Committee for Risk Assessment

RAC

Annex 1

Background document

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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

11 September 2015

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: Methylhydrazine (MH)

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

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

Index Number:

Contact details for dossier submitter:

Bureau REACH, RIVM,

The Netherlands

bureau-reach@rivm.nl

Version number: 0.3 **Date: August 2014**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Hydrazine, methyl-***
EC number:	***200-471-4***
CAS number:	***60-34-4***
Annex VI Index number:	**-**
Degree of purity:	**-**
Impurities:	***confidential***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Carc. 1B - H350
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 1B - H350

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	None		None	Not evaluated
	Acute toxicity - dermal	None		None	Not evaluated
	Acute toxicity - inhalation	None		None	Not evaluated
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	None		None	Not evaluated
3.5.	Germ cell mutagenicity	None		None	Not evaluated
3.6.	Carcinogenicity	Carc. 1B; H350	None	None	
3.7.	Reproductive toxicity	None		None	Not evaluated
3.8.	Specific target organ toxicity –single exposure	None		None	Not evaluated
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not evaluated
3.10.	Aspiration hazard	None		None	Not evaluated

4.1.	Hazardous to the aquatic environment	None		None	Not evaluated
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Hazard statements: H350: May cause cancer
Precautionary statements: not harmonised

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

MH has not previously been assessed for harmonised classification by RAC or TC C&L.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal is based on the information as available in the registration dossiers of MH and the evaluation of the Health Council of the Netherlands (2012). MH has shown carcinogenicity in available animal experiments. Oral and inhalation exposure to MH is followed by an increased incidence of tumors (e.g. lung tumors, tumors of liver, tumors of cecum, nasal tumors, adenomas and adenomatous) and this effect has been observed in mice and hamsters (Toth B. 1972, Toth B. and Shimizu H. 1973). Oral administration of 0.01% MH over the entire lifespan led to development of: lung tumors with an incidence of 24% in female mice and 22% in male mice; liver tumors with an incidence of 32% in female hamsters and 54% in male hamsters; tumors of cecum in female hamsters with an incidence of 18% and 14% in males. Similar results have been seen in the lungs and livers in another 1-year MH inhalation study with hamster and mice. Incidence for nasal tumors was also significantly increased in MH-treated mice and hamsters following inhalation exposure. The association between MH exposure and cancer is considered as causal. Data from epidemiological studies on carcinogenicity are not available.

2.3 Current harmonised classification and labelling

MH has currently no harmonised classification (Annex VI, CLP Regulation).

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The self-classification as available from the C&L Inventory Database includes self-classification of a total of 167 notifiers for flammability, acute toxicity, skin irritation, skin corrosion, skin sensitisation, serious eye damage/eye irritation, respiratory sensitisation, specific target organ toxicity (single exposure), carcinogenicity and aquatic toxicity.

Self-classification for carcinogenicity was done by 164 notifiers. These notifications included 9 self-classifications for Carc. 1A, 132 self-classification for Carc. 1B and 23 self-classification for Carc. 2. A summary is provided in the table below.

Table 4: Summary of CLP self-classifications

Type of hazard	Hazard class	Number of notifiers classifying in the hazard class (percentage of total notification)
Physical hazards	Flam. Liq. 2 (H225)	165 (99%)
Human health hazards	Carc. 1A (H350)	9 (5%)
	Carc. 1B (H350)	132 (79%)
	Carc. 2 (H351)	23 (14%)
	Acute Tox. 1 (H330)	166 (99%)
	Acute Tox. 2 (H300)	164 (98%)
	Acute Tox. 2 (H301)	4 (2%)
	Acute Tox. 2 (H310)	142 (85%)
	Acute Tox. 2 (H311)	1 (1%)
	Acute Tox. 3 (H311)	26 (16%)
	STOT SE 1 (H370)	1 (1%)
	STOT SE 3 (H335)	9 (5%)
	Skin Irrit. 2 (H315)	9 (5%)
	Skin Corr. 1B (H314)	155 (93%
	Skin Sens. 1 (H317)	138 (83%)
	Eye Irrit. 2 (H319)	9 (5%)
	Eye Dam. 1 (H318)	95 (57%)
	Resp. Sens. 1 (H334)	44 (26%)
Environmental hazards	Aquatic Acute 1 (H400)	93 (56%)
	Aquatic Chronic 1 (H410)	99 (59%)
	Aquatic Chronic 2 (H411)	66 (40%)

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A substance with the classification of Carc. 1B; H350 is normally subject to harmonised classification (CLP article 36.1.b). MH is currently not classified according to Annex VI of CLP. However, based on the experimental animal data, a classification as Carc. 1B; H350 for the endpoint carcinogenicity is warranted to MH.

Repeated-dose toxicity and genotoxicity data of MH are also presented in this report as supportive information, as they may provide relevant data for the assessment of carcinogenicity of MH. However, the classification of MH regarding germ cell mutagenicity and repeated-dose toxicity is

not discussed in this report.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

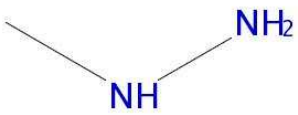
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	200-471-4
EC name:	Methylhydrazine
CAS number (EC inventory):	60-34-4
CAS number:	
CAS name:	Hydrazine, methyl-
IUPAC name:	Methylhydrazine
CLP Annex VI Index number:	
Molecular formula:	CH_6N_2
Molecular weight range:	46.0 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
methylhydrazine EC no.: 200-471-4		confidential	

Current Annex VI entry: no harmonized classification

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential (see IUCLID)			The impurities do not warrant classification.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No data concerning the additives of methylhydrazine are available				

1.2.1 Composition of test material

There is no information on the purity of the methylhydrazine that was used for the carcinogenicity studies.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Colorless liquid		
Melting/freezing point	Not applicable	According to column II of Annex VII, liquid is a waiver for the endpoint study record: Melting point.	
Boiling point	87.5°C	Merck, 2001	
Relative density	0.874	Merck, 2001	No unit
Vapour pressure	50 mm Hg	Boublik, 1984	Measured at 25°C
Surface tension	Not applicable	In accordance with column 2 of REACH Annex VII, the surface tension study does not need to be conducted as due to its chemical structure, no surface activity is predicted.	
Water solubility	>10% (HSDB) or 1 kg/L	Secondary literature experimental data: >10% (no details but peer-reviewed, K2, Handbook of Data on Organic Compounds cited by HSDB) Secondary literature experimental data: 1 kg/L (no details but peer-reviewed, K2, Merck index cited by EPIsuite) QSAR: 1 kg/L (reliable, K2, from log Kow) QSAR: 1 kg/L (reliable, K2, from fragments method)	For water solubility data were highly consistent: >10% (HSDB) or 1 kg/L (Merck and EPIsuite estimates, from log Kow and from fragments method). As a worst-case the highest one (leading to maximal exposure of aquatic organisms) should be retained for risk assessment. However 1 kg/L is not-realistic and probably rounded off: 1kg of liquid MMH alone already occupies more than one liter (due to the density), and addition of 1L of water cannot reduce the total volume to one liter. A more appropriate conclusion is that water solubility of MMH will never be a limiting factor for hazard or exposure.
Partition coefficient n-octanol/water	Log Kow = -1.00	QSAR: -1.00 (reliable, K2) (HSDB and EPIsuite database; Hansch C. *et al.*, 1995) Secondary literature experimental data: -1.05 (no details but peer-reviewed, K2)	Two octanol-water partition coefficients obtained by two different methods (QSAR, experiment) were very consistent: Log Kow of – 1.00 and -1.05 i.e. a difference of only 5% As a worst-case the highest one (leading to maximal exposure of fat tissues, organism, soil) is retained for risk assessment: Log Kow = -1.00
Flash point	-8°C	Anonymous study report 2010	Measured at 98.2 kPa
Flammability	Not applicable	study scientifically unjustified ; In accordance with section 1 of REACH Annex XI, the flammability	

		study does not need to be conducted as the flammability is deduced from flash point and boiling point.	
Explosive properties	Not applicable	Study scientifically unjustified; In accordance with column 2 of REACH Annex VII, explosive properties does not need to be investigated as the substance does not contain any chemical groups associated with explosion risk (chemical groups as described in ECHA Guidance R.7a, Table R.7.1-28).	
Self-ignition temperature	Not available		
Oxidising properties	Not applicable	In accordance with column 2 of REACH Annex VII, the oxidising properties study does not need to be conducted as the substance is incapable of reacting exothermically with combustible materials on the basis of the chemical structure.	
Granulometry	Not applicable	According to column II of Annex VII, this endpoint study record is a waiver for the form of this substance is liquid.	
Stability in organic solvents and identity of relevant degradation products	Not available		
Dissociation constant	Not available		
Viscosity	0.775 cP	Kirk-Othmer Encyclopedia of Chemical Technology. cited by HSDB	At 25°C

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this report

2.2 Identified uses

MH is manufactured in Europe and mainly used as a solvent, as an organic intermediate, and as a rocket propellant, either as a single constituent, or mixed with other hydrazines (Spacecraft maximum allowable concentrations for selected airborne contaminants (B5) (2002)). It is used as a solvent and as a chemical intermediate (REACH registration dossier, Health Council of the Netherlands 2002). PROCs: 1, 2, 3, 8b, 9, 15 and 16 were assigned by the registrants which indicates that MH is mainly used in closed systems. The amount of MH produced in the EU is approximately 100 – 1000 tonnes per annum for the full registration plus an unknown amount for the intermediate registrations.

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.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

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 ^{14}C-methylhydrazine at doses of 22 mg/kg (mice), 15 mg/kg (rats), and 10 mg/kg (monkeys and dogs). At 2, 4, 8 and 24 hours after exposure, representative samples of approximately 20 tissues from each animal were processed for ^{14}C assay using liquid scintillation counting techniques. Both blood and urine samples were simultaneously analysed by a chemical colorimetric method for unchanged MH, and the results were correlated with total ^{14}C content. Tissue distribution of ^{14}C showed the highest concentrations in liver, kidney, bladder, pancreas, and blood serum. Results of the ^{14}C assays indicated that the mouse, rat and monkey excreted twice as much as the dog in the first 2 hours. Among the tested species, peak tissue levels in dog and mouse were found at 4 hours; monkey showed its highest values at 2 hours post-exposure and in rat there was no apparent consistent pattern relative to time. 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. At 24 hours post-exposure, detectable amounts were still present, but with a clear decline over time (except in mice where a decline was less clear). MH was excreted via urine (26% in dogs, 31% in monkey, 40% in rat, 9% in mice). There was no explanation for the low values for mice. Approximately 50% of the total ^{14}C excretion, at all experimental times, was apparently unchanged MH as implied by the colorimetric results. Faeces and exhaled air were not monitored. 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.

In another study (Dost F.N. *et al.,* 1966), the respiratory and urinary excretion by rats of MH and its metabolites has been studied by means of radiotracer techniques. Rats given 0.12 m-mole ^{14}C-methylhydrazine /kg i.p. respired approximately 45% of the ^{14}C during the following 24 hr. Of the respired radioactivity, 20% to 25% was $^{14}CO_2$; the remainder was $^{14}CH_4$. At sub-convulsive doses, 40% administered radioactivity in ^{14}C-methylhydrazine was excreted in urine. The percentage of urinary excretion of ^{14}C from higher doses of ^{14}C-methylhydrazine was less, but the net amount excreted was slightly higher.

4.1.2 Human information

No relevant information is available.

4.1.3 Summary and discussion on toxicokinetics

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.

