Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

1,4-dichloro-2-nitrobenzene

EC Number: 201-923-3
CAS Number: 89-61-2

CLH-O-0000007202-85-01/F

Adopted

1 December 2022

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 1,4-DICHLORO-2-NITROBENZENE

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: 1,4-dichloro-2-nitrobenzene
EC number: 201-923-3
CAS number: 89-61-2
Dossier submitter: The Netherlands

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2022	France		MemberState	1
Comment received				
Two studies carried out according to OECD TG 453, one in rats and the other in mice, in both sexes, are available. A statistically significant increase in the incidence of hepatocellular adenoma as well as hepatocellular adenoma and carcinoma combined were observed in male rats, whereas no increase tumor incidence was seen in females. The study also shows an increase of the incidence of renal cell adenoma and carcinoma in male rats. In mice, significantly increased incidences of liver adenoma in female mice, liver carcinoma in both sexes, and liver adenoma, carcinoma, and hepatoblastomas combined in both sexes were observed. Two OECD TG 408 studies conducted in rats and mice of both sexes show an increase of the incidences of histopathological lesions in both sexes in the liver. In the kidney, an increase of incidence of histopathological lesions was only seen in males and females rats. These results consequently confirm that liver and kidney are target organs. This supports the evidence of the hepatocarcinogenicity of 2-5-dichloronitrobenzene. However, the exact MoA is not known and there is no information to explain the difference of effects between male and female rats. Regarding the potential carcinogenicity of the substance on the kidney, the data do not allow to determine if the mode of action is specific to rats. Therefore, by default, the effect is considered relevant to human. Based on these data, FR agrees with the proposal to classify the substance as Carcinogen 1B, H350 and supports the T25 and the generic concentration limit of 0.1%.				
Dossier Submitter's Response				
Thank you for agreeing to the classification proposal for carcinogenicity.				
RAC's response				
RAC agrees with the DS's response.				

Date	Country	Organisation	Type of Organisation	Comment number
03.02.2022	Germany		MemberState	2
Comment received				

Classification for (germ cell) mutagenicity is not warranted due to insufficient data.

Nevertheless, there is clearly a concern for the potential of 1,4-dichloro-2-nitrobenzene (DCNB) to cause gene mutations from in vitro data provided by the dossier submitter. This is in agreement with additional in silico analyses (using QSAR TB 4.5, Derek Nexus 6.1.1, Sarah Nexus 3.1.1, Leadscope Model Applier LSMA 3.1.0-40) performed by the DE CA. No suitable in vivo follow-up study for DCNB was identified by the dossier submitter or the DE CA, nor for any of the 17 analogues the DE CA selected using the OECD TB with a similarity of 0.8 or more (based on Tanimoto score).

The concern for mutagenicity and/or genotoxicity is based on following information:

1) IUCLID, REACH registration dossier: positive in vitro genotoxicity (Ames), no other studies available, no in vitro or in vivo follow-up (this would be a case for dossier evaluation. On the other hand, in vivo follow-up is not necessary after harmonised classification as Carc. 1B, H350).
2) OECD QSAR Toolbox (version 4.5) lists several positive in vitro studies outside the ECHA domain (Ames test, in vitro mammalian gene mutation, in vitro mammalian chromosome aberration).
3) QSAR prediction
a. OECD QSAR Toolbox: in vitro genotoxicity (alert for genotox.: nitro aromates)
b. Derek Nexus: plausible bacterial mutagenicity (Ames, alert 329 aromatic nitro compounds)
c. Sarah Nexus: positive for bacterial mutagenicity (Ames, based on example in the training set)
d. LSMA (positive probabilities given in brackets): positive for bacterial mutagenicity (0.913), CA in vitro (0.977, 0.655), MN in vivo (0.558), mixed predictions for gene mutation in mammalian cells/in vivo

The structural properties and possible metabolites give strong indications for genotoxicity and/or mutagenicity. This is confirmed by available experimental evidence from in vitro studies (Ames, in vitro mammalian gene mutation and chromosome aberration). Note that the dossier submitter points out that the majority of these studies are limited in their reliability and considers the evidence rather ambiguous.

In the CLH dossier, several positive Ames tests (positive mainly without metabolic activation) were identified, as well as negative or equivocal mammalian cell chromosome aberration studies and a negative HPRT mutagenicity test. This is contrasted by information in the OECD QSAR toolbox that lists a positive in vitro mutagenicity test in mammalian cells; however, the source of this information (listed as JRC, ECVAM) could not be verified by the DE CA.

