Additional information

For a Substance under Harmonised Classification and Labelling Process

Substance Name: Methylhydrazine

EC Number: 200-471-4
CAS Number: 60-34-4

Interested parties are invited to comment on the possible classification of methylhydrazine for carcinogenicity, taking into consideration the read-across information summarised by the dossier submitter (RIVM) below and the original CLH report (section 4.10) which is available on ECHA website.
Read-across information to support the carcinogenicity assessment of methylhydrazine in a weight of evidence approach.

RIVM, July 2015

1. Introduction

The Netherlands proposed harmonised classification of methylhydrazine (CAS 60-34-4) in category 1B for carcinogenicity (Carc. 1B). During the RAC assessment of the proposal, additional information was requested on the mutagenic and carcinogenic properties of structural analogues of methylhydrazine which could support the proposed classification. This document provides information on a number of structurally related hydrazine compounds and compares this information with the properties of methylhydrazine. The results for other hydrazines are considered to support the classification of methylhydrazine as Carc. 1B (H350: May cause cancer) under the CLP Regulation. The final conclusion is based on the information for methylhydrazine itself and the information available for structurally similar substances. Therefore, this is considered a weight-of-evidence assessment. The approach for grouping and read-across in this document is partly based on the Read-across Assessment Framework (RAAF, Reference ECHA-15-R-07-EB).

This document contains information on the identity of the target substance (chapter 2), a description of the selection of the source substances and their identity (chapter 3), a comparison of the target and source substances of the chemical/physical properties (chapter 4), metabolism (chapter 5), mechanism of action (chapter 6), mutagenicity (chapter 7) and carcinogenicity (chapter 8) and a conclusion (chapter 9).

2. Identity of the target substance

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

\[
\begin{align*}
\text{H} & \quad \text{H}_2\text{N} \quad \text{N} \quad \text{CH}_3 \\
\end{align*}
\]

The purity of this mono-constituent substance is confidential, as well as the impurities (see IUCLID). However, the impurities do not affect the classification.
3. Selection of source substances

Source substances selection was based on structural analogs having the same central N-N moiety and either -H or -CH3 attached to the nitrogen atoms, as these are the closest possible analogs. The source hydrazines should have one N-group similar to methylhydrazine (either -NH2 or -NH-CH3). Of the four possible analogs fulfilling these requirements (hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine and trimethylhydrazine), no relevant information regarding mutagenicity and carcinogenicity could be retrieved for trimethylhydrazine. Therefore, this substance was not included. Hydrazine compounds with larger alkylgroups (ethylhydrazine) were not considered relevant as the main mutagenicity mechanism was DNA methylation.

As this is a proposal for classification for carcinogenicity, the difference in potency is considered less relevant (taking into account differences in potency would be scenario 4 of RAAF). Therefore, this is considered scenario 2 of the RAAF; an analogue approach for which the read-across hypothesis is based on different compounds having the same effect.

The following source substances were identified:

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

\[
\begin{array}{c}
\text{H} \\
\text{N-H-H}
\end{array}
\]

Mono-constituent substance. Publicly available information on purity (93-99.0%) is available for one of the registered substances. Ammonium hydroxide (CAS 1336-21-6, EC 215-647-6) was stated as an impurity.

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

\[
\begin{array}{c}
\text{H}_3\text{C} \\
\text{H} \\
\text{N-CH}_3
\end{array}
\]

No information on purity as this substance is not registered.

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

\[
\begin{array}{c}
\text{H} \\
\text{N-CH}_3 \\
\text{H-CH}_3
\end{array}
\]

Mono-constituent substance. No publicly available information on purity and impurities.
Information on physical/chemical properties, toxicokinetics, mutagenicity and carcinogenicity was collected using information from the registrations, summaries of the properties of the source hydrazines and via Scopus for more recent information.

The reliability of the studies with the source substances could not be fully evaluated because most summaries and study reports do not contain information on purity and impurity profiles. However, most studies are considered acceptable for classification and labeling because they have been used in the past to support the harmonised classification of the source hydrazine compounds. Also, as no specific study is used to read-across from the source substances to the target substance, absence of purity/impurity data is considered less relevant.