4.2 Acute toxicity

Not evaluated in this report

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this report

4.4 Irritation

Not evaluated in this report

4.5 Corrosivity

Not evaluated in this report

4.6 Sensitisation

Not evaluated in this report

4.7 Repeated dose toxicity

Table 10: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
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. Experiment I: 10 Macaca mulatta monkeys (5 males and 5 females) Ip injections of 5 mg/kg MH were given to 8 monkeys for 3 days, followed by 2.5 mg/kg for 20 days (group 1) or 2.5 mg/kg for 8 days plus 5 mg/kg for 12 days (group 2). Injections were given 5 days/week for 4 weeks. 2 animals were used as saline controls Experiment II: 5 male Macaque monkeys were used. 2 served as controls and only received saline injection. 3 received various intraperitoneal (i.p.) doses of MH (alternating 7 or 10 mg/kg daily until death). Blood samples were taken at 2 day intervals, 3 times per animal. The clinical laboratory measurements included complete blood count, serum glucose, alkaline phosphatase, and glutamic oxaloacetic transaminase. At the end of the exposures, necropsies were performed on all animals.	Preliminary study: ≥5 mg/kg: emesis on day 2 At 10 mg/kg: vomiting on day 3, convulsions on day 3-5, death on day 5. Experiment I: Group 1 and 2: vomiting on day 2 (all) and 3 (4:8), convulsions on day 3 (2:8). Group 1: emesis on day 19 and 24 (1:4) Group 2: vomiting on day 16, emesis on day 18 (3:4), 24 and 25 (1:4) Experiment II: death (on day 2, 3 and 4), preceded by convulsions. significant differences were found in the liver, with moderate fatty infiltration.	$LOEL_{monkey, injection}$ 5 mg/kg	Back KC and Pinkerton MK, (1967)
A series of 12 Beagle dogs received 15 mg/kg MH plus 200 mg pyridoxine HCL for 6 days via inhalation or injection (not specified in the study). The clinical evidence of renal damage was examined at 12 hours, 24 hours, 48 hours, 72 hours and 6 days post exposure.	12 and 24 hours: markedly swollen and deep purple-red kidneys with a somewhat greenish sheen. Hemoglobin casts and sometimes hemoglobin crystals in tubular lumina. Marked erythrophagocytosis by the Kupffer cells in the sinusoids of the liver. 48 hours: the kidneys are less swollen and hyperemic. Many proximal epithelial cells are necrotic and desquamating into the tubular lumen. The ingested red cells have mostly been broken down into hemosiderin and this	$LOEL_{dog, inhalation or injection}$=15 mg/kg	Sopher R.L. et al. (1968)

	process is essentially complete by 6 days. 72 hours: the kidneys are normal size and showed slight brownish pigmentation. Hyaline droplets were absent and desquamation of cells nearly absent. 6 days: the kidneys were virtually normal.		
20 monkeys macaca mulatta (10/sex) were exposed to MH intra-peritoneally. The left kidney of each monkey was transplanted to a subcutaneous pocket. Eight weeks after surgery, baseline renal function tests and a needle biopsy were performed. Six weeks after needle biopsy, monkeys were divided into 5 groups. G1 (controls): injected ip with saline for 14 days G 2: a single injection of 7.5 mg/kg MH G 3: 2.5 mg/kg MH daily for 14 days G 4: 5.0 mg/kg every other day for 14 days G 5: 5.0 mg/kg daily for 5, 7 or 10 days. Renal function tests were performed 24 hours after the final injection.	All animals exposed to MH lost their appetite and subsequently lost weight. Group 4: emesis after 3rd injection (2:4) Group 5: emesis after the third injection (all), which continued intermittently. All became weak and lethargic. Convulsions (2:4) on day 4 and 6. Hematuria and hemoglobinuria (1:4) All exposed groups: changes in the morphology of both proximal and distal tubule cells. These changes consisted primarily of cellular vacuolization, mitochondrial swelling with a loss of density in the mitochondrial matrix, and partial disappearance of cristae. Changes were most pronounced in Group 2.	$LOEL_{monkey, intraperitoneal}$=2.5 mg/kg	George ME, (1968)
6-month inhalation exposures were conducted on a 6-hour/day 5-day/week basis at air concentrations of 0.2, 1, 2 and 5 ppm MH in four experiments and were conducted on a basis of continuous exposure of 0.2 ppm to animals in another experiment. Each of the experimental animal groups, as well as the controls, consists of 8 beagle dogs, 4 rhesus monkeys, 50 Wistar rats and 40 ICR mice. All animals were female except for rats. The experimental animals were weighed biweekly during the studies and a series of 15 clinical chemistry and eight hematology tests was conducted on the same schedule. Bone marrow studies on dogs were	<u>Mice:</u> Increased mortality (15% and 27% at 2 and 5 ppm) <u>Rats:</u> Decreased body weight gain at ≥2 ppm. <u>Dogs:</u> All doses: increase in methemoglobin, decrease of red blood cell counts. Increased serum bilirubin and alkaline phosphatase levels, presence of Heinz bodies, decrease in M/E ratio with increasing erythropoietic activity <u>Monkeys:</u> All doses: decrease of red blood cell counts, presence of Heinz bodies	$LOEL_{dog, monkey, rat, mouse; inhalation}$= 0.2 ppm	MacEwen J.D. and Haun C.C. (1971)

also performed.			
Inhalation route Experiment I: Groups of 4 female monkeys, 8 female dogs, male rats and male mice were exposed to 5 ppm or 2 ppm MH for 6-month (6h/day, 5d/week). Microsections of lungs, hearts, livers, spleens, and kidneys were examined from all large animals and from 10 rats and 10 mice in each experimental group. Microsections of brains and endocrine glands were examined from monkeys and dogs. Experiment II: Groups of 4 male monkeys, 8 male dogs, 10 male rats and 10 female mice were exposed to each of the four species consisted of three exposure groups: (1) continuous 0.2 ppm MH for 6 months (2) intermittent 1 ppm MH for 144 days (6h/day, 5d/week) (3) intermittent 0.2 ppm MH for 145 days (6h/day, 5d/week).	Experiment I: monkeys and rats: no histo-pathological lesions observed. Dogs: ≥2 ppm: cholestasis, hepatic and renal tubular hemosiderosis. Mice: ≥2 ppm: periportal cholestasis, bile duct proliferation, and renal tubular and splenic hemosiderosis.5 ppm: centrilobular cholestasis, bile duct proliferation, and centrilobular hemosiderosis. Experiment II: monkeys and rats: no histo-pathological lesions observed dogs: all exposed groups: periportal intracanalicular cholestasis, moderate lymphoid hyperplasia. mice: hepatic, splenic and renal tubular hemosiderosis which is most severe for the continuous 0.2 ppm and intermittent 1 ppm conditions.	$NOEL_{monkey, rat; inhalation}$=5 ppm $LOEL_{dog, mice; inhalation}$= 0.2 ppm	Kroe, D.J. (1971)
Inhalation route Groups of 8 female beagle dogs, 4 female rhesus monkeys, and 80 male albino rats (Sprague-Dawley strain CFE) were continuously exposed for atmospheric concentrations of 0.1 and 0.04 ppm MH for 90 days	Rats: 0.1 ppm: significantly decreased body weight gain. ≥0.04 ppm: HCT, HGB, RBC significantly ↓ after 45 days but not 90 days. Dogs: 0.1 ppm: Significant increases in serum phosphorus and alkaline phosphatase levels. HCT, HGB, RBC significantly ↓. nutmeg appearance of livers consistent with passive congestion. Monkeys: no significant differences observed	One monkey in the 0.04 ppm exposure group died on the 10th day of exposure. At necropsy a preexisting condition of amyloidosis was observed. There was no evidence of any relationship of MH exposure to death, and the monkey was excluded from the experimental group. $NOEL_{dog,}$	Darmer K.I. and MacEwen J.D. (1973)

		$_{rat;inhalation}$ = 0.04 ppm NOEL $_{monkey, inhalation}$ = 0.1ppm	

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

No relevant information is available.

4.7.1.2 Repeated dose toxicity: inhalation

In the study of Sopher and co-workers (Sopher R.L. et al., 1968), MH (15 mg/kg) plus 200 mg pyridoxine HCL (to prevent the CNS effects caused by MH) were administrated for 6 days to 12 beagle dogs via inhalation or injection (not further specified in the study) The animals were clinically analysed after 12h, 24h, 48h, 72h and 6 days exposure. The first clinical evidence of renal damage is gross haematuria and hemoglobinuria at 12 to 24 post-exposure. This continues for about 14 hours and the tested dogs are somewhat oliguric for several days. At 12 hours post-exposure the kidneys are markedly swollen and are deep purple-red with a somewhat greenish sheen. At 48 hours the kidneys are less swollen and hyperemic. After 72 hours the kidneys are essentially normal sized and show only slight brownish pigmentation. At 6 days the kidneys are virtually normal. MH has primary nephrotoxicity. However, the mechanism of this nephrotoxicity is not known. The prominent erythorphagocytosis gives ample evidence to the fact that MH is hematotoxic.

The results in the experiments performed by MacEwen and co-workers (MacEwen J.D. and Haun C.C., 1971) in beagle dogs, rhesus monkeys, Wistar rats and ICR mice have shown that MH produces a dose-related haemolytic anaemia with Heinz body formation in dogs and monkeys after 6 month exposure. This was demonstrated by the dramatic changes observed in the dogs and monkeys exposed to 5 ppm and 2 ppm of MH on methemoglobin formation, red blood cell counts, haematocrit levels, serum bilirubin and alkaline phosphatase levels. The effects were greatest in dogs but also occurred in monkeys. The anaemia is reversible with removal from further exposure at least up to a level of 5 ppm intermittent exposure. It suggests that LOEL for MH in this study is 0.2 ppm. Rat growth was significantly depressed in the 2 and 5 ppm MH exposures. Deaths occurred in mice when exposed to 2 and 5 ppm MH. The mortality was 27% at 5 ppm and 15% for the 2 ppm MH exposure group. Mortality in mice at lower MH exposure concentrations was comparable to that of the control groups.

In another study (Kroe, D.J., 1971), the toxic effects of intermittent or continuous chronic exposure of monkeys, dogs, rats, and mice to lower levels of MH were investigated. This study demonstrated that continuous exposure of monkeys or rats at a concentration of 0.2, 1, 2 and 5 ppm MH did not induce histopathological lesions at the light microscopic level. The same exposure levels and exposure periods did induce pathological lesions in livers and kidneys of dogs and livers, kidneys, and spleens of mice. Mice showed hepatic, splenic, and renal tubular hemosiderosis under all conditions of exposure to MH, and the degree of hemosiderosis showed a dose-related pattern. Lymphoid hyperplasia was observed in some exposed dogs; however, the limited sampling precludes definitive interpretation of this observation.

After continuously exposure to atmospheric concentrations of 0.1 ppm and 0.04 PPM MH for 90 days, measureable effects have been observed in exposed groups of rats, dogs and monkeys

(Darmer K.I. and MacEwen J.D., 1973). Exposure to MH significantly decreased the growth of rats at the high dose. Rat haematology values (HCT, HGB, RBC) were slightly lower in both exposure groups in rats, suggesting some haemolytic effects. This change was statistically significant after 45 days but was not significant at 90 days of exposure. Dogs showed significant increases in serum phosphorus and alkaline phosphatase levels and significant haemolytic effects were noted only at the 0.1 ppm level. The red blood cells in the dogs exposed at 0.1 ppm MH level demonstrated increased osmotic fragility. No significant change occurred at 0.04 ppm level for this test. Gross pathologic changes were observed in dogs at 0.1 ppm level. The livers of the exposed dogs had a nutmeg appearance consistent with the passive congestion previously seen at higher dose levels. No gross pathology differences were observed in monkeys. Continuous MH exposure at an atmospheric concentration of 0.04 ppm did not significantly alter the haematology of the test animals (except in rats) and had no effect on rat growth.

4.7.1.3 Repeated dose toxicity: dermal

No relevant information is available.

4.7.1.4 Repeated dose toxicity: other routes

Back KC and Pinkerton MK (1967) investigated the toxicological effects of MH in Macaca-mulatta-monkeys. In the preliminary experiment (2 animals, receiving 5 or 10 mg/kg ip for 5 days), no symptoms were noted on day 1. On day 2, both animals displayed emesis at approximately 2 hours post injection. The monkey on 5 mg/kg showed no further symptoms on the remaining days. The monkey receiving 10 mg/kg vomited on day 3, convulsed on day 3-5 and died on day 5. Injections of 5 mg/kg MMH were given to 8 monkeys for 3 days. This was followed by administration of 2.5 mg/kg for 20 days, 2.5 mg/kg for 8 days followed by 5 mg/kg for 12 days. Injections were given 5 days/week for 4 weeks. All animals vomited on the first day. Several animals showed convulsions at the 2nd and 3rd day. At the end of the investigation, no significant differences were seen in serum glucose, serum glutamic-oxaloacetic-transaminase, or serum alkaline-phosphatase. No pathological alterations occurred in the organs of treated monkeys compared with untreated controls. In three monkeys given 7 to 10 mg/kg MH until death (at day 2, 3 and 4), significant differences were found in the liver, with moderate fatty infiltration.

