Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

m-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether

EC Number: 202-987-5
CAS Number: 101-90-6

CLH-O-0000001412-86-250/F

Adopted

30 November 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON *M*-BIS(2,3-EPOXYPROPOXY)BENZENE; RESORCINOL DIGLYCIDYL ETHER

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: m-bis(2,3-epoxypropoxy)benzene; resorcinol diglycidyl ether
EC number: 202-987-5
CAS number: 101-90-6
Dossier submitter: Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
03.05.2018	Germany		MemberState	1
Comment received				
The German CA does not support a change in classification from Carc. 2 to Carc. 1B. Physicochemical properties: In section 7 of the CLH-report the vapour pressure is stated as "low, 4x10-5 mm Hg at 25°C" and a reference to NTP 2011. Based on internet research we found the value 40.7 mm Hg at 25°C (reference: NTP 1992, https://pubchem.ncbi.nlm.nih.gov/compound/7586#section=Density; https://cameochemicals.noaa.gov/chemical/18114) As the melting point and boiling point are not very high, we were wondering if the value in the report is reliable.				
Dossier Submitter's Response				
The comments are noted. - With respect to the comment on the proposed classification for carcinogenicity, see our response to comment nr 2. - It is noted that different values for the vapour pressure of resorcinol diglycidyl ether are provided by various sources. The Dossier Submitter performed an additional search and it is not clear which value would actually correspond to the chemical under current evaluation. It is noted that, in general, a higher vapour pressure would be expected for chemicals with a lower boiling point. On the other hand, higher molecular weight compounds have lower vapour pressures.				
RAC's response				
Thank you for the comments. Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
03.05.2018	Germany		MemberState	2

Comment received

The NTP 1986 study shows that oral gavage of resorcinol diglycidyl ether results in forestomach squamous cell carcinoma and papilloma in rats and mice of both sexes. The current harmonised classification as carcinogenic Category 2 reflects these findings. With the present dossier, a classification in Category 1B is aimed at.

We would like to thank the Netherlands CA for providing the present CLH dossier. We agree with the dossier submitters, that the criteria for animal carcinogenicity of resorcinol diglycidyl ether are fulfilled, evidenced by benign and malignant neoplasms in two species and both sexes. Two modes of action, or their combination, are plausibly suggested:
• direct, evidenced by in vivo somatic cell genotoxicity and in vitro mutagenicity;
• indirect, evidenced by chronic irritation/inflammation of the forestomach.

The positive results from the NTP 2-year studies in two rodent species, both sexes, would formally present sufficient evidence of carcinogenicity (according to CLP regulation, Annex I, 3.6.2.2.3 (b)), allowing a classification of Carcinogenicity, Category 1B.

However, the case is complicated by several aspects:
• The rodent tumours are limited to the forestomach. The forestomach has no direct counterpart in humans, although the human oral cavity and the oesophagus have comparable squamous epithelial tissues. The residence time of food in the human oesophagus (short) and the rodent forestomach (long) should also be considered.
• No non-neoplastic lesions (treatment related) outside the forestomach were found in sub-chronic or chronic studies.
• The forestomach presents the site of first contact in the oral gavage studies.
• The substance is classified as skin/eye irritant, and in the 2-year but also in 90-day studies, forestomach inflammation and hyperplasia are evident.
• Positive evidence for in vivo genotoxicity (micronucleus test) stems from single, high-dose intraperitoneal application only; negative results stem from an oral study with a similar or higher dose.
• Toxicokinetic data suggest a rapid inactivation of the DNA-reactive epoxy-groups, at least in vitro with S9 liver homogenate.

