Committee for Risk Assessment

RAC

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

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11 September 2015

CLH-O-0000001412-86-75/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: **Methylhydrazine**

EC Number: **200-471-4**

CAS Number: **60-34-4**

The proposal was submitted by the **Netherlands** and received by RAC on **29 October 2014.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Dossier Submitter 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 November 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 January 2015.**

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Normunds Kadikis**

Co-Rapporteur, appointed by RAC: **Jolanta Stasko**

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 **11 September 2015** by **consensus.**

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	612-RST-00-Y	methylhydrazine	200-471-4	60-34-4	Carc. 1B	H350	GHS08 Dgr	H350			
RAC opinion	612-RST-00-Y	methylhydrazine	200-471-4	60-34-4	Carc. 1B	H350	GHS08 Dgr	H350			
Resulting Annex VI entry if agreed by COM	612-RST-00-Y	methylhydrazine	200-471-4	60-34-4	Carc. 1B	H350	GHS08 Dgr	H350			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

During the RAC opinion development process the Dossier Submitter (DS) submitted additional documentation containing information related to the carcinogenicity of structurally similar hydrazines. This additional documentation was then subject to a second, targeted public consultation (PC) and subsequent evaluation by RAC.

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No information from humans on the carcinogenity of methylhydrazine is available. Five carcinogenicity studies were provided in the CLH report, one via the inhalation route, and the others via oral administration.

Based on the increased incidence of various tumours in mice and hamsters exposed to methylhydrazine via drinking water or inhalation for either their entire lifespan or one year, classification as Carc. 1B – H350 (may cause cancer) was proposed by the DS with no specific route of exposure stated.

In one study with CDF1 mice, no tumour formation was observed after 8 weeks treatment with methylhydrazine (once weekly) by gavage or intraperitoneal injection (i.p.), when compared to the controls. The DS considered that 8 weeks was too short to reveal any carcinogenic properties of the substance.

Daily treatement of Swiss mice with 0.01% methylhydrazine via drinking water for their life span resulted in a large increase in the incidence of tumours (lung tumours, malignant lymphomas etc.) compared to the untreated control group.

In a different study, a large increase in the incidence of liver tumours and tumours of the caecum as well as other types of tumours at low incidences was found in Golden Syrian hamsters following application of 0.01% methylhydrazine via drinking water for their life span compared to the untreated control group. However, this study with Golden Syrian hamsters was repeated by a different laboratory under similar test conditions and no marked difference in the incidence of tumours between the treated groups and untreated control groups were found.

The carcinogenicity of methylhydrazine by the inhalation route was also tested over a period of one year (6 hours/day, 5 days/week, a number of different doses from 0 ppm (control group) up to 2.0 ppm or 5.0 ppm) in Fischer 344 rats, Golden Syrian hamsters, C57BL/6J mice and Beagle dogs. Significant oncogenic changes were noted in the respiratory, hepatic and vascular systems of mice and hamsters, but not in rats or dogs. However, as only four dogs per dose and sex were exposed to methylhydrazine, the number of tested dogs was considered too small to draw conclusions on the absence of carcinogenic potential.

The mode of action leading to the carcinogenicity of methylhydrazine is unknown. The mutagenicity data, which were quite contradictory, were included only as support for the carcinogenicity classification and were considered not to be sufficient for a classification for germ cell mutagenicity in its own right. The same applies to the repeated dose toxicity data – they were only included as supportive data for the assessment of the carcinogenicity of methylhydrazine. Detailed discussion on whether the observed (non-neoplastic) repeated dose toxicity effects were adverse or not is considered relevant.

Comments received during public consultation

Two public consultations were launched for methylhydrazine. The first was on the CLH report (standard part of the CLH process) and the second was a targeted PC seeking additional information on read-across considerations submitted by the DS during the process of opinion development (see RAC general comments).

During the first public consultation, a number of Member State Competent Authorities (MSCAs) supported the proposal submitted by the DS to classify methylhydrazine as Carc. 1B, H350 based on the different types of tumours found in mice and hamsters following exposure via both the oral and inhalation routes, as well as in both males and females. In addition, one MSCA referred to conflicting results on mutagenicity in the Ames test and a weakly positive response *in vivo* in the host-mediated assay and stressed that genotoxic potential cannot definitively be excluded. This MSCA also called on the DS to prepare a proposal to harmonise the germ cell mutagenicity classification, since the relevant data were provided in the CLH report. The MSCA also considered that the toxicokinetic data were very poor and not sufficient to describe the toxicokinetic profile of the compound. They noted that the metabolism of methylhydrazine should also be addressed.
One MSCA considered that the reported 8-week study on mice was too short to demonstrate an absence of tumourigenicity. In addition, the MSCA stressed that data on repeated dose toxicity were incomplete and mainly based on old studies not sufficient to describe the toxicity of the compound.