The nephrotoxic effects of MH were studied in macaca-mulatta-monkeys via intraperitoneal administration (George M.E., 1968). The monkeys were divided in 5 groups: Group 1 (controls) was injected ip with saline for 14 days; Group 2 was exposed to a single injection of 7.5 mg/kg MMH; Group 3 to 2.5 mg/kg daily for 14 days; Group 4 to 5.0 mg/kg every other day for 14 days; and Group 5 to 5.0 mg/kg daily for 5 to 10 days. Renal function tests were performed 24 hours after the final injection. All animals exposed to MH lost their appetite and subsequently lost weight. All monkeys in Group 5 had emesis after the third injection, which continued intermittently, and all became weak and lethargic. Two out of 4 animals from group 5 showed convulsions (on day 4 and 6). There was no significant difference in renal function between controls and MH exposed animals. There were changes in the morphology of both proximal and distal tubule cells after MH exposure, consisting primarily of cellular vacuolization, mitochondrial swelling with a loss of density in the mitochondrial matrix, and partial disappearance of cristae. Changes were most pronounced in Group 2. In conclusion, there was no statistically significant change in the renal function tests in any group. However, examination of the renal biopsy samples revealed major changes in the subcellular morphology in all groups of monkeys following MH exposure.

4.7.1.5 Human information

No relevant information is available.

4.7.1.6 Other relevant information

No relevant information is available.

4.7.1.7 Summary and discussion of repeated dose toxicity

Repeated dose toxicity studies are presented as they may provide relevant data for assessment of carcinogenicity. Classification however is not discussed for this endpoint.

Repeated dose toxicity of MH has been investigated in several species such as dogs, monkeys, rats and mice via inhalation or intraperitoneal administration. It has been found that MH induces red cell damage, nephrotoxic changes, and hemoglobinuria in dogs despite prophylactic treatment with pyridoxine after 6 days exposure to 15 mg/kg MH via inhalation (George M.E., 1968). In two 6-month inhalation studies (MacEwen J.D. and Haun C.C., 1971, Kroe, D.J., 1971), MH showed toxicity by inducing pathological lesions in livers and kidneys of dogs and in livers, kidneys, and spleens of mice. Anaemia was also found in exposed dogs. However, MH did not induce histopathological lesions at the light microscopic level in rats and monkeys. In a 90 days inhalation study (Darmer K.I. and MacEwen J.D., 1973) in dogs, monkeys and rats, 0.04 ppm MH increased serum phosphorus and alkaline phosphatase levels in dogs and significant haemolytic effects were noted in dogs and rats at 0.1 ppm. Exposure to 0.1 ppm MH caused gross pathologic changes in dogs. No changes have been found for rat growth. But in other studies, it was found that rat growth is largely depressed by administration of MH (MacEwen J.D. and Haun C.C., 1971; Darmer K.I. and MacEwen J.D., 1973). The fact that the MH exposure conditions of these experiments induce histopathological changes in dogs and mice but less or not in monkeys and rats is most probably explained by species susceptibility to MH induced hemolysis and species capability for clearing the products of hemolysis. The repeated dose toxicity of MH has been also tested in monkeys by intraperitoneal administration in a 4-week study (Back K.C. and Pinkerton M.K., 1967) and a 14-days study (George M.E., 1968). No pathological alterations occurred in the organs of treated monkeys. However, in the monkeys given 7 to 10 mg/kg MH until death, significant differences were found in the liver, with moderate fatty infiltration. In addition, in two studies in monkeys, convulsions were observed after exposure to MH. There is an extremely narrow limit between a no effect and a lethal dose of MH in monkeys.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not evaluated in this report

4.9 Germ cell mutagenicity (Mutagenicity)

The results of available genotoxicity studies are summarized in Table 11 for *in vitro* prokaryotic test systems, Table 12 for *in vitro* eukaryotic test systems and Table 13 for *in vivo* genotoxicity tests with mammals.

Table 11: Summary table of relevant *in vitro* prokaryotic test systems

Type of test	Species	Method	Concentration	Remarks	Results	Reference
Bacterial, gene mutation	Salmonella typhimurium TA102	Ames test (with liquid incubation assay)	0, 0.5, 1, 2 µmole / plate Test solutions were prepared in distilled water.	No metabolic activation MH compared with hydrazine, 1,2-dimethylhydrazinium, 1, l-dimethylhydrazine	+	Poso A *et al* (1995)
Bacterial, gene mutation	Salmonella typhimurium TA102 and TA100	Ames test (with modified preculturing procedure)	Six doses to a maximum of 2 µmol/plate for TA100 and a maximum of 10 µmol/plate for TA102 MH was dissolved in sterilized water.	+/- S9 of rat livers microsomes or bovine serum albumin (BSA) Benzo[a]pyrene (pure grade) and 2-nitrofluorene (pure grade) used a positive controls for TA100 and 2-Aminoanthracene and bleomycin for TA102.	+ (- S9) TA100 and TA102 - (+ S9) TA100 and TA102 - (BSA) TA100	Matsushita H Jr *et al* (1993)
Bacterial, gene mutation	Salmonella typhimurium TA1535, TA1537	Ames test (revertants survivors was corrected for the percentage of surviving bacteria)	0, 100, 200, 500, 1000 µg/plate	+/- S9 of rat liver microsomes MNNG positive control for TA1535 without activation, DMN positive control for TA1535 with activation	+ (- S9) TA1535 - (- S9) TA1537 + (+ S9) TA1535 + (+ S9) TA1537	Rogan E *et al* (1982)
Bacterial, gene mutation	Salmonella typhimurium TA100	Ames test	0, 1, 2 and 3 µmol in aqueous solutions.	+/- S9 of mouse liver microsomes Aflatoxin B1as a positive control for the activity of S9.	- (- S9) - (+ S9)	von Wright A and Tikkanen L (1980b)
Bacterial, gene mutation	Salmonella typhimurium TA100, TA98	Ames test	Spot test: 0, 2.0, 5.0, 10.0 µmol/plate in aqueous	+/- S9 of mouse liver microsomes Hydrazine sulfate as a	Spot test (-S9): - TA 98 and TA 1950 + TA 100	von Wright A *et al* (1978)

	and TA1950		solutions. Plate test: 0, 10, 50, 100, 200 µg/plate in aqueous solutions.	positive control Aflatoxin B as a positive control for the activity of S9.	Plate test (TA 100): - (- S9) - (+ S9)	
Bacterial, gene mutation	Salmonella Typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100, G-46 and E. coli WP2 uvr A	Ames test Spot test/plate test and for TA1535 also suspension test	0, 0.0001, 0.001, 0.01, 0.1, 1 µL/plate (- S9) 0, 0.01, 0.1, 1 and 5 µL/plate (+ S9) Test compounds diluted in dimethylsulfoxide (DMSO). Suspension test (TA 1535 + S9): 1 and 5 µL/mL	+/- S9 of mouse liver microsomes Dimethylnitrosamine as a positive control for TA-1535.	- (- S9) - (+ S9) + TA1535 (+ S9)	Brusick D, Matheson DW (1976)
Bacterial, gene mutation	E. coli WP2 trpE56 with CM871	Repair test			repair deficient strain more sensitive to MH than corresponding repair-proficient strain	Poso A *et al* (1995)
Bacterial, gene mutation	Escherichia coli WP2 uvrA	Direct bacterial tests (spot test and 'treat and plate')	0, 0.5, 1 and 2 µmol in aqueous solutions		+	Von Wright A and Tikkanen L (1980b)
Bacterial, gene mutation	Escherichia coli WP2B/r trp with Escherichai coli WP2 uvrA, trp Escherichia coli CM871 uvrA, recA, lexA, trp	Modified spot test	0, 0.5, 1.0 and 2.0 µmol/plate	Hydrazine sulfate as a positive control	+ repair-proficient strain WP2 produced less revertants in spot tests than the repair-deficient strains WP2 *uvrA* or GM871 *uvrA, recA, lexA.*	Von Wright A and Tikkanen L (1980a)

Bacterial, gene mutation	Escherichia coli WP2B/r trp with Escherichai coli WP2 uvrA, trp and Escherichia coli CM871 uvrA, recA, lexA, trp	Liquid-incubation test ("treat and plate)	0, 0.5 and 1.0 µmol/ml	Hydrazine sulfate as a positive control	+	Von Wright A and Tikkanen L (1980a)
Bacterial, gene mutation	E. coli, WP2 try, hcr	Toxicity test	0, 10, 20, 30, 40 and 50 µg		+ (strongly bacteriocidic)	Von Wright A *et al* (1977)
Bacterial, gene mutation	E. coli W 3110 thy, polA with its $polA_1^+$ revertant and E. coli, WP2 try, hcr with E. coli B/r WP2 try	Repair test	0, 0.5 or 1.0 mg in aqueous solutions	positive controls, methyl methanesulphonate (MMS) for $polA_1$ and $poIA^+_1$ strains and	repair deficient strains more sensitive to MH than corresponding repair-proficient strains	Von Wright A *et al* (1977)
Bacterial, gene mutation	E. coli, WP2 try, hcr	Ames test Modification of the 'treat and plate'	0, 5, 10, 20 µg/ml in aqueous solutions	mitomycin C for hcr and hcr^+ strains	+	Von Wright A *et al* (1977)
Yeast, non-specific DNA damage	Saccharomyces cerevisiae D4	Equivalent or similar to OECD Guideline 481 (Genetic Toxicology: Saccharomyces cerevisiae, Mitotic Recombination Assay)	0, 0.0001, 0.001, 0.01, 0.1, 1 µL/plate (- S9) 0, 0.01, 0.1, 1 and 5 µL/plate (+ S9)	+/- S9 of mouse liver microsomes	- (- S9) - (+ S9)	Brusick D, Beng DW (1976)

Table 12: Summary table of relevant *in vitro* eukaryotic test systems

Type of test	Species	Method	Concentration	Remarks	Results	Reference
Mammalian Cells, DNA damage	Rat hepatocytes	Alkaline elution assay Fluorimetric analysis of DNA single strand brakes (SSBs)	0, 0.03, 0.3 and 3 mM Chemicals were dissolved in water. Those compounds not sufficiently soluble in water were dissolved in ethanol, dimethyl sulfoxide (DMSO), or acetone.		-	Sina JF (1983)
Mammalian Cells, forward mutations	Mouse lymphoma L5178Y cells	Forward mutation to ouabain , thymidine, thioguanine and cytosine arabinoside resistance	0, 0.1, 1, 2.5 and 5 mM	40% survival was the lowest acceptable survival rate	-	Rogers A and Back K (1981)
Mammalian Cells, forward gene mutation	Mouse lymphoma L5178Y cells	TK +/- assay	0, 0.0005, 0.001, 0.05 and 0.1 µL/mL in DSMO (- S9) 0, 0.001, 0.005, 0.01 and 0.05 µL/mL in DSMO (+ S9)	+/- S9 of mouse liver microsomes	- (- S9) - (+ S9)	Brusick D, Matheson DW (1976)
Mamalian cells, non-specific DNA damage	Human diploid embryonic lung WI-38 cells	Unscheduled DNA Synthesis Equivalent or similar to OECD Guideline 482	0, 0.1, 0.5 and 1.0 µg/mL - but top-dose sample was lost in the +S9 test	+/- S9 of mouse liver microsomes MNNG as a positive control for –S9 2AAF as a positive control for +S9	- (- S9) - (+ S9)	Brusick D, Matheson DW (1976)

Table 13: Summary table of relevant *in vivo* genotoxicity tests

Type of test	**Species**	**Method**	**Concentration**	**Remarks**	**Results**	**Reference**
Host-mediated assay	Male NMRI mice	Single dose via stomach intubation	0 and 33 mg/kg in aqueous solutions	TA 1950 was used as indicator strain	± (marginal mutagenic activity)	Von Wright and Tikkanen L (1980b)
Host-mediated assay	Male NMRI mice, 3/dose	Single dose via stomach intubation	0 and 0.7 mmol or 30 mg/kg in physiological saline	TA 1950 was used as indicator strain	-	Von Wright A *et al* (1978)
Dominant Lethal Assays, sperm genotoxicity	Random bred ICR mice	5 day i.p. injection	0, 2.6, 0.86, 0.26 mg/kg	low number of pregnant females in the low and intermediate dosage groups and in the negative control group. Triethylenemelamine (TEM) as a positive control. Negative control IP injection of corn oil or water solvents.	-	Brusick D, Matheson DW (1976)
Dominant lethal assays, sperm genotoxicity	Sprague Dawley rats	5 day i.p. injection	0, 2.15, 0.72, 0.215 mg/kg	Triethylenemelamine (TEM) as a positive control. Negative control IP injection of corn oil or water solvents.	-	Brusick D, Matheson DW (1976)

4.9.1 Non-human information

4.9.1.1 In vitro data

MH was mutagenic in the Salmonella typhimurium strain TA102 when tested without metabolic activation at concentrations between 0.5 and 2.0 µg/plate, and caused DNA lesions in the Escherichia coli DNA repair-assay (Poso A *et al*. 1995).