4. Comparison of the physical/chemical properties

Table 1. Physical and chemical properties of the selected hydrazines.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>methylhydrazine</th>
<th>hydrazine</th>
<th>1,1-dimethylhydrazine</th>
<th>1,2-dimethylhydrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td>liquid</td>
<td>liquid</td>
<td>liquid</td>
<td>liquid</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>6.65 kPa</td>
<td>1.9 kPa</td>
<td>17-22 kPa</td>
<td>9 kPa</td>
</tr>
<tr>
<td>Water solubility</td>
<td>&gt;10%</td>
<td>miscible</td>
<td>10-100%</td>
<td>miscible</td>
</tr>
<tr>
<td>Log Kow</td>
<td>-1</td>
<td>-0.16</td>
<td>-1.19</td>
<td>-0.54</td>
</tr>
<tr>
<td>Boiling point</td>
<td>87.5 °C</td>
<td>113.5°C</td>
<td>63.9°C</td>
<td>81°C</td>
</tr>
</tbody>
</table>
5. Metabolism

Table 2. Metabolites of the selected hydrazines.

<table>
<thead>
<tr>
<th>Identified metabolites</th>
<th>methylhydrazone</th>
<th>hydrazine</th>
<th>1,1-dimethylhydrazone</th>
<th>1,2-dimethylhydrazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Carbon dioxide</td>
<td>Nitrogen</td>
<td>Carbon dioxide</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td></td>
<td>methane</td>
<td>Acetyl/diacetylhydrazone</td>
<td>Glucose hydrazone</td>
<td>Azomethane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pyruvate hydrazone</td>
<td></td>
<td>methanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhalation</td>
<td>Acetyl hydrazone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Diacetyl hydrazone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro</td>
<td>Methyl radicals</td>
<td>Free radical formation</td>
<td>Methyl radicals</td>
<td>Methyl radicals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Free radical formation</td>
<td>Formaldehyde</td>
</tr>
<tr>
<td>In vivo DNA adducts</td>
<td>Methylanducts</td>
<td></td>
<td>N7-methylguanine</td>
<td>N7-methylguanine</td>
</tr>
<tr>
<td></td>
<td>N7-methylguanine</td>
<td></td>
<td>and O6-methylguanine</td>
<td>and O6-methylguanine</td>
</tr>
<tr>
<td></td>
<td>and O6-methylguanine</td>
<td></td>
<td>in liver of mice, rats</td>
<td>(Perse, 2011)</td>
</tr>
<tr>
<td></td>
<td>in liver of mice, rats and hamsters treated in vivo.</td>
<td></td>
<td>and hamsters treated in vivo.</td>
<td></td>
</tr>
<tr>
<td>Additional references</td>
<td>ATSDR, 1997 and SCOEL, 2010</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is very limited data on the metabolism of methylhydrazine. The available information shows that 45% of the radioactive labeled carbon is exhaled as CO₂ and methane after i.p. injection and approximately 40% was excreted in the urine (CLH report).

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 that methylhydrazine could also form methyl adducts comes from an in vitro study using isolated hepatocytes and liver microsomes (Albano, 1989). In vitro incubation of methylhydrazine, 1,1-DMH and 1,2-DMH resulted in free radical intermediates which were detected by ESR spectroscopy of the reaction with a spin trapping agent (4-POBN). All three hydrazines produced spin adducts with spectral features reported for the methyl free radical adduct of 4-POBN. This is supported by the formation of methane as indirect evidence.

6. Mechanism of action
The mechanisms by which hydrazines produce adverse health effects are described in detail by ATSDR (1997) and SCOEL (2010). Below copies of the summaries of these studies are given.

- **ATSDR (1997):**

  “A number of studies have investigated the mechanisms by which hydrazines produce adverse health effects. These data suggest there are at least two distinct mechanisms of action for hydrazines: one involving the direct binding of those hydrazines with a free amino group (hydrazine and 1,1-dimethylhydrazine) to key cellular molecules, and the other involving the generation of reactive species such as free radical intermediates or methylidiazonium ions as a result of metabolism. Studies which support the existence of these mechanisms are discussed below.