No in vivo studies were identified by the dossier submitter or the DE CA. Therefore, the concern for germ cell mutagenicity could not be clarified definitively. A legal classification as Muta. 1B, H340, is not possible without in vivo confirmation. For a classification as Muta. 2, H341, at least a clear positive signal in mammalian cells is needed. The dossier submitter concludes that "for bacteria, genotoxicity assays suggest some mutagenic potential of 1,4-dichloro-2-nitrobenzene. A chromosome aberration study conducted in

mammalian cells gave an equivocal result.”
Dossier Submitter’s Response
Thank you for your comments and additional information from in silico analyses. These data support the current proposal. We agree with you that a genotoxic potential of 1,4-dichloro-2-nitrobenzene cannot be excluded but that the available information is not sufficient for classification.
RAC’s response
Thank you for your comments and additional information from the in silico analyses. These data support the current proposal. We agree with you that a genotoxic potential of 1,4-dichloro-2-nitrobenzene cannot be excluded but that the available information is not sufficient for classification.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2022	France		MemberState	3
Comment received				
FR agrees with the conclusions stated in the CLH report. There is no data from human epidemiological studies, no in vivo studies conducted on germ cells or somatic cells available. The only study conducted in Non-mammalian experimental systems regarded as reliable, an Ames test, showed a positive result in the salmonella typhimurium strain TA100. Also, the only reliable study conducted in mammalian cells (a chromosome aberration study on Chinese hamster lung Cells) has equivocal results, as it has shown increase in structural aberrations at a cytotoxic concentration. FR supports the conclusion that there is no enough data to classify the substance regarding mutagenicity, but the results obtained do not exclude the fact that the substance may be mutagenic.				
Dossier Submitter’s Response				
Thank you for agreeing to the classification proposal for germ cell mutagenicity.				
RAC’s response				
RAC agrees with the DS’s response.				

Date	Country	Organisation	Type of Organisation	Comment number
03.02.2022	Germany		MemberState	4
Comment received				
The proposal to classify 1,4-dichloro-2-nitrobenzene as Carc. 1B, H350, is supported. The substance was studied in chronic toxicity and carcinogenicity oral dietary studies in rats and mice (Yamazaki et al., 2006). No neoplastic lesions occurred in female rats. In male rats, tumours were found in liver, kidney and Zymbal’s gland. In liver, dose-dependent cell foci, and in high dose animals (2000 ppm or 109 mg/kg bw/d) statistically significant adenomas or adenomas and carcinomas combined were observed. In kidney and Zymbal’s gland, adenomas and/or carcinomas were observed with a statistically significant trend and an increase at the high dose. Incidences for these				

neoplasms at the high dose were above or at the upper end of the historical control range. Urothelial hyperplasia in the renal pelvis and mineralisation of the renal papilla were observed as non-neoplastic lesions in male rats at all dose levels. Since the pelvic area is not the primary site of (cortical) renal cell tumours, these effects are unlikely to represent pre-neoplastic lesions. Chronic progressive nephropathy has been observed in almost all males and more than half of the females of the control and treatment dose groups. As hyaline droplets in the proximal tubular cells and immunohistochemical evidence of a species-specific alpha2u-globulin nephropathy was reported for treated male rats (without any details on incidences and severity) in a 90-day study, this could indicate a male rat-specific mode of action that may have contributed to the overall limited increases in incidences of the renal cortical tumours. However, uncertainties remain as alpha2u-globulin-negative eosinophilic droplets were reported in female dose groups and no dose-relationship was seen in the high dose groups for hyaline droplets in males and females and for cytoplasmic tubular basophilia for the two highest dose groups in males (see Table 5 in Yamazaki et al. 2005). Thus, the relevance of the observed kidney tumours in male rats for humans is unclear. Species-specific effects may also be discussed for the Zymbal´s gland tumours. In mice, the target organ for neoplastic lesions was the liver (both males and females), with a strong dose-dependent increase of hepatocellular carcinomas in males and females, and in addition, a statistically significant increase of hepatoblastomas in all dose groups in males and at the high dose above historical control range also in females. Hepatoblastomas rarely occur spontaneously in mice. Taken together, the data provided by the dossier submitter allows to conclude that 1,4-dichloro-2-nitrobenzene is clearly a carcinogen in rodents and that the criteria for classification as Carc. 1B, H350 1B are fulfilled. This conclusion is based on substance-induced hepatocellular adenoma and carcinoma in two species and two sexes. In addition, hepatoblastoma was observed as a further tumour type in mice. A progression of malignancy of the tumours was reported in that the liver tumours metastasised in mice and the animals died due to the liver tumours before the end of the study. These tumours and the mechanism of action are considered relevant to humans and thus relevant for classification as presumed human carcinogen. A genotoxic mode of action cannot be excluded.
Dossier Submitter's Response
Thank you for your comprehensive comment and agreeing to the classification proposal for carcinogenicity.
RAC's response
RAC agrees with the DS's response.