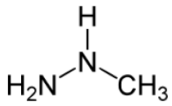
During the second targeted public consultation two MSCAs supported the proposed classification of methylhydrazine as Carc. 1B (H350), based on the information provided to support read-across from the following substances: hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine.

Summary and assessment of the Dossier submitter's additional information on read-across substances

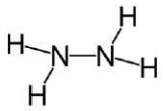
Read-across information on structurally related hydrazine compounds similar to methylhydrazine was provided by the dossier submitter (DS). The DS chose the following substances: hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine based on structures having the same central N-N moiety and either -H or -CH3 attached to the nitrogen atoms, as these are the closest possible chemical analogs.

Selected hydrazine compounds:

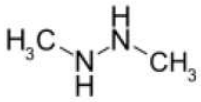
Methylhydrazine
(CAS 60-34-4, EC 200-471-4)



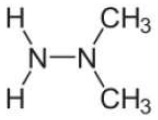
Hydrazine
(CAS 302-01-2, EC 206-114-9)



1,2-dimethylhydrazine
(CAS 540-73-8, no EC number)



1,1-dimethylhydrazine
(CAS 57-14-7, EC 200-316-0)



No relevant information regarding mutagenicity or carcinogenicity could be retrieved for trimethylhydrazine. Therefore, this substance was not included. The main mutagenicity mechanism is considered to be DNA methylation.
The four hydrazines are all liquids with a high vapor pressure, are miscible with water and have a negative Log Kow and thus can be considered to have comparable physical/chemical properties for the purpose of the mutagenicity/carcinogenicity assessment.

There are very limited data on the metabolism of methylhydrazine. The available information shows that 45% of the radioactive labeled carbon is exhaled as CO_2 and methane after i.p. injection and approximately 40% was excreted in the urine. The source hydrazine compounds seem to have different metabolic pathways. However, this difference is likely to be caused by differences in the available information on metabolites. The available information on DNA adducts shows that methyl adducts are formed by all three source hydrazine compounds. The only information indicating that methylhydrazine could also form methyl adducts comes from one *in vitro* study using isolated hepatocytes and liver microsomes.

DNA and RNA adducts may be responsible for gene mutations observed in a number of *in vitro* studies and may also serve as the initiating event for cancers induced by hydrazines *in vivo*.

It was suggested that administration of hydrazine to rodents results in the formation of N7-methylguanine and O6-methylguanine in liver DNA. It has therefore been proposed that the methylation mechanism involves the reaction of hydrazine with endogenous formaldehyde to yield formaldehyde hydrazone, which could be metabolized to the potent methylating agent diazomethane. The data supported the proposal that formaldehyde-hydrazone, the condensation product of hydrazine and formaldehyde, is rapidly transformed in various (liver) cell fractions to a DNA-methylating agent. The reaction of hydrazine with formaldehyde resulting in the formation of a hydrazone could also occur since methylhydrazine has a free amino group.

The metabolites of 1,2-DMH (azoxymethane and methylazoxymethanol) can form methyldiazonium which can methylate DNA. The metabolic pathway of 1,2-DMH could be considered as not relevant for methylhydrazine because it requires a methyl group on each of the two nitrogen atoms, but methylhydrazine contains only one methylated nitrogen atom.

In general, the available information shows that hydrazines are oxidised at the N-N moiety, resulting in azo (N=N) compounds and following further metabolism, ultimately resulting in formation of nitrogen gas (N2) and a methyl radical. In one *in vitro* study is was shown that methylhydrazine can also be metabolised to substances that can form methyl radicals and *in vivo* methane formation was observed. Therefore, it is expected that methylhydrazine can also form methyl DNA adducts and is (therefore) mutagenic.

The "source" hydrazine compounds have no harmonised classification for mutagenicity However, the available data show that these source compounds are all mutagenic *in vitro*. *In vivo* mutagenicity in somatic cells was seen in all "source" hydrazine compounds though not always in all studies and organs. Overall, comparison of the mutagenicity does not support read-across from the source hydrazines to methylhydrazine.

The available carcinogenicity studies with hydrazine compounds show that there are clear differences between species but almost all studies were positive (see Table 1 below). Blood vessel tumours observed in mice following methylhydrazine exposure via the oral and inhalation routes are also observed with both "source" methylated hydrazines and caecum tumours are also found with 1,2-DMH. Therefore, the results with the "source" hydrazine compounds support the relevance of these tumours for classification.