In an Ames test with deviations in growth periods, MH was mutagenic in TA 100 and TA 102 without S9. The mutagenicity of MH disappeared with S9 mix or BSA (Matsushita H Jr *et al*. 1993). Matsushita H Jr *et al*. (1993) observed that the mutagenicity of alkylhydrazines was best seen in the TA 102 after a 5h growth period followed by incubation with the presence of MH. The mutagenicity of MH in TA 102 decreased as the growth period increased.

MH was highly mutagenic in TA 1535 with activation, but marginally active in TA1537 (Rogan EG *et al*. 1982). MH was not mutagenic without activation. Rogan E *et al* (1982) determined the percentage of survival at each dose level, and revertants survivors were corrected for the percentage of surviving bacteria.

MH gave positive results in spot tests and "treat and plate" tests with Escherichia coli WP2 uvr A trp (Von Wright A and Tikkanen L, 1980b). MH was recA-independent mutagen to E. coli, which suggest that its mutagenicity might result from chemical modifications of DNA-bases, resulting in mistakes in pairing.

MH gave negative results in Ames tests in Salmonella typhimurium TA100 (Von Wright A and Tikkanen L, 1980b). The toxicity of MH made it impossible to use higher concentrations than 3 µmol/plate without causing bacterial growth inhibition.

Von Wright A and Tikkanen L (1980a) reported MH was mutagenic in Escherichia coli WP2B/r trp WP2 uvrA, trp and CM871 uvrA, uvrA recA, lexA.

In a spot test the TA 100 reverted to some extent with MH (Von Wright A *et al*. 1978). Neither TA 98 and TA 1950 reverted. In a plate-incorporation test the highest possible non-toxic amounts of MH were applied and these apparently were too small to cause a detectable increase of revertants.

MH was positive in revertant tests (Von Wright A *et al*. 1977). MH is toxic to bacteria, and in the liquid tests the mutagenicity of MH can be detected when strong bacteriocidic concentrations are applied and the test bacteria are concentrated 10 times after the treatment (Von Wright A *et al* 1977).

There were no clear indications of mutagenic activity by MH in any of the microbial assays if conducted as standard plate tests in standard Salmonella typhimurium tester strains reported by Brusick D and Matheson DW (1976). MH was negative in E. coli WP2 uvrA- and in the Saccharomyces cereuisiae strain D4 (Brusick D and Matheson DW, 1976). The toxicity of MH for bacteria and yeast was high and concentrations of 10 µL/plate were consistently too toxic to use. However, MH was positive in a suspension assay.

MH did not induce unscheduled DNA synthesis in WI-38 cells and proved negative in the BUdR-selective system of L5178Y mouse-lymphoma cells. Dominant lethality induced by MH was not demonstrated in rats or mice (Brusick D and Matheson DW, 1976). Treatment of L5178Y cells with MH (0.1 mM, 1mM, 2.5 and 5 mM) resulted in no significant mutation induction in any of the 4 selective systems (Rogers AM and Back KC, 1981). MH was toxic for L5178Y cells. A dose of 10 mM resulted in less than 40% of survival.

Negative results were obtained regarding DNA damage in isolated rat hepatocytes using DNA alkaline elution techniques (Sina JF *et al*. 1983).

4.9.1.2 In vivo data

In vivo, no dominant lethal mutations were induced in rats and mice given five daily intraperitoneal injections of up to 2.15 and 2.6 mg/kg bw, respectively (Brusick D, Matheson DW, 1976).

In a host-mediated assay MH showed marginal mutagenic activity (Von Wright A and Tikkanen L, 1980b). Although, in a previous study with a similar dose MH was negative in the host mediated assay (Von Wright A *et al*., 1978). In the negative host-mediated assay it was concluded that the number of bacteria in the peritoneal fluid was apparently too small, owing to the growth inhibition caused by MH, to allow the increase of the number of revertants to be detected (Von Wright A *et al* 1978). It was suggested that the marginally positive effect was most probably a result of the relatively large amounts of intact MH that can be detected in the peritoneal fluids of mice treated with this compound (Von Wright A and Tikkanen L, 1980b).

4.9.2 Human information

No human data available.

4.9.3 Other relevant information

QSAR (Quantitative Structure Activity Relationships) was used to develop a model to describe the genotoxic mechanism of MH, taking advantage of the results of previous mutagenicity studies. Energy of the lowest unoccupied molecular orbital together with octanol-water partition coefficient explains nearly completely the mutagenic activity of alkylated hydrazine compounds included in the analysis (Poso A *et al* 1995). The chemical nature of these DNA-lesions is (as detected in repair test), at present, unknown, but methylation of DNA-bases is an obvious possibility.

4.9.4 Summary and discussion of mutagenicity

Conflicting results were observed with respect to the mutagenicity of MH in different strains of Salmonella typhimurium. MH was reported to have a positive response in the Salmonella typhimurium strain TA 100 and TA 102 without metabolic activation (Poso A *et al* 1995; Matsushita H Jr *et al*. 1993) and in TA 1535 and TA 1537 with and without activation (Rogan EG *et al* 1982). MH was also positive in spot test with TA 100 and in a suspension assay with TA 1535 (Von Wright A *et al*. 1978; Brusick D and Matheson DW, 1976).

However, in other studies, no mutagenic effects of MH were reported in Salmonella typhimurium TA 98, TA100, TA 1535, TA 1537, TA 1538, TA 1950, with or without metabolic activation system (Von Wright A and Tikkanen L, 1980b; Brusick D and Matheson DW, 1976; Von Wright A *et al*., 1978).

There is evidence for mutagenic activity of MH in Escherichia coli strains. MH was positive in Escherichia coli pol A assay and weakly positive responses in Escherichia coli WP2 hcr- (Von Wright A and Tikkanen L, 1980b; Von Wright A and Tikkanen L, 1980a; Von Wright A *et al*., 1977). MH caused DNA lesions in the Escherichia coli DNA repair-assay (Poso A *et al*, 1995). However, MH was negative in E. coli WP2 uvrA- and in the Saccharomyces cereuisiae strain D4 (Brusick D and Matheson DW, 1976).

The conflicting results of MH in tests designed to measure mutagenic activity could be related to the strong bacteriocidic effects of MH. The toxicity of MH makes high concentrations impracticable in the plate-incorporation tests and so the negative results obtained with MH may simply reflect too small amounts of the test agent in the test system (Von Wright A *et al*., 1980b). Form the results it can be established that bacteria in liquid incubation assays are more sensitive to MH than in the standard plate assay (Rogan E *et al*., 1982). With that respect, MH resembles nitrosamines, which are only weak mutagens in plate-incorporation tests but highly mutagenic in liquid-incubation test with microsomes (Bruce NA *et al*., 1973). Nitrosamines need activation before becoming an alkylating agent. Poso et at (1995) suggested that based on the chemical nature of these DNA-lesions is (as detected in repair test), at present, unknown, but methylation of DNA-bases is an obvious possibility. Further, the ability to detect the mutagenicity of MH can be enhanced by inclusion of survival factors in calculation of mutation frequency (Von Wright A and Tikkanen L, 1978, Von Wright A *et al*., 1977).

Two *in vitro* L5178Y mouse lymphoma assays (Rogers A and Back K, 1981; Brusick D and Matheson DW, 1976) and two *in vitro* DNA damage and repair assays in respectively rat hepatocytes (Sina JF, 1983) and human diploid embryonic lung WI-38 cells (Brusick D and Matheson DW, 1976) were available. There were no indications of mutagenic activity by MH in any of the mammalian cell tests.

An *in vivo* dominant lethal test performed in male ICR mice and SD rats was available. MH was not considered genotoxic in this study (Brusick D and Matheson DW, 1976). In mice no significant effects were observed. In rats, a high ratio of death to total implants was observed in week 7, but this was associated with an absence of death implants in controls (Brusick D and Matheson DW, 1976).

MH has no or a weakly positive response in the host-mediated assay (Von Wright A *et al*., 1978; Von Wright A and Tikkanen L, 1980b). The marginally positive result obtained with MH in the host-mediated assay is most probably a result of the relatively large amounts of intact MH that can be detected in the peritoneal fluids of mice treated with MH (Von Wright A and Tikkanen L, 1980b). It is questionable whether the positive result is related to mutagenic activity or that the result is positive because of the growth inhibition caused by MH in conditions of the host-mediated assay.

There is some evidence for mutagenicity in liquid incubation assays in *in vitro* bacterial systems. However, as in most cases there is no data available of mutagenicity in the germ cells of humans. Further the results from *in vivo* inheritable germ cell mutagenicity tests in rats and mice were negative. Also the results from *in vitro* mutagenicity tests in mouse lymphoma cells and human diploid embryonic lung cells were negative. Conflicting results were obtained from host-mediated assays.

4.9.5 Comparison with criteria

The CLP criteria for classification in germ cell mutagenicity category 1 are as follows:

“Category 1: substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.

Category 1A: The classification is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.

Category 1B: The classification in is based on:

- positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people."

According to these criteria, a classification in germ cell mutagenicity category 1 is not warranted since there is no data available on MH of mutagenicity in the germ cells of humans.

The CLP criteria for classification in germ cell mutagenicity category 2 are as follows:

"Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

- somatic cell mutagenicity tests in vivo, in mammals; or
- other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."

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.

4.9.6 Conclusions on classification and labelling

Information regarding mutagenicity is displayed as supporting evidence for carcinogenicity. Classification is not discussed for this endpoint for MH.

