CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: dichloromethane

EC Number: **200-838-9**

CAS Number: **75-09-2**

Index Number: **602-004-00-3**

Contact details for dossier submitter:
MSCA Italy National Institute of Health on behalf of Ministry of Health
Viale Regina Elena, 299 - 00161 Rome, Italy.
leonello.attias@iss.it

Version number: 2 **Date: 21/07/2023**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Dichloromethane
Other names (usual name, trade name, abbreviation)	Methane, dichloro- Methane, dichloro-
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	200-838-9
EC name (if available and appropriate)	dichloromethane
CAS number (if available)	75-09-2
Other identity code (if available)	*[For example CIPAC number]*
Molecular formula	CH2Cl2
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	84.933
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**
dichloromethane	>99.5 - 100 % (w/w)		

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

[Please insert rows according to the number of impurities in the substance. If impurities are confidential information it is sufficient to state whether they contribute to the classification and labelling.]

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Not relevant				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Classification table

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	602-004-00-3	dichloromethane	200-838-9	75-09-2	Carc. 2	H351	GHS08 Wng	H351			
Dossier submitters proposal					**Add** Muta 2 **Modify** Carc 1B	**Add** H341 **Modify** H350	**Retain** GHS08 **Modify** Dgr	**Add** H341 **Modify** H350			
Resulting Annex VI entry if agreed by RAC and COM					Carc 1B Muta 2	H350 H341	GHS08 Dgr	H350 H341			

Table 7: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Harmonised classification proposed	Yes
Carcinogenicity	Harmonised classification proposed	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Endocrine disruption for HH	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Endocrine disruption for ENV	Hazard class not assessed in this dossier	No
PBT/vPvB	Hazard class not assessed in this dossier	No
PMT/vPvM	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Dichloromethane (DCM) is currently listed on Annex VI of CLP Regulation ((EC) No 1272/2008) as a substance suspected of causing cancer. The substance was originally selected for substance evaluation in CoRAP 2016 in order to clarify concerns about:

- Carcinogen
- Suspected mutagen
- Suspected reprotoxic
- Suspected sensitiser
- Potential endocrine disruptor
- High (aggregated) tonnage.

On the basis of the available information, an harmonized classification of the substance is envisaged by eMSCA, as a follow-up at EU level by adding the following hazard categories: Carc 1B H350 and Muta category 2 H341.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

This substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 100 000 tonnes per annum.

This substance is used by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

In particular for consumer use, the substance is used in the following products: adhesives and sealants, plant protection products, washing & cleaning products, biocides (e.g. disinfectants, pest control products) and coating products. Widespread uses by professional workers are also available for the substance in the following products: coating products, washing & cleaning products, adhesives and sealants, biocides (e.g. disinfectants, pest control products) and plant protection products.

This substance is used at industrial site in the following products: washing & cleaning products, extraction agents, adhesives and sealants, coating products and heat transfer fluids.

This substance has an industrial use resulting in manufacture of another substance (use of intermediates).

This substance is used for the manufacture of: chemicals, textile, leather or fur, plastic products and rubber products.

6 DATA SOURCES

Sources: PUBMED, SCOPUS, WEB OF SCIENCE, ScienceDIRECT, ECHA dissemination site, IUCLID (Reg data), OECD sids, IARC.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	liquid	(ECHA, 2023)	
Melting/freezing point	178 K at 101325 Pa	(ECHA, 2023)	
Boiling point	313 K at 101325 Pa	(ECHA, 2023)	
Relative density	1.32 g/cm^3 at 25 °C	(ECHA, 2023)	
Vapour pressure	584 hPa at 25°C (352 mm Hg)	(ECHA, 2023)	
Surface tension	Data waiving	(ECHA, 2023)	
Water solubility	13.2 g/L at 25°C and pH 7	(ECHA, 2023)	
Partition coefficient n-octanol/water (KOW)	Log K_{ow} 1.25 at 20°C and pH 7	(ECHA, 2023)	This value was supported by the CODATA LOGKOW database (recommended value of 1.25) and the calculated log Kow of 1.34 (EPISUITE 4.0)
Partition coefficient n-octanol/air (K_{OA})	-	-	-
Flash point	The substance is not flammable.	(ECHA, 2023)	Relevant literature sources and studies indicate that this substance has no flashpoint. However, under certain conditions the substance can form flammable vapour/air mixtures (13-22 % Vol at 20 °C) which under normal circumstances are difficult to ignite (under optimum conditions of 18 % Vol in air at 20 °C the minimum energy needed for ignition is 9300 mJ, which is many 10000-folds higher than for vapours of other common flammable solvents. Classification as flammable is thus not required.

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	The substance is not flammable.	(ECHA, 2023)	Relevant literature sources and studies indicate that this substance has no flashpoint. However, under certain conditions the substance can form flammable vapour/air mixtures (13-22 % Vol at 20 °C) which under normal circumstances are difficult to ignite (under optimum conditions of 18 % Vol in air at 20 °C the minimum energy needed for ignition is 9300 mJ, which is many 10000 fold higher than for vapours of other common flammable solvents. Classification as flammable is thus not required. Water reactivity and pyrophoricity are not expected based on the structural properties and experience in handling the substance. The substance does not form aerosols.
Explosive properties	Non explosive	(ECHA, 2023)	
Self-ignition temperature	878 K at 101 325 Pa	(ECHA, 2023)	
Oxidising properties	No	(ECHA, 2023)	
Granulometry	D50	(ECHA, 2023)	
Stability in organic solvents and identity of relevant degradation products	Data waiving	(ECHA, 2023)	
Dissociation constant	Data waiving	(ECHA, 2023)	Study technically not feasible
Viscosity	0.42 mPa.s at 25 °C	(ECHA, 2023)	

8 EVALUATION OF PHYSICAL HAZARDS

The substance is not classified for the physico-chemical aspect. See table of summary of physico-chemical properties above. Physical hazards are not further assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

ADME

Absorption

Due to its lipophilicity (Log Kow 1.25 at 20°C and pH 7) and to its low relative molecular mass (84.93 g/mol), DCM can readily cross biological membranes. After inhalation exposure, pulmonary uptake is rapid, approaching steady state within a few hours after the exposure both in humans and in animals (IARC, 2017).

Limited data on oral absorption in humans suggest that DCM is also readily absorbed by this route of exposure (Hughes & Tracey, 1993; Vetro, 2012). Oral bioavailability studies in humans are not available, only case reports of accidental ingestion (quantitative estimates of the ingested amounts, in these cases, are not known precisely). In animals, absorption from the gut after oral doses is rapid and

nearly complete, according to reports of several studies with radiolabel in mice and rats (McKenna & Zempel, 1981; Angelo, 1986a, b). In a study performed in rats was reported that on average 97% of the radiolabel was recovered in expired air as DCM, CO, and carbon dioxide (CO_2) in the 24 hours after each repeated oral dose of 50 or 200 mg/kg per day in rats. In mice, reported absorption is more rapid (but equally extensive) with an aqueous vehicle than with an oil-based vehicle, consistent with studies on other chlorinated solvents (Angelo, 1986a).
The permeability of human skin to DCM is 24 g/m^2 per hour (Ursin, 1995). Various studies on the rate of absorption through animal skin and subsequent pharmacokinetics have been reported. Tissue concentrations of DCM were measured in various organs (lung, liver, brain, kidney, heart and fat) of 128 white rats, using gas chromatography, following immersion of two-thirds of their tails in the solvent for 1, 2, 3 or 4 h. Small increases were seen in most tissues after 1 or 2 h of exposure, and DCM concentrations in fatty tissues increased markedly after 3 h of exposure. After 4 h of exposure, DCM concentrations remained elevated in fatty tissues and were increased in all other tissues studied (Makisimov & Mamleyeva, 1977).

Distribution

DCM after absorption enters blood circulation and undergoes a rapid systemic distribution to tissues, with the highest concentrations expected in adipose tissue and other fatty tissues (due to the lipophilicity of the compound).
DCM is distributed to many organs, including liver, kidney, lungs, brain, muscle and adipose tissue, epididymal fat and testes after respiratory and oral exposure (US EPA, 2011). It is quite rapidly excreted after oral exposure, mostly via the lungs in the exhaled air. It can cross the blood-brain barrier and be transferred across the placenta, and small amounts can be excreted in urine or in milk. Exhalation of DCM after inhalation exposure increases when exposed to higher concentrations. The remainder is metabolized to carbon monoxide, carbon dioxide and inorganic chloride (US EPA, 2011).

Metabolism

Two pathways compete for metabolism of DCM*:* CYP450 (CYP2E1-mediated reductive dehalogenation, also Mixed-Function Oxidases (MFO) pathway), and GST-mediated metabolism (conjugation of DCM to GSH) (see Figure 1. - Proposed pathways for DCM metabolism).
However, both pathways are expected to operate even at low exposures. DCM binds to the CYP reaction site with higher affinity than to the GST site, therefore DCM is metabolized by CYP at lower exposure levels. When the available CYP enzyme is saturated (at higher levels of exposure or in case of poor metabolizer) more DCM is available for binding to the lower-affinity GST metabolic site, and the proportion of DCM metabolized by GST increases.

CYP2E1 Pathway

Exposure to DCM, regardless of exposure route, results in the formation of CO, as assessed by measurement of elevated levels of CO in expired air and increased levels of COHb in the blood, in the CYP2E1 metabolic pathway in studies in animals and humans (IRIS, 2011).
After the formation of formyl chloride during the first step in the CYP2E1 pathway, it is demonstrated the formation of a marginal quote of S-formyl GSH from formyl chloride in the presence of GSH (3% maximum at pH 9) with most (>97%) of the formyl chloride metabolized further to CO (CO formation from formyl chloride was independent of GSH presence in the assay) (Watanabe, 2006).
In some cases the oxidation by CYP2E1 may be considered a detoxication reaction, as it removes the potential carcinogen from other pathways which can activate it to genotoxic materials. Such is probably the case for DCM. The balance between bioactivation and detoxication should be kept in mind when the benefits of high or low expressions of CYP2E1 are being considered (Guengerich, 1991). Moreover, it should be considered that subgroups of population, express low metabolism of

CYP2E1 as demonstrated in the study conducted by Wu (2013) in which it is presented a systematic analysis of genotype combinations and functional combinations of CYP450 across whole Chinese population: in this study, the authors claim that ultrarapid metabolizer (UM) phenotype did not feature for CYP2E1 (and CYP2C9).

GST Pathway

The GST pathway for DCM metabolism involves conjugation with GSH, forming S-chloromethyl GSH. The conjugation is catalysed by GSTT1 (glutathione S-transferase T1), the most active GSTs isoform (Mainwaring, 1996; Sherratt, 1997). Dose-dependent COHb formation was readily demonstrated, with the single-day exposures resulting in peak COHb saturations of 1.9%, 3.4%, 5.3%, and 6.8%, respectively, at 0, 50, 100, and 200 ppm (DiVincenzo, 1981). Mainwaring (1996) determined mRNA and protein expression of GSTT1 in cells from human liver and lung, both of which are target organs for DCM in the mouse. While expression of GSTT1 was readily detected in the liver, very low levels were detected in the lungs. Furthermore, GSTT1 activity with DCM was measured in three samples of lung: it was about one order of magnitude less than that in human liver. The product is the S-Chloromethyl GSH is reactive and is believed to be one of the DCM metabolites responsible for DNA binding and mutagenicity (Graves, 1996). S-chloromethyl GSH can also be hydrolysed to form hydroxymethyl GSH, which can either decompose to release formaldehyde or be oxidized by formaldehyde dehydrogenase to form S-formyl GSH. The latter is subsequently hydrolysed to release formic acid and GSH. Formic acid further decomposes to release CO_2. Thus, while both the CYP and GST pathways can generate CO_2, only the CYP pathway produces CO from DCM.