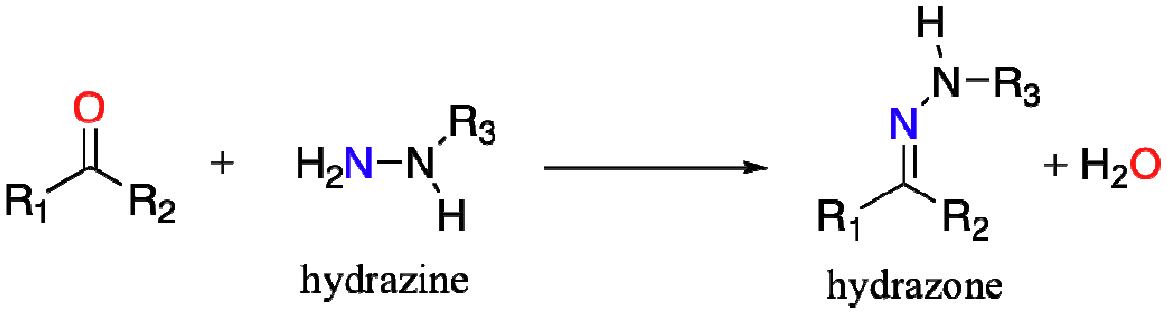
Table 1: Carcinogenicity data of the selected hydrazines (taken from background document, modified)

Route/ species	Methylhydrazine	Hydrazine (incl. hydrate and sulfate)	1,1-dimethyl-hydrazine	1,2-dimethyl-hydrazine
Oral: rat		Hepatocellular adenomas, carcinomas and cholangiomas Lung tumours		Colon tumours (single dose) Liver angiosarcoma, cholangioma, hepatocellular carcinoma, bowel adenocarcinoma, ear canal papilloma, colon carcinoma (intermediate exposure) Angio(sarco)mas
Oral: mouse	Lung adenoma Angio(sarco)ma Cholangio(sarco)ma	Lung tumours Hepatoma Breast One study negative	Blood vessel, lung, kidney, and liver tumours Lung adeno(carcino)mas	Blood vessel tumours Angio(sarco)ma, lung adeno(carcino)ma and colon tumours
Oral: hamster	Malignant histiocytoma caecum tumours Second study negative	Hepatocellular carcinomas		Blood vessel tumours

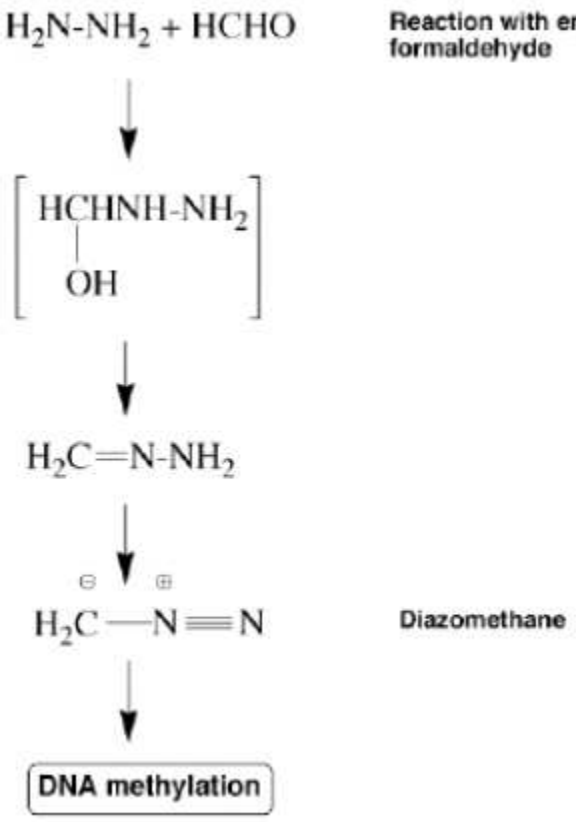
Inhalation:rat	Negative	Nasal adenomatous polyps and malignant nasal epithelial tumours Thyroid carcinoma	Pancreas, pituitary tumours	
Inhalation:mouse	Lung adenoma Liver adenoma and carcinoma Hemangio(sarco)ma Nasal adenomatous polyps and adenomas	Lung adenoma	Lung, liver, nasal cavity, bone and blood vessels tumours	
Inhalation:hamster	Nasal polyps and adenomas	Benign nasal polyps Colon neoplasms Thyroid parafolicullar cell adenoma		
CLH		Carc 1B	Carc 1B	Carc 1B