4.10 Carcinogenicity

Table 14: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
MH was dissolved in water and was given once a week for 8 weeks to (BALB/c x DBA/2) F1 (CDF1) mice, either by gavage (0.2 mg in 0.2 ml) to females or by intraperitoneal injection (0.23 mg in 0.1 ml) to males. All mice were autopsied. The lungs were inspected for tumor modules, and the surface nodules per lung were counted. The gross observation of pulmonary tumors and leukemia was verified by microscopic examination of paraffin sections of lung, liver, thymus, spleen, kidney, lymph nodes, and other organs.	No increase in tumor incidence in the MH treated group has been observed compared to the controls.	Negative	Kelly et al. (1969).
Swiss randomly bred and C3H inbred mice MH dissolved in drinking water as a 0.01 % solution and was given continuously for the life span of 50 female and 50 male Swiss mice (5 and 6 weeks old at the start). The average daily MH dose per animal was 0.71 mg for females and 0.66 mg for males. The tumor incidences in MH-treated mice were examined.	MH shortened the survival in Swiss mice (50 % survival 30 weeks for male and ±45 weeks for female MH treated animals, compared to 80 weeks for male and 100 weeks for female controls). Lung adenomas: Females: incidence 24% Males, incidence 22% Control incidences not included The average latent period for tumors in both females and males was 51 weeks. Malignant lymphomas: Females: incidence of 4% (lymphocytic type), observed at 37/43 wk. Control incidences not included Other tumors: A few benign and malignant liver cell tumors, chloangiomas and cholangiocarcinomas were seen in both sexes. A number of other types of neoplasms were also found. However, incidences were not significantly different from controls.	Positive	Toth, B. (1972)
MH was dissolved in drinking water as a 0.01% solution and was given continuously for life to 50 female and 50 male Golden Syrian hamsters (6 weeks old at the start). The average daily intake of MH was 1.3	MH treatment reduced the survival of the hamsters compared to controls: 0% for MH treated animal against 7% female and 22% males in controls in week 100	Positive	Toth B and Shimizu H (1973)

mg for females and 1.1 mg for males. As a control, 100 females and 100 males were kept untreated. Complete necropsies were performed on all animals. All organs were examined macroscopically. Histological studies were done on the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testis, brain, nasal turbinate, and at least 4 lobes of the lungs. For electron microscopic examination, tumor tissues of 10 different animals were taken.	Malignant histiocytoma of liver: females: incidence 32%.Average latent period 70 weeks. Males: incidence 54%.Average latent period 78 wk. Incidence in male and female controls: 0% Tumors of cecum: Females: 9 hamster (18%) developed 13 lesions (7 with 9 polypoid adenomas, 1 with a polypoid adenoma and an adenocarcinoma and 1 with 2 adenocarcinomas). Average latent period 64 weeks. Males: 7 hamsters (14%) developed 9 tumors (5 animals with 6 polypoid adenomas, 1 with 1 polypoid adenoma and an adenocarcinoma, 1 with an adenocarcinoma). Average latent period 77 wk. Incidence in controls: 1% Other types of tumors also occurred but in low incidences not significantly different from controls.		
Three groups of Golden Syrian male hamsters (30 hamsters per group) were given: G1 (Control) – drinking water adjusted to pH 3.5 with HCl G2 – 0.1% MH in drinking water adjusted to pH 3.5 with HCl G3 – 0.01% MH in drinking water Treatment was daily for 2 years. For the first 11 months of the experiment, the nominal average daily dose of MH was 7.5 mg/kg bw/day for G2 and 7.3 mg/kg bw/day for G3. Animals were given a complete necropsy at termination of the study. Histologic examinations were performed on tissues from the lung, heart, oesophagus, trachea, thyroid, liver, spleen, kidney, urocyst and testes plus any lesions seen at necropsy.	Neither the incidence, degree of severity, nor age at onset of non-neoplastic pathologic changes was markedly different in animals drinking aqueous MH and control animals. Incidence of adrenocortical tumors: 23% in G1versus 4% in G3 and 12% in G2.	Negative	MacEwen JD, Vernot EH (1975)

Inhalation exposure to MH for 1 year (6 hours/day, 5 days/week). Fischer 344 rats (CDF[F344]/CrlBR), 100/sex/dose, 150/sex/dose for control group Exposure concentrations: 0, 0.02, 0.2, 2.0 and 5.0 ppm Golden Syrian hamsters (Lak:LVG[SYR]), 200 males/dose Exposure concentrations: 0, 0.2, 2.0 and 5.0 ppm C57BL/6J mice400 females/dose Exposure concentrations: 0, 0.02, 0.2 and 2.0 ppm beagle dogs, 4/sex/dose Exposure concentrations: 0, 0.2 and 2.0 ppm	Rats: The overall tumor incidence (both benign and malignant) was comparable in all groups of rats. Hamsters: ≥0.2 ppm: increased incidence of submucosal cysts (29, 31 and 26% vs 18% in controls) and rhinitis (12, 14 and 16% vs 6% in controls) in nasal cavity ≥2 ppm: increased incidence polyps in nasal cavity(5 and 6 % vs 0% in controls) ≥2 ppm: significant increased incidence of focal collapse of the lung (3 and 4% vs 0% in controls) 5 ppm: increased incidence adenomas in nasal cavity (4% vs 0.5% in controls) Mice: ≥ 0.2 ppm MH: marked, dose dependent increases in lung adenomas, significant at 2 ppm 2 ppm: Adenomas and adenomatous polyps were seen in the nasal mucosa of a few mice at highest MMH exposure levels. 2 ppm: A small number of unusual neoplasms (osteomas) were observed in nasal tissue. Dogs: no MH induced lesions	Positive in hamsters and mice Negative in rats and dogs	Kinkead, E.R. et al. (1985)

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

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. Kelly reported that oral administration aqueous solutions of MH at a total dose of 3.7 mg/mouse (0.46 mg/ administration, 1x/week, for 8 weeks) to female CDF1 mice, and i.p. administration of total dose of 1.8 mg/mouse (0.23 mg/ injection, 1x/week, for 8 weeks) in male mice of the same strain produced no more lung adenomas or leukemias than were found in untreated controls after 8 weeks of treatment. The incidence of pulmonary tumors and leukemia in the controls and the MH treated mice is summarized in Table 15 below.

Table 15: Carcinogenic activity (leukemia and lung adenomas) of MH in CDF1 mice

Group	Total dose (mg/mouse)	Route	Schedules	Pulmonary tumors				Leukemia	
				no. of mice with tumors/ total no. of mice	%	Mean nodule count	Median latent period in weeks & (range)	No. of mice with leukemia	Median latent period (week)
Saline controls	(0.2 ml)	Oral	1/wk x 8	1/10	10	0.1	33 (33)	0	-
	(0.1ml)	Intraperitoneal	1/wk x 8	1/9	11	0.1	32 (32)	0	-
MH	3.7	Oral	1/wk x 8	0/9	0	-	-	0	-
	1.8	Intraperitoneal	1/wk x 8	3/30	10	0.1	33 (33)	0	-

A solution 0.01 % MH was given daily in drinking water to 5- and 6- week old randomly bred Swiss mice for their entire lifetimes in the study of Toth (Toth B 1972). In the MH-treated animals, 12 females developed 17 lung tumors (adenomas) with an incidence of 24%. The average latent period for these tumors was 51 weeks, the first was found at the 36th week and the last at the 67th week of age. In the males of this group, 11 animals developed 12 lung tumors (adenomas) with an incidence of 22%. The average latent period for tumor was 51 weeks, the first was observed at the 39th week and the last at the 70th week. Only two malignant lymphomas (lymphocytic type) with an incidence of 4% were seen in the females. They were observed at the 37th and 43rd weeks of age. In addition, a few benign and malignant liver cell tumors, cholangiomas and cholangiocarcinomas and a number of other types of neoplasms were seen in both sexes. The survival and tumor incidences in MH-treated Swiss mice are presented in the tables below (Table 16 and Table 17). Control incidences and latencies of lung adenomas and malignant lymphomas are not presented in the publication, but were reported in Toth B 1969, where Swiss mice were treated with hydrazine sulphate (it is unclear whether this control group represents a concurrent control or a historic control). Seen the increased incidence and the reduced latency period, the increase in lung adenomas is considered evidence for the carcinogenicity of MH.

Table 16: survival rates in MH-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 17: Tumor distribution in MH-treated and control Swiss mice

Group	No. and sex	Lung adenomas		Malignant lymphomas		Other tumors (Latent period in wk)
		Incidence	Average latent period in weeks	Incidence	Latent period in weeks	
MH-treated	50 ♀	24%	51 (36-67)	4%	37, 43	1 cholangiocarcinoma (49) 1 angioma of adrenal (61) 3 hepatomas (48, 51, 61) 6 choloangiomas (35, 47, 48, 51, 53, 62) 4 angiomas of liver (43, 47, 48, 55) 2 angiosarcomas of liver (48, 60)
	50 ♂	22%	51 (39-70)	-	-	2 cholangiomas (49, 52) 1 cholangiocarcinoma (45) 3 hepatomas (59, 66, 70) 1 angioma of liver (66) 1 angiosarcoma of liver (70) 1 liver cell carcinoma (67)
Control *	110 ♀	12.7%	90 (64-119)	15%	39-115	1 luteoma (99) 1 granulosa cell tumor (65) 1 hemangioma of ovary (42) 1 subcutaneous fibroma (87) 1 papilloma of forestomach (112) 1 malignant plasmacytoma (71) 3 subcutaneous sarcomas (68, 82, 82) 3 hemangiomas of liver (69, 77, 84) 1 sex cord mesenchymal tumor (99)
	110 ♂	10.0%	74 (47-110)	2%	73, 82	2 hemangiomas of liver (72, 80)

* As presented in Toth B 1969.

In another study, Toth and coworkers (1973) showed that malignant histiocytomas (Kupffer cell sarcomas) were observed in the livers of 32% of female and 54% of the male Golden Syrian hamsters received 0.01% MH in drinking water ad libitum for life, while such tumors were not observed in the control groups. The incidence of tumors of cecum was 18% in females and 14% in males compared to 1% in the controls. The tumor distribution in MH-treated and control hamsters is presented in Table 18 below. MH also shortened the survival period of the hamsters (Table 19).

Table 18: Tumor distribution in MH-treated and controls hamsters

Group	Effective no. and sex	Animals with						
		Malignant histiocytomas			Tumors of cecum			Other tumors
		No.	%	Latent periods (age in wk)	No.	%	Latent periods (age in wk)	
MH-treated	49 ♀	16	32	70 (46-92)	9 (7 with 9 polypoid adenomas 1 with a polypoid adenoma and an adenocarcinoma and 1 with 2 adenocarcinomas)	18 14 2 2	64 (50-76)	3 polypoid adenomas of colon (54, 70, 82) 2 dermal melanocytomas (68, 76) 2 angiosarcomas of liver (72, 92) 2 leiomyosarcomas of uterus (76, 80) 1 cholangioma (76) 1 hepatoma (70) 1 angiosarcom of lung and heart (41) 1 adenoma of parathyroid (63) 1 angioma of liver (70) 1 carcinoma of forestomach (46) 1 adenocarcinoma of sebaceous gland (76) 1 malignant schwannoma (64) 1 angioma of fat and muscle (40)
	50 ♂	27	54	78 (47-103)	7 (5 anomals with 6 polypoid adenomas, 1 with 1 polypoid adenoma and an adenocarcinoma, 1 with an adenocarcinoma).	14	77 (64-94)	6 papillomas of forestomach (51, 67, 76, 90, 103) 2 adenocarcinomas of glandular stomach (51, 76) 2 leiomyosarcomas of glandular stomach (76, 80) 2 adrenal cortical carcinomas (81, 83) 1 angioma of spleen (65) 1 polypoid adenoma of colon (90) 1 carcinoma of salivary gland (100)

								1 adrenal cortical adenoma (78) 1 anitschkow cell sarcoma of heart (63) 1 squamous cell carcinoma of nasal cavity (47)
Control	99 ♀	0	0		1	1	53	7 malignant lymphomas (74, 79, 81, 93, 94, 99, 110) 3 adrenal cortical carcinomas (79, 94, 110) 3 leiomyosarcomas of uterus (35, 92, 100) 3 dermal melanocytomas (57, 66, 73) 2 papillomas of forestomach (80, 92) 1 adenocarcinoma of uterus (115) 1 adrenal cortical adenoma (100) 1 adenocarcinoma of ovary (80) 1 adenoma of Langerhans islands (99) 1 adenocarcinoma of kidney (64) 1 adenoma of thyroid (84) 1 sarcoma, s.c. (74)
	97 ♂	0	0		1	1	84	7 adrenal cortical carcinomas (80, 101, 111, 114, 121, 126) 6 papillomas of forestomach (66, 81, 89, 121, 124) 4 malignant lymphomas (73, 89, 90, 98) 3 adrenal cortical adenomas (74, 101, 123) 1 dermal melanocytoma (116) 1 carcinoma of forestomach (82) 1 papilloma of gallbladder (123) 1 leiomyosarcoma, abdominal (81)

								1 hepatoma (82)

Table 19 Survival rate in MH-treated and control golden 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	

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. The mean weight of hamsters receiving the unbuffered MH solution paralleled the control group mean body weight until the 15th month when weight losses occurred. The group of hamsters receiving the buffered MH solution had significantly lower mean body weights than the control group throughout the study after 3rd month of treatment. After 11th month of the study, all groups exhibited a gradual but steady loss of weight. Predominately adrenocortical tumors were found: incidences were 23% in control animals versus 4% in the hamsters treated with MH in tap water and 12% in the groups treated with MH in pH 3.5 water. This can be due to the small numbers of control animals that were suitable for histologic examination. In addition, a few neoplasms were observed only in the experimental groups, with an incidence of 1-2 animals. Table 20 lists the number and types of neoplasms found in this study. The overall tumor incidence for the group administrated with MH in tap water was 16%, with MH in pH 3.5 water was 24% and control was 31%. These findings are in contrast to the findings of Toth and Shimizu (1973).