  *In vitro* studies have shown that hydrazine reacts with alpha-keto acids to form hydrazones compounds (O’Leary and Oikemus, 1956).”

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**Explanation (UCDavis, Chemwiki):** Hydrazones are formed in reactions between aldehydes/ketones and hydrazines.

```
\[
\begin{align*}
\text{R}_1\text{R}_2\text{O} + \text{H}_2\text{N} &=& \text{H}_2\text{N} - \text{N}\rightarrow \text{R}_3 \\
\text{hydrazine} &\rightarrow & \text{R}_1\text{R}_2\text{N} - \text{N}\rightarrow \text{R}_3 + \text{H}_2\text{O} \\
\text{hydrazone} & &
\end{align*}
\]
```

“By binding to keto acids and forming hydrazones, hydrazine inhibited oxygen consumption with mitochondrial substrates *in vitro* (Fortney 1967). This mechanism may well account for the hyperlactemic and hypoglycemic effects of hydrazine observed in humans (Ochoa *et al.* 1975) and dogs *in vivo* (Fortney 1967). Hydrazine and 1,1-dimethylhydrazine can form hydrazones with vitamin B6 derivatives (Comish 1969). By binding to vitamin B6 derivatives, hydrazine and 1,1-dimethylhydrazine are able to inhibit reactions that require vitamin B6 as a cofactor. These reactions include transamination reactions, decarboxylation and other transformations of amino acids, the metabolism of lipids and nucleic acids, and glycogen phosphorylation (NRC 1989). Deficiency of vitamin B6 can produce convulsions, dermatitis, and anemia. These data suggest that the convulsions and anemia observed in animal studies are the result of the formation of hydrazone derivatives of vitamin B6. In addition, some authors have proposed that a free amino group, as found in hydrazine and 1,1-dimethylhydrazine, is required for hydrazone formation (Comish 1969). This would explain why convulsions are associated with exposures to hydrazine and
1,1-dimethylhydrazine, and not 1,2-dimethylhydrazine. It should be noted that pyridoxine (one of the forms of vitamin B6) is commonly used to treat humans exposed to hydrazine or 1,1-dimethylhydrazine.

A number of in vitro studies have reported the production of reactive intermediates during the metabolism of hydrazines (see Section 2.3.3). Evidence for the production of radicals including methyl, acetyl, hydroxyl, and hydrogen radicals has been observed during the metabolism of hydrazine (Ito et al. 1992; Noda et al. 1988; Runge-Morris et al. 1988; Sinha 1987), 1,1-dimethylhydrazine (Albano et al. 1989; Tomasi et al. 1987), and 1,2-dimethylhydrazine (Albano et al. 1989; Augusto et al. 1985; Netto et al. 1987; Tomasi et al. 1987). Multiple pathways, both enzymatic and nonenzymatic, appear to be involved in free radical generation. Free radicals have been implicated in protein (hemoglobin) damage associated with hydrazine in human erythrocytes (Runge-Morris et al. 1988), suggesting that free radicals may be involved in the anemic effects of hydrazines observed in animals in vivo (Haun and Kinkead 1973; Rinehart et al. 1960). It has also been proposed that metabolism of 1,2-dimethylhydrazine yields a reactive, methylidiazonium ion (Feinberg and Zedeck 1980; Sohn et al. 1991). The production of reactive species during the metabolism of hydrazines may also explain their genotoxic effects, such as the formation of DNA and RNA adducts in vivo (Becker et al. 1981; Beranek et al. 1983; Bolognesi et al. 1988; Bosan et al. 1986; Netto et al. 1992; Pozharisski et al. 1975; Quintero-Ruiz et al. 1981). DNA and RNA adducts may well be responsible for gene mutations observed in a number of in vitro studies (DeFlora and Mugnoli 1981; Hawks and Magee 1974; Kang 1994; Kerklaan et al. 1983; Levi et al. 1986; Malaveille et al. 1983; Noda et al. 1986; Oravec et al. 1986; Parodi et al. 1981; Rogers and Back 1981; Sedgwick 1992; Wilpart et al. 1983) and may also serve as the initiating event for cancers induced by hydrazines in vivo.”