Organ-specific metabolism of GSTT1.

GSTT1-1 activity has not been detected in the erythrocytes of mice, rats, cattle, sheep, pigs and rhesus monkeys. However, it is expressed in humans depending on genetic polymorphism. In rats and mice, this enzyme activity has been found in the liver, lungs, and kidneys, and in hamsters in the liver and kidneys. In humans, particularly high levels of the mRNA for GSTT1-1 were found in the liver, kidneys, skeletal muscle and pancreas, moderate levels in the prostate, ovaries, and colon, and moderate to low levels in the heart, brain, and spleen. In the placenta, lungs and thymus, only very low levels of the mRNA for GSTT1-1 were detected.

Another important issue is the subcellular localization and the absolute level of the expressed GSTT-1 enzyme. While GSTT-1 in mouse liver is readily found in cytoplasm and nuclei of hepatocytes, it is found at lower levels in nuclei of bile-duct epithelial cells, and in cytoplasm and nuclei of some human hepatocytes (Sherratt, 2002). This less intense nuclear localization is thought to be of significance for carcinogenic risk because less S-chloromethyl GSH and formaldehyde will be generated near DNA.

The possibility of a switch of the CYP2E1 pathway towards the GSTT1 pathway should be taken into account in case of co-exposure to competitive substances for the MFO pathway and in sub-populations expressing low levels of CYP2E1 (Wu, 2013).

An *in vitro* study (MAK, 2016) highlighted that differences in susceptibility to the genotoxic effects of DCM in the blood cells of persons are associated with different GSTT-1 polymorphisms. This observation was also reported in other two studies (Hallier, 1993; Olvera-Bello, 2010).

In conclusion, the *in vitro* rate constants for the two enzyme systems are consistent with the hypothesis that metabolism of DCM occurs *in vivo* by two competing pathways: a high-affinity saturable pathway (identified as MFO) and a low-affinity first-order pathway (identified as GST). The metabolic rate constants for GST obtained from the studies are also consistent with the hypothesis of Andersen (1987) that production of large quantities of glutathione/DCM conjugates *in vivo* may

increase the frequency with which lung and liver tumours develop in some species of animals (e.g., B6C3F1 mouse).
Both pathways can generate reactive and unstable metabolites, mechanistically linked to DCM-induced genotoxicity and carcinogenesis, but it is thought that these come primarily from the GST pathway (Andersen, 1987). In this work the authos (Andersen, 1987) argue that tumour incidence did not correlate with the amount of DCM metabolized by the CYP450 pathway: consequently, metabolism of DCM by GST appears to be important in carcinogenesis. Moreover, humans are polymorphic for GSTT1, with a proportion of the population showing no activity towards DCM. CYP2E1 catalytic activity predominates at relatively low concentrations of substrate, but there is ample evidence that GST-mediated metabolism eventually predominates at higher concentrations (Gargas, 1986; Clewell, 1995; Bos, 2006). Such higher concentrations of DCM are readily observed in occupational settings and in some environmental exposures. Moreover, with continued exposure to DCM, even at relatively low concentrations, CYP2E1 readily becomes saturated. As reported in the IARC monograph 110 (IARC, 2017), the evidence strongly supports qualitative similarities in both oxidative and GST-mediated metabolism of DCM between humans and rodents. Differences in activity levels and tissue and cellular distributions of GSTT1 and CYP2E1 across species could explain the different target organ for the observed carcinogenicity.

Excretion
Exhalation is the main route of excretion of DCM in humans being its primary metabolites CO_2 and CO, with lesser amounts as DCM excreted in the urine. Only 5% of absorbed DCM is exhaled unchanged, 25–34% excreted converted as CO, and the balance excreted as CO_2. After cessation of exposure, the half-life of DCM in the blood has been estimated to be about 40 minutes, with concentrations of parent and metabolites returning the pre-exposure levels within a few days. Urinary excretion occurs mostly during and/or within the first hour after cessation of exposure, and in total accounts for less than 0.1% of uptake (IARC, 2017).

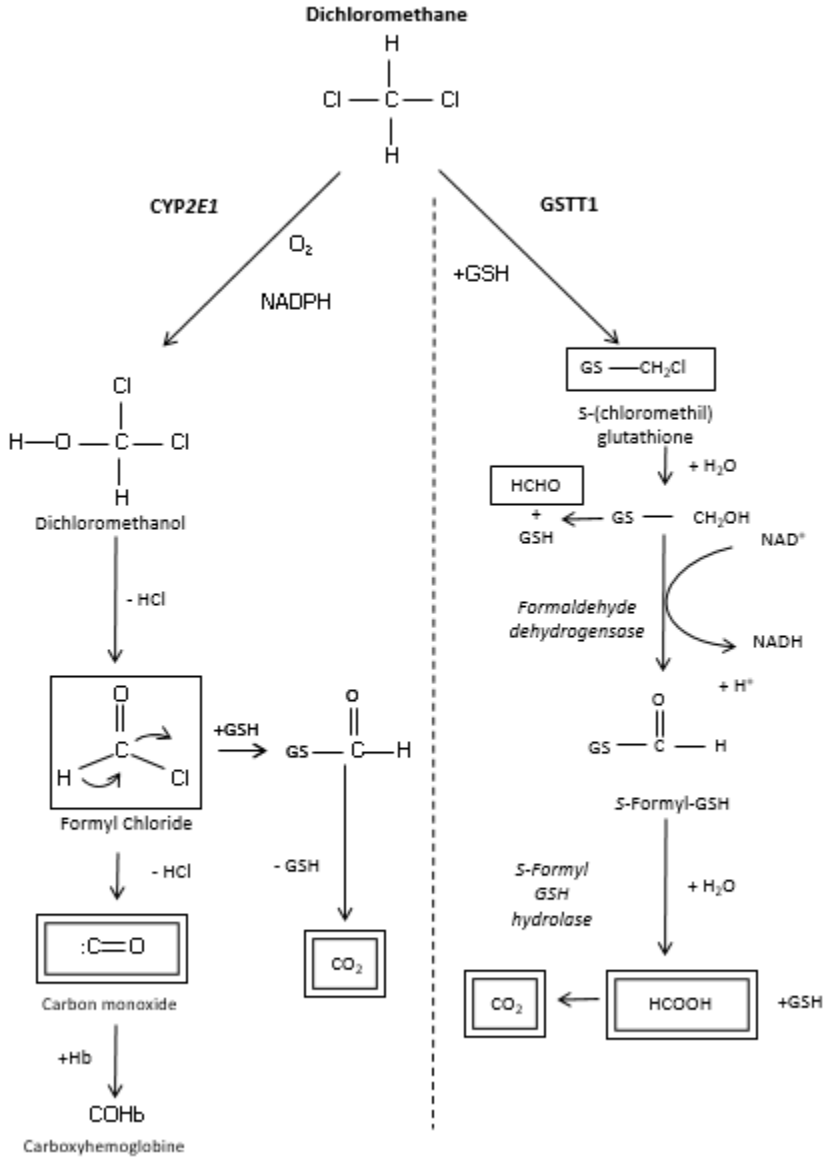

Figure 1. - Proposed pathways for DCM metabolism: CYP2E1-mediated metabolism is shown on the left. GST-mediated metabolism is shown to the right

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Due to its lipophilic properties and low relative molecular mass, DCM can readily cross biological membranes. After inhalation the blood-air partition coefficient measured *in vivo* in humans ranges from 8 to 10. These data might be influenced by GSTT1, enzyme present in the human erythrocytes and involved in the metabolism of DCM. In animals the blood-air partition coefficient measured *in vivo* ranges from 19 to 23 (in rodents).

While there are no quantitative data on oral absorption in humans, in a study is reported an average value of 97% in radioactive expired air as DCM, carbon monoxide (CO), and carbon dioxide (CO_2) in the 24 hours after each repeated oral dose of 50 or 200 mg/kg per day in rats. In the same study, it was reported that the absorption in mice is equally extensive (Angelo, 1986b).

Regarding the permeability of human skin to DCM, is reported the value of 24 g/m^2 (Ursin, 1995).

In humans, once absorbed, DCM enters in circulation and is rapidly distributed to tissues. Due to the lipophilic properties of DCM, the highest concentrations are expected in adipose tissues.
In animals DCM is also rapidly distributed to tissues after *in vivo* and intravenous exposure: DCM has been measured in liver, kidney, lung, and whole carcass. The highest concentration was found in kidney (Angelo, 1986a)
One pathway for metabolism of DCM is a reductive dehalogenation catalysed by cytochrome P450 2E1 (CYP2E1), the MFO pathway. The initial product of the reaction is chloromethanol that spontaneously rearranges to form formyl chloride that, in turn can spontaneously generate CO or react with glutathione (GSH) to generate formylglutathione that rearranges to form CO_2. In this pathway, CO (produced only by this pathway), that has a great affinity for hemoglobin, forms carboxyhemoglobin (COHb).
Another pathway is via conjugation with GSH. The first product of the reaction is S-chloromethyl GSH. The conjugation is catalysed by GSTT1, the most active GSTs isoform (Mainwaring, 1996; Sherratt, 1997). S-Chloromethyl GSH is believed to be one of the DCM metabolites responsible for DNA binding and mutagenicity (Graves, 1996). *S*-chloromethyl GSH can also be hydrolysed to form hydroxymethyl GSH, which can decompose to release formaldehyde or can be oxidized (by formaldehyde dehydrogenase) to form *S*-formyl GSH. By hydroxylation *S*-formyl GSH releases formic acid and GSH. Formic acid further decomposes to release CO_2. Both metabolic pathways of DCM involve polymorphic and variously distributed enzymes in human tissues. The different distribution of these enzymes, particularly GSTT1, plays an important role in the definition of the susceptible populations.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated.

10.2 Acute toxicity - dermal route

Not evaluated.

10.3 Acute toxicity - inhalation route

Not evaluated.

10.4 Skin corrosion/irritation

Not evaluated.

10.5 Serious eye damage/eye irritation

Not evaluated.

10.6 Respiratory sensitisation

Not evaluated.

10.7 Skin sensitisation

Not evaluated.