Adverse health effects by hydrazines; underlying mechanism

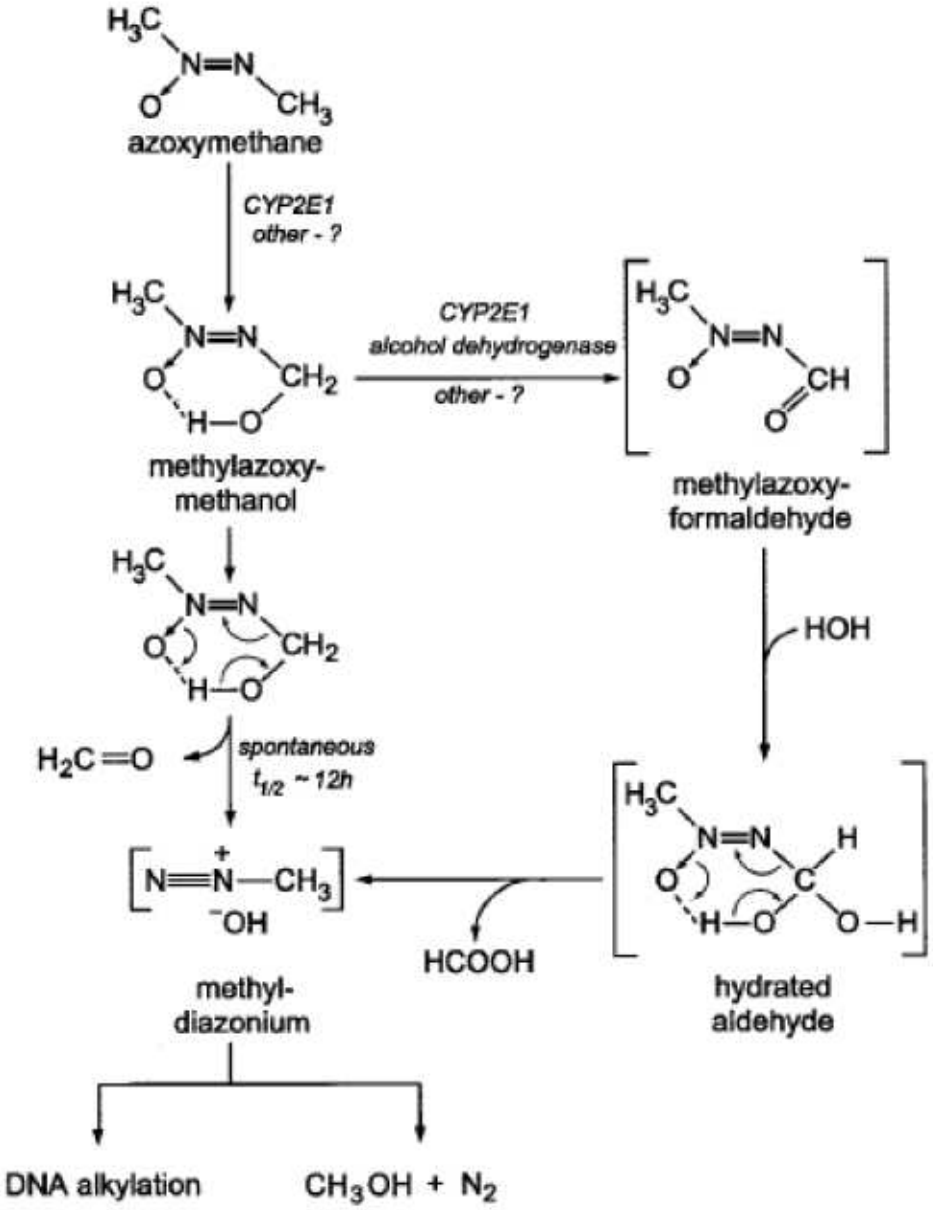
A. Hydrazones are formed in reactions between aldehydes/ketones and hydrazines.



B. Mechanistic concept of hydrazine-induced DNA methylation



C. Metabolic pathways of azoxymethane and methylazoxymethanol activation (Sohn, 2001).



According to the Read-Across Assessment Framework (RAAF) (European Chemicals Agency, 2015), two main approaches can be applied – the analogue approach and the category approach. The analogue approach is based on read-across from a single source substance to a single structurally similar target substance. The prediction of properties relies essentially on the structural similarity between the source and target substances. In a category approach, read-across is used among a number of structurally similar substances. Within this category, as a result of the structural similarity, the physico-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern.

The source substances: hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine have been selected by the DS due to structural analogy based on same central N-N moiety and either -H or -CH3 attached to the nitrogen atoms, as the closest possible analogs. The four hydrazines (target and source substances) show similar physico-chemical properties concerning physical state at room temperature, vapor pressure, water solubility and Log Kow. All three source hydrazines show induction of tumours resulting in a harmonised classification of category 1B for

carcinogenicity. However, there is some inconsistency in the tumour sites and the occurance of mutagenicity *in vivo*. This might be explained by the differences in metabolism between the source hydrazine compounds. Formaldehyde-hydrazone, the condensation product of hydrazine and formaldehyde, is rapidly transformed in various (liver) cell fractions to a DNA-methylating. The reaction of hydrazine with formaldehyde resulting in the formation of a hydrazone could also occur with methylhydrazine having a free amino group. The metabolic pathway of 1,2-dimethylhydrazine could be considered as not relevant for methylhydrazine, because it requires a methyl group on each of the two nitrogen atoms in order to form azoxymethane and methylazoxymethanol leading to formation of methyldiazonium which can methylate DNA, but methylhydrazine contains only one methylated nitrogen atom.

The DS stresses that the source substances are considered as one homogeneous group and there are difficulties to indentify the most relevant source substance for methylhydrazine. Therefore, the category approach for read-across is used.

DNA methylation is considered as the main mutagenicity mechanism leading to initiation of cancer. Available information on DNA adducts shows that methyl adducts are formed by all three source hydrazine compounds. However, this information is not available for methylhydrazine (Table 2 below). Nevertheless, is was shown *in vivo* that methylhydrazine can be metabolised to substances that can form methyl radicals wich could lead to methyl adducts.