- SCOEL, 2010 (hydrazine only)

“Administration of hydrazine to rodents results in the formation of N7-methylguanine and O6-methylguanine in liver DNA. Co-administration of L-[methyl-14C] methionine or [14C] formate with the hydrazine led to labelling of the methylguanines, suggesting involvement of the one-carbon pool for the methylation process (Quintero-Ruiz et al., 1981). It has been proposed that the methylation mechanism involves reaction of hydrazine with endogenous formaldehyde to yield formaldehyde hydrazone, which could be metabolized to the potent methylating agent diazomethane (Bosan & Shank, 1983; Bosan et al., 1986; see Figure 1). According to the data of Barrows et al. (1983), there was no increased direct incorporation of the tritium-labelled methyl group of methionine into 5-methyl-cytosine in hydrazine treated rats. In experiments using postmitochondrial (S9), microsomal, cytosolic or mitochondrial cell fractions from rat liver in vitro, methylation of DNA guanine occurred, S9 being the most active fraction. Neither the P450 monooxygenase nor flavin monooxygenase systems appeared to be important in hydrazine/formaldehyde-induced methylation of
DNA. However, sodium azide, cyanamide and carbon monoxide all inhibited S9-supported DNA methylation. Bovine liver catalase, a haem-containing cytochrome, readily transformed hydrazine/formaldehyde to a methylating agent. 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 (Lambert & Shark, 1988). This metabolic concept is summarised in Figure 1.

![Mechanistic concept of hydrazine-induced DNA methylation](image)

**Figure 1.** Mechanistic concept of hydrazine-induced DNA methylation (Bosan and Shank, 1983 as presented by SCOEL, 2010).

Later, van Delft et al. (1977) further examined the pattern of DNA methylation. The induction of N7- and O6-methylguanine was studied in the liver DNA of rats, 16 hr after treatment with various doses of hydrazine. After DNA isolation, the presence of N7-methylguanine in DNA was assessed with an immunochemical method and with a physicochemical technique (HPLC with electrochemical detection). Application of these two methods resulted in almost identical patterns of dose-dependent induction of guanine N7-methylation in rats dosed orally with 0.1 to 10 mg hydrazine per kilogram of bodyweight, increasing from 1.1-1.3 to 39-45 N7-methylguanine per 10^6 nucleotides. At lower dosages a constant adduct level was observed, equivalent to that in untreated rats (background level). The O6-MeGua level was analysed by a combination of HPLC separation and competitive radioimmunoassay. Background level was observed for untreated rats and no increase was visible up to the 0.2 mg/kg dose group. After hydrazine doses from 0.2 to 10 mg/kg, O6-methylguanine increased from 0.29 to 134 per 10^9 nucleotides. The data were interpreted to show that even at dosages below the maximum tolerated dose (0.6 mg/kg/day), for which carcinogenic effects have not been described experimentally, methyl DNA adducts are formed. The authors also concluded that their results were consistent with the
aforementioned mechanistic concept of hydrazine-induced DNA methylation (Figure 1).”

In addition to the SCOEL evaluation, a diagram showing the metabolic pathways of azoxymethane and methylazoxymethanol, two metabolites identified in vivo of 1,2-DMH, was presented by Sohn (2001). Figure 2, shows that the metabolites of 1,2-DMH can form methylidiazonium which can methylate DNA.

![Diagram showing metabolic pathways of azoxymethane and methylazoxymethanol activation. Sohn, 2001.](image)

**Figure 2.** Diagram showing metabolic pathways of azoxymethane and methylazoxymethanol activation. Sohn, 2001.

The type of mutations in tumours of rats and mice treated with 1,2-DMH is consistent with the O6-MeG mutagenic properties. Also overexpression of O6-alkylguanine DNA-alkyltransferase (MGMT), the DNA repair protein that removes these types of adducts, reduces mutations and tumours induced by methylating agents (Povey, 2002).

Overall, there seem to be two mechanisms proposed for the DNA methylating properties of the source hydrazine compounds.