A classification in Category 2 (or even no classification) should not be dismissed prior to weighing of arguments. According to CLP regulation, Annex I, 3.6.2.2.3 (b) Carcinogenicity in experimental animals: "- limited evidence of carcinogenicity: […] (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.". The CLP guidance (section 3.6.2.3.2, a) further states for tissues without human equivalent, that "tumours observed only in these tissues, with no other observed tumours, are unlikely to lead to classification".
It is further stated in the CLP guidance (section 3.6.2.3.2, j) that "[…] tumours occurring only at sites of contact and/or only at excessive doses need to be carefully evaluated for human relevance for carcinogenic hazard. For example […] forestomach tumours, following administration by gavage of an irritating or corrosive, non-mutagenic chemical, may be of questionable relevance, both due to the lack of a corresponding tissue in humans, but importantly, due to the high dose direct effect on the tissue. However, such determinations must be evaluated carefully in justifying the carcinogenic potential for humans; any occurrence of other tumours at distant sites must also be considered.
It is recognised, that the relevance for humans is given mainly due to the genotoxicity of

the substance. A forestomach inflammation-based proliferation alone is most likely not very relevant for human carcinogenicity. The genotoxicity seems to have - because of rapid metabolisation / inactivation –a rather local, not a systemic effect, which could contribute to local tumour growth.
Given the differences in passage time between human oesophagus and rodent forestomach, would the irritating properties of the substance lead to inflammation in the human oesophagus? Would the genotoxic properties be able to affect the human oesophagus, given passage time differences, possible inactivation of the epoxy groups, and a possible lack inflammation/proliferation? What is the relevance of the studies for other routes of exposure, i.e. inhalation or skin contact?

A more thorough discussion of the relevance of the outcome of the animal studies for humans and a weighing of evidence for either adapting the classification to Category 1B or for keeping the current Category 2 would be desired to make clear, why Category 1B is the most appropriate measure.

In addition, the results from the key study (NTP 1986) should be evaluated more critically:
• Data from 50 mg/kg bw/d dosage in rats (m/f) should not be taken into account at all for an assessment of carcinogenicity because of low survival of 0/50 (m) or 1/50(f) accompanied with reduced body weight / body weight gain compared to control groups. Mortality is not associated with occurrence of forestomach tumours.
• In rat NTP 90 day oral gavage studies, at 12.5 and 25 mg/kg bw/d histological findings from the forestomach included inflammation (6/10 to 9/10), basal cell hyperplasia (2/10 to 5/10) and fibrosis (up to 2/10) without ulceration. These rather early occurring non-neoplastic effects should be considered and discussed in their relevance for humans.
• The purity of the substance in the NTP study is only about 82%. The identity of the impurities and a potential influence on the outcome of the studies should be discussed.
• The reduction in body weight gain comprises 23 % in total in the dose group 25 mg/kg bw/d of the NTP study 1 (rat). However, the maximal tolerated dose (MTD) is characterised by approximately 10 % reduction in body weight gain (CLP guidance section 3.6.2.3.2 j.).

The CLP guidance states (section 3.6.2.3.2 j.):
• "Excessive toxicity, for instance toxicity at doses exceeding the MTD, can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which can lead to tumour development as a secondary consequence unrelated to the intrinsic potential of the substance itself to cause tumours at lower less toxic doses."

Dossier Submitter's Response

The comments are noted.

- *Excessive toxicity*: In the rat main study, an increased incidence of forestomach tumours (squamous cell papilloma and carcinoma) was noticed at 25 and 50 mg/kg bw/d, though the effects were not as striking at the high dose. A reduced survival and a reduction in body weight was observed as well at these dose levels. Survival was 42/50, 5/50 and 0/50 in males and 37/50, 16/50 and 1/50 in females at 0, 25 and 50 mg/kg bw/d, respectively. These tested dose levels could be considered too high and it is agreed that the result at these dose levels needs to be evaluated carefully.
  A supplemental study was subsequently performed using a dose level of 12 mg/kg bw/d. No decrease in body weight or reduced survival was noticed at this lower dose level. An increase in incidences of forestomach tumours was however noticed. The forestomach tumour incidences at this dose were statistically significantly increased

(squamous cell papilloma: 32% (m) and 38% (f) at 12 mg/kg bw/d versus 0% in controls; squamous cell carcinoma: 78% (m) and 54% (f) at 12 mg/kg bw/d versus 0% in controls) and outside the range of historical control data (see also our response to comment nr 3). The Dossier Submitter considers the rat study adequate at least at one dose level for determining the carcinogenicity. The Dossier Submitter considers that the forestomach tumours as observed in F344/N rats of both sexes should be taken forward for classification of resorcinol diglycidyl ether for the endpoint carcinogenicity (see also further below, our response with respect to the MoA).