Table 2: Metabolites of the selected hydrazines

Identified metabolites	methylhydrazine	hydrazine	1,1-dimethylhydrazine	1,2-dimethylhydrazine
Oral	Carbon dioxide methane	Nitrogen Acetyl/diacetylhydrazine Pyruvate hydrazone Urea 1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid	Carbon dioxide Glucose hydrazone	Carbon dioxide Azomethane ethane
Inhalation		Acetyl hydrazine Diacetyl hydrazine		
Other				Azoxymethane methylazoxymethane
In vitro	Methyl radicals (Albano, 1989)	Free radical formation	Methyl radicals (Albano, 1989) Free radical formation	Methyl radicals (Albano, 1989) Formaldehyde
In vivo DNA adducts		Methyladducts N7-methylguanine and O6-methylguanine in liver of mice, rats and hamsters treated *in vivo*.	N7-methylguanine (Sagelsdorff, 1988)	N7-methylguanine and O6-methylguanine (Perse, 2011)
Additional references	ATSDR, 1997 and SCOEL, 2010			

In general, the available information shows that hydrazines are oxidised at the N-N moiety, resulting in azo (N=N) compounds and following further metabolism, ultimately resulting in formation of nitrogen gas (N2) and a methyl radical. Methylhydrazine can also be metabolised to substances that can form methyl radicals (*in vitro*). Available information on methylhydrazine

show further that 45% of the radioactively labelled carbon is exhaled as CO_2 and methane after i.p. injection and approximately 40% is excreted in the urine.

The available data show that all source compounds are mutagenic *in vitro* and partly *in vivo* in somatic cells.

The available carcinogenicity studies with hydrazine source compounds show that there are clear species differences but in almost all studies tumour formation was observed.

Nevertheless, according to the Read-Across Assessment Framework (RAAF) (European Chemicals Agency, 2015), "'read-across and grouping', or 'read-across', is one of the most commonly used alternative approaches for data gap filling in registrations submitted under the REACH Regulation" (underlining by RAC).

Assessment and comparison with the classification criteria

No relevant information is available from humans regarding carcinogenicity from exposure to methylhydrazine.

The DS summarised 5 carcinogenicity studies performed on animals which were published from 1969 to 1985 and which were not in accordance with the relevant OECD guidelines. For example, the testing time for inhalation exposure in rats according to OECD guideline 451 should be 2 years instead of 1 year, as was the case even in the most recent study (form 1985) reported in the CLH report. A summary of the animal tests reported in the background document is included in the Appendix below.

In general, the results obtained from oral, intraperitoneal injection and inhalation studies on mice, rats, hamsters and dogs are contradictory.

In CDF1 mice treated for 8 weeks with methylhydrazine (once per week) by gavage or intraperitoneal injection, no tumour formation was observed. Since the exposure time is very short RAC suggests discarding this study from any further weight of evidence analysis.

Daily administration of 0.01% methylhydrazine to Swiss mice mice via drinking water for the entire life span resulted in large increases in the incidence of lung adenomas (24 % for females compared to 12.7 % seen in earlier non-concurrent controls from an older study by the same author and 22 % for males compared to 10 % in non-concurrent controls). No information is provided on the statistical significance and also no historical control database is available for the Eppley Swiss Webster mice (randomly bred). Methylhydrazine shortened the survival time of mice - 50 % survival was at 30 weeks for males and approximately 45 weeks for females, compared to 60 and 80 weeks for male and female controls, respectively (Table below), indicating that the maximum tolerated dose (MTD) had probably been exceeded. The reported malignant lymphomas in Swiss mice revealed a generally higher incidence in the untreated (not concurrent) control group.

Tests on Golden Syrian hamsters (application of 0.01% methylhydrazine via drinking water for the life span) demonstrated elevated levels of malignant histiocytomas (32 % for females and 54 % for males compared to 0 % in controls) as well as tumours of the caecum (18 % for females and 14 % for males compared to 1 % in controls). No information was provided on the statistical significance of these results and no relevant historical control database was available. The DS

provided additional information on Syrian hamsters, which referred to small background incidences of hepatic tumours (up to 2 %) and tumours in the caecum in this species. Other types of tumours also occurred in the reported long-term studies in Swiss mice and Syrian hamsters, but at low incidences and these were not significantly different from controls. Again, a shortened survival time of the Syrian hamsters was also detected in comparison to untreated control groups, possibly indicating that the MTD had been exceeded (Tables 3 and 4 below).