**Mechanism 1**

The metabolic pathway of hydrazine as shown in Figure 1 seems to be relevant for all substituted hydrazines with a free amino group, including
methylhydrazine. The reaction of hydrazine with formaldehyde resulting in the formation of a hydrazone could also occur with methylhydrazine.

Mechanism 2

The metabolic pathway of 1,2-DMH as shown in Figure 2 could be considered as not relevant for methylhydrazine because it requires a methyl group on each of the two nitrogen atoms. Methylhydrazine contains only one methylated nitrogen atom.

The initiating steps in the formation of the ultimate reactive species of 1,2-DMH is the formation of the azomethane (CH3-N=N-CH3) and azoxymethane. These oxidations could also occur in methylhydrazine. However, further metabolism of this product is unknown.

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. The in vitro study by Albano shows that methylhydrazine can also be metabolised to substances that can form methyl radicals and in vivo methane formation is shown. Therefore, it is expected that methylhydrazine can also form methyl DNA adducts and is (therefore) mutagenic.

7. Mutagenicity

Table 3. Mutagenicity data of the selected hydrazines.

<table>
<thead>
<tr>
<th></th>
<th>methylhydrazine</th>
<th>hydrazine</th>
<th>1,1-dimethylhydrazine</th>
<th>1,2-dimethylhydrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro: Ames</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard:</td>
<td>Positive with</td>
<td>Without</td>
<td>Without activation</td>
<td>Without activation</td>
</tr>
<tr>
<td>negative</td>
<td>and without</td>
<td>3/6 positive</td>
<td>3/6 positive</td>
<td>mostly positive</td>
</tr>
<tr>
<td>Liquid</td>
<td>activation</td>
<td>With activation</td>
<td>With activation 50%</td>
<td>With activation 50%</td>
</tr>
<tr>
<td>incubation:</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In vitro:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mammalian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cells</td>
<td></td>
<td></td>
<td>usable</td>
<td>usable</td>
</tr>
<tr>
<td>Gene mutation:</td>
<td></td>
<td></td>
<td>usable</td>
<td>usable</td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
<td>usable</td>
<td>usable</td>
</tr>
<tr>
<td>UDS: negative</td>
<td></td>
<td></td>
<td>usable</td>
<td>usable</td>
</tr>
<tr>
<td>Gene mutation:</td>
<td></td>
<td></td>
<td>usable</td>
<td>usable</td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
<td>part positive</td>
<td>positive</td>
</tr>
<tr>
<td>UDS: positive</td>
<td></td>
<td></td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>micronucleus</td>
<td>Weakly positive</td>
<td>Negative in bone marrow but positive</td>
<td>Rat: negative bone marrow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in 1/3 studies</td>
<td>in spermatids and</td>
<td>Rat: positive colon</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>liver (ECHA registration</td>
<td>(Okada, 2013)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>vivo.001)</td>
<td>Mouse: positive</td>
<td></td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td>Negative in lung</td>
<td></td>
<td>Positive: colon, intestine (Newell, 2004)</td>
<td></td>
</tr>
<tr>
<td>gene mutation</td>
<td>liver and bone marrow (Douglas, 1995)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone marrow positive (Carlsen, 2009)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td>Liver and lung</td>
<td></td>
<td>Comet assay positive</td>
<td></td>
</tr>
<tr>
<td>chromosomal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aberration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>strand</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>breaks/Comet assay</td>
<td>positive Positive in several organs</td>
<td>positive in several organs in mice (Sasaki, 1997)</td>
<td>in liver and negative in stomach (Hobbs, 2015)</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------------</td>
<td>--------------------------------------------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>In vivo UDS</td>
<td>Mouse sperm negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo dominant lethal</td>
<td>negative</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>References</td>
<td>All references not stated are as summarised in ATSDR, 1997 and CLH report for methylhydrazine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The source hydrazine compounds have no harmonised classification for mutagenicity (but do have classifications for carcinogenicity, see below). However, the available data show that these source compounds are all mutagenic in vitro and form methyl DNA adducts in vivo. In vivo mutagenicity in somatic cells was shown for all source hydrazine compounds though not always in all studies and organs. The site of in vivo mutagenicity varies between the source compounds. The study by Sasaki (1998) compares the three source hydrazine compounds in a mouse model and shows that Comet formation varies between the hydrazine compounds and depends on route of exposure. The site of mutagenicity shows some relationship with the carcinogenicity. However, other factors such as repair of the methyl adducts and apoptosis or necrosis followed by proliferation could also be relevant. The absence of a harmonised classification for mutagenicity for the source hydrazine compounds may be related to the data available at the time the harmonisation was performed and/or the criteria that were applicable.