- *Purity resorcinol diglycidyl ether.* The purity of resorcinol diglycidyl ether in the NTP (1986)-studies, as determined with epoxide titration, was 81%. Thin-layer chromatography by one system indicated five trace impurities. A second system indicated nine trace impurities and one slight trace impurity. By gas-liquid chromatography, 30 impurities were detected with a total area of approximately 14% of the major peak area. One of the impurities had an area that was 3.7% of the major peak area, and two groups of unresolved impurities had a combined area of 3.7% and 2.0% of the major peak area. The remaining impurities had a combined area of less than 4% of the major peak area. However, NTP (1986) stated that the identity of the impurities was not determined.
  Whether or not the impurities had an influence on the outcome of the NTP carcinogenicity studies is unknown.

- *Relevance of forestomach tumours for humans*: It is noted that the carcinogenic effects of resorcinol diglycidyl ether are limited to the forestomach, i.e. the site of contact. Humans do not have a forestomach. However, humans do have comparable squamous epithelial tissues in the oral cavity and the upper two-third of the oesophagus. Rodent tumours occurring in tissues with no human equivalent may provide information on the carcinogenic potential of a chemical. It cannot automatically be ruled out that the substance could cause similar tumours of comparable cell/tissue origin (e.g. squamous cell tumours at other epithelial tissues) in humans (cf. CLP-Guidance section 3.6.2.3.2-a).
  The CLP-Guidance also considers that forestomach tumours in rodents following administration by gavage of irritating or corrosive, non-genotoxic chemicals are considered not relevant for humans (cf. CLP-Guidance section 3.6.2.3.2-k).
  The Dossier Submitter acknowledges that the available skin irritation, the repeated dose studies as well as the carcinogenicity studies point towards and irritative effect at the site of contact upon exposure to resorcinol diglycidyl ether. Taking into account all these data, this might suggest that chronic tissue damage with resultant hyperplasia may have contributed to the carcinogenic response.
  However, based on the available in vitro and in vivo mutagenicity studies, resorcinol diglycidyl ether can be considered a mutagenic substance. In vitro studies showed positive genotoxic effects, though it is noted that not all in vivo mutagenicity results were conclusive. Resorcinol diglycidyl ether-induced micronucleus could only be revealed upon intraperitoneal exposure and not upon oral exposure in vivo. This might be explained by the results of the the toxicokinetic study performed by Seiler (1984a) showing that resorcinol diglycidyl ether is rapidly inactivated within the body. This rapid metabolization to genetically inactive substances and the in vitro results indicating that this substance does not require metabolic activation might also explain why resorcinol diglycidyl ether-induced tumours were observed only at the site of contact (the forestomach) in the oral (gavage) carcinogenicity studies performed by the NTP; the active substance is not distributed to other tissues in significant amounts. As there are currently no data which can exlude a genotoxic mode of action, the

Dossier Submitter assumed that the (local) genotoxicity contributed to the observed tumour response.

Based on these data (positive in 2 studies, 2 species, both sexes), it can be concluded that there is sufficient evidence of carcinogenicity. We conclude that resorcinol diglycidyl ether should be classified in category 1B.

RAC's response

Thank you for the comments, which have been taken under consideration. RAC agrees with the dossier submitter, that the classification criteria for Carc. 1B are met, as discussed in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2018	France		MemberState	3

Comment received

In the NTP study performed in rats, the survival was less than 30% in the highest dose groups in both males and females. Therefore, the dose of 50 mg/kg bw/day is expected to be higher than the maximal tolerable dose and findings obtained at this dose should be taken with caution. In addition, the survival in both treated and control groups in the female mice, is very low, questioning the reliability of the study.

Do you have any historical control data for the occurrence of forestomach tumours in the NTP studies? If yes, are the incidences reported in the tested groups outside the HCD?

We agree that the increase of tumours, consistently found in different studies and species, fulfils criteria for a category 1B. However, the fact that the increased tumours are only observed in the forestomach with a substance with both irritating and mutagenic properties is more in favour of a category 2.

