regarding assessment of developmental effects, we support the dossier submitter’s evaluation to focus mainly on the two developmental toxicity studies performed on eugenol (and not on the developmental toxicity studies using clove oil) and agree that eugenol does not warrant classification for developmental effects since they were observed at doses which maternal toxicity was also seen. Overall, the current evidence does not suffice to classify eugenol as a reproductive toxicant.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number

15.08.2023	United Kingdom	Health and Safety Executive	National Authority	5
Comment received				
Acute Toxicity (oral) The DS considered the in vivo acute toxicity oral study in rats (Sober, H.A. et al, 1950) as the only acceptable study for classification. However, it is unclear why this study is deemed more acceptable than other studies in the dataset. The LD50 value, which is only just within criteria for category 4, established in this study could also have been skewed by the high mortality rate seen at 1.6 ml/kg bw. Looking at the rest of the dataset, LD50 values > 2000 mg/kg bw/d are frequently reported. It would be useful if the DS could further explain their reasoning for excluding the rest of the dataset from their evaluation of the classification.				

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	6
Comment received				
We support the dossier submitter's proposal of classifying eugenol as Acute (Oral) Tox. 4 (H302) based on the reported LD50 of 1930 mg/kg in the acute oral toxicity study in rats (Sober et al., 1950).				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
15.08.2023	United Kingdom	Health and Safety Executive	National Authority	7
Comment received				
Skin corrosion/irritation The DS includes 3 in vivo studies performed by Motoyoshi et al (1979), which gave irritancy scores ranging from 1 ('mildly irritating') to 3 ('severely irritating'). All of these suffer from significant limitations including no observation period, a non-standard scoring system and the substance was applied for a long period of time (24-48 hours compared to the 4 hours required by OECD TG). The REACH data, which has not been assessed by the RMS, appears to be of better quality and is negative for skin irritation. It would be useful if the DS could further explain their reasoning for excluding the REACH data from their evaluation of the classification.				

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	8
Comment received				
We could support the dossier submitter's proposal of classifying eugenol as Skin Irrit. 2 (H315); however, we question the dossier submitter's justification for the classification as it is not entirely logical in the CLH report. The dataset on skin irritation of eugenol stems from 2 studies: a non-standard assay on skin irritation on rabbits, guinea pigs, miniature swines and humans (Motoyoshi et al., 1979) and skin irritation data from the REACH registration dossier that was not available for the dossier submitter to evaluate. However, the dossier submitter concluded that data from neither studies were suitable for classification purposes because either of major study deficiencies or lack of data for evaluation; nevertheless, a proposal for classification was				

made.
Therefore, it would be helpful if the dossier submitter can further clarify the data basis (to support the weight-of-evidence approach) of classifying eugenol as Skin Irrit. 2.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
15.08.2023	United Kingdom	Health and Safety Executive	National Authority	9
Comment received				
Serious eye damage/irritation Classification for Serious eye damage/irritation was based on the DS assessment of skin irritation. It was noted that eugenol may cause irritation in the eye and serious damage cannot be ruled out. However, there is no mention in the CLP guidance to suggest that when a substance is irritating to the skin, that it should also be classified for eye irritation. It is also noted that the available eye irritation study from the REACH registration dossier was not considered by the DS in their evaluation of the data; it would be useful if the DS could further explain their reasoning for not including this study.				

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	10
Comment received				
We do not support the dossier submitter's proposal of classifying eugenol as Eye Irrit. 2 (H319) by the approach of extrapolating the Skin Irrit. 2 (H315) classification for this hazard class. In the CLP Guidance (Section 3.3), this approach is mainly applied if the substance is classified for Skin Corr. 1 (H314) but not for Skin Irrit. 2. Furthermore, as commented under skin corrosion/irritation, it is not entirely clear to us in the CLH report the data basis for the classification of eugenol as a skin irritant (see respective comment under Skin corrosion/irritation). Even if our concern on skin irritation is resolved, more evidence and justification would be required to support the eye irritation classification by extrapolation.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
15.08.2023	United Kingdom	Health and Safety Executive	National Authority	11
Comment received				
STOT SE The available studies suggest that eugenol can elicit a narcotic effect; e.g., weakness of hindlegs, paralysis, lower jaw relaxation and ataxia. It should be noted that these narcotic effects coincide with animal deaths. Therefore, if Acute toxicity 4 (oral) is considered to be appropriate, then it should also be considered whether an additional classification for narcotic effects is justified.				

Date	Country	Organisation	Type of Organisation	Comment

				number
09.08.2023	Germany		MemberState	12
Comment received				
Generally, we support the STOT SE 3 classification proposal. However, effects observed after oral exposure in rats and dogs are already covered by Acute Tox. 4 classification as they occur at doses falling within the guidance values for this hazard class (300 – 2000 mg/kg bw). Moreover, the effects can be considered as causal for (dogs) or at least associated with mortality (rats) and the narrow dose spacing in the rat study does not provide evidence of effects at doses significantly lower than the LD50 (although they cannot be excluded either). Effects seen in the inhalation toxicity study, on the other hand, do warrant classification as STOT SE 3 since they were transient in nature and not associated with mortality.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.08.2023	Germany		MemberState	13
Comment received				
We agree with the dossier submitter's evaluation that the existing dataset provides no convincing evidence to classify eugenol for STOT RE.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
11.08.2023	United Kingdom	Health and Safety Executive	National Authority	14
Comment received				
Eugenol; 2-methoxy-4-(prop-2-en-1-yl)phenol (EC: 202-589-1; CAS: 97-53-0). The chronic Aquatic 2 hazard classification is based on a Daphnia magna 21-day NOEC of 0.0959 mg/L (mm) (Anon., 2021) which reflects the highest treatment (0.125 mg/L nominal). The CLH report notes that a dose-response effect was not observed (a positive effect on reproduction was observed at some treatments), an EC10 was not possible due to the lack of effects and the LOEC is considered >0.0959 mg/L. This indicates that the 0.0959 mg/L NOEC is unbounded and is not applicable in terms of meeting hazard classification criteria given it is not a true NOEC and a statistically significant reduction in reproduction was not observed. We note that the study treatments do not cover the full range of quoted water solubility of eugenol which is a limitation. Was a range finder test used to determine the exposure concentrations and are the results available to help interpret this study? The EU REACH registration (ECHA, 2023) includes a predicted long term Daphnia NOEC of 7.07 mg/L which is within the quoted range of solubility. Should additional information become available, the hazard classification should be reassessed. Also, during the study various 'handling' and 'inadvertent' test organism deaths were recorded. It would be useful to clarify if these occurrences impacted the statistical power of the study. Finally, given eugenol is considered rapidly degradable and does not meet bioaccumulation hazard criteria, the surrogate approach is not applicable in this instance. ECHA (2023) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13694/6/2/5/?documentUUID=af4c77ca-7a94-4841-95a3-4a4770f82954. Accessed				

08/08/2023.