10.8 Germ cell mutagenicity

Table 9: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
bacterial reverse mutation assay The study was performed before the publication of the OECD TG 471, but whose conduct was compatible with OECD recommendations. TA 102 strain or E.Coli WP2 is missing. Key study	dichloromethane 75-09-2 200-838-9	S. typhimurium, other: TA98, TA100, TA1535, TA1537, TA 1538 (with and without met. act.) Test concentrations: 125, 250, 500, and 750 µl in 9 liter desiccator The lowest effective dose is 18 µg/mL for TA100 and 72 µg/mL for TA98 (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg /mL in atmosphere).	Test results: positive for S. typhimurium: TA98, TA100; in both with and without met. act. genotoxicity: positive cytotoxicity: not specified	Gocke, 1981
Gene mutation in Chinese hamster lung V79 and CHO cells –S9 no OECD TG limits of the study:	dichloromethane 75-09-2 200-838-9,	Test concentrations: 0.5 - 5% 5% is equivalent to 65000 µg/mL	Negative for Chinese hamster lung fibroblasts (V79); met. act.: without S9 genotoxicity: negative cytotoxicity: no cytotoxicity	Jongen, 1981

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
-short exposure -lack of metabolic activation				
Gene mutation, Chinese hamster ovary cells (*hprt* locus) No OECD TG	dichloromethane 75-09-2 200-838-9,	Test concentrations: 0.3 and 0.5% (v/v) equivalent to 3000 and 5000 ppm 5000 ppm is equivalent to 3975 µg/mL Only with mouse liver cytosol (S 100 fraction); GST-mediated metabolism Positive controls :not available Negative control: yes	Positive at the HPRT locus of CHO cells in the presence of metabolic activation (GST-mediated metabolism) genotoxicity: positive cytotoxicity: no	Graves & Green, 1996
Gene mutation mouse lymphoma L5178Y cells, Tk locus equivalent or similar to OECD TG 490	dichloromethane 75-09-2 200-838-9,	Test concentrations: 2000 and 2500 nl/ml without S9 2000, 2500 and 3000 nl/ml with S9 The highest concentration tested 3000 nl/ml is equivalent to 3300 µg/mL Negative control: yes	Genotoxicity: inconclusive results are reported in the study cytotoxicity: no	Myhr, 1990
Chromosome Aberration in CHO-K1 cells equivalent or similar to OECD TG 473	dichloromethane 75-09-2 200-838-9,	Test concentrations: 0-2-5-10 µl/ml (+ and – S9) The highest concentration tested 10µl/ml is equivalent to 6500 µg/mL Positive control substance(s): Cyclophosphamide (1 µg/ml) Triethylenemelamine (50 µg/ml)	Positive +/- S9; met. act.: with and without genotoxicity: positive cytotoxicity: yes negative controls: valid positive controls: valid	Thilagar, 1983
Chromosome Aberration in CHO	dichloromethane 75-09-2 200-838-9,	Test concentration: Doses: 0, 160, 500, 1600 and 5000 µg/mL	Negative +/- S9 met. act.: with and without genotoxicity: negative	Anderson, 1990

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Similar to OECD 473 GL		Maximum concentration tested 5000 µg/mL Positive control substance(s): mytomycin C in trial +S9 cyclophosphamide in trial –S9	cytotoxicity: yes vehicle controls: not applicable negative controls: valid positive controls: valid	
In vitro mammalian micronucleus test with Kinetochore labelling Similar to OECD TG 487	dichloromethane 75-09-2 200-838-9,	In AHH-1, MCL-5 and h2El human lymphoblastoid cell lines The AHH-1 cell line is a human B lymphoblastoid Tk+/- line with native CYP1A1 activity. The MCL-5 cell line was produced by transfection of L3 cells (AHH-1-derived cells which possess elevated CYP1AI activity with cDNAs encoding four human cytochrome P450 isoenzymes and microsomal epoxide hydrolase). The h2El cell line contains the pH441 vector with a cDNA for human CYP2E. Test concentration: from 2 to 10 mM DCM Positive controls: not available Negative control: yes	Positive in MCL-5 and h2El cells (statistically significant dose responses of a similar magnitude, 3-fold at the top dose). Kinetochore staining indicated a similar induction of K+ve and K-ve micronuclei in both cell lines (MCL-5 and h2E1). Positive in MCL-5, h2E1 cell lines, increasing with increasing concentrations. Negative in AHH-1 cultures. These results indicate that the cytochrome P450 pathway may produce both aneugenic and clastogenic metabolites. The lowest effective dose in MCL-5 and h2El cells was 2.5 mM, equivalent to 200 µg/mL. The highest ineffective dose in AHH-1 cultures was 10mM equivalent to 850 µg/mL.	Doherty, 1996

Table 10: Summary table of mutagenicity/genotoxicity tests in mammalian somatic cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus assay Similar to OECD TG 474	dichloromethane 75-09-2 200-838-9,	NMRI mouse bone marrow intraperitoneal injection (i.p.) x2 (0 and 24h) 4 animals (2 male and 2 female) per dose group Tested concentrations: 0, 425, 850 and 1700 mg/kg Mice were sacrificed after 30h and bone marrow smears were prepared for MN test. Positive control: none Negative control: olive oil.	Negative at 1700 mg/kg (the highest ineffective dose) Cytotoxicity: PCE/NCE not present.	Gocke, 1981
Micronucleus assay Similar to OECD TG 474 The study was performed before the publication of the OECD TG.	dichloromethane 75-09-2 200-838-9,	C57BL/6J/Alpk mouse bone marrow by gavage, single dose 5 male and 5 female C57BL/6J/Alpk mice were exposed, Tested concentration: 4000, 2500 and 1250 mg/kg DMC in corn oil. Bone marrow samples were taken 24, 36, 48 and 72 h after dosing The highest dose-level being selected to be the maximum tolerated dose. Positive control: Cyclophosphamide 65 mg/kg Negative control: corn oil	Negative also at 4000 mg/kg (the highest ineffective dose). The incidences of micronucleated polychromatic erythrocytes (MPEs) in test animals at 24, 36, 48 and 72 h after exposure to MC showed no significant increases over control values at any of the dose levels or time points in either sex. Cytotoxicity: The percentage of PCEs was also determined as a measure of cytotoxicity. Reductions in the percentage of PCEs were observed with MC in both male and female mice at dose levels of 2500 and 4000 mg/kg at 24 h after dosing.	Sheldon, 1987
Micronucleus test Similar to OECD TG 474 The study was performed before the publication of the OECD TG.	dichloromethane 75-09-2 200-838-9	Mouse peripheral red blood cells (B6C3F1) Mouse peripheral red blood cells were collected from tail of B6C3F1 mice Five female mice 8-9 weeks old for each group Inhalation 6 hr/d, 5 d/wk, 0, 4000, 8000 ppm 2 weeks Positive control: none Negative control: corn oil	Positive at 4,000 and 8,000 ppm. A dose-related response was observed for MN in PCEs but statistically significant only at the highest dose (8000 ppm). Significant increase in MN in NCEs both at 4000 and 8000 ppm. Cytotoxicity: a significant reduction of % of PCEs was observed at the highest dose (% of PCE in the control was 10.8 and % of PCE 8000 ppm	Allen, 1990

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			was 8.3).	
Micronucleus test Similar to OECD TG 474 The study was performed before the publication of the OECD TG.	dichloromethane 75-09-2 200-838-9	Mouse peripheral red blood cells (B6C3F1) Mouse peripheral red blood cells were collected from tail of B6C3F1 mice Inhalation, 6 hr/d, 5 d/wk, 0, 2000 ppm 12 wks	Positive The % of MN in PCE and NCE in peripheral blood lymphocytes statistically increased at 2000 ppm. Cytotoxicity: no change in the frequency of PCE after exposure. (% of PCE in the control was 4.83 and % of PCE 2000 ppm was 5).	Allen, 1990
Micronucleus test Similar to OECD TG 474	dichloromethane 75-09-2 200-838-9	CD-1 mouse bone marrow Six male mice, 8-10 week-old were used. i.p. 0, 430, 860 and 1720 mg/kg of DCM single dose The highest dose was fixed by the preliminary dose-finding test, and the micronucleus assay was performed at three levels – the highest dose and 1/2 and 1/4 of the highest dose. 1000 immature erythrocytes were analyzed per animal. When the control data were acceptable, the increase in micronucleus frequency against the concurrent negative control data was evaluated using a conditional binomial test. Positive control: mitomycin C, 0.5 mg/kg, single i.p. treatment, sampled at 24 h for bone marrow Negative control: vehicle control.	Negative There was no micronuclei induction in male CD-1 mouse bone marrow cells after single intraperitoneal treatments of up to 1720 mg/kg, which was 80% of the LD_{50} Cytotoxicity: not evaluated.	Morita, 1997
Micronucleus test Similar to OECD TG 474	dichloromethane 75-09-2 200-838-9	Mouse reticulocytes and normochromatic erythrocytes of B6C3F1 mouse 8-10 male mice of eight to ten 8-week-old were used. inh., 6 h/days, 5 days/wk, 6 wk Doses: 400, 800 and 1,600 ppm The frequencies of micronucleated reticulocytes (MN-RETs) and	Negative The MN incidences in RETs and NCEs were not significantly increased by inhalation of DCM (400, 800 and 1,600 ppm). DCM did not display clastogenicity/aneugenicity or adverse effects on hematopoiesis in bone marrow	Suzuki, 2014

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		micronucleated normochromatic erythrocytes (MN-NCEs) were determined in blood specimens collected in week 6 using an Epics XL-MCL flow cytometer, and following the protocol for the in vivo Mouse MicroFlow PLUS Kit. The frequencies of MN-RETs and MN-NCEs were determined by acquisition of about 20,000 RETs and about 1,000,000 NCEs for each animal. Negative control: the control group was exposed to filtered air only. Positive control: none.	cells. Cytotoxicity: no reduction of NCE was reported at any DCM dose.	
Chromosomal aberration Similar to OECD TG 475 The study was performed before the publication of the OECD TG.	dichloromethane 75-09-2 200-838-9	CA in Sprague-Dawley rat bone marrow 5 male rats 8 weeks-old for each group inh. 6 h/day, 5 days/wk, 6 months Doses: 0, 500, 1500, 3500 ppm Bone marrow cells were collected from 5 rats/sex/group for cytogenetic evaluation after 6 months of exposure. Bone marrow samples were processed by conventional techniques and examined for evidence of cytogenetic effects. Negative control: untreated Positive control: none	Negative No increased cytogenetic aberrations were observed in rats exposed to 500, 1500, or 3500 ppm of methylene chloride for 6 months when compared to their respective control groups. Cytotoxicity: no PCE/NCE reported	Burek, 1984
Chromosomal aberration Similar to OECD TG 475 The study was performed before the publication of the OECD GL.	dichloromethane 75-09-2 200-838-9	CA in bone marrow (B6C3F1) Subcutaneous exposure: single dose Doses: 0, 2500 and 5000 mg/kg in corn oil. Treated mice were 8-9 weeks old. Only female were treated. 8 animals were scored for bone marrow CA. 400 cells of each dosage were counted (50 first-division cells for culture). Aberration was categorized by type as deletion or rearrangement events, gaps were not included as aberration data. Positive control: DMBA 2,5 mg/kg	Negative at 2500 and 5000 mg/kg. No significantly increase in the mean of percent aberrant cells were observed at any dose levels (mean of 8 animal at 2500 mg/kg and 7 animals at 5000 mg/kg). Cytotoxicity: Mitotix index (MI) and Replicative index (RI) was calculated, no significant effect was observed.	Allen, 1990