Dossier Submitter's Response

The comments are noted.

- *Rat study*: In the main study, an increased incidence of forestomach tumours (squamous cell papilloma and carcinoma) was noticed at 25 and 50 mg/kg bw/d, though the effects were not as striking at the high dose. A reduced survival and a reduction in body weight was observed as well at these dose levels. Survival was 42/50, 5/50 and 0/50 in males and 37/50, 16/50 and 1/50 in females at 0, 25 and 50 mg/kg bw/d, respectively. These tested dose levels could be considered too high and it is agreed that the result at these dose levels needs to be evaluated carefully.
  A supplemental study was performed using a dose level of 12 mg/kg bw/d. No decrease in body weight or reduced survival was noticed at this lower dose level. An increase in incidences of forestomach tumours was however noticed. The forestomach tumour incidences at this dose were statistically significantly increased (squamous cell papilloma: 32% (m) and 38% (f) at 12 mg/kg bw/d versus 0% in controls; squamous cell carcinoma: 78% (m) and 54% (f) at 12 mg/kg bw/d versus 0% in controls) and outside the range of historical control data (see below). The Dossier Submitter considers the rat study adequate at least at one dose level for determining the carcinogenicity. The Dossier Submitter considers that the forestomach tumours as observed in F344/N rats of both sexes should be taken forward for classification of resorcinol diglycidyl ether for the endpoint carcinogenicity (see also further below, our response with respect to the MoA).

- *Mouse study*: A reduction in body weight in high dose (100 mg/kg bw/d) female animals was observed. Survival was not significantly affected but was indeed low in all female groups (control and resorcinol diglycidyl ether treated animals). However, an increase in incidences of forestomach tumours was noticed (papilloma(tosis): 0%, 8% and 20% in males, 0%, 10% and 20% in females, and carcinomas: 0%, 29% and 50% in males, 0%, 24% and 47% in females at 0, 50 and 100 mg/kg bw/d, respectively). The increased incidence of these tumours showed a positive trend and the incidences in the high dose were significantly higher than those in controls (and outside the range of historical control data (see below)). The Dossier Submitter considers that the forestomach tumours as observed in B6C3F1 mice of both sexes should be taken forward for classification of resorcinol diglycidyl ether for the endpoint carcinogenicity (see also further below, our response with respect to the MoA).

- *Historical control data*: Historical control data for forestomach tumours in NTP studies are available. See tables 1 and 2 for the historical incidence of stomach tumours in F344/N rats and B6C3F$_1$ mice, respectively.
  The historical incidences of (fore)stomach tumours are close to zero. In comparison with the historical control data, the incidence in rats and mice dosed with resorcinol diglycidyl ether was higher than that in historical control data.
  It is noted that historical control data should preferably be taken from the same laboratory and the same strain, using a time period that is close to the time period at which the study under consideration is conducted. According to the information available in the NTP-publication, the 2-year NTP studies of resorcinol diglycidyl ether were conducted at EG&G Mason Research Institute under a subcontract to Tracor Jitco, Inc., the prime contractor for the Carcinogenesis Testing Program. The 2-year studies in rats were begun in April 1979 and completed in April 1981. The supplemental rat studies were started in April 1980 and completed in April 1982. The 2-year studies in mice were begun in March 1979 and completed in March 1981. It is stated in the NTP-report (1986) that the historical control data of F344/N rats and B6C3F1 mice were taken as from March 16, 1983. These included data of the Mason lab which was the performing laboratory of the NTP carcinogenicity studies on resorcinol diglycidyl ether. It is noted that that the exact time period has not been specified for the individual historical studies. No further details are available to the Dossier Submitter on the historical control data from NTP.