The results of the Ames test seem to be comparable between the source and the target substances as most hydrazines show an inconsistent result in this test. The result of the gene mutation tests and the UDS test differ between methylhydrazine and the source hydrazines as these tests are negative for methylhydrazine and positive for the source substances. However, considering the indication that methylhydrazine can form methyl radicals which are considered to be the mechanism for mutagenicity and carcinogenicity of the other hydrazines, this may also indicate that these negative in vitro tests may not be predictive of absence of mutagenicity for methylhydrazine. The absence of information on the mutagenicity in vivo for methylhydrazine limits the assessment of the suitability of read-across for carcinogenicity.

Overall, comparison of the mutagenicity does not support read-across from the source hydrazines to methylhydrazine.

### 8. Carcinogenicity

**Table 4.** Carcinogenicity data of the selected hydrazine.

<table>
<thead>
<tr>
<th>Route/species</th>
<th>methylhydrazine</th>
<th>Hydrazine (including</th>
<th>1,1-</th>
<th>1,2-dimethylhydrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>----------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>Negative (Steinhoff, 1990)</td>
<td>Blood vessel, lung, kidney, and liver tumours (Toth, 1973a)</td>
<td>Angio(sarco)mas (Toth and Pattil, 1982)</td>
<td>Lung adenoma and carcinoma (Kinkead, 1985)</td>
<td></td>
</tr>
<tr>
<td>Oral: hamster</td>
<td>Oral: mouse</td>
<td>Inhalation: rat</td>
<td>Inhalation: mouse</td>
<td></td>
</tr>
<tr>
<td>Malignant histiocytoma</td>
<td>Cecum tumours (Toth and Shimizu, 1973) Second study negative (MacEwan, 1975)</td>
<td>Negative (Kinkead, 1985)</td>
<td>Lung adenoma (Kinkead, 1985)</td>
<td></td>
</tr>
<tr>
<td>Blood vessel tumours (Toth and Pattil, 1982)</td>
<td>Angio(sarco)ma, lung adenocarcinoma and colon tumours (Izumi, 1979)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Inhalation: hamster | Nasal polyps and adenomas (Kinkead, 1985) | Benign nasal polyps Colon neoplasms Thyroid parafollicular cell adenoma (Vernot, 1985) | CLH | Carc 1B | Carc 1B | Carc 1B | Reference: All references are as summarized in ATSDR, 1997, SCOEL report on hydrazine, 2010 and CLH proposal methylhydrazine

Besides the common routes of exposure shown in Table 4 above, there are many more studies especially for 1,2-DMH using other routes such as subcutaneous and intra-peritoneal injection which are not summarised here.

The increase in tumours observed in some studies with methylhydrazine is also seen with the source hydrazine compounds in the same species. The increase in blood vessel and lung tumours observed in the oral mouse study are also observed with 1,1-DMH and 1,2-DMH. However, hydrazine induced lung tumours but no blood vessel tumours. Blood vessel tumours are also seen in several other species and routes for the source hydrazine compounds.

The cecum tumours observed in the oral hamster study were not observed with the source hydrazine compounds in this species. However, colon tumours were induced in mice and rats with 1,2-DMH. Treatment of rats and mice with 1,2-DMH is used as a model for colon carcinogenesis and it has been shown that the metabolite azoxymethane also induces colon tumours (Perse, 2011).

The negative inhalation study in the rat with MH is not consistent with comparable studies with hydrazine and 1,1-DMH. However, in these studies no consistent type of tumours was observed.