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Negative control: corn oil		
Chromosomal aberration Similar to OECD TG 475 GL The study was performed before the publication of the OECD GL.	dichloromethane 75-09-2 200-838-9	CA in lung and bone marrow cells (B6C3F1) Five female mice 8-9 weeks old for each group Inhalation 6 hr/d, 5 d/wk, 0, 4000, 8000 ppm 2 weeks Positive control: none Negative control: corn oil	Positive only at 8000 ppm in bone marrow Positive at 4000 and 8000 ppm in lung cells. Baseline of CAs in lung is higher than BM, this is characteristic of lung cells in this experimental conditions. A dose-related increase of CA in lung cells was observed both at 4000 and 8000ppm, but was statistically significant only at 8000 ppm. A dose-related increase of CA in BM was observed only at 8000ppm. Cytotoxicity: the MI was not statistically reduced by DCM exposure. Replication Index was statistically depressed only at 8000 ppm in lung cells but at any dose in BM cells.	Allen, 1990
Chromosomal aberration Similar to OECD TG 475 The study was performed before the publication of the OECD TG.	dichloromethane 75-09-2 200-838-9	CA in mice BM (C57BL/6J) Four male mice 3-5-month-old for each group Intraperitoneal, 100, 1000, 1500, 2000 mg/kg Single dose Positive control: Cyclophosphamide 50 mg/kg Negative control: corn oil and untreated mice	Negative for all types of aberrations scored. Animal death occurred at higher doses, leaving two mice for analysis at the 1500 mg/kg dose and only one at the 2000 mg/kg dose. None of the mice at any DCM dose revealed evidence of a significant elevation in either aberrations per cell or percent aberrant cells. Cytotoxicity: Replicative indices appeared to be unaffected by DCM exposure.	Westbrook-Collins, 1990
Gene mutation, Pig-a assay No OECD TG available	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse inh. 6 h/day, 5 days/wk 6 wk Eight to ten 8-week-old male B6C3F1 mice doses: 400, 800 and 1,600 ppm	Negative The Pig-a mutant frequencies in total RBCs was analysed after 3 and 6 weeks after initial inhalation. The mutations induced by the three different doses of DCM (400, 800 and 1,600 ppm) were not statistically different from those in the control at	Suzuki, 2014

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		The selected exposure concentrations were based on a reproductive experiment in the factory B6C3F1 mice were euthanized under anesthesia 18 hours after the last exposure. Blood was collected from each animal in weeks 3 and 6 after inhalation of DCM. A flow cytometer and the EXPO32 analysis software were used for data acquisition. After gating for the single cell population, about 1,000,000 TER-119-positive cells were analyzed to determine the frequency of CD24-negative red blood cells (RBCs). Positive control: mice i.p. administered a single dose of with N-ethyl-N-nitrosourea at 70 mg/kg. Negative control: the control group was exposed to filtered air only	weeks 3 and 6. Positive control showed the Pig-a mutant frequencies at 3 and 6 weeks were significantly higher than those of the control (222 ± 81 × 10−6 at 3 weeks, p=0.0076; 265 ± 312 × 10−6 at 6 weeks, p=0.0102, the Steel test, n=3).	
Gene mutation, transgenic rodent, Similar to OECD TG 488 TG	dichloromethane 75-09-2 200-838-9	Gpt Delta in liver C57BL/6J mouse liver inh. 6 h/day, 5 days/wk, 4 wk 5 8-week-old male gpt Delta C57BL/6J mice were used in each of the three exposure groups and in the control group The mice were euthanized under anaesthesia 7 days after the final inhalation to fix mutation. Doses: 800 ppm, Negative control: the control group was exposed to filtered air only.	Negative For the gpt assay, more than 1,500,000 colonies derived from the rescued phages per liver per mouse were analysed. Mutant frequencies are shown as means ± SD. *p<0.05 vs. control (Dunnett's test). The p-values for the Dunnett's test: DCM800, p=0.999 Weakness of the study: the mutagenicity in the liver was examined at a single concentration.	Suzuki, 2014
Unscheduled DNA synthesis, Similar to OECD TG 482 TG	dichloromethane 75-09-2 200-838-9	A1pk:AP rat Gavage × 1 2-3 animals of 9-13 weeks-old male Alpk:AP rats were used for each treatment and time point. Doses first experiment: 100, 500 mg/kg DCM; Doses second experiment: 500 and 1000 mg/kg DCM Hepatocytes were assessed for UDS via autoradiography 4 (100 and 500	Negative The oral gavage study gave negative results at the 4-hour sampling time, which is considered to be the most appropriate period of exposure for chemicals whose physical form is unlikely to lead to their retention in the gastrointestinal trac. The rats exposed to 1000 mg/kg DCM were also examined for UDS 12 hours	Trueman & Ashby, 1987

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		mg/kg DCM) and 12 hours later (500 and 1000 mg/kg DCM) Negative control: Negative control animals were similarly exposed to the same laboratory air supply used for the test animals. Positive control: animals were treated with 40 mg/kg of 6BT (6-dimethylaminophenylazobenzthiazol).	after dosing, and no evidence of activity was apparent.	
Unscheduled DNA synthesis, Similar to OECD TG 486 The assay was performed before the publication of the TG.	dichloromethane 75-09-2 200-838-9	F344 rat 5-6 Adult 7-8 weeks-old male rats were used for each treatment and time point. Whole body inh., 2h or 6 h doses: 2000 and 4000 ppm of DCM At least 25, but normally 50, morphologically unaltered cells were examined per slide and where possible 3 slides per animal. Negative control: control animals were similarly exposed to the same laboratory air supply used for the test animals. Positive control: animals were treated with diethylnitrosamine (DEN) at 10^{-2} M concentration and tritiated thymidine	Negative The data for the inhalation experiments represent the pooling of two identical studies for rats (1st exp 2000 and 4000 ppm DCM at 2 and 6 hours after exposure) for levels and timing of exposure. DCM failed to induce UDS under the conditions of exposure employed.	Trueman & Ashby, 1987
Unscheduled DNA synthesis,	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse liver 5-6 adult 6 weeks-old male mice were used for each treatment and time point. Whole body inh., 2h or 6 h doses: 2000 and 4000 ppm of DCM At least 25, but normally 50, morphologically unaltered cells were examined per slide and where possible 3 slides per animal. Negative control: control animals were similarly exposed to the same laboratory air supply used for the test animals. Positive control: animals were treated with diethylnitrosamine (DEN) at 10^{-2} M concentration and tritiated thymidine	Negative The data for the inhalation experiments represent the pooling of two identical studies for mice (1st exp 2000 and 4000 ppm DCM at 2 and 6 hour after exposure) for levels and timing of exposure. DCM failed to induce UDS under the conditions of exposure employed.	Trueman & Ashby, 1987

Mechanistic studies *in vitro* and *in vivo*

Table 91: Summary table of mechanistic studies *in vitro* (role of GST or CYP pathway in bacteria)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Bacterial reverse mutation assay in TA 100 The study is not OECD TG 471 compliant	dichloromethane 75-09-2 200-838-9	Mutagenic activity enhanced with rat liver microsomes (CYP metabolism) or cytosolic fraction (GST metabolism). Enzymic system were prepared from 3months-old Wistar rats. The homogenates were centrifuged, and the supernatant and microsomal fraction stored separately. 6-hr exposure in enclosed 37°C system. Doses: 0, 3500, 7000, and 14000 ppm of DCM	Positive +/- S9 in TA 100 Dichloromethane was directly mutagenic in S. typhimurium TA100, mutagenic activity was enhanced by addition of rat liver microsomes or cytosolic fraction (i.e. enhanced metabolism of dichloromethane by CYP and GST, respectively).	Jongen, 1982
Bacterial reverse mutation Assay in TA 100 The study is not OECD TG 471 compliant	dichloromethane 75-09-2 200-838-9	The mutagenic activity was enhanced only when rat liver post-mitochondrial S9 fraction (glutathione conjugation of DCM) was added and not rat liver microsomes. 3-day exposure in sealed jars. Doses 0 up to 84,000 ppm Peak response at 12 h. Exogenous GST or GSH had no effect.	Positive +/- S9 in TA 100 A significant increase in dichloromethane mutagenicity could only be achieved by increasing the concentration of post-mitochondrial supernatant. Under these conditions the increase in mutagenicity was derived solely from glutathione conjugation of dichloromethane.	Green, 1983
Bacterial reverse mutation Assay in TA 100 GSH wt and TA 100 GSH-deficient strain (NG54) The study is not OECD TG 471 compliant	dichloromethane 75-09-2 200-838-9	The NG54 strain was slightly less responsive to dichloromethane exposure, addition of rat liver cytosol marginally increased the mutagenic response to dichloromethane, but addition of GSH had little effect	Positive +/- S9 in TA 100	Dillon, 1992
Bacterial reverse mutation Assay in Salmonella TA1535 strain that had	dichloromethane 75-09-2 200-838-9	This modified strain, showed a positive mutagenic response to dichloromethane that was predominantly (96–100%) due to mutations that were GC→AT transitions. Only 15% of the mutations were	Positive in TA 1535 stain –S9	De Marini, 1997

been modified by the cloning of the rat gene for GSTT1 into its genome The study is not OECD TG 471 compliant		GC→AT transitions in the TA100 strain, a homologue strain that lacks the rat GSTT1 gene.		

Table 12: DNA damage in mammalian systems in *in vitro* studies

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
DNA single-strand breaks, in B6C3F1 mouse hepatocytes	dichloromethane 75-09-2 200-838-9	Maximum concentration tested 34 µg/mL	Positive + S9; –S9 NT	Graves, 1994a
DNA SSB (single strand breaks) in Chinese hamster ovary cells	dichloromethane 75-09-2 200-838-9	Maximum concentration tested 5100 µg/mL	Positive + S9, negative –S9	Graves, 1994a
DNA SSB (single strand breaks) in Chinese hamster ovary cells	dichloromethane 75-09-2 200-838-9	Maximum concentration tested 3975 µg/mL Doses: 0, 0.25, 0.5, 1 mM DCM in the presence of mouse liver S100 fraction (20% v/v).	Positive +/- S9 Stronger effects +S9 Weakness of the study: the results are from a single experiment.	Graves and Green, 1996
DNA–protein cross-links	dichloromethane 75-09-2 200-838-9	in hepatocytes of: • B6C3F1 mouse • F344 rats, • Syrian golden hamsters • human cells (expressing GSTT1)	Positive –S9 (+S9 NT) in mouse hepatocytes at 43 µg/mL Negative –S9 in F344 rats at 425 µg/mL Negative in hamster at 425 µg/mL Negative –S9 in human cells at 425 µg/mL	Casanova, 1997
DNA–protein cross-links, Chinese hamster ovary cells (CHO)	dichloromethane 75-09-2 200-838-9	Maximum concentration tested 3975 µg/mL Doses: 0, 0.25, 0.5, 1 mM DCM in the presence of mouse liver S100 fraction (20% v/v).	Positive +/- S9 Stronger effects +S9 Weakness of the study: the results are from a single experiment.	Graves and Green, 1996
DNA–protein crosslinks, V79 cells	dichloromethane 75-09-2 200-838-9	The highest ineffective concentration was 850 µg/mL (10mM) Doses: 0, 2.5, 5, 10 mM of DCM	Negative - S9	Hu, 2006