Table 20: Neoplasms found in hamsters receiving 0.01% MH in drinking water

Group	**Effective no. of animals**	**Neoplasms**	
		Total number of tumors	**Type of tumors**
pH 3.5 water (Control)	17	4	a) Adenoma, adrenal cortex b) Adenoma, adrenal cortex (left adrenal) Carcinoma, adrenal cortex (right adrenal) c) Carcinoma, adrenal cortex
MH in tap water	30	4	a) Carcinoma, adrenal cortex, metastatic to lung b) Hemangioendothelioma of liver c) Hepatocellular carcinoma d) Hepatocellular carcinoma
MH in pH 3.5 water	30	6	a) Carcinoma, adrenal cortex b) Carcinoma, adrenal cortex, metastatic to lung c) Carcinoma, adrenal cortex, bilateral d) Histiocytoma, skin of thorax e) Melanoma, skin of ear

4.10.1.2 Carcinogenicity: inhalation

A 1-year inhalation study was undertaken to determine oncogenic effects of MH in rats, hamsters, mice and dogs (Kinkead E.R. 1985). MH exposure caused a dose related depression of growth rate in male rats (particularly at 5 ppm exposure concentration). Mean body weights of female rats fluctuated more than those of males, but the weights of the two highest exposure concentration groups remained significantly below the control group. The mean weight of the 5 ppm MH

exposure groups of hamsters showed a definite depression compared to the controls which were able to gain weight and finally overtake the control group during the postexposure phase of the study. The red blood cell count, hemoglobin and hematocrit values were depressed in exposed dog groups. There were no adverse MH exposure-related lesions in either male or female rats (Table 21). But as frequently happens with stressed rodents, there were dose related decreases in the incidence of leukemia and in pituitary adenomas at the highest dose. The presence of nasal tumors (adenomas and polyps) in the hamsters exposed to the higher levels is significant (Table 22).

Table 21: Neoplastic lesions found in rats (incidence ratio and percentages)

Sex	**Type of tumor**	**Controls**		**0.02 ppm MH**		**0.2 ppm MH**		**2 ppm MH**		**5 ppm MH**	
		Incidence ratio	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**
Male	Lung carcinoma	7/150	4.7	6/100	6	0/100	0	3/99	3	1/99	1
	Mononuclear cell leukemia	18/150	12	9/100	9	3/100 [b]	3	3/99 [b]	3	4/99 [b]	4
	Pituitary adenoma	44/150	29	34/100	34	32/100	32	23/99	23	18/99	18
	Testicular interstitial cell tumor	125/149	84	86/100 [a]	86	89/100 [a]	89	73/95	77	80/96	83
	Tyroid “C” cell adenoma	22/150	15	17/100	17	18/100	18	15/99	15	3/99 [a]	3
Female	Lung adenoma	1/149	1	1/99	1	2/100	2	1/99	1	1/99	1
	Lung carcinoma	3/149	2	5/99	5	1/100	1	3/99	3	0/99	0
	Mononuclear cell leukemia	19/149	13	6/99	6	5/100 [b]	5	1/99 [a]	1	0/99 [a]	0
	Pituitary adenoma	43/149	29	45/99 [a]	45	43/100 [a]	43	48/99 [a]	48	26/99	26
	Mammary hyperplasia	10/149	7	9/99	9	10/100	10	18/99	18	9/99	9
	Mammary adenoma	15/149	10	10/99	10	10/100	10	9/99	7	9/99	9
	Mammary adenocarcinoma	5/149	3	1/99	1	0/100	0	0/99	0	2/99	2

[a] Different from controls, $p<0.05$

[b] Different from controls, $p<0.01$

Table 22: Lesions observed in hamsters (males) following the inhalation of MH vapor (incidence ratio and percentages)

Organ	**Type of tumor**	**Controls**		**0.2 ppm MH**		**2 ppm MH**		**5 ppm MH**	
		Incidence ratio	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**
Nares	adenoma	1/190	0.5	0/177	0	0/180	0	7/177 [a]	4

	polyp	0/190	0	0/177	0	9/180 [b]	5	11/177 [b]	6
Lung	Bronchogenic adenoma	0/189	0	0/177	0	0/174	0	1/174	0.5
	Alveolar adenoma	0/189	0	0/177	0	0/174	0	1/174	0.6
Adrenals	Cortical adenoma (benign)	16/191	8	16/173	9	10/172	6	23/176 [b]	13
	Cortical adenoma (malignant)	11/191	6	14/173	8	11/172	6	10/176	6

[a] Different from controls, $p<0.05$

[b] Different from controls, $p<0.01$

In mice, there were significant increases in irritation of nasal cavity such as nasal inflammation, plasmacytosis, and hemorrhage in the mandibular lymph nodes. A number of changes were seen in the liver with marked increases in incidence of cysts, bile duct hyperplasia, hepatocellular pleomorphism and gallbladder crystals in the high exposure group. Statistically significant increases in angiectasis were also seen in the highest MH exposure group of mice. Neoplastic lesions found in mice are presented in Table 23. Adenomas and adenomatous polyps were found in the nasal mucosa of a few mice at the highest MH exposure level. Although the numbers are not large, they are considered significant since none were found in the controls. Statistically significant increases in liver adenomas and carcinomas were also seen in mice exposed to 2 ppm MH and parallel pleomorphic changes were seen in hepatocytes with a significant increase at the highest dose level. Neoplastic vascular lesions (hemangiomas) were markedly increased in the high exposure level.

Table 23: Neoplastic lesions found in mice (females) following inhalation of MH vapor (incidence ratio and percentages)

Organ	**Type of tumor**	**Controls**		**0.02 ppm MH**		**0.2 ppm MH**		**2 ppm MH**	
		Incidence ratio	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**	**Incidence ratio**	**%**
Nasal mucosa	Adenoma	0/367	0	1/354	0.3	0/349	0	1/355	0.3
	Adenomatous polyp	0/367	0	0/354	0	0/349	0	4/355	1.1
	Osteoma	0/367	0	0/354	0	0/349	0	3/355	0.8
	Epithelial neoplasms (nasal and respiratory mucosa)	0/367	0	2/354	0.6	1/349	0.3	4/355	1.1
Lung	Adenoma	13/364	4	16/354	5	23/347	7	56/360 [b]	16
	Carcinoma	0/364	0	1/354	0.3	2/347	0.6	3/360	0.8
Liver	Adenoma	6/373	2	2/357	0.6	5/357	1	20/363 [b]	5.5
	Carcinoma	2/373	0.5	4/357	1	4/357	1	14/363 [b]	4

Duodenum adenoma	1/310	0.3	5/303	2	7/309 [a]	2	5/308	2
Hemangioma	5/387	1	9/371	2	5/368	1	22/371 [b]	6
Hemangiosarcoma	1/387	0.3	4/371	1	4/368	1	5/371	1

[a] Different from controls, p<0.05

[b] Different from controls, p<0.01

No MH induced lesions were found in any of the MH exposed dogs.

4.10.1.3 Carcinogenicity: dermal

No relevant information is available.

4.10.2 Human information

No relevant information is available.

4.10.3 Other relevant information

NIOSH considers MH to be a potential occupational carcinogen as defined by the OSHA carcinogen policy [29 CFR 1990] and therefore exposure should be minimized to the lowest feasible level. The NIOSH recommended exposure limit (REL) is 0.04 ppm (0.08 mg/m3) as a ceiling concentration determined over any 120- min sampling period (NIOSH-Documentation for IDLHs-Methyl hydrazine, 1994).

MH (and its salts) are considered as A3 carcinogens by ACGIH (ACGIH-Threshold Limit Values for Chemicals Substances and Physical Agents and Biological Exposure Indices, 2008) and were listed on July 1, 1992 as chemicals known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 et seq.)

In 2002, at request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluated the carcinogenic properties of MH and proposed a classification with reference to the EU-directive (DECOS; Health Council of the Netherlands 07: 24, 2002). It was concluded that MH should be considered as carcinogenic to humans (comparable with EU-category 2). Although no data on humans were available, there was sufficient evidence for the carcinogenicity of MH in experimental animals. Inhalation of MH induced benign and malignant tumors in mice and hamsters and oral (drinking water) exposure caused benign tumors in mice and malignant tumors in hamsters in one experiment. No tumors were found in rats and dogs following inhalation, but the exposure time in rats may have been too short; that is 1 year instead of 2 years as recommended in OECD guideline 451. The evaluation committee was of the opinion that MH should be considered as carcinogenic to humans.

4.10.4 Summary and discussion of carcinogenicity

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. However, the

available evidence has clearly showed that exposure to MH causes increase of tumor incidence in animals. A overview on the available studies and results is given in Table 11.

Toth (Toth B., 1972) has clearly demonstrated that daily administration of 0.01% MH via drinking water largely increased the incidence of tumors (such as lung tumors, malignant lymphomas etc.) in Swiss mice. In a later study in Golden Syrian hamsters (Toth B and Shimiza H 1973), MH was also found to increase the incidence of liver tumors and tumors of cecum dramatically and induce other types of tumors in low incidence. However, this result could not be repeated in a comparable study in male Golden Syrian Hamsters (MacEwen and Vernot, 1975). In another study (Kinkead ER 1985), the carcinogenicity of MH was tested in rats, hamsters, mice and dogs. Significant oncogenic changes were noted in the respiratory, hepatic, and vascular systems of mice and hamsters, but not in rats and dogs. However, as only four dogs per dose and sex were exposed, the number of tested dogs is considered too small to conclude the absence of a carcinogenic potential. Further, testing was limited to one year for all species. These findings also indicated the variation in sensitivity of different animal species to MH. 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.

Nevertheless, contradictive results have been also observed in some other studies. Kelly and co-workers have found that MH administrated by gavage or intraperitoneal injection did not increase the incidence of tumors in mice (Kelly et al. 1969). These contradictive findings can be due to the different mice strain and different exposure routes used. Also the limited exposure period of 8 weeks may have influenced the results.

The mechanisms through which MH elicits carcinogenicity is still unknown. However, the reported investigations above present evidence that MH is carcinogenic. Since there are no reasons to conclude that the effects observed in the animal studies are not relevant to humans, it is concluded that MH may also pose a hazard to humans.

4.10.5 Comparison with criteria

The CLP criteria for classification in Carc. 1 are as follows:

“Known or presumed human carcinogens

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

Category 1B: Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be

derived from:

- *human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or*
- *animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies*

showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals."

In the CLP, sufficient evidence of carcinogenicity is defined as when "*a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;"*

Limited evidence of carcinogenicity is defined as when "*the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."*

According to these criteria, a classification in Carc. Cat. 1A is not warranted since there is no human data (epidemiological studies) available on carcinogenicity endpoint for MH.

However, 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 2 animal species (mice and hamsters) and in males as well as females. Although some negative results were found in other studies on carcinogenicity of MH, these negative results may be generated by differences in animal strains or exposure level and/or duration. According to the dossier submitter, classification Carc. 1B –H350 is therefore warranted. As no data are available by dermal route, it is proposed not to specify route of exposure in the hazard statement.

The CLP criteria for classification in Carc. 2 are as follows:

"Suspected human carcinogens

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either rom limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."

Classification as Carc. 2 is not appropriate as the available animal studies showed sufficient evidence that exposure to MH can increase the incidence of tumors in several organs in mice and hamsters.

4.10.6 Conclusions on classification and labelling

Based on the increased incidence of various tumors in mice and hamsters exposed to MH via drinking water or inhalation for entire lifespan or one year, a classification as Carc. 1B – H350: May cause cancer is proposed for MH, with no specific route of exposure added.

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.

Additional key elements

One MSCA pointed out an error in the 8-week study on mice. This error occurred in the summary in Table 14 of the CLH report with respect to the study by McEwan and Vernot (1975). In their response, the DS explained that the study included three dose groups, namely, G1 - control: drinking water pH=3.5, G2: 0.01% methylhydrazine in drinking water pH=3.5 and G3: 0.01% methylhydrazine in (not pH adjusted) drinking water. The value of 0.1% in Table 14 of the CLH report should be 0.01% for both experimental test groups.