Table 3: Survival rates in methylhydrazine-treated and control Swiss mice

Treatment	Initial no. and sex of mice	No. of survivors (age in weeks)												
		10	20	30	40	50	60	70	80	90	100	110	120	130
MH	50 ♀	41	41	39	33	13	8	-	-	-	-	-	-	-
	50 ♂	41	37	24	15	6	3	1	-	-	-	-	-	-
Control	110 ♀	109	109	107	104	96	89	73	57	41	23	11	1	-
	110 ♂	110	95	91	86	67	55	41	22	6	1	1	-	-

Table 4: Survival rates in methylhydrazine-treated and control Golden Syrian hamsters

Treatment	Initial no. and sex	No. of survivors at week												
		10	20	30	40	50	60	70	80	90	100	110	120	130
0.01 % MH in drinking water daily for life	50 ♀	49	48	48	47	39	27	16	4	1				
	50 ♂	50	49	48	48	43	39	30	18	8	2			
Untreated control	100 ♀	100	100	100	92	74	61	46	31	20	7	4		
	100 ♂	96	93	90	87	80	74	57	42	32	22	15	10	

The tumour-incidence results in the first Syrian hamsters study were not repeated in a second, similar 2-year study on male Golden Syrian hamsters, conducted a few years later. In this second study, adrenocortical tumours were the most frequently seen tumour-type, and occurred more often in controls than in treated animals (incidences were 31 % in the pH 3.5 adjusted drinking water control group versus 16 % for the test group receiving 0.01% methylhydrazine via tap water and 24 % in the group receiving the substance via drinking water adjusted to pH 3.5). The large difference between treated and control animals could be partly explained by the fact that only 17 control group animals were suitable for histologic examination out of 30 specimens used initially for all experimental groups. Nevertheless, these results are considered as negative. However, this second study was performed with hamsters 5 months of age at the start of treatment whereas the hamsters in the first study were 6 weeks (44 days) old. In the second study no significant differences in survival rates were detected between treated and control animals (see Table 5 below).

Table 5: Survival rates in methylhydrazine-treated and control Golden Syrian hamster

Weeks of Treatment	Percentage of Survivors		
	Control	Buffered MMH	Unbuffered MMH
10	100%	100%	100%
20	100	100	100
30	100	100	100
40	100	100	93
50	94	97	80
60	64	70	77
70	52	43	47
80	24	17	17
90	12	0*	3

*Two remaining survivors were moribund and were killed at 83 weeks.

Data on carcinogenicity tests with methylhydrazine by the inhalation route for 1 year (6 hours/day, 5 days/week, a number of different doses from 0 ppm (control group) up to 2.0 ppm or 5.0 ppm) in Fischer 344 rats, Golden Syrian hamsters, C57BL/6J mice and Beagle dogs have been also reported.

These inhalation exposure experiments with rats revealed no dose-effect relationships or clear differences between treated groups and controls either for male or female animals (Figures below). However, the exposure times can be considered too short to conclude with confidence on the absence of carcinogenic potential.

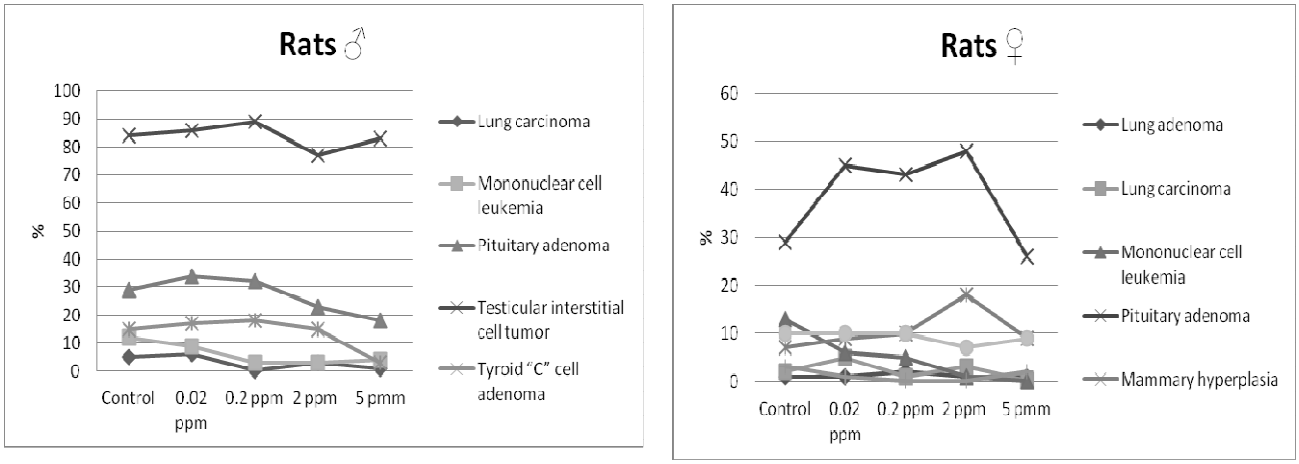


Figure: Neoplastic lesions found in male and female rats after one year inhalaltion exposure

Tests on Beagle dogs (4 animals per sex and dose) demonstrated similarly negative results as no methylhydrazine induced lesions were found in any of the exposed dogs. However, the number of tested dogs and exposure time could be considered too small and too short, respectively, to conclude with confidence on the absence of carcinogenic potential.

