Committee for Risk Assessment

RAC

Annex 2a

Response to comments document (RCOM)

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

Methylhydrazine

EC number: 200-471-4

CAS number: 60-34-4

CLH-O-0000001412-86-75/F

Adopted

11 September 2015

ANNEX 2A - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON METHYLHYDRAZINE

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 attachments including confidential documents received during the public consultation have been provided in full to the dossier submitter, to RAC members and to the Commission (after adoption of the RAC opinion). 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.

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Substance name: **methylhydrazine**
EC number: **200-471-4**
CAS number: **60-34-4**
Dossier submitter: **Netherlands**

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
08.12.2014	Norway		MemberState	1
Comment received				
Norway would like to thank the Netherlands for the proposal for harmonised classification and labeling of methylhydrazine, CAS-no. 60-34-4. We support the proposal to classify methylhydrazine with Carc. 1B, H350 based on the observed findings. This includes increased incidence of malignant and benign tumors in lung, liver, nose and adrenals in 2 animal species and in males as well as females. Classification with Carc. 1B – H350 is therefore warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC considers that the classification of methylhydrazine as Carc. 1B, H350 is justified. This conclusion is based on the weight of evidence from carcinogenicity animal studies and taking into account the fact that the mode of action leading to the carcinogenicity of methylhydrazine could be formation of methyl radicals followed by DNA methyl adduct formation.				

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2014	France		MemberState	2
Comment received				
FR does support the proposed classification of MH for carcinogenicity. Indeed, the data presented in animals (mice and hamsters) showing increased incidence of adenomas and adenocarcinomas in different organs (lungs, liver, caecum, nose...) are in favour of the carcinogenicity of MH. Tumors have been observed in these 2 species after oral exposure (drinking water at 0.01%) or by inhalation. Not all studies in hamsters are positive and studies in rats and dogs by inhalation are negative. No relevant information is available in humans regarding carcinogenicity. Therefore, the effects observed in mice and hamster can be considered as appropriate for human. Finally, the substance has shown conflicting results on mutagenicity in the Ames test; MH is not mutagenic in vitro in any of the mammalian cell				

tests. There is some evidence of a weakly positive response in vivo in the host-mediated assay.

FR would appreciate to know more about the mode of action leading to the carcinogenicity of the MH. In particular, based on the available data on genotoxicity/mutagenicity in vitro and in vivo, which are not very recent ones, a genotoxic potential cannot definitively be excluded. As genotoxicity may also be related to carcinogenicity, it would be very helpful to have the results of an in vivo mutagenicty test following current guidelines to conclude on the mutagenicity of HP and therefore if a genotoxic potential is confirmed, it would be another argument to support the classification on carcinogenicity.

Comments regarding Part A. Chapter 3: justification that action is needed at community level:
It is said in the dossier that genotoxicity data are presented in the report as supportive evidence but that the classification of MH regarding germ cell mutagenicity is not discussed. However in part B section 4.9.5, the classification criteria for mutagenicity are discussed for MH and a conclusion is given. FR would appreciate the dossier submitter to harmonize these sections.

Comments regarding Human Health Hazard Assessment – Toxicokinetics part:
4.1.1. p.14: DS states that " The toxicokinetics of MH via intravenous route was studied by Pinkerton and co-workers (Pinkerton M.K. et al, 1967). In this study a total of 20 mice, 20 rats, 17 dogs and 16 monkeys received intra-peritoneal injections of 14C-methylhydrazine at doses of 22 mg/kg (mice), 15 mg/kg (rats), and 10 mg/kg (monkeys and dogs)." FR would appreciate that DS checked the route of exposure (either iv or ip).

DS states: "This might due to the fact that the different tested species clear the material in a different way which may be due either to difference in rate or metabolic pathway". The metabolism of MH is not addressed in the report; FR would appreciate if the DS could develop a little bit on the metabolism of MH in order to enable RAC to discuss this statement.

DS states: "MH was excreted via urine (26% in dogs, 31% in monkey, 40% in rat, 9% in mice)." FR would appreciate if the DS could precise if the % reflect total absorbed dose or excreted dose.

