Committee for Risk Assessment

RAC

Annex 2
Response to comments document (RCOM) to the Opinion proposing harmonised classification and labelling at EU level of

2,2-bis(bromomethyl)propane-1,3-diol

EC Number: 221-967-7
CAS Number: 3296-90-0

CLH-O-0000001412-86-212/F

Adopted

8 June 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-BIS(BROMOMETHYL) PROPANE-1,3-DIOL

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: 2,2-bis(bromomethyl)propane-1,3-diol
EC number: 221-967-7
CAS number: 3296-90-0
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Sweden		MemberState	1
Comment received				
The Swedish CA proposes that the hazard class reproductive toxicity should be addressed in the CLH-dossier. The dossier submitter states that "BMP is not a selective reproductive toxicant, because the findings are concomitant with general toxicity". However, based on the effects seen on litter size, number of pups born alive, pup weight and the number of primary and growing follicles (Treinen, K. A. et al., 1989), it is possible that a different assessment could be made.				
Dossier Submitter's Response				
"Because there were reproductive effects only in groups with lowered body weight, one could question the specificity of BMP as a reproductive toxicant. The contribution of decreased body weight to the reproductive impairment produced by BMP cannot be assessed by these data alone; studies are currently ongoing to evaluate the effect of diet restriction on fertility in Swiss mice" (Treinen, K. A. et al., 1989). New data mentioned by Treinen not found.				
RAC's response				
Thank you for your comment. As no corrected body weights were available, it is likely that lower increases in body weights were related to lower numbers of pups per litter. No other clinical sign of toxicity has been identified, some effects on the kidneys were mainly seen in male animals. However, reproductive toxicity is not covered in the scope of the CLH proposal, thus this endpoints is not subject to any further evaluation.				

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	Germany		MemberState	2
Comment received				
The German CA supports the CLH proposal of the Norwegian CA. However, there is some doubt, if the data presented are sufficient to fulfil the criteria of Muta 1B.				
Dossier Submitter's Response				
Thank you for your support. See comments below.				
RAC's response				
Thank you for your comment. Your position is noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2017	Netherlands		MemberState	3
Comment received				
There is clear evidence from a reliable study that BMP induces tumours in multiple sites in both males and females in both rats and mice. Also preneoplastic lesions are observed in several organs. In addition, BMP shows mutagenic activity. Several of the tumours may also be relevant for humans. We therefore agree that BMP should be classified as Carc. 1B; H350. It is noted that a part of table 17 seems to be missing (i.e. the clear neoplastic effects in mice as well as part of the clear neoplastic effects in rats).				
Dossier Submitter's Response				
Thank you for your support. The missing page is inserted at the end of this document (NTP, full study report p. 11).				
RAC's response				
Thank you for your comment. Your position is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	4
Comment received				
FR agrees with the classification proposal. However, FR disagrees with the following statement: "Clear exposure-related carcinogenic effects were observed at 17 sites in male rats (skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas)". In the pancreas for instance, the incidences are: 1/51, 2/53, 4/51, 3/53, and 3/59, and in the seminal vesicle: 0/51, 0/53, 0/51, 0/55, and 2/60. Regarding those results, it can't be stated that those effects are clearly exposure related. Comparison with historical controls data would maybe allow a more robust assessment of those effects. Moreover, no mention is made in the CLH to the statistical significance of those effects, which could have been very helpful for the assessment. 28: in the last sentence, it is stated that the 90-day study (Elwell et al., 1989) is supportive evidence for carcinogenic effect seen in Dunnick et al. study. However, in				

Dunnick et al. study, urinary bladder and kidney neoplastic effects are equivocal or uncertain, and, contrary to the 90-day study, rats are more sensitive than mice.
P25-26: a part of the table is missing.

Table 15: could you explain why the 2-year study from Ton et al. (2004) is in this table of "other studies relevant for carcinogenicity" and not in table 14?

Dossier Submitter's Response

Thank you for the comments. According to Dunnick et al 1997 p. 542: "An exposure-related carcinogenic effect was observed at 17 sites in male rats, and 4-6 sites in female rats and male and female mice." For clarity tables with statistics have been inserted in the end of this document (Dunnick et al 1997, table III, IV, V, VI).

Although the concurrent control group is always the first and most appropriate control group used for evaluation. We agree that historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Neoplasm incidences from the NTP historical control database (Haseman *et al.,* 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects. Hence historical control data is available in the NTP report 452 (NTP, 1996).

We agree that the sentence in page 28 could be rephrased, and suggest the following sentence: "The 90-day study (Elwell et al., 1989) is supportive evidence for kidney and bladder lesions in mice and rats."

P25-26: The missing page is inserted in the end of this document (NTP, full study report p. 11).

We think that Ton et al 2004 is best placed in table 15 as this is an additional investigation of the NTP material and not a separate carcinogenicity study.