DNA–protein cross-link, murine GSTT1 transfected V79 cells	dichloromethane 75-09-2 200-838-9	The lowest effective concentration was at 212 µg/mL (2.5 mM) Doses: 0, 2.5, 5, 10 mM of DCM	Positive - S9 after treatment with proteinase K	Hu, 2006
Single-strand breaks, human primary hepatocytes	dichloromethane 75-09-2 200-838-9	The highest ineffective concentration was tested 5100 µg/mL Doses: 0- 90 mM of DCM	Negative - S9	Graves, 1995
DNA–protein cross-link, human hepatocytes (expressing GSTT1)	dichloromethane 75-09-2 200-838-9	Maximum concentration tested 425 µg/mL Doses: 0-5mM	Negative - S9	Casanova, 1997
DNA damage by comet assay Primary human lung epithelial cells	dichloromethane 75-09-2 200-838-9	10, 100, 1,000 µM	Positive, weak trend, independent of GST activity (GST enzymatic activity not present in the cultured cells)	Landi, 2003

Table 13: DNA damage in mammalian systems in *in vivo* studies

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
DNA single strand breaks by alkaline elution No OECD TG available	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse liver inh., 6h doses: 0, 4831 ppm	Positive	Graves, 1994
DNA single strand breaks, by alkaline elution No OECD TG available	dichloromethane 75-09-2 200-838-9	AP rat liver inh., 6h doses: 0, 4527 ppm	Negative	Graves, 1994
DNA single strand breaks, by alkaline elution No OECD TG available	dichloromethane 75-09-2 200-838-9	CD rat liver Po, 1 administration 1275 µg/mL	Positive	Kitchin & Brown, 1994
DNA single strand breaks, by alkaline elution No OECD TG available	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse liver inh., 6h doses: 0, 2000, 6000 and 8000 ppm an increasing dose-dependent SSBs were observed from 4000 ppm onwards. 5 animals for control group 3-4 animals for each treated group To achieve depletion of reduced GHS in the liver,	Positive Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage	Graves, 1995

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		mice were injected with 1g/Kg BSO (i.p.) immediately before DCM exposure.		
DNA single strand breaks by alkaline elution No OECD TG available	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse lung inh., 3h doses: 0, 1000, 2000 and 4000 ppm, An increasing dose-dependent SSBs were observed from 2000 ppm onwards. To achieve depletion of reduced GHS in the lung, mice were injected with 1g/Kg BSO (i.p.) immediately before DCM exposure.	Positive Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage	Graves, 1995
DNA single strand breaks by alkaline elution No OECD TG available	dichloromethane 75-09-2 200-838-9	AP rat lung inh., 3h doses: 0, 4000 ppm	Negative	Graves, 1995
DNA damage, measured with Comet assay conducted by using the protocol recommended by the Japanese Center for the Validation of Alternative Methods	dichloromethane 75-09-2 200-838-9	male B6C3F1 mouse liver comet assay Eight to ten 8-week-old male B6C3F1 for each group inh., 6h/day, 5 days/wk, 6wk doses:0, 400, 800 and 1600 ppm, For each sample, at least 100 cells were scored. The tail intensity (TI) was measured for each nucleus scored.	Negative	Suzuki, 2014
DNA–protein cross-links	dichloromethane 75-09-2 200-838-9	B6C3F1/CrlBR mouse Liver and lung Groups of three mice were pre-exposed for 2 days (6 hr/day) to 4000 ppm (for four experiments) of unlabelled DCM. On the third day, the animals were exposed for 6 hr to [^{14}C]DCM.	Positive in liver Negative in lung	Casanova, 1992

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		inh., 6 h/day, 3 days doses: 0, 4000 ppm,		
DNA–protein cross-links,	dichloromethane 75-09-2 200-838-9	Syrian hamster, liver and lung one hamster were pre-exposed for 2 days (6 hr/day), On the third day, the animals were exposed for 6 hr to [^{14}C]DCM. inh., 6 h/day, 3 days, doses: 0, 4000 ppm,	Negative in liver and lung	Casanova, 1992
DNA–protein cross-links,	dichloromethane 75-09-2 200-838-9	male B6C3F1/CrlBR mouse Liver Groups of three mice and one hamster or groups of nine mice were preexposed for 2 days (6 hr/day) to selected concentrations of unlabelled DCM. Preexposure concentrations were 144, 491, 1518, 2587 and 4017 ppm. On the third day, the animals were exposed for 6 hr to [^{14}C]DCM at a concentration very similar to that used for the preexposures. Concentrations of [14C]DCM used in the final exposure were: 146, 498, 1553, 2599, and 3923 ppm. inh., 6 h/day, 3 days	Positive A concentration-dependent increase in DPX formation was observed at concentrations ranging from 498 to 3923 ppm.	Casanova, 1996
DNA–protein cross-links,	dichloromethane 75-09-2 200-838-9	Syrian golden hamster, Liver Preexposure concentrations were: 491, 1518 and 4017 ppm. On the third day, the animals were exposed for 6 hr to [^{14}C]DCM at a concentration very similar to that used for the preexposures. Concentrations of [^{14}C]DCM used in the final exposure were: 498, 1553 and 3923 ppm.	Negative DNA-protein cross-links were not detected in the livers of hamsters exposed to the same exposure atmosphere as mice.	Casanova, 1996

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		inh., 6 h/d, 3 days		
Sister-chromatid exchange,	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse lung cells inh., 6 h/day, 5 days/ wk; 12wk doses: 0, 2000 ppm	Positive	Allen, 1990
Sister-chromatid exchange,	dichloromethane 75-09-2 200-838-9	B6C3F1 mouse bone marrow Doses: 0, 2,500, or 5,000 mg/kg DCM in corn oil, sc × 1	Negative	Allen, 1990
Sister-chromatid exchange,	dichloromethane 75-09-2 200-838-9	C57BL/6J mouse bone marrow 4 Male 3-5-month-old C57B1/6J mice for each dose group Doses: 0, 100, 1000, 1500 and 2000 mg/kg (µg/mL), ip × 1	Negative	Westbrook-Collins, 1990

No data are available for germ cell mutagenicity.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Summary of the *in vitro* data

DCM was mutagenic in *S. typhimurium* strains TA 98 and TA 100 with and without metabolic activation, but not in strains TA 1535, 1537, and 1538, in a key study performed before the publication of the OECD 471, but whose conduct was compatible with OECD recommendations (Gocke, 1981). Some in vitro mechanistic studies were conducted with the aim to clarify the role of metabolism in the activation of the formation of the reactive intermediate(s). These studies showed that the mutagenic effect is expressed also when GSTT1 pathway is not prevalent or is even absent (Green, 1983; Jongen, 1982; Gocke, 1981; Jongen, 1978).

The induction of gene mutations was also analysed in mammalian cell systems. No increase in the mutant frequency was found in Chinese hamster epithelial (V79) or ovary (CHO) cells in a HPRT assay after one hour exposure to 0.5 -5% (v/v) DCM without metabolic activation. The reliability of this study was limited by a short exposure time and the lack of metabolic activation (Jongen, 1981). DCM was mutagenic in CHO cells at the *Hprt* locus in one study, in the presence of exogenous metabolic activation (Graves & Green, 1996), and gave equivocal results in the mouse lymphoma $Tk^{+/-}$ assay in another study (Myhr, 1990). DNA sequence analysis of the *Hprt* mutants of CHO cells treated with DCM indicated that 4 out of 8 mutations were GC→AT transitions, two were GC→CG transversions and two AT→TA transversions. This pattern was more similar to that of 1,2-dibromoethane (ethylene dibromide) (IARC,1999) (7 out of 9 being GC→AT transitions) than that of formaldehyde, a metabolite of DCM, that has been identified *in vitro*, for which all mutations were single base transversions and 5 out of 6 arose from AT base pairs (Graves, 1996). The only gene mutation study available in mouse lymphoma L5178Y cells showed ambiguous results (Myhr, 1990).

Chromosomal aberrations were observed in CHO cells in the presence and absence of an exogenous metabolic system (Thilagar & Kumaroo 1983), while negative results were reported in an other study (Anderson, 1990) (see table 9).

Induction of micronuclei was reported in several *in vitro* studies. In a study (Doherty, 1996) micronuclei induced by DCM were both kinetochore-positive and negative, which is an indication of a mixed mechanism, including both aneuploidy and clastogenicity. On the contrary, a prevalence of kinetochore-negative micronuclei (clastogenicity) were reported in human MCL-5 cells that stably express cDNA encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1 and epoxide hydrolase, and in h2E1 cells, which contains a cDNA for CYP2E1. An increased frequency of micronucleus formation was observed in MCL-5 and h2E1 cell lines but not in the parental cell line AHH-1 (only expressing CYP1A1). This study shows that metabolically competent cell lines expressing human cytochrome P450 isoenzymes can metabolize halogenated hydrocarbons, such as DCM to genotoxic species (Doherty, 1996).

Summary of the *in vivo* data

DCM did not induce micronucleus formation *in vivo* in the bone marrow of mice treated by gavage or intraperitoneal injection (Gocke, 1981; Sheldon, 1987; Morita, 1997). Mice treated with DCM trough inhalation at 2000 ppm (6940 mg/m^3) for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of micronuclei in peripheral blood erythrocytes (Allen, 1990). The highest dose tested (8000 ppm, 6 hours per day, 5 days per week, for 2 weeks) gave positive results in erythrocytes and lung cells, but negative results in bone marrow. On the other hand, DCM did not cause micronucleus formation in male B6C3F1 mice exposed at 400, 800 and 1600 ppm by inhalation for 6 weeks (6 hours per day, 5 days per week) (Suzuki, 2014).

DCM did not cause chromosomal aberration *in vivo* in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins, 1990; Allen, 1990). A small increase in the frequency of chromosomal aberration in mouse bone marrow and lung cells was reported after exposure to DCM at 8000 ppm by inhalation for 6 hours per day, 5 days per week, for 2 weeks (Allen, 1990). Negative results were also reported in an assay for chromosomal aberration in rat bone marrow (Burek, 1984). No gene mutations were observed in the following two experiments after inhalation exposure to DCM: a Pig-a assay in the erythrocytes of peripheral blood of male B6C3F1 mice exposed to DCM at 400, 800, or 1600 ppm for 6 weeks (6 hours per day, 5 days per week); and a transgenic rodent gene mutation assay on Gpt Delta C57BL/6J mice treated for 4 weeks (6 hours per day, 5 days per week) with DCM at 800 ppm (Suzuki, 2014) where liver cells were analysed.
DCM did not induce unscheduled DNA synthesis *in vivo* in Fischer 344 rats treated by gavage or inhalation, or in B6C3F1 mouse hepatocytes treated by inhalation (Trueman & Ashby, 1987).

Mechanistic studies *in vitro* and *in vivo*

Two major metabolic pathways for the metabolism of DCM have been characterized in humans and experimental animals (as reported in the Toxicokinetic section). One pathway is CYP2E1-mediated reductive dehalogenation, which ultimately generates CO and CO_2 as stable end products. One of the intermediates, formyl chloride, can react with nucleophiles. GSH conjugation, catalysed primarily by GSTT1, is another important metabolic pathway of DCM, resulting in the formation of reactive metabolites, including formaldehyde and S-chloromethyl GSH.
The relationship between the metabolism (CYP and GST pathways) of DCM and mutagenicity has been examined in several studies with various assays for bacterial mutation as also reported in the IARC monograph 110 (IARC, 2017). In a study (Jongen, 1982), for example, it is showed that while DCM was directly mutagenic in *S. typhimurium* TA100, the mutagenicity was enhanced by addition of rat liver microsomes or cytosolic fraction. This implicates enhanced metabolism of DCM by CYP and GST, respectively. In contrast, in another study (Green, 1983) the mutagenicity of DCM was tested in the same *S. typhimurium* strain and an increase in mutagenic activity was observed only

when rat liver post-mitochondrial S9 fraction was added, but not when rat liver microsomes were used.