The DS provided additional information on methylhydrazine classifications suggested or adopted in some countries. The USA National Institute for Occupational Safety and Health (NIOSH) has considered methylhydrazine to be a potential occupational carcinogen as defined by the Occupational Safety & Health Administration (OSHA) carcinogen policy [29 CFR 1990 (USA)] and therefore exposure should be minimised to the lowest feasible level. The NIOSH recommended exposure limit (REL) is 0.04 ppm (0.08 mg/m^3) as a ceiling concentration determined over any 120 min sampling period (NIOSH-Documentation for IDLHs-Methyl hydrazine, 1994).

The American Conference of Governmental Industrial Hygienists (ACGIH) considered methylhydrazine and its salts as A3 carcinogens (ACGIH-Threshold Limit Values for Chemicals Substances and Physical Agents and Biological Exposure Indices, 2008).

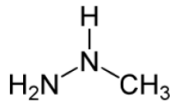
In 2002, at the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluated the carcinogenic properties of methylhydrazine and concluded that it should be considered as carcinogenic to humans (comparable to EU-category 2 according to the now superseded Dangerous Substances Directive).

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

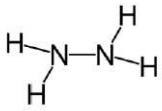
Read-across information on structurally related hydrazine compounds similar to methylhydrazine was provided by the dossier submitter (DS). The DS chose the following substances: hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine based on structures having the same central N-N moiety and either -H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

Selected hydrazine compounds:

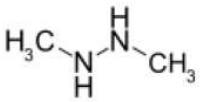
Methylhydrazine
(CAS 60-34-4, EC 200-471-4)



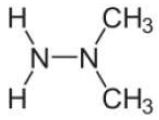
Hydrazine
(CAS 302-01-2, EC 206-114-9)



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.

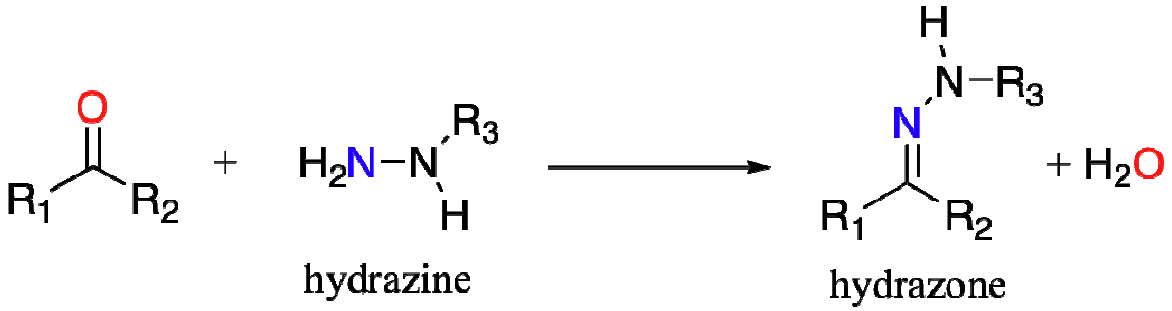
Table 1: Carcinogenicity data of the selected hydrazines (taken from background document, modified)

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	

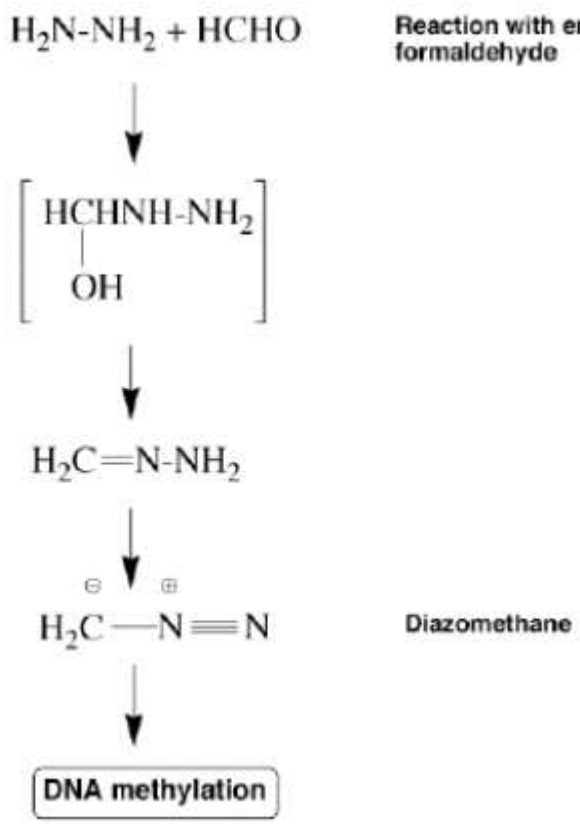
Inhalation:mouse	Lung adenoma Liver adenoma and carcinoma Hemangio(sarco)ma Nasal adenomatous polyps and adenomas	Lung adenoma	Lung, liver, nasal cavity, bone and blood vessels tumours	
Inhalation:hamster	Nasal polyps and adenomas	Benign nasal polyps Colon neoplasms Thyroid parafolicullar cell adenoma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

Adverse health effects by hydrazines; underlying mechanism

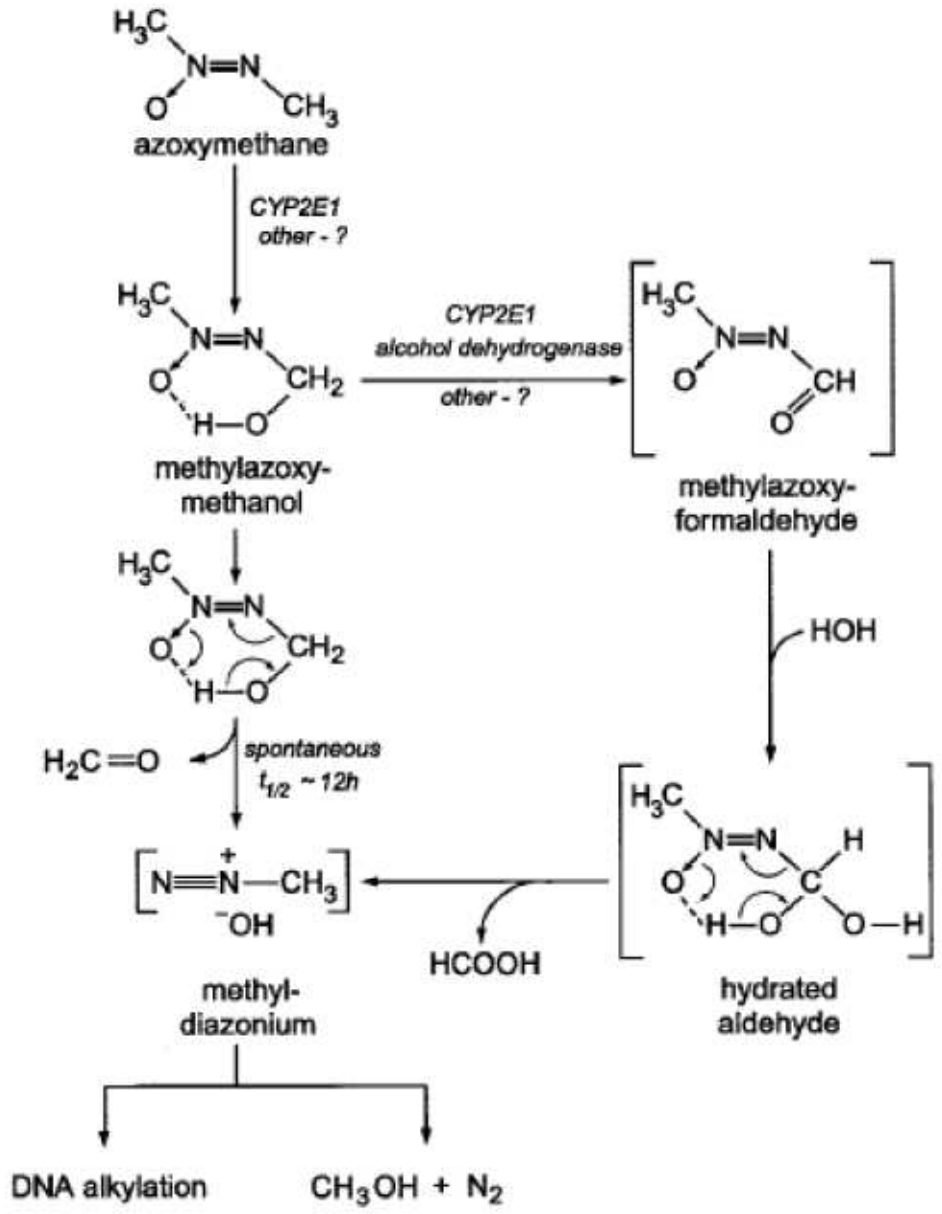
A. Hydrazones are formed in reactions between aldehydes/ketones and hydrazines.



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 – analogue approach and 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 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 carried out 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.

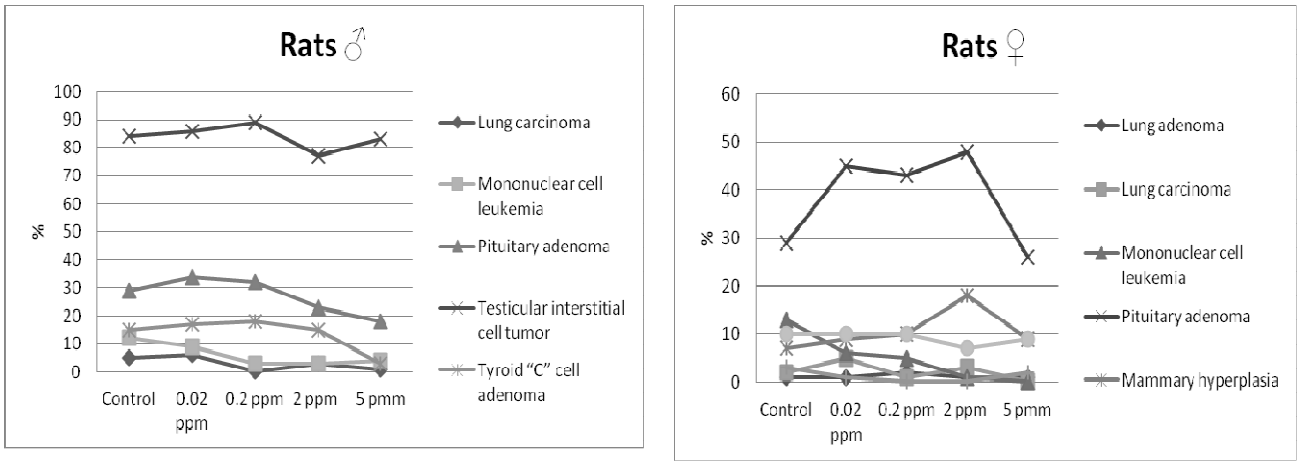


Figure: Neoplastic lesions found in male and female rats after one year inhalaltion exposure

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.

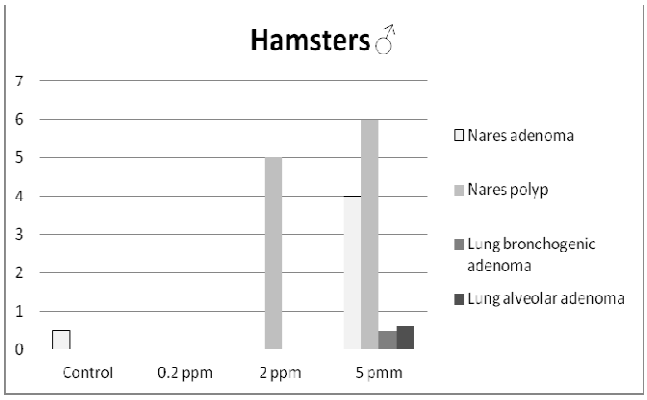


Figure:. Nares and lung neoplastic lesions found in male hamsters (incidence in %)

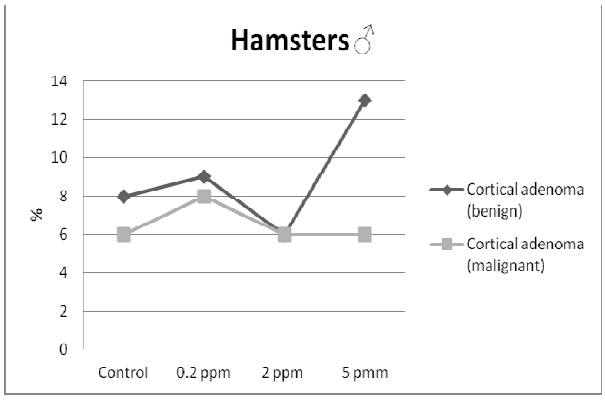


Figure: Cortical adenomas found in male hamsters (incidence in %)

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).

