Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

N,N'-methylenediacrylamide

EC Number: 203-750-9
CAS Number: 110-26-9

CLH-O-0000007157-72-01/F

Adopted

15 September 2022

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CLH-O-0000007157-72-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***N,N'*-methylenediacrylamide**

EC Number: **203-750-9**

CAS Number: **110-26-9**

The proposal was submitted by **Sweden** and received by RAC on **28 September 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **13 December 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 February 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Betty Hakkert**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 September 2022** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	*N,N'*-methylenediacrylamide	203-750-9	110-26-9	Muta. 1B	H340	GHS08 Dgr	H340			
RAC opinion	TBD	*N,N'*-methylenediacrylamide	203-750-9	110-26-9	Muta. 1B	H340	GHS08 Dgr	H340			
Resulting Annex VI entry if agreed by COM	TBD	*N,N'*-methylenediacrylamide	203-750-9	110-26-9	Muta. 1B	H340	GHS08 Dgr	H340			

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The substance *N,N*'-methylenediacrylamide (MBA) is used as a crosslinking agent and as a monomer for polymerisation. It is used by professional workers in the production of electrophoresis gels.

The scope of the current CLH report and this opinion is focussed on germ cell mutagenicity.

The DS proposed Muta. 1B for MBA since:

- two studies with MBA demonstrated positive results from heritable germ cell mutagenicity tests in mammals (one study after intraperitoneal (i.p). exposure, the other after oral exposure);
- supporting evidence of the mutagenic effect of MBA is available from *in vivo* studies, including increased micronuclei in bone marrow after i.p. injection and positive comet assays in testicular cells after oral exposure;
- the available data provided clear evidence that MBA reaches the germ cells and interacts with germ cell DNA;
- the mutagenic effect of MBA can be passed on to the progeny.

The DS also considered MBA to be structurally similar to acrylamide, a chemical which has a harmonised classification as Muta. 1B and whose mutagenic effects are well known. For the assessment of germ cell mutagenicity the DS considers the information on MBA as sufficient for classification and the information on acrylamide is only used as supporting evidence.

Comments received during consultation

Comments were received from two MSCAs.

Both MSCAs supported Muta. 1B based on positive results from *in vivo* heritable germ cell mutagenicity tests in mammals, supporting positive results seen in other genotoxicity studies and the similarity with acrylamide.

In response to a comment from one MSCA concerning the summary of one of the *in vitro* studies (Hashimoto and Tanii, 1985), the DS confirmed that considering the context, the text should be read as "No cytotoxicity" instead of "Cytotoxicity was not specified".

Assessment and comparison with the classification criteria

The following table provides a summary of the available data on MBA for the endpoint germ cell mutagenicity:

Study	Reference	Result	Remarks
In vitro			
Bacterial reverse mutation assay *S. typhimurium* TA 1535, 1537, 1538, 98 and 100 0, 5, 50, 500, 1000 and 5000 µg/plate MBA purity >95%, in water	Hashimoto and Tanii, 1985	Negative (+/- S9)	Details on experimental design not provided. *S. typhimurium* TA 102 or *E. coli* WP2 uvrA not tested. No cytotoxicity.
Bacterial reverse mutation assay *S. typhimurium* TA 1535, 97, 98 and 100 0, 100, 333, 1000, 3333, 10000 µg/plate MBA purity not known, in DMSO	Zeiger *et al*., 1988 (NTP)	Positive in TA1535 (+S9) and TA100 (+S9). Negative in TA1535 (-S9), TA100 (-S9), TA97 (+/-S9) and TA98 (+/-S9).	*S. typhimurium* TA 102 or *E. coli* WP2 uvrA not tested. Cytotoxicity was not specified.
In vivo			
Dominant lethal and heritable reciprocal translocation assay, mouse, i.p. 0 and 225 mg/kg bw, single exposure 0 and 90 mg/kg bw, 5 consecutive days MBA purity 99.9%	Rutledge *et al*., 1990 (NTP)	Positive for dominant lethal effects and heritable reciprocal translocations	Information on general toxicity of parental generation not reported.
Micronucleus assay, mouse, bone marrow (OECD TG 474), i.p. 0, 25, 50 and 100 mg/kg bw, 2 consecutive days MBA purity not specified	NTP, 1988	Positive Statistically significant increased number of MN in PCE at all dose levels	
Comet assay, mouse, testis, oral (gavage) 0, 50, 100 and 190 mg/kg bw, 2 consecutive days MBA purity not specified	Hansen *et al*., 2014	Positive Statistically significant increased mean % tail DNA in high dose group	Animals were observed during study period twice daily for abnormalities in clinical appearance. Information on adverse effects not reported.