DS states: "Both clinically and pathologically, the dog was apparently much more susceptible than the other species tested to the toxic effects of MH and to severe kidney damage". Does the DS has any hypothesis to explain these species differences? If so then it would be useful to have s short development on that as species differences are also observed for carcinogenic effects.

Page 15, DS states " Rats given 0.12 m-mole 14C-methylhydrazine /kg i.p. respired approximately 45% of the 14C during the following 24 hr. Of the respired radioactivity, 20% to 25% was 14CO2; the remainder was 14CH4.." FR would appreciate if the DS could also express the dose in mg/kg to compare exposure levels with the previous studies. FR would appreciate if the applicant could explain what "respire "means exactly in this context: expire or inhale?

Page 15, DS states "At sub-convulsive doses, 40% administered radioactivity in 14C-methylhydrazine was excreted in urine." FR would appreciate if the applicant could explain what are sub-convulsive doses: which level?

Page 15, DS states "The percentage of urinary excretion of 14C from higher doses of 14C-

methylhydrazine was less, but the net amount excreted was slightly higher". FR find this sentence difficult to understand. Could it be reworded?

Section 4.1.3 page 15. DS states " The available information on toxicokinetics of MH indicates that MH distributes mainly to liver, kidney and bladder. In rats, it was found that approximately 45% MH was respired and 40% MH was excreted in urine within 24 hours." FR would appreciate if the DS could add a first sentence saying that the data on toxicokinetic are very poor and not sufficient to describe the TK profile of the compound.

Dossier Submitter's Response

Thank you for the support for the proposed classification.

We agree that it would be relevant to know more about the mode of action of MH and that an in vivo mutagenicity test would have been helpful in assessing the mode of action. A request for such a study could be considered when RAC does not agree with the proposed classification.

We have long doubted whether to include the mutagenicity data only as support for the carcinogenicity classification or also for classification for mutagenicity. Finally we concluded not to propose classification for mutagenicity, because of the limited data available for this hazard class. As a result of these last minute changes, the CLH dossier is not consistent with respect to mutagenicity and the comparison of the mutagenicity data with the classification criteria should have been removed. However, the dossier cannot be changed after submission.

The toxicokinetic study by Pinkerton et al (1967) was performed via the intraperitoneal route.

The statement on the possible explanation of the difference in Tmax between the species is already theoretical. Seen the limited data on metabolism for this substance it is not possible to provide an additional explanation.

The fraction excreted in the urine is determined as the percentage of the amount in the urine after 24 hours divided by the injected amount and representing the excreted fraction.

We have no explanation for the clinical and pathological species differences.

The intraperitoneal dose in the studies by Dost et al (1966) of 0.12 mmol per kg body weight corresponds with 5.5 mg/kg bw based on a molecular weight of 46 g/mol. Respire means expire.

The wording "sub-convulsive dose" was only used in the summary of the study report. However, there were three dose levels (0.12, 0.24 and 0.48 mmol/kg bw with a median lethal dose of 0.6 mmol/kg bw) tested of which the "sub-convulsive dose" was the middle dose. Therefore, the sub-convulsive dose level corresponds with 11 mg/kg bw.

The percentage of radioactivity excreted via urine at intraperitoneal dose levels of 0.12 mmol/kg bw, 0.24 mmol/kg bw and 0.48 mmol/kg bw was 41%, 39% and 22% respectively showing a decrease in the percentage excreted at the highest dose level. However, the absolute excreted amounts of 2.26 mg/kg bw, 4.29 mg/kg bw and 4.84 mg/kg bw respectively, did increase although not dose dependently.

Although we agree with the proposed sentence, a CLH dossier cannot be changed after submission.

RAC's response
The questions raised and responses provided by the DS are noted. RAC considers that the classification of methylhydrazine as Carc. 1B, H350 is justified.