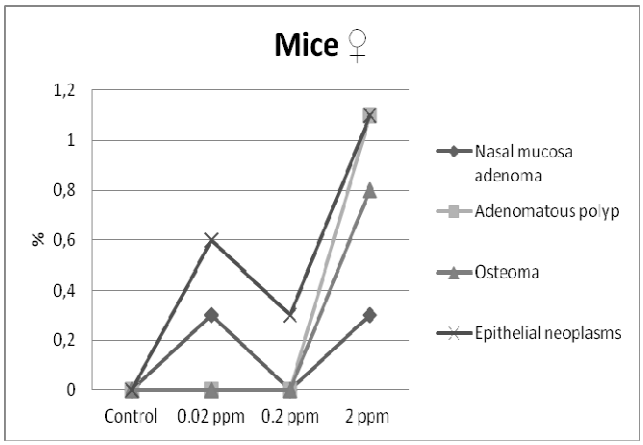


Figure: Nasal mucosa adenomas, adenomatous polyps, osteomas and epithelial neoplasms found in female mice (incidence in %)

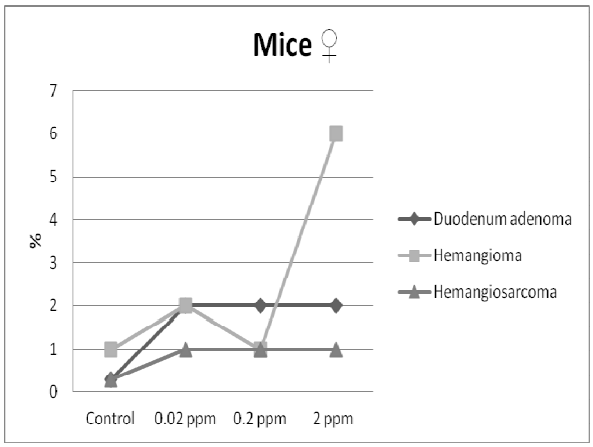


Figure: Duodenum adenomas, hemangiomas and hemangiosarcomas found in female mice (incidence in %)

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.

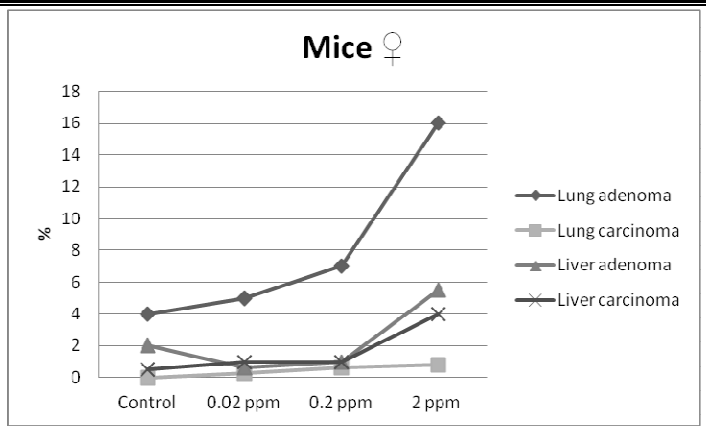


Figure: Lung and liver adenomas and carcinomas found in female mice (incidence in %)

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 and 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**.**

Supplemental information - In depth analyses by RAC

A summary of carcinogenicity studies of methylhydrazine and an in depth analysis is provided in the table of the Appendix below.

Appendix

Summary of carcinogenicity studies of methylhydrazine

Study	Test object	Route	Dose	Exposure time	Incidence, count (%)		Type of tumor	Effect	Notes
					Treated group	Control group			
Kelly et al. (1969)	CDF1 mice ♀	Oral gavage	3.7 mg (0.46 mg / administration)	1x/week, for 8 weeks	0/9 (**0 %**)	1/10 (**10 %**)	Lung adenomas	-	Short exposure time, shall be excluded from the analysis
					0%	**0%**	Leukemia	-	
	CDF1 mice ♂	i.p.	1.8 mg (0.23 mg / injection)	1x/week, for 8 weeks	3/30 (**10 %**)	1/9 (**11 %**)	Lung adenomas	-	
					0%	**0%**	Leukemia	-	
Toth (1972)	Swiss mice	Oral	0.01 % in water	lifetime	12/50 (**24%**) ♀	14/110 (**12.7%**) ♀	Lung adenomas	++	Control from Toth, 1969
					11/50 (**22%**) ♂	11/110 (**10%**) ♂			
					2/50 (**4%**) ♀	17/110 (**15%**) ♀	Malignant lymphomas	-	
						2/110 (**2%**) ♂			

Toth and Shimizu (1973)	Golden Syrian hamsters	Oral	0.01 % in water	lifetime	16/49 (**32%**) ♀	0/99 (**0%**) ♀	Malignant histiocytomas	**++**	Animals were 44 days old at the start of exposure. Not repeated in similar MacEwen and Vernot (1975) study

4.11 Toxicity for reproduction

Not evaluated in this report

4.12 Other effects

Not evaluated in this report

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this report

6 OTHER INFORMATION

Not evaluated in this report

7 REFERENCES

ACGIH (2008) - Threshold Limit Values for Chemicals Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH, p 41

Ames BN, Durston WE, Yamasaki E, Lee FD (1973) Proc. Nat. Acad. Sci. (USA) Vol 70, No 8, pp 2281-2285.

Back K.C. and Pinkerton M.K. (1967) Toxicology and pathology of repeated doses of monomethylhydrazine in monkeys. AMRL-TR-66-199

Brusick D. and Matheson D.W. (1976) Mutagen and oncogen study of methylhydrazine. Testing laboratory: Litton Bionetics, Inc. Report no.: AMRL-TR-76-80. Owner company: Aerospace Medical Research Laboratory. Report date: 1976-12-01.

Darmer K.I., MacEwen J.D. (1973) Monomethylhydrazine - Chronic low level exposures and 24-hour emergency exposure limits. Proceedings of the fourth annual conference on environmental toxicology held at Fairborn, Ohio on 16, 17, and 18 october 1973. Report no.: AMRL-TR-73-125. Owner company: Aerospace Medical Research Laboratory. Report date: 1973-12-01.

Dost, F.N., Reed, D.J., and Wang, C. H. (1966) The metabolic fate of monomethylhydrazine and unsymmetrical dimethylhydrazine. Biochemical Parmacology 15: 1325-1332

George M.E., Mautner W, Back K.C. (1968) Nephrotoxic effects of monomethylhydrazine in monkeys. Aerospace Medical Research Laboratory, Report date: AMRL-TR-68-110

Hansch C., Leo A., Hoekman D. (1995) Exploring QSAR: Hydrophobic, electronic, and steric constants. American Chemical Society, Washington, DC.

Haun, C.C. (1970) Chronic exposure to low concentrations of monomethylhydrazine. Proceedings of the First Annual Conference on Environmental Toxicology, AMRL-TR-70-102, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio

Health Council of the Netherlands (2002) Evaluation of the carcinogenicity and genotoxicity of N-methylhydrazine.

Kelly, M.G., O'Gara R.W., Yancey S.T., Gadekar K., Botkin C. and Oliviero V.T. (1969) Comparative carcinogenicity of N-isopropyl-alpha-(2-methylhydrazine)p-toluamide HCl (procarbazine hydrochloride), its degradation products, other hydrazines, and isonicotinic acid hydrazide. J. Nat. Cancer Inst. 42:337-344

Kinkead, E.R., Haun C.C., Vernot E.H., and MacEwen J.D. (1985) A chronic inhalation toxicity study on monomethylhydrazine. AF AMRL-TR-85-025. Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH.

Kroe, D.J. (1971) Animal Pathology Resulting from Long-Term Exposure to Low Levels of Monomethylhydrazine. Report No. AMRL-AD-751-120. Aerospace Medical Research Laboratory, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio.

MacEwen J.D., Haun C.C. (1971) Chronic exposure studies with monomethylhydrazine. Testing laboratory: Not specified (probably SysteMed Corporation). Report no.: AMRL-TR-71-120. Owner company: Aerospace Medical Research Laboratory. Report date: 1971-12-01

MacEwen J.D., Vernot E.H. (1975) Studies on the effect of monomethylhydrazine in drinking water on Golden Syrian Hamsters. Toxic Hazard Research Unit Annual Report , AMRL-TR-75-57, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio

Matsushita H. Jr, Endo O., Matsushita H., Yamamoto M. and Mochizuki M. (1993) Mutagenicity of alkylhydrazine oxalates in Salmonella typhimurium TA 100 and TA102 demonstrated by modifying the growth conditions of the bacteria. Mutation Research, 301, 213-222.

NIOSH-Documentation for IDLHs-Methyl hydrazine (1994)
http://www.cdc.gov/niosh/idlh/60344.html

Pinkerton M.K., Hagano E.A., Back K.C. (1967) Distribution and excretion of 14C-monomethylhydrazine. Testing laboratory: Aerospace Medical Research Laboratories. Report no.: AMRL-TR-67-175. Owner company: Aerospace Medical Research Laboratories. Report date: 1967-11-01.

Poso A., von Wright A. and Gynker J., (1995) An empirical and theoretical study on mechanisms of mutagenic activity of hydrazine compounds. Mutation Research vol. 332, pp 63-71

REACH registration dossier: methylhydrazine (CAS no. 60-34-4)

Rogan E.G., Walker B.A., Gingell R., Nagel D.L. and Toth B. (1982) Microbial mutagenicity of selected hydrazines. Mutation Research, 102, 413-424.

Rogers A.M. and Back K.C., (1981) Comparative mutagenicity of hydrazine and 3 methylated derivatives in L5178Y mouse lymphoma cells. Mutation Research, 89, 321-328.

Sopher R.L., Esparza A.R., Robinson F.R. (1968) The effect of methylhydrazine by inhalation or injection in dog's kidneys. AMRL-TR-68-175. AMRL TR Dec:159-74

Sopher R.L., Esparza A.R., Robinson F.R. (1969) Renal pathology of acute methylhydrazine intoxication in dogs. Aerosp Med. Jan; 40(1): 55-61

Spacecraft maximum allowable concentrations for selected airborne contaminants (book) (2002) Volume 4 B5

Toth, B. (1969) Lung tumor induction and inhibition of breast adenocarcinomas by hydrazine sulfate in mice. J. Nat. Cancer Inst., 42, 469-475.

Toth, B. (1972) Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. Int. J. Cencer, 9, 109-118.

Toth, B. & Shimizu, H. (1973) Methylhydrazine tumorigeniesis in Syrian golden hamsters and the morphology of malignant histiocytomas. Cancer Res., 33, 2744-2753.

Toth B. (1977) The Large Bowel Carcinogenic Effects of Hydrazines and Related Compounds Occurring in Nature and the Environment. Cancer, 40, 2427-2431.

Von Wright A. and Tikkanen L. (1980a) The comparative mutagenicities of hydrazine and its mono- and dimethyl derivatives in bacterial test systems, Mutation Research, 78, 17-23.

Von Wright A. and Tikkanen L. (1980b) Hydrazine and methylhydrazine as recA-independent mutagens in Escherichia coli. Mutation Research, 71, 269-271.

Von Wright, A., Niskanen, A. & Pyysalo, H. (1978) Mutagenic propoerties of ethylidene gyromitrin and its metabolites in microsomal activiation tests and in the host mediated assay. Mutat. Res., 54, 167-173.

Von Wright, A., Niskanen, A. & Pyysalo, H. (1977) The toxicities and mutagenic propoerties of ethylidene gyromitrin and N-methylhydrazine with Escherichia coli as test organism. Mutat. Res., 56, 105-110.

8 ANNEXES