Table 1. Historical incidence of stomach tumours in F344/N rats receiving corn oil by gavage (NTP 1986) [(a)]

A. Male animals

Laboratory	**Incidence**	**Site**	**Diagnosis**
Batelle	1/100	Cardiac stomach	Squamous cell papilloma
Gulf South	1/269	Stomach, NOS	Squamous cell papilloma
	1/269	Stomach, NOS	Squamous cell carcinoma
Litton	0/147		
Mason	1/200	Forestomach	Squamous cell papilloma
Papnicolaou	0/50		
Southern	1/299	Forestomach	Squamous cell papilloma
total	*5/1065 (0.5%)*		

B. Female animals

Laboratory	**Incidence**	**Site**	**Diagnosis**
Batelle	0/99		
Gulf South	1/276	Stomach, NOS	Squamous cell carcinoma
Litton	1/150	Stomach, NOS	Squamous cell papilloma
Mason	0/199		
Papnicolaou	0/50		
Southern	1/299	Stomach	Squamous cell papilloma
	1/299	Gastric mucosa	Squamous cell papilloma

	1/299	Forestomach	Squamous cell papilloma
total	*5/1073 (0.5%)*		

(a) data as of March 16, 1983

Table 2. Historical incidence of stomach tumours in B6C3F1 mice receiving corn oil by gavage (NTP 1986) [(a)]

A. Male animals

Laboratory	**Incidence**	**Site**	**Diagnosis**
Batelle	0/100		
Gulf South	1/224	Stomach, NOS	Papilloma, NOS
Litton	1/147	Forestomach	Papilloma, NOS
	1/147	Stomach, NOS	Squamous cell papilloma
Mason	1/196	Forestomach	Squamous cell carcinoma
Papnicolaou	1/48	Stomach, NOS	Squamous cell carcinoma
Southern	1/296	Stomach, NOS	Squamous cell papilloma
	1/296	Stomach, NOS	Squamous cell carcinoma
total	*7/1011 (0.7%)*		

B. Female animals

Laboratory	**Incidence**	**Site**	**Diagnosis**
Batelle	0/99		
Gulf South	2/245	Stomach, NOS	Squamous cell papilloma
	1/245	Stomach, NOS	Adenocarcinoma, NOS
Litton	0/145		
Mason	0/197		
Papnicolaou	0/47		
Southern	1/297	Gastric mucosa	Squamous cell papilloma
	1/297	Gastric mucosa	Adenoma, NOS
	1/297	Forestomach	Squamous cell papilloma
total	*6/1030 (0.6%)*		

(a) data as of March 16, 1983

- *MoA:* The Dossier Submitter acknowledges that the available skin irritation, the repeated dose studies as well as the carcinogenicity studies point towards and irritative effect at the site of contact upon exposure to resorcinol diglycidyl ether. Taking into account all these data, this might suggest that chronic tissue damage with resultant hyperplasia may have contributed to the carcinogenic response. However, resorcinol diglycidyl ether is also a mutagenic substance. As there are currently no data which can exlude a genotoxic mode of action, the Dossier Submitter assumed that the (local) genotoxicity contributed to the observed tumour response. Based on these data (positive in 2 studies, 2 species, both sexes), it can be concluded that there is sufficient evidence of carcinogenicity. We therefore conclude that resorcinol diglycidyl ether should be classified in category 1B.

RAC's response

Thank you for the comments. Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2018	France		MemberState	4

Comment received

The reported studies are difficult to analyse due to the limited information on the protocols. In the absence of more reliable data, we agree with the proposed categories and ATE.

Regarding acute dermal toxicity, the high LD50 in the first study could also be explained

by the short duration of exposure (7 hours instead of 24 hours as recommended in the OECD guideline).
Dossier Submitter's Response
Thank you for your support. The dossier Submitter acknowledges that only minimal information with respect to the study characteristics of the acute toxicity studies is available. All information available in the publications is presented by the Dossier Submitter in the CLH-report plus its accompanying Annex I. The Dossier Submitter notes that the presented acute toxicity data have previously been used by TC C&L to conclude on the classification for acute toxicity of resorcinol diglycidyl ether (i.e. R22 (Harmful if swallowed), R21 (Harmful in contact with skin)). Therefore, the Dossier Submitter considered that these data should again be included in current evaluation for classification, though acknowledging that the data might not fully fulfill current standards. The deviation from OECD test guidelines is noted for the various studies, including the acute dermal toxicity study with a 7 hours duration of exposure. This might have an impact on the outcome of this study.
RAC's response
Thank you for the comments. Noted.