Treated male Golden Syrian hamsters showed quite mild carcinogenic effects presenting as nares adenomas and polyps as well as lung bronchogenic and alveolar adenomas (highest incidence 4-6 % for nares tumours at the highest doses, see Figures below). No clear dose-effect relationships

was shown for benign and malignant cortical adenomas. Again, the shorter exposure period (1 year instead of 2 years) needs to be considered.

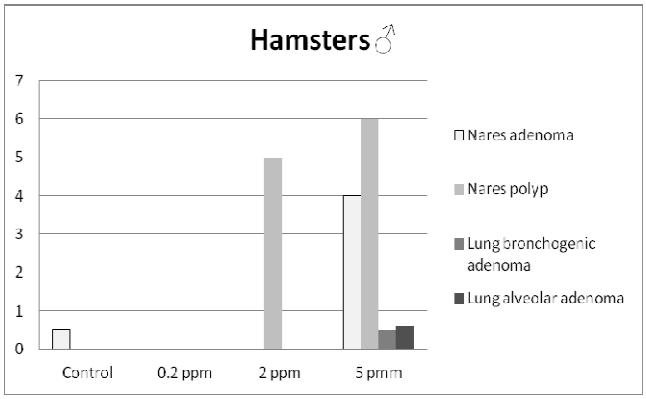


Figure:. Nares and lung neoplastic lesions found in male hamsters (incidence in %)

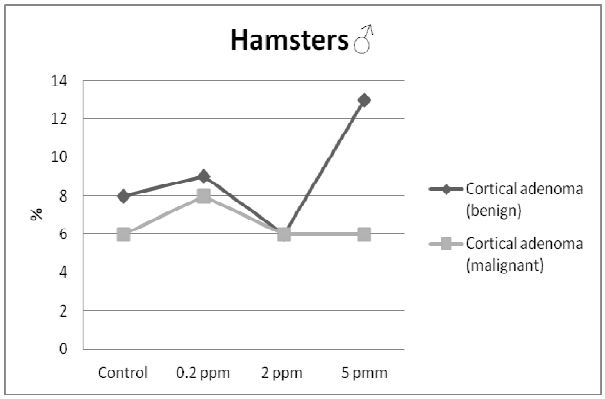


Figure: Cortical adenomas found in male hamsters (incidence in %)

In mice, mild carcinogenic effects or no clear dose-effect relationships were revealed for nasal mucosa adenomas, adenomatous polyps, osteomas and epithelial neoplasms as well as for duodenum adenomas, hemangiomas and hemangiosarcomas (see Figures below).

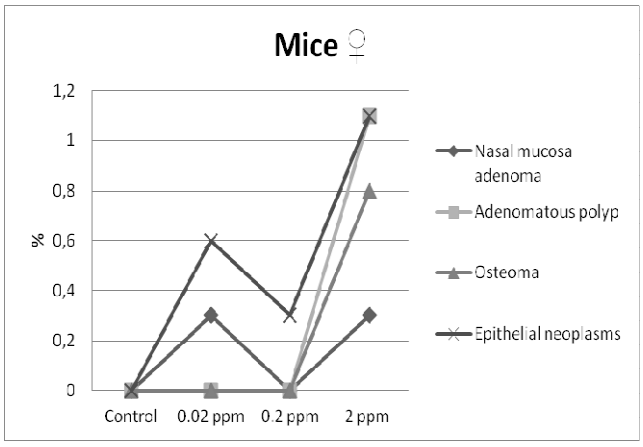


Figure: Nasal mucosa adenomas, adenomatous polyps, osteomas and epithelial neoplasms found in female mice (incidence in %)

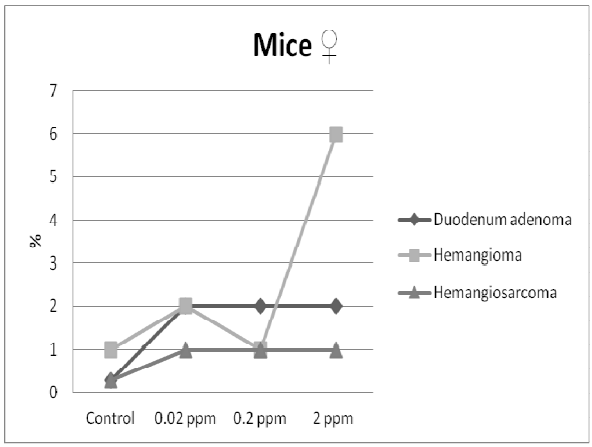


Figure: Duodenum adenomas, hemangiomas and hemangiosarcomas found in female mice (incidence in %)

In contrast, lung adenomas in mice showed a remarkable dose-effect relationship and a high incidence, expressed to a lesser extent in relation to liver carcinomas and lung carcinomas (Figure below). As regards liver adenomas, no clear dose-effect was demonstrated.