RAC's response

Thank you for your comment. Your position is noted. RAC agrees with your comment on "Clear exposure-related carcinogenic effects". The underlying data will be presented in the RAC opinion together with historical control data. RAC considers the lesions in kidney and urinary bladder as supportive evidence for neoplasms in the kidney and bladder in the carcinogenicity study, though of limited value for the carcinogenicity assessment as a whole.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Sweden		MemberState	5

Comment received

Based on the information available in the CLH report, we agree with the proposed classification Carc. 1B.

The results from the NTP study show a carcinogenic response in both sexes of rats and mice which is further supported by findings in the 90-day study performed in the same species. The lack of a carcinogenic response in the study performed with Sprague-Dawley rats is not considered to weaken the strength of evidence for category 1B, taking into account the lower doses used.

Dossier Submitter's Response

Thank you for your support.

RAC's response
Thank you for your comment. Your position is noted. RAC agrees that the carcinogenicity study in SD-rats is of limited weight given the lower doses used in that study.

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	Germany		MemberState	6
Comment received				
NO presented sufficient evidence to propose classification and labelling as Carc 1B.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment. Your position is noted.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2017	Netherlands		MemberState	7
Comment received				
Since positive findings have been reported in the in vivo tests (oral and i.p. micronucleus tests in rats and a comet assay in rats), together with several positive in vitro tests and because it cannot be excluded that BMP has a direct mutagenic effect on the germ cells (as shown by the reduction in follicles in the study by Bolon, 1997), we agree that BMP should be classified as Muta. 1B; H340				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted and considered for the opinion document.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	8
Comment received				
FR is of the opinion that category 2 would be more appropriate. Concerning criteria of category 1B, there is positive results from in vivo somatic cell mutagenicity test in mammals, but the evidence that substance has potential to cause mutation in germ cell is not sufficiently robust. The decrease in follicles counts seen in Bolon et al. (1997) study could be the consequence of many phenomena, like a disruption in the hypothalamic-pituitary axis. Without any other information (such as kinetics data), FR is of the opinion that data are too sparse to attribute the decrease of follicles to the presence of BMP in germ cells. P19: It is stated that there are three Ames tests available while four tests are listed in table 11. Please clarify. P19: it is indicated that the sister chromatid exchange assay (Galloway et al., 1987) is negative and in table 11 and P21, it is indicated that the results from this test are equivocal. Please clarify.				
Dossier Submitter's Response				
Thank you for the comment. See our response to comment number 11 below.				

We propose to rephrase the first paragraph in p. 19:
In vitro studies: In the in vitro assays, four Ames tests are included, with different purities of the compound. Three tests show clear concentration-related positive results in the presence of 30% Syrian hamster liver S9-mix (unknown author, 1996 (98.63% purity); unknown author, 1996 (99.5% purity));(Zeiger et al., 1992)) and one test showing a negative result with the S9-mix concentrations limited to 10% (Mortelmans et al., 1986). In summary, positive findings were obtained when using high concentrations of hamster S9-mix (30%). No mutagenic activity was detected when using rat liver S9-mix, or low concentrations of hamster S9-mix for metabolic activation.

P19: For the SCE assay of Galloway et al 1987: In table 11 the conclusion is: Equivocal in the presence of rat S9 and negative without rat S9. We suggest that the final sentence in the second paragraph in page 19 is rephrased to: "The same authors also conducted sister chromatide exchange assay that was equivocal in the presence of rat S9 and negative without rat S9."

RAC's response

Noted, in support of the DS's response. The arguments for Cat. 2 or 1B have been documented in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Sweden		MemberState	9

Comment received

The line of reasoning behind the classification proposed is clearly presented. However, for transparency and to allow for an independent assessment, we would have appreciated the results from the individual studies tabulated (information on frequency, statistical significance etc).
Based on the information available in the CLH report, we agree with the proposed classification Muta. 1B.
The majority of results from in vitro and in vivo studies in somatic cells clearly demonstrate a genotoxic potential of the substance. Although there are no germ cell studies available and no supportive toxicokinetic information with respect to levels of BMP in gonads for the species used in the micronucleus tests (i.e. B6C3F1), the effects on follicles in the Bolon (1997) study demonstrates target tissue exposure. Similarly, the effects noted in the Treinen (1989) study support exposure of gonads in rat and consequently that effects noted in the in vivo (rat) mammalian alkaline comet assay may occur in gonads.

Dossier Submitter's Response

Thank you for the support. The data is tabulated in table 11 and 12, although specific frequencies and statistical significance is not specifically stated for all studies.

RAC's response

Noted and considered in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Finland		MemberState	10

Comment received

FI CA agrees with the dossier submitter that BMP is a mutagen and based on available data should be classified at least in category 2.

FI CA is concerned that data from reproductive toxicity studies may not be sufficient to show that BMP has potential to cause mutations in germ cells, which is one of the requirements for the classification in category Muta. 1B. Adverse reproductive effects, which were observed only in females, occurred simultaneously with general toxicity (decreased body weight). Therefore, FI CA considers that it cannot be excluded that these adverse reproductive effects may be caused by general toxicity.