In summary, the observed *in vitro* mutagenicity of DCM cannot be univocally attributed to a specific metabolic pathway (Jongen, 1982; Green, 1983; Dillon, 1992; De Marini, 1997, see table 11).

DCM was also tested for its ability to induce DNA damage measured by comet assay *in vitro* (see table 12). The frequency of DNA single-strand breaks was increased in mice B6C3F1 hepatocytes without metabolic activation (Graves, 1994) and in CHO cells cultured with DCM in the presence, but not in the absence, of an exogenous metabolic activation system (Graves, 1994). In the Graves and Green study, the effects were stronger with metabolic activation. Conversely, DNA single-strand breaks were not induced in Syrian hamster hepatocytes (Graves, 1995).

DCM induced DNA–protein cross-links *in vitro* in hepatocytes of male B6C3F1 mice, but not in hepatocytes of Fischer 344 rats or Syrian hamsters (Casanova, 1997). DNA–protein cross-links were also induced in CHO cells exposed to DCM with or without exogenous metabolic activation, with DNA damage being greater in the presence of metabolic activation (Graves & Green, 1996). A standard and proteinase K-modified comet assay to measure DNA damage and DNA–protein crosslinks in V79 cells transfected with the murine GSTT1 gene (V79 mGSTT1) and in parental V79 cells is also available. DCM induced DNA damage in both cell types. However, the study showed the presence of DCM-induced DNA–protein crosslinks in the V79 mGSTT1 cell line and not in standard V79 cell line, which indicates that the induction of DNA–protein crosslinks is associated to GSTT1 pathway (Hu, 2006).

Genotoxicity data are also available in human cells. DCM did not induce DNA single strand breaks in human primary hepatocytes (Graves, 1995); no induction of DNA–protein cross-links *in vitro* was observed in human hepatocytes with functional GSTT1 genes after treatment with DCM (Casanova, 1997).
The induction of SCEs was investigated in a study conducted in human peripheral blood lymphocyte cultures, showing a role of GSTT1 (Landi; 2003).
In addition, several studies to detect DNA damage also *in vivo* are available for DCM.
DNA–protein cross-links were induced *in vivo* in the liver, but not in the lung of B6C3F1/CrlBR mice exposed trough inhalation to DCM (Casanova, 1992). No DNA–protein cross-links were detected in Syrian hamster liver or lung after inhalation of DCM (Casanova, 1992). DNA–protein cross-links were not induced in the liver of Syrian golden hamsters but were observed in the liver of B6C3F1/CrlBR mice treated with DCM by inhalation (Casanova, 1996).
In a study *in vivo*, mice treated with DCM at 2000 ppm [6940 mg/m^3] for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of SCEs in lung cells (Allen, 1990). Exposure to higher concentrations (8000 ppm -27800 mg/m^3- for 2 weeks) also induced an increase in the frequency of SCE in peripheral blood erythrocytes. DCM did not induce SCE in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins, 1990; Allen, 1990).

10.8.2 Comparison with the CLP criteria

Table 14: Results of *in vitro* and *in vivo* mutagenicity data in comparison to the CLP criteria

Toxicological results	**CLP criteria**
No evidence is available in human. Thus, a classification category 1A is not appropriate for DCM.	*The classification in Category 1A is based on positive evidence from human epidemiological studies.*
Testing *in vitro*: Bacterial mutation assays: positive Tests involving mammalian cells: negative for gene mutation; positive for clastogenicity, preferentially liked to GST-mediated metabolism although a role of P450-mediated metabolism cannot be excluded (Casanova, 1997), positive MN and SCE results were reported in human cell lines or isolated cells, in particular in one study, the extent of SCE was greater in cells from individuals without GST activity (Hallier, 1993), in another study, by contrast, the extent of SCE was greater in cells from individuals with high GSTT1 activity (Olvera-Bello, 2010); DNA damage measured as DNA-protein crosslinks, SSBs and UDS gave negative results. **Testing *in vivo* (experiments in mammals):** In somatic cells (MN assays): - DCM was not able to induce MN *in vivo* in bone marrow. - Positive results were reported at high concentration in MN *in vivo* in erythrocytes and lung cells, after treatment via several routes of exposure (oral, inhalation) (Allen, 1990). - Negative results were also reported in chromosomal aberration in BM after ip administration or subcutaneous injection in mice and in rat (Westbrook-Collins, 1990; Allen, 1990; Burek, 1984). - A small increase in the frequency of chromosomal aberration in mouse bone marrow and lung cells was reported after exposure to DCM at 8000 ppm by inhalation for 6 hours per day, 5 days per week, for 2 weeks (Allen, 1990). - No gene mutations were observed in the following two experiments after inhalation exposure to DCM: a Pig-a assay in the erythrocytes of peripheral blood of male B6C3F1 mice exposed to DCM at 400, 800, or 1600 ppm for 6 weeks (6 hours per day, 5 days per week); and a transgenic rodent gene mutation assay on Gpt Delta C57BL/6J mice treated for 4 weeks (6 hours per day, 5 days per week) with DCM at 800 ppm (Suzuki, 2014) where liver cells were analysed. - The UDS *in vivo* in Fischer 344 rats treated by gavage or inhalation, and in B6C3F1 mouse hepatocytes treated by inhalation (Trueman & Ashby, 1987) after DCM treatment were also negative.	*The classification in Category 1B 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.*

Due to the absence of studies showing positive results in germ cells, the classification as Muta cat 1B is not appropriate for DCM.	
Mechanistic studies As reported in the mechanistic studies, the GST or CYP metabolism mediated pathways could affect differently the genotoxicity through species. In general, in the *in vivo* genotoxicity studies the strongest responses were observed in mouse lung and liver tissues with the greatest rates of GST metabolism and the highest susceptibility to DCM-induced tumours. The available data demonstrated a clear correlation between the observed genotoxicity *in vitro* and *in vivo* and the activity of GST pathway, but a role of P450 metabolic pathway in the induction of genotoxic effects cannot be ruled out. Moreover, it is important to note that, as reported in a study (Crebelli, 1999), the halogenated hydrocarbons (such as DCM) are not very effective in inducing micronucleus formation in mouse bone marrow, therefore a negative bone marrow micronucleus assay is not sufficient to rule out the concern raised by the consistently positive *in vitro* results. In conclusion, the available data show evidence of genotoxicity both *in vitro* and *in vivo*. In particular, it is noted that the effects observed *in vivo* were in association with metabolic pathway operative also in humans. Thus, based on these results, the classification mutagen category 2 is considered appropriate for DCM.	*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.* *Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.*

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

DCM has been assessed for genotoxicity in a variety of *in vitro* assays in bacterial and mammalian cells. DCM induces gene mutations in bacteria, but not in mammalian cells *in vitro*. Evidence of clastogenicity *in vitro* was reported. This evidence was preferentially linked to GST-mediated metabolism, although a role of P450 mediated metabolism cannot be excluded (Casanova, 1997). In human cell lines or cells isolated *ex vivo* DCM induced micronucleus formation and SCEs (Hallier, 1993; Doherty, 1996; Olvera-Bello, 2010), while studies on DNA–protein cross-links, DNA single-strand binding proteins (SSBs), and unscheduled DNA synthesis gave negative results (Jongen, 1981; Graves, 1995; Casanova, 1997). In one study, the extent of SCEs was greater in cells from individuals without GST activity (Hallier, 1993). In another study, by contrast, the extent of SCEs was greater in cells from individuals with high GSTT1 activity (Olvera-Bello, 2010).
DCM was also tested in several *in vivo* studies. DCM was not able to induce MN *in vivo* in bone marrow. Positive results were reported at high concentration in erythrocytes and lung cells, after treatment *via* several routes of exposure (oral, inhalation) (Allen, 1990). Moreover, it is important to note that, as reported in a study (Crebelli, 1999), the halogenated hydrocarbons (such as DCM) are not very effective in inducing micronucleus formation in mouse bone marrow, therefore a negative

bone marrow micronucleus assay is not sufficient to rule out the concern raised by the consistently positive *in vitro* results.
As reported in the mechanistic studies, the GST or CYP metabolism mediated pathway could affect differently the genotoxicity trough species. In general, in the *in vivo* genotoxicity studies the strongest responses were observed in mouse lung and liver tissues with the greatest rates of GST metabolism and the highest susceptibility to DCM-induced tumours.
The available data demonstrated a clear correlation between the observed genotoxicity *in vitro* and *in vivo* and the activity of GST pathway, but a role of P450 metabolic pathway in the induction of genotoxic effects cannot be ruled out.

Altogether, the available data show evidence of genotoxicity both *in vitro* and *in vivo*. In particular, it is noted that the effects observed *in vivo* were in association with metabolic pathway operative also in humans. Then, a classification as mutagen category 2, H341 is warranted.

10.9 Carcinogenicity

Table 15: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Carcinogenicity study mouse (B6C3F1) male/female

50 male/50 female per dose group
Age: 7 weeks

Equivalent or similar to OECD TG 451

Reliability 2 with restrictions (Klimisch score) supporting study, experimental study</td>
<td>dichloromethane

75-09-2;200-838-9

Purity 99%

oral: drinking water

Doses/Concentrations: 0, 5, 50, 125, 185, 250 (recovery, 18 months exposure), Basis: nominal in water

Vehicle: water

Doses: 0, 60, 125, 185, 250 mg/kg/bw/day (recovery, 18 months exposure)

Basis:

nominal conc.

Doses/Concentrations: 0, 61, 124, 177, 234 mg/kg bw/day (males) Basis: actual ingested

Doses/Concentrations: 0, 59, 118, 172, 238 mg/kg bw/day (females) Basis: actual ingested

Exposure: 104 weeks (daily)</td>
<td>Males:
An increased incidence of hepatocellular carcinoma at the highest dose compared with the first control group was observed. All tumours were in the range of the historical control.

<table>
<tr><th>Doses (mg/kg bw)</th><th>0 (1st)</th><th>0 (2nd)</th><th>60</th><th>125</th><th>185</th><th>250</th></tr>
<tr><td>Hepatocellular adenoma (%)</td><td>6/60 (10)</td><td>4/65 (6)</td><td>20/200 (10)</td><td>14/100 (14)</td><td>14/99 (14)</td><td>15/125 (12)</td></tr>
<tr><td>Hepatocellular carcinoma (%)</td><td>5/60 (8)</td><td>9/65 (14)</td><td>33/200 (17)</td><td>18/100 (18)</td><td>17/99 (17)</td><td>23/125 (18)*</td></tr>
<tr><td>Combined (%)</td><td>11/60 (18)</td><td>13/65 (20)</td><td>51/200 (26)</td><td>30/100 (30)</td><td>31/99 (31)</td><td>35/125 (28)</td></tr>
</table>
*p=0.0114 (250 mg/kg vs control 1)

No significant exposure related trend in survival was found in males.