Date	Country	Organisation	Type of Organisation	Comment number
16.12.2014	Germany		MemberState	3
Comment received				
The German CA supports the CLH proposal of methylhydrazine.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC considers that the classification of methylhydrazine as Carc. 1B, H350 is justified.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2014	France		MemberState	4
Comment received				
Section 4.10 Table 14 : DS states for the results of the Toth et al. (1972) study : "Lung adenomas: Females: incidence 24% Males, incidence 22% Control incidences not included. FR noticed that the incidence is given in table 17. same for the statement "Malignant lymphomas: Females: incidence of 4% (lymphocytic type), observed at 37/43 wk. Control incidences not included" Table 14 page 32 description of the study of MacEwen JD, Vernot EH (1975) ; DS states "G2 – 0.1% MH in drinking water ». FR noticed that results for this group are not described in the table 20. Table 14 page 33 description of results of the study of Kinkead, E.R. et al. (1985). For mice no figures are given which makes the comparisons with hamster and rats difficult. FR would appreciate if the DS could give the incidence of tumors in mice. Section 4.10.1.1 Carcinogenicity: oral page 33. DS states "An early study (Kelly et al. 1969) did not demonstrate any increase in tumor incidence in the group of mice received MH over control animals." FR noticed that this study was only a 8 weeks. The duration of exposure is not sufficient to demonstrate an absence of tumors. FR would appreciate if the DS could add a sentence saying that. Page 39: DS states "In 1975 MacEwen and Vernot designed a study to test the reproducibility of the carcinogenic activity of MH administered in drinking water of male Golden Syrian hamsters (MacEwen J.D. and Vernot E. H., 1975). This 2-year drinking water study hamsters received untreated or acidified drinking water (pH 3.5) containing 0.01% MH, and acidified water only in unexposed controls. Neither the incidence, degree of severity, nor age at onset of non-neoplastic pathologic changes was markedly different in animals drinking aqueous MH in comparison to control animals." FR noticed that in table 14 page 32 another group was mentioned: G2 0.1% MH in drinking water for which the results are missing.				

Section 4.10.4 page 42: DS states :"The carcinogenicity of MH has been studied specially in the aviation sector as MH is commonly used as fuel for aircrafts. Both positive and negative results have been found." This last sentence is confusing as it suggest that human data on carcinogicity are available whch is not the case. Therefor FR would appreciate if the DS couls add at the beginning of this sentence: "in animals…"
Dossier Submitter's Response
As indicated with the * behind the controls in table 17 and as described in the text above table 17, the control incidences were from a different study. We did not include the control incidence in the summary table (table 14) because this would require an explanation regarding the relevance of the controls. This would not be proportional for a summary table. The decrease in adrenocortical tumours is described in the text above table 20 and the individual tumours are presented in the last column of table 20 including the adrenocortical tumours. Information on the neoplastic lesions in mice following inhalation exposure (Kinkead, 1985) are available in table 23. We agree that an 8-week exposure period is not sufficient to demonstrate the absence of tumour formation. This statement is already included in the summary of carcinogenicity on page 43. There is an error in table 14 as the study by McEwan and Vernot (1975) included three dose groups namely G1: control: drinking water pH=3.5, G2: 0.01% MH in drinking water pH=3.5 and G3: 0.01% MH in unadjusted drinking water. The value of 0.1% in table 14 should be 0.01%. We agree that it would have been clearer if we stated in the summary that positive and negative results were found in animal studies. However, we cannot change the CLH dossier.
RAC's response
The questions raised and responses and corrections provided by the DS are noted. The characterization of particular studies emphasised is reflected in the RAC assessment.

