COMPILED COMMENTS ON CLH CONSULTATION

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Last data extracted on 24.08.2020

Substance name: 4-Nitrosomorpholine
CAS number: 627-564-6
EC number: 627-564-6
Dossier submitter: Germany

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.08.2020	Sweden		MemberState	1
Comment received				
The Swedish CA supports classification of 4-nitrosomorpholine (CAS No. 59-89-2) as specified in the proposal. SE agrees with the proposal to set the SCL to 0.001% based on the high potency of the substance.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.08.2020	France		MemberState	2
Comment received				
There are appropriate in vitro and in vivo studies available, which, in a weight of evidence, clearly show that 4-nitrosomorpholine is a mutagen in liver. 4-Nitrosomorpholine is known to be a hepatotoxicant and has liver as a target organ. The substance is clastogen in the presence of S9 in vitro, the liver should be a target for in vivo follow up. Liver clinical effects were reported in only one genotoxicity/repeated dose toxicity study (Hayashi et al, 2015). Both in vivo micronucleus tests available using hepatocytes (Ashby and Lefevre, 1989, Hayashi et al., 2015) were positive. Even if there is no validated OECD TG available for liver as target tissue, there is ongoing work to develop an OECD guideline for liver MN. Thus, test results can be regarded as relevant for classification. Hayashi et al. (2015) and Ashby and Lefevre (1989) did not report positive controls or historical controls for the published tests, however the studies gave positive results. In Hayashi et al. (2015), toxic effects in liver (single cell necrosis) related to treatment have been detected already at the lowest dose tested (5 mg/kg bw) of-N-nitrosomorpholine in 80% of rats orally treated for 14 days. We agree with DS that the influence of high liver toxicity on the test outcome (MNHEPs) in liver cells remains still unclear in terms of dose. The liver micronucleus trial results indicate a high sensitivity for the repeat dose liver micronucleus assay in detecting hepatocarcinogen (Sui et al, 2015). In order to reach a conclusion, hepatocarcinogenicity of 4 Nitrosomorpholine needs to be discussed in order to evaluate the liver toxicity data from Hayashi et al. (2015) for reliability in terms of a possible classification. Despite negative results in in vivo heritable germ cell mutagenicity test, namely a dominant lethal test, an in vivo experimental for 4 NMOR in which some aspects of				

toxicokinetic like distribution have been examined separately shows the presence of 4 NMOR in testis (28 dpm/mg wet tissue of radioactivity in testis).

The classification of mutagens in Category 2 is based on positive evidence obtained from
(i) in vivo mammalian somatic cell mutagenicity tests or
(ii) other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Classification based on weight of evidence:
There is an in vivo database for 4-nitrosomorpholine that might be worth discussing. All of the available in vivo genotoxicity studies are publications and despite limitations in test design and reporting, those studies could be considered reliable. There is one available in vivo genotoxicity study which is positive and which can be identified as key study and as reliable even if the positive controls are missing. We agree that in all studies with positive results higher dose levels from 100 mg/kg bw and above were applied. Dose levels above MTD could interfere with the validity of the results of a genotoxicity study and could lead to false (positive) results. For 4-nitrosomorpholine a LOAEL (14 days) of 5 mg/kg bw/d was derived for oral administration in rats. The positive MN liver test by Hayashi et al. (2015) is to be explored as reported/measured toxicity and clinical effects were observed and liver is the target organ. There is ongoing work on OECD TG on liver MN. There is, in our opinion, no reason to disregard the studies. It is possible to assess, based on all reported positive and negative in vivo genotoxicity studies, if the positive effect following oral administration was robust and valid. Most of the positive studies were performed using intraperitoneal administration.

Contrary to DS, we disagree to say that the entire database is contradictory. The available key study like the MN test (Hayashi et al., 2015) is to be considered by RAC. In summary, a robust classification in Category 2 based on weight of evidence can be warranted if the data of the MN test is considered despite limitations as well as the whole results of available in vivo genotoxicity studies.

Conclusions on classification and labelling
There are mutagenicity assays with positive evidences for 4-nitrosomorpholine and the current data of the MN test could be sufficient to fulfil the classification criteria for mutagenicity in Category 2. Hence, at present, a classification and labelling of 4-nitrosomorpholine as mutagenic is to be discussed.

Date	Country	Organisation	Type of Organisation	Comment number
20.08.2020	Sweden		MemberState	3
Comment received				
We propose to consider whether a more holistic weight of evidence assessment could be useful in concluding on the mutagenicity classification. This would include the results of all the mutagenicity studies, in combination with carcinogenic studies indicating that a mutagenic mechanism may be involved. Although the mutagenicity studies are judged to be of low reliability, there are several positive studies as well as tumor data that point to a mutagenic property of this substance that may warrant classification as Muta 2.				