Females:
Hepatocellular adenoma or hepatocellular adenocarcinoma significantly increased, but in the range of historical controls.
Significant trend towards longer survival was observed in females.

Historical controls for hepatocellular adenoma or carcinoma (combined): mean, 32.1%; range, 7–58%</td>
<td>Serota, 1986</td>
</tr>
<tr>
<td>Carcinogenicity study in mice (Swiss)

Male/female

Age: 9 weeks

50 or 60 mice/ group</td>
<td>dichloromethane

75-09-2

200-838-9

Purity 99%

oral: gavage</td>
<td>Males:
Pulmonary adenomas or adenocarcinomas (combined) in mice that died at 78 weeks: 1/14 (7%), 4/21 (19%), 7/24 (29%)*
Pulmonary adenomas or adenocarcinomas (combined) at end of experiment: 5/50 (10%),5/50 (10%), 9/50 (18%).
*p<0.05
Excess mortality (P < 0.01) was observed in male mice exposed to the lowest and highest dose.</td>
<td>Maltoni, 1988</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Equivalent to carcinogenicity test (lifetime)

Reliability 3 (Klimisch score) supporting study, experimental study</td><td>Doses/concentration: 0, 100 or 500 mg/kg bw/day in olive oil by gavage

Vehicle: olive oil

Groups of 60 male and 60 female mice

Exposure: once per day, for 4 or 5 days per week, for 64 weeks (daily).

Kept under observation for lifespan</td><td>Females:
No treatment-related increase in the incidence of any tumour type in females.

Excess mortality was observed in female mice exposed at lowest and highest dose.

Limits:

Due to an excess of mortality, at the highest dose the time of exposure was only 64 weeks and the study was interrupted at 78 weeks (instead of 104).</td><td></td></tr>
<tr><td>Carcinogenicity study in mice (B6C3F1)

Age: 8-9 weeks old

male/female

Groups of 50 male and 50 female

Equivalent or similar to OECD TG 451

Reliability 2; key study</td><td>dichloromethane
75-09-2
200-838-9

Purity 99%

inhalation: vapour (whole body)

Doses/Concentrations: 0, 2000, and 4000 ppm

Doses/Concentrations: 0, 2009, and 3982 ppm (analytical conc.)

Vehicle: no vehicle

Exposure: 102 weeks (6 h/d, 5 d/w)</td><td>Males:
<table><tr><th>Concentration (ppm)</th><th>0</th><th>2000</th><th>4000</th></tr><tr><td>Bronchiolo-alveolar adenoma (%)</td><td>3/50 (6%)*</td><td>19/50 (38%)**</td><td>24/50 (48%)**</td></tr><tr><td>Bronchiolo-alveolar carcinoma (%)</td><td>2/50 (4%)*</td><td>10/50 (20%)***</td><td>28/50 (56%)**</td></tr><tr><td>Hepatocellular adenoma (%)</td><td>10/50 (20%)</td><td>14/49 (29%)</td><td>14/49 (29%)</td></tr><tr><td>Hepatocellular carcinoma (%)</td><td>13/50 (26%)</td><td>15/49 (31%)</td><td>26/49 (53%)***</td></tr><tr><td>Hepatocellular adenoma or carcinoma (Combined) (%)</td><td>22/50 (44%)*</td><td>24/49 (49%)</td><td>33/49 (67%)***</td></tr></table>*P < 0.001 (trend)[a]
**P < 0.001
***P < 0.05
[a]Incidental tumour test
Females:
<table><tr><th>Concentration (ppm)</th><th>0</th><th>2000</th><th>4000</th></tr><tr><td>Bronchiolo-alveolar adenoma (%)</td><td>2/50 (4%)*</td><td>23/48 (48%)**</td><td>28/48 (58%)***</td></tr><tr><td>Bronchiolo-alveolar carcinoma (%)</td><td>1/50 (2%)*</td><td>13/48 (26%)**</td><td>29/48 (58%)**</td></tr><tr><td>Hepatocellular adenoma (%)</td><td>2/50 (4%)*</td><td>6/48 (13%)</td><td>22/48 (46%)**</td></tr><tr><td>Hepatocellular carcinoma(%)</td><td>1/50 (2%)*</td><td>11/48 (23%)***</td><td>32/48 (67%)**</td></tr><tr><td>Hepatocellular adenoma or carcinoma (Combined) (%)</td><td>3/50 (6%)*</td><td>16/48 (33%)***</td><td>40/48 (83%)**</td></tr></table>*P < 0.001 (trend)[a]
**P < 0.001
***P < 0.04
[a]Incidental tumour test
Survival of male and female mice exposed to methylene chloride was reduced during the second year of the studies.</td><td>NTP, 1986
Mennear, 1988</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Carcinogenicity study in mouse: B6C3F$_1$

Age: 8-9 weeks old

Female

68 mice/group

inhalation: vapour (whole body)

Equivalent or similar to OECD TG 451

Reliability 2; key study</td>
<td>dichloromethane

75-09-2

200-838-9

Purity 99%

Inhalation:

6 h/days, 5 days/wk:

0 ppm for 104 wk

2000 ppm 26 wk/0 ppm 78 wk

0 ppm 78 wk/2000 ppm 26 wk

2000 ppm, 52 wk/0 ppm, 52 wk

0 ppm, 52 wk/2000 ppm, 52 wk

2000 ppm, 78 wk/0 ppm, 26 wk

0 ppm, 26 wk/2000 ppm, 78 wk

2000 ppm, 104 wk</td>
<td>Females
<table>
<tr><th rowspan="2">Concentration/ time of exposure</th><th colspan="3">Lung effects</th></tr>
<tr><th>Bronchiolo-alveolar adenoma</th><th>Bronchiolo-alveolar carcinoma</th><th>Combined</th></tr>
<tr><td>0 ppm for 104 wk</td><td>1/67 (1%)</td><td>4/67 (6%)</td><td>5/67 (7%)</td></tr>
<tr><td>2000 ppm 26 wk/0 ppm 78 wk</td><td>8/68(12%)</td><td>17/68 (25%)</td><td>21/68 (31%)*</td></tr>
<tr><td>0 ppm 78wk/2000 ppm 26 wk</td><td>0/67</td><td>3/67 (4%)</td><td>3/67 (4%)</td></tr>
<tr><td>2000 ppm 52 wk/0 ppm 52 wk</td><td>12/63 (19%)</td><td>36/63 (57%)</td><td>40/63 (63%)*</td></tr>
<tr><td>0 ppm 52 wk/2000 ppm 52 wk</td><td>5/67 (7%)</td><td>6/67 (9%)</td><td>10/67 (15%)</td></tr>
<tr><td>2000 ppm 78 wk/0 ppm 26 wk</td><td>19/68 (28%)</td><td>25/68 (37%),</td><td>38/68 (56%)*</td></tr>
<tr><td>0 ppm 26 wk/2000 ppm 78 wk</td><td>7/67 (10%)</td><td>7/67 (10%),</td><td>13/67 (19%)**</td></tr>
<tr><td>2000 ppm 104 wk</td><td>18/67 (27%)</td><td>31/67 (46%)</td><td>42/67 (63%)*</td></tr>
</table>
<table>
<tr><th rowspan="2">Concentration/ time of exposure</th><th colspan="3">Liver effects</th></tr>
<tr><th>Hepatocellular adenoma</th><th>Hepatocellular carcinoma</th><th>Combined</th></tr>
<tr><td>0 ppm for 104 wk</td><td>8/67 (12%)</td><td>11/67 (16%)</td><td>18/67 (27%)</td></tr>
<tr><td>2000 ppm 26 wk/0 ppm 78 wk</td><td>16/68 (24%)</td><td>14/67 (21%)</td><td>27/67 (40%)</td></tr>
<tr><td>0 ppm 78wk/2000 ppm 26 wk</td><td>16/67 (24%)</td><td>13/67 (19%)</td><td>23/67 (34%)</td></tr>
<tr><td>2000 ppm 52 wk/0 ppm 52 wk</td><td>14/64 (22%)</td><td>18/64 (28%)</td><td>28/64 (44%)**</td></tr>
<tr><td>0 ppm 52 wk/2000 ppm 52 wk</td><td>9/67 (13%)</td><td>12/67 (18%)</td><td>21/67 (31%)</td></tr>
<tr><td>2000 ppm 78 wk/0 ppm 26 wk</td><td>28/68 (41%)</td><td>25/68 (37%)</td><td>42/68 (62%)*</td></tr>
<tr><td>0 ppm 26 wk/2000 ppm 78 wk</td><td>17/67 (25%)</td><td>20/67 (30%)</td><td>32/67 (48%)**</td></tr>
<tr><td>2000 ppm 104 wk</td><td>24/68 (35%)</td><td>35/68 (51%)</td><td>47/68 (69%)*</td></tr>
</table>
*P < 0.01[b]
**P < 0.05
b Likelihood ratio score test
Statistical analysis applied to combined incidence only.</td>
<td>Kari, 1993</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>Reduced latency of liver and lung carcinomas is reported in the table below:
<table>
<tr><th rowspan="2">weeks of exposure</th><th colspan="4">Carcinomas incidence</th></tr>
<tr><th colspan="2">Lung</th><th colspan="2">Liver</th></tr>
<tr><th>Treated</th><th>Control</th><th>Treated</th><th>control</th></tr>
<tr><td>13</td><td>0/20</td><td>0/10</td><td>0/10</td><td>0/10</td></tr>
<tr><td>26</td><td>0/20</td><td>0/10</td><td>0/20</td><td>0/10</td></tr>
<tr><td>52</td><td>1/20</td><td>0/10</td><td>6/20</td><td>0/10</td></tr>
<tr><td>68</td><td>2/20</td><td>0/11</td><td>4/26</td><td>0/11</td></tr>
<tr><td>75</td><td>3/20</td><td>1/10</td><td>9/20</td><td>0/10</td></tr>
<tr><td>78</td><td>4/19</td><td>1/10</td><td>7/19</td><td>0/10</td></tr>
<tr><td>83</td><td>4/20</td><td>2/10</td><td>6/20</td><td>1/10</td></tr>
<tr><td>91</td><td>8/30</td><td>0/15</td><td>10/30</td><td>1/15</td></tr>
</table>
The numbers in bold indicate the reduced latency for lung and liver tumour in mice. The first observation of tumour occurrence in lung was at 52 weeks compared to 75 weeks reported in the control; also in the liver, the first tumour appeared at 52 weeks compared to 83 weeks reported in the control mice.