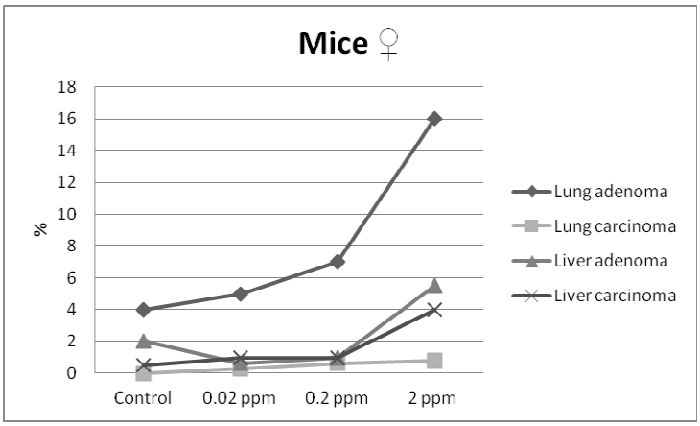


Figure: Lung and liver adenomas and carcinomas found in female mice (incidence in %)

The mode of action leading to the carcinogenicity of methylhydrazine is unknown, but mutagenic potential cannot be excluded. The mutagenicity data provided as support for the carcinogenicity classification were contradictory. Methylhydrazine showed no mutagenicity in *in vivo* inheritable germ cell mutagenicity tests in rats and mice, or in *in vitro* mutagenicity tests in mouse lymphoma cells and human diploid embryonic lung cells. In contrast, liquid incubation assays in *in vitro* bacterial systems (Ames test with liquid incubation assay) revealed mutagenic activity, which should be considered. However, there were no clear indications of mutagenic activity of methylhydrazine in any of the microbial assays which were conducted as standard plate tests in standard *Salmonella typhimurium* tester strains. In addition, the related substance 1,2-dimethylhydrazine is considered to be an alkylating agent with potential to induce large intestine tumours in rats following administration by gavage.

Repeated dose toxicity of methylhydrazine has been investigated in several species, including dogs, monkeys, rats and mice via inhalation or intraperitoneal administration. It has been found that methylhydrazine induces red cell damage, nephrotoxic changes, and hemoglobinuria in dogs, as well as pathological lesions in the liver and kidney in dogs and in the liver, kidney, and spleen in mice. Methylhydrazine did not induce histopathological lesions in rats and monkeys.

Comparison with the classification criteria

RAC concluded that since there are no human data with methylhydrazine available, classification in Category 1A can be excluded.

RAC considers that the studies with mice revealed pronounced carcinogenic effects. Lung adenomas were reported in one oral and one inhalation (one year exposure) long-term study, showing clear a dose-effect relationship; only mild effects for lung and liver carcinomas were seen in the inhalation study.

In hamsters, the studies reported were contradictory, with one study positive for malignant histiocytomas and tumours of the caecum and a second with negative results, although the test conditions were almost the same, with the exception of the age of animals at the start of exposure. The DS indicated in the additionally provided explanations that the first study with positive results should be considered more relevant as OECD TG 451 and 452 require testing to begin as soon as possible after weaning and acclimatisation and preferably before the animals are 8 weeks old. However, in the positive hamster study and in the Swiss mice mice study (treated via drinking

water for entire life span), a shortened survival time of the animals was detected in comparison to untreated control groups, possibly indicating that the MTD may have been exceeded , but clear evidence of that was not available.

Via the inhalation route, carcinogenic effects were found in hamsters, which presented as nares adenomas and polyps. For other effects in hamsters no clear dose – effect relationship was demonstrated. No carcinogenic effects were found in rats. Nevertheless, the inhalation studies showing negative outcomes or only mild effects should be considered to be not fully adequate for carcinogenicity testing due to the short exposure times employed.

Additionally, RAC concludes that mutagenic activity as the mode of action leading to the carcinogenicity of methylhydrazine cannot completely be excluded due to the potential for formation of methyl DNA adducts, which was indirectly demonstrated. Also in a "read-across" assessment, source hydrazines showed both mutagenic and carcinogenic properties

Finally, RAC concluded that based on the positive results in two species of animals (mouse and hamster) and several independent studies in one species, and taking also into account that mutagenic activity as the mode of action for carcinogenicity of methylhydrazine cannot be excluded, then classification is therefore warranted.

Based on the weight of evidence for carcinogenicity from the animal studies conducted with methylhydrazine, as well as evidence for mutagenicity of methylhydrazine and supported by data from source hydrazines used in a read-across assessment, showing both mutagenic and carcinogenic properties, RAC is of the opinion that there is sufficient evidence to classify **methylhydrazine in Category 1B (H350: May cause cancer)** according to the CLP criteria**.**

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

Annex 2b Comments received during the targeted public consultation on the additional information provided by the Dossier Submitter, response to comments provided by the Dossier Submitter and by RAC (excluding confidential information).