In addition, FI CA considers that decreased follicle numbers without any toxicokinetic data may not be sufficient to demonstrate that BMP can interact with genetic material of germ cells. No mechanistic data or explanation is provided regarding the mechanism causing the decreased follicle numbers. Therefore, it cannot be excluded that the decreased follicle numbers could be due to a mechanism that does not involve BMP to be present in germ cells. Available toxicokinetic data provides evidence that BMP does not reach germ cells in males (Hoehle et al. 2009), but no toxicokinetic data for females is available.

Dossier Submitter's Response
Thank you for the comment. See our response to comment number 11 below.
RAC's response
Noted and in line with the discussion in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	Germany		MemberState	11

Comment received

The mutagenicity in somatic cells is sufficiently presented. However, to fulfill the criteria of Muta 1B it is necessary to show some evidence that the substance has the potential to cause mutations to germ cells.

Page 10:
The toxicokinetic study on male rats does not present convincing data, that the compound reaches the testes. At four different time points male rats are investigated. Only at one time point (1 administration, unfasted) 0.01 % of dose applied are detected in testes. At the other three time points nothing is detected at all.
No data were presented for female rats.
Therefore there is some doubt, that the compound reaches the germ cells at all.

Page 17:
In table 13 the dossier submitter presents a study with a continuous breeding protocol in Swiss mice. BMP exposure significantly decreases the numbers of litters per pair, pubs born alive per litter, and pup weight when adjusted for litter size. Sperm concentration, motility, morphology, and oestrual cyclicity are unaffected by BMP. Histopathology in the F0 animals reveals specific kidney lesions in both sexes. The dossier submitter concludes that the impaired fertility in BMP-treated female mice occurs in the absence of effects on reproductive organ weights and oestrual cyclicity. However, it does not support the assumption that this effect is due to mutations in germ cells.

Page 18:
In table 13 the dossier submitter presents data on the follicle counts in the ovaries of animals from the above mentioned continuous breeding study. The number of follicles in the ovaries is clearly reduced. These results suggest that BMP reaches the ovaries. However, it does not support the assumption that this effect is due to mutations in germ

cells. In conclusion, there are doubts whether a classification as Muta 1B is appropriate.
Dossier Submitter's Response
Thank you for the comment. We agree with you that the data is not clearly indicating Muta 1B. However, to fulfill the criteria of Muta 1B it is only necessary to show some evidence that the substance has the potential to cause mutations to germ cells. In our view such evidence has been presented in the CLH-report.
RAC's response
Noted.

Appendix – see Comment number 3 – missing page of Table 17 referred to in the Dossier Submitter's response is below.

2,2-Bis(bromomethyl)-1,3-propanediol, NTP TR 452 **11**

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Skin: squamous cell papilloma, keratoacanthoma, trichoepithelioma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma (4/51, 6/53, 14/51, 24/55, 21/60) Skin, subcutaneous tissue: fibroma, fibrosarcoma, or sarcoma (2/51, 9/53, 13/51, 16/55, 10/60) Mammary gland: fibroadenoma or adenoma (0/51, 4/53, 7/51, 7/55, 5/60) Zymbal's gland: adenoma or carcinoma (2/51, 1/53, 4/51, 5/55, 15/60) Oral cavity (pharynx, tongue, or gingiva): squamous cell papilloma or carcinoma (0/51, 4/53, 9/51, 10/55, 13/60) Esophagus: squamous cell papilloma (0/51, 0/53, 1/51, 5/55, 0/60) Forestomach: squamous cell papilloma (0/51, 0/53, 0/51, 1/55, 5/60) Large intestine: adenoma or carcinoma (0/51, 0/53, 3/51, 4/55, 11/59) Small intestine: adenoma or carcinoma (0/51, 0/53, 0/51, 2/53, 5/59) Malignant mesothelioma: (0/51, 3/53, 8/51, 9/55, 26/60) Urinary bladder: transitional cell papilloma or carcinoma (0/51, 0/53, 1/51, 3/55, 2/59)	Oral cavity: squamous cell papilloma or carcinoma (2/50, 3/51, 5/53, 6/52) Esophagus: squamous cell papilloma (0/50, 0/51, 1/53, 10/52) Mammary gland: fibroadenoma (25/50, 45/51, 46/53, 45/52) Thyroid gland: follicular cell adenoma or carcinoma (0/50, 0/51, 2/53, 4/52)	Harderian gland: adenoma or carcinoma (4/50, 7/51, 16/50, 22/49) Lung: alveolar/ bronchiolar adenoma or carcinoma (15/50, 11/51, 16/50, 25/49) Kidney (renal tubule): adenoma (0/50, 0/51, 3/50, 2/49)	Harderian gland: adenoma or carcinoma (3/52, 12/50, 13/51, 19/50) Lung: alveolar/ bronchiolar adenoma or carcinoma (5/52, 5/50, 15/51, 19/50) Skin (subcutaneous tissue): sarcoma (0/52, 1/50, 4/51, 11/50)