Date	Country	Organisation	Type of Organisation	Comment number
16.12.2014	Germany		MemberState	5
Comment received				
The CLH report demonstrates that the criteria for classification of methylhydrazine (MH) as a carcinogen of category 1B are met. Based on available experimental studies, a causal relationship between the oral and inhalation exposure to MH and the increased incidence of malignant and benign tumors, e.g. malignant histiocytomas, cecum tumors (adenoma and carcinoma), lung (adenomas), liver (adenomas and carcinomas), nose (adenomas and polyps) and adrenals (benign adenomas) has been demonstrated in two animal species (mice and hamsters) and in males as well as females.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC considers that the classification of methylhydrazine as Carc. 1B, H350 is justified.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2014	France		MemberState	6
Comment received				
Table 11 page 23: Results for the study of Poso et al. (1995) are not given. Table 11 page 24: Results for the study of Von Wright A et al (1977) are not given. Section 4.9.5 page 30: DS states "According to these criteria, a classification in germ cell mutagenicity category 2 is not appropriated for MH as MH showed no mutagenicity in in vivo inheritable germ cell mutagenicity tests in rats and mice, and also no mutagenicity from in vitro mutagenicity tests in mouse lymphoma cells and human diploid embryonic lung cells. Although there is some evidence for mutagenicity of MH in liquid incubation assays in in vitro bacterial systems, conflicting results were obtained from different host-mediated assays. Therefore, the classification of MH as a germ cell mutagen is not proposed." FR considers that the in vivo data base on mutagenicity is not sufficient to definitively exclude a mutagenic potential of MH. The in vivo tests mentioned are not recent one and there is no in vivo test based on the current guidelines for testing in vivo mutagenicity. As genotoxicity may also be responsible for carcinogenicity, it would be very helpful to have the results of an in vivo mutagenicity test following current guidelines to conclude on the mutagenicity of HP and therefore if a genotoxic potential is confirmed, it would be another argument to support the classification on carcinogenicity.				
Dossier Submitter's Response				
The results of the tests by Poso et al (1995) and Von Wright et al (1977) should be considered as positive as described in 4.9.1.1. Reduced survival of (DNA) repair deficient strains compared to repair proficient strains indicates the formation of DNA damage. We agree that it would be relevant to know more about the mode of action of MH and that an in vivo mutagenicity test would have been helpful in assessing the mode of action. A request for such a study could be considered when RAC does not agree with the proposed classification.				
RAC's response				
The explanation provided is noted. RAC agrees with the comment that new in vivo mutagenicity test performed according to current guidelines would be helpful in assessing the mode of action and giving (probably) additional evidence for the classification adopted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
20.12.2014	France		MemberState	7
Comment received				
P 16 in the table 10 in the thirds columns, the DS provides figures for the different LOEL. FR would appreciate if the applicant could discuss if this values are indeed LOEL or LOAEL (for adverse effects)? P16 in the table 10, DS states in the first column " Preliminary establishment of dose level:1 male and 1 female animal received daily ip injections of 5 or 10 mg/kg MH for 5 days."FR would appreciate if the DS could add the specie.				

Page 19: DS states: The prominent erythorphagocytosis gives ample evidence to the fact that MH is hematotoxic"; "erythorphagocytosis" should be erythrophagocytosis. FR would know if the kidneys were the only organs investigated in this study, if not, FR would appreciate if the DS could mention that other organs were not studied. Page 19: DS states: "It suggests that LOEL for MH in this study is 0.2 ppm." FR would appreciate if the DS could explain why it doesn't consider this value as a LOAEL. Section 4.7.1.7 page 21: FR would appreciate if the DS could add a first sentence saying that the data on repeated toxicity are incomplete and mainly based on old studies not sufficient to describe the toxicity of the compound.
Dossier Submitter's Response
The information on the repeated dose toxicity are only included to provide relevant data for assessment of the carcinogenicity. Detailed discussion on the adversity of the observed effects is considered not relevant for the proposed classification for carcinogenicity. The statement "Preliminary establishment of dose level:1 male and 1 female animal received daily ip injections of 5 or 10 mg/kg MH for 5 days." refer to monkeys. We agree that it should have been erythrophagocytosis. The publication by Sopher et al (1968) is not clear on the studied parameters but seems to be limited to the kidney, the liver and urine. We agree that the LOEL of 0.2 ppm could also be considered a LOAEL. We agree that the data on repeated dose toxicity are incomplete and mainly based on old studies. However, we cannot change the current text of the CLH dossier.
RAC's response
The questions raised and responses and corrections provided by the DS are noted.