Additional			
Reproductive Assessment by Continuous Breeding (RACB) study, mouse, oral (drinking water) 0, 1.6, 4.7 and 9.3 mg/kg bw/d MBA purity 97-99%	Chapin *et al*., 1995 (NTP)	Slightly, statistically significant, increased number of early resorptions and increased post-implantation loss noticed in high dose group	This study included a dominant lethal segment with pre-mating exposure of the male animals (control and high dose only). Only the results of the dominant lethal segment are reported in this table.
Sperm count, morphology and testicular histopathology; mouse; oral 0, 50, 100 and 200 mg/kg bw, single exposure MBA of "specific reagent grade"	Sakamato and Hashimoto, 1988	Dose-dependent effect on sperm count and histopathology	
Sex-linked recessive lethal test, Drosophila melanogaster (OECD TG 477) 0 and 600 ppm MBA purity >99%	Foureman *et al*., 1994	Positive for sex-linked recessive lethal mutations. Negative for reciprocal translocations.	

Since classification in category 1A requires human evidence, this category is not applicable in this case as no human data are available for MBA. Classification in category 2 is also not appropriate as the experimental results clearly point towards a mutagenic effect of MBA in the germ cells as pointed out below.

One *in vivo* heritable germ cell mutagenicity test in mammals is presented in the dossier and provides clear positive results. The dominant lethal assay of Rutledge *et al*. (1990), involved four experiments, three focussing on dominant lethal effects and one on heritable translocation effects. In the first experiment, focusing on the effect of MBA at various stages of spermatogenesis, the number of implantations/pregnant females and the number of live embryos per pregnant females was statistically significantly reduced compared to controls, and the percent dead implants was subsequently increased after single i.p. pre-mating treatment (225 mg/kg bw) of male mice with MBA. The dominant lethal effects were observed during the first 4 days post-treatment. Also, a reduced number of pregnant females and of living embryos were observed in females mated 36.5-43.5 days post-treatment to exposed males, which the study authors considered an effect caused by cytotoxicity in differentiating spermatogonia. The occurrence of dominant lethal effects as observed during the first 4 days post-treatment was verified in a second experiment using single exposure (225 mg/kg bw) with matings during 1-9 days post-treatment and in a third experiment after 5× repeated i.p. treatment (90 mg/kg bw per day) with matings during 1-12 days post-treatment and using two different strains of untreated female animals. The results of these second and third experiments confirmed the findings of the first experiment with dominant lethal effects during 0.5-3.5 days post-treatment. The fourth experiment of Rutledge *et al*. (1990) was a heritable translocation assay upon 5× repeated i.p. treatment. The results of this experiment demonstrated an increased incidence of male offspring with reduced fertility and

heritable reciprocal translocations in these animals after treatment of the male parental generation with MBA.

The DS indicated that the effects observed from matings during the first four days and during days 36.5-43.5 post-treatment in the dominant lethal study of Rutledge *et al*. (1990) matched in time with effects seen in sperm count and sperm morphology in the mouse study of Sakamoto and Hashimoto (1988). After single oral treatment with MBA, resting spermatocytes were either absent or reduced 1-3 days post-treatment, whereas at 35 days post-treatment a decrease in the number of sperm was observed in the caput epididymis (Sakamoto and Hashimoto, 1988).

Overall, the results of the dominant lethal study of Rutledge *et al*. (1990) provide clear evidence of an effect of MBA on the germ cells. This is supported by the Reproductive Assessment by Continuous Breeding (RACB) study of Chapin *et al*. (1995) which included a dominant lethal segment. In this specific part of the study, dominant lethal reproductive effects were observed, i.e. a slightly increased number of early resorptions resulting in an increased post-implantation loss, after oral (drinking water) pre-mating treatment of males with MBA.

Further support is provided by positive results from *in vivo* somatic cell mutagenicity and genotoxicity testing. An *in vivo* mouse micronucleus test with i.p. administration of MBA demonstrated formation of micronuclei in the bone marrow (NTP, 1988). RAC notes that the i.p. administration, as applied in this *in vivo* mouse micronucleus study as well as in the dominant lethal study of Rutledge *et al*. (1990), is not a regular route of human exposure. However, the *in vivo* comet assay by Hansen *et al*. (2014) demonstrated DNA damage in testicular cells after oral (gavage) treatment of mice, indicating that MBA can also reach the gonads upon oral exposure. RAC further notes that in the above-described dominant lethal segment of the RACB study of Chapin *et al*. (1995), treatment with MBA was via the oral route.

Finally, two bacterial mutagenicity tests were presented in the dossier of which the study of Hashimoto and Tanii (1985) showed negative results with and without metabolic activation, while in the study of Zeiger *et al*. (1988), MBA was found to be mutagenic with metabolic activation in two (TA100 and TA1535) out of four included strains. MBA was further shown to be mutagenic in a sex-linked recessive lethal test in Drosophila melanogaster, though negative for reciprocal translocation in this test (Foureman *et al*., 1994). RAC notes the structural similarity with acrylamide, a known mutagenic chemical, and considers that the data on acrylamide gives additional support for the proposed classification.

Overall, these data provide clear evidence of germ cell mutagenicity. Therefore, for *N,N'*-methylenediacrylamide (MBA), RAC concurs with the DS that **Muta. 1B (H340) is warranted**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).