The lung tumours observed in the mouse with MH were confirmed in comparable studies with hydrazine and 1,1-DMH (1,2-DMH was not tested by inhalation). The increase in liver, blood vessel and nasal tumours with methylhydrazine after inhalation in mice was only confirmed in the study with 1,1-DMH.

The increase in nasal tumours in hamsters was confirmed in the only other nasal study with hamsters using hydrazine.

Overall, the available carcinogenicity studies with hydrazine compounds show that there are clear differences between species but almost all studies were positive. The blood vessel tumours observed in mice following methylhydrazine exposure via the oral and inhalation routes are also observed with both source methylated hydrazines and the cecum tumours are also seen with 1,2-DMH. Therefore, the results with the source hydrazine compounds support the relevance of these tumours for classification. Histiocytomas were not observed with the source hydrazine compounds. These tumours may be related to iron accumulation after anemia as iron accumulation was observed in the histiocytes and is known to induce tumours via free radicals through the Fenton reaction.

9. Conclusion
The available data for methylhydrazine and the three closest analogues clearly show that the physical and chemical properties of all four substances are comparable.

The available data on metabolism is limited for several of the source and target hydrazines making it difficult to compare. However, the available information indicates that there are differences in the metabolism between the four hydrazine compounds. From a chemical perspective, methylhydrazine could be comparable to hydrazine as it contains a free amino group (comparable to hydrazine and 1,1-methylhydrazine) but also to the methyl hydrazines as it contains a methylated amino group (comparable to 1,2-dimethylhydrazine). In general, all hydrazines seem to be oxidized at the N-N bond into N=N and further resulting in formation of nitrogen gas (N₂) and a remaining methyl radical. The proposed mechanism for the three other hydrazine compounds is the formation of methyl DNA adducts resulting in mutations and carcinogenicity. The formation of methyl adducts is shown in vivo for the three source hydrazines but are only indicated for methylhydrazine based on the formation of methyl radicals in vitro.

The mutagenicity data in vitro indicate a difference between the source and the target substances as the target substances are positive for gene mutation and UDS in vitro whereas methylhydrazine is negative. However, considering the likely similarity in metabolism and indicated formation of methyl radicals by methylhydrazine, it could also be considered that the available in vitro data are not predictive of the mutagenic potency of methylhydrazine.

All three source hydrazines show induction of tumours resulting in a harmonised classification of all close analogues in category 1B for carcinogenicity. However, there is some inconsistency in the tumour sites and the sites where mutagenicity is observed in vivo between the three substances. This may partly depend on the animal species and strain used and the route of exposure, but is also substantiated by the differences in metabolism between the source hydrazine compounds. Therefore, the source substances cannot be considered as one homogeneous group and it is difficult to assess which of the source substances is most relevant for methylhydrazine. The relevance of the tumours observed with methylhydrazine is supported by the occurrence of comparable tumours (blood vessel and colon) with some of the source methylated hydrazines. Although there are some differences in tumour sites between the different hydrazines, all three source hydrazines are classified as carcinogenic 1B and show at least some type of tumours (blood vessel and colon) also found with most hydrazines.

Based on the comparable chemical structure, physical and chemical properties, metabolism and sites of carcinogenicity, read-across of the carcinogenic properties is warranted. The available information on mutagenicity indicates that there may be differences but this may also indicate that the available in vitro data are not predictive of the mutagenic potency of methylhydrazine. Overall, read-across of the carcinogenic properties of the three source hydrazine compounds to methylhydrazine is considered acceptable as supporting evidence.
Based on a weight of evidence assessment consisting of the positive carcinogenicity in most studies with methylhydrazine, supported with the clear structural relation with the three source hydrazines which are already classified as carcinogens, classification of methylhydrazine as Carc. 1B (H350: May cause cancer) according to CLP is warranted.

10. References


(http://www.atsdr.cdc.gov/toxprofiles/tp100.pdf)


Registration data methylhydrazine (http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d85d808-3e5b-0d44-e044-00144f67d249/DISS-9d85d808-3e5b-0d44-e044-