Survival was reduced compared with controls in groups exposed to DCM for 52, 78, or 104 weeks.</td><td></td></tr>
<tr><td>Carcinogenicity study in mouse: Crj:BDF1

Age: 8-9 weeks old

Males and females

50 mice/group

Equivalent or similar to OECD Guideline 451

Reliability 2, key study</td><td>dichloromethane

75-09-2

200-838-9

Purity 99%

Doses/Concentrations: 0, 1000, 2000, and 4000 ppm

Inhalation

Exposure: 104 weeks (6 h/d, 5 d/w)</td><td>Males
<table>
<tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr>
<tr><th>0</th><th>1000</th><th>2000</th><th>4000</th></tr>
<tr><td>Bronchiolo-alveolar adenoma (%)</td><td>7/50 (14%)*</td><td>3/50 (6%)</td><td>4/50 (8%)</td><td>14/50 (28%)</td></tr>
<tr><td>Bronchiolo-alveolar carcinoma (%)</td><td>1/50 (2%)*</td><td>14/50 (28%)**</td><td>22/50 (44%)**</td><td>39/50 (78%)**</td></tr>
<tr><td>Bronchiolo-alveolar adenoma or carcinoma (Combined)</td><td>8/50 (16%)*</td><td>17/50 (34%)***</td><td>26/50 (52%)**</td><td>42/50 (84%)**</td></tr>
<tr><td>Hepatocellular adenoma (%)</td><td>10/50 (20%)*</td><td>13/50 (26%)</td><td>14/50 (28%)</td><td>15/50 (30%)</td></tr>
<tr><td>Hepatocellular carcinoma(%)</td><td>10/50 (20%)*</td><td>9/50 (18%)</td><td>14/50 (28%)</td><td>20/50 (40%)***</td></tr>
<tr><td>Hepatocellular adenoma or carcinoma (Combined) (%)</td><td>15/50 (30%)*</td><td>20/50 (40%)</td><td>25/50 (50%)***</td><td>29/50 (58%)***</td></tr>
<tr><td>Liver haemangioma (%)</td><td>0/50</td><td>4/50 (8%)</td><td>3/50 (6%)</td><td>5/50 (10%)***</td></tr>
<tr><td>Adrenal gland pheochromocytoma (%)</td><td>1/50 (2%)****</td><td>0/50</td><td>1/50 (2%)</td><td>3/50 (6%)</td></tr>
<tr><td>Haemangioma (all organs) (%)</td><td>1/50 (2%)****</td><td>5/50 (10%)</td><td>6/50 (12%)</td><td>7/50 (14%)***</td></tr>
</table>
*P < 0.001 (trend)c
**P < 0.001
***P < 0.05</td><td>JBRC, 2000a, Aiso, 2014</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>****P < 0.05 (trend)
Survival: survival rates in males exposed to 2000 and 4000 ppm were decreased (76%, 70%, 52% and 40 respectively at 0, 1000, 2000 and 4000 ppm, no statistical analysis reported).

Females
<table>
<tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr>
<tr><th>0</th><th>1000</th><th>2000</th><th>4000</th></tr>
<tr><td>Bronchiolo-alveolar adenoma (%)</td><td>2/50 (4%)</td><td>4/50 (8%)</td><td>5/49 (10%)</td><td>12/50 (24%)**</td></tr>
<tr><td>Bronchiolo-alveolar carcinoma (%)</td><td>3/50 (6%)*</td><td>1/50 (2%),</td><td>8/49 (16%),</td><td>20/50 (40%)**</td></tr>
<tr><td>Bronchiolo-alveolar adenoma or carcinoma (Combined)</td><td>5/50 (10%)*</td><td>5/50 (12%)</td><td>12/49 (24%)***</td><td>30/50 (60%)**</td></tr>
<tr><td>Hepatocellular adenoma (%)</td><td>1/50 (2%)*</td><td>7/49 (9%)***</td><td>4/49 (8%)</td><td>16/50 (32%)**</td></tr>
<tr><td>Hepatocellular carcinoma(%)</td><td>1/50 (2%)*</td><td>1/49 (2%)</td><td>5/49 (10%)</td><td>19/50 (38%)**</td></tr>
<tr><td>Hepatocellular adenoma or carcinoma (Combined) (%)</td><td>2/50 (4%)*</td><td>8/49 (16%)***</td><td>9/49 (18%)***</td><td>30/50 (60%)**</td></tr>
<tr><td>Liver haemangioma or heamangiosarcoma (combined) (%)</td><td>3/50 (6%)****</td><td>2/49 (4%),</td><td>0/49</td><td>7/50 (14%)</td></tr>
</table>
*P < 0.001 (trend)c
**P < 0.001
***P < 0.05
****P < 0.01 (trend)
Note: the trend test for Liver haemangioma or heamangiosarcoma is statistically significant (P < 0.01).

Survival: survival rates in females exposed to 2000 and 4000 ppm were decreased (52 %, 52%, 34% and 42% respectively at 0, 1000, 2000 and 4000 ppm, no statistical analysis reported).</td><td></td></tr>
<tr><td>Carcinogenicity study in mice strain A

Males

Age: 6–8 weeks

20 or 50 mice/group

Reliability 4</td><td>dichloromethane

75-09-2

200-838-9

Purity >95%

Doses/Concentrations: 0, 160, 400 and 800 mg/kg bw

Intraperitoneally injection

Exposure: 3x wk; 24, 17, 17 or 16 times</td><td>Multiplicity of bronchiolo-alveolar tumours: 0.27, 0.94, 0.80, 0.50, Not Statistically significant.

No tumour incidence provided.

Histopathological examination of the lung only. Full histopathology not performed.

Survival: 47/50, 18/20, 5/20, 12/20.</td><td>Theiss, 1977</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Carcinogenicity study in rat (Fischer 344)

male/female

25–85 male and female Fischer 344 rats

age:7 weeks

Exposure: 104 weeks (daily) according to OECD TG 451

Reliability 2, key study</td>
<td>dichloromethane

75-09-2

200-838-9
Purity 99%

oral: drinking water

Doses/Concentrations: 0, 0, 5, 50, 125, 250 (highest dose) 250 (recovery group 18 months exposure)
Basis: nominal conc. in water

Doses /Concentrations: 0, 6, 52, 125, 235, 232 (recovery, 18 months exposure) mg/kg bw/day (males)
Basis: actual ingested

Doses / Concentrations: 0, 6, 58, 136, 263, 269 (recovery, 18 months exposure) mg/kg bw/day (females)
Basis: actual ingested

Interim terminations were carried out at 26, 52, and 78 weeks in control group 1 and in the groups at the lowest, intermediate, and highest dose, such that 50 males and 50 females per group received</td>
<td>Survival: No significant exposure related trend in survival was found in males and females.

Males:
<table>
<tr><th rowspan="2">Concentration mg/kg bw</th><th colspan="3">Liver effects</th></tr>
<tr><th>Hepatocellular adenoma</th><th>Hepatocellular carcinoma</th><th>Combined</th></tr>
<tr><td>0 (control 1)</td><td>4/85 (5%)</td><td>2/85 (2%)</td><td>6/85 (7%)</td></tr>
<tr><td>0 (control 2)</td><td>5/50 (10%)</td><td>2/50 (4%)</td><td>7/50 (14%)</td></tr>
<tr><td>5</td><td>2/85 (2%)</td><td>0/85</td><td>2/85 (2%)</td></tr>
<tr><td>50</td><td>3/84 (3%)</td><td>0/84</td><td>3/84 (3%)</td></tr>
<tr><td>125</td><td>3/85 (3%)</td><td>1/85 (1%)</td><td>3/85 (3%)</td></tr>
<tr><td>250 (highest dose)</td><td>1/85 (1%)</td><td>0/25</td><td>2/85 (2%)</td></tr>
<tr><td>250 (recovery dose)</td><td>4/25 (16%)</td><td></td><td>4/25 (16%)</td></tr>
</table>
NS[a]
[a] Cochran-Armitage, χ^2 test

Two vehicle-control groups were run concurrently.

8 wk followed by 26 wk without DCM treatment, to determine whether any toxicity was reversible with time.

Females:
<table>
<tr><th rowspan="2">Concentration mg/kg bw</th><th colspan="3">Liver effects</th></tr>
<tr><th>Hepatocellular adenoma</th><th>Hepatocellular carcinoma</th><th>Combined</th></tr>
<tr><td>0 (control 1)</td><td>0/85</td><td>0/85</td><td>0/85*</td></tr>
<tr><td>0 (control 2)</td><td>0/50</td><td>0/50</td><td>0/50</td></tr>
<tr><td>5</td><td>1/85 (1%)</td><td>0/85</td><td>1/85 (1%)</td></tr>
<tr><td>50</td><td>2/83 (2%)</td><td>2/83 (2%)</td><td>4/83 (5%)**</td></tr>
<tr><td>125</td><td>1/85 (1%)</td><td>0/85</td><td>1/85 (1%)</td></tr>
<tr><td>250 (highest dose)</td><td>4/85 (4%)</td><td>2/85 (2%)</td><td>6/85 (7%)**</td></tr>
<tr><td>250 (recovery dose)</td><td>2/25 (8%)</td><td>0/25</td><td>2/25 (8%)**</td></tr>
</table>
NS[a]
[a] Cochran-Armitage, χ^2 test
NS
*P=0.041 (trend)
** P<0.05

Hepatocellular adenoma or hepatocellular adenocarcinoma significantly increased, but in the range of historical controls.</td>
<td>Serota, 1986b</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>treatment for 104 weeks.

Exposure: 104 weeks (daily)</td><td>The average historical incidences of neoplastic nodules and hepatocellular carcinomas were 6.3 and 1.7%, respectively.</td><td></td></tr>
<tr><td>Carcinogenicity study in rats (Sprague-Dawley)
Male/female

Age: 13 weeks

54–70 male and female rats/ group

Equivalent to carcinogenicity test (lifetime)</td><td>dichloromethane

75-09-2

200-838-9
Purity 99.9%

Oral: gavage in olive oil.

Doses/Concentrations: 0 (untreated), 0 (olive oil), 100, 500 mg/kg bw

Exposure: 4-5 days/wk for 64 weeks</td><td>Males and females:

No significant differences in tumour incidence between control and treated rats both in males and females.

Survival:

Excess mortality was observed both in females and male rats at the highest dose (P < 0.01).

Comments:

The period of treatment was short and reporting of data was inadequate.</td><td>Maltoni, 1988</td></tr>
<tr><td>Carcinogenicity study in rats (Sprague-Dawley)

Male/female

Age: 8 weeks

92–97 rats/group</td><td>dichloromethane

75-09-2

200-838-9
Purity 99%

Inhalation
0, 500, 1500, 3500 ppm

Exposure:6 h/day, 5 days/wk, for 104 wk</td><td>Males

<table><tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr><tr><th>0</th><th>500</th><th>1500</th><th>3500</th></tr><tr><td>Salivary gland sarcoma: (%)</td><td>1/92 (1%)</td><td>0/95</td><td>5/95 (5%)</td><td>11/97 (11%)*</td></tr><tr><td>Total number of benign mammary gland tumours</td><td>8/92</td><td>6/95</td><td>11/95</td><td>17/97</td></tr></table>*P = 0.002, Fisher exact test
NR

Females

<table><tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr><tr><th>0</th><th>500</th><th>1500</th><th>3500</th></tr><tr><td>Total number of rats with a benign mammary tumours</td><td>79/96</td><td>81/95</td><td>80/96</td><td>83/97</td></tr><tr><td>Total number of benign mammary gland tumours</td><td>165</td><td>218</td><td>245</td><td>287</td></tr></table>NR

Survival:
No exposure-related effect on mortality was observed in male rats while mortality was significantly increased among females at the highest dose.</td><td>Burek, 1984 and EPA, 1985</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Carcinogenicity study in rats (F344)

Male/female

Age: 7–8 weeks

50 rats/group

Reliability 2, key study</td><td>dichloromethane

75-09-2

200-838-9
Purity 99%

Inhalation: 0, 1000, 2000, 4000 ppm (0, 3470, 6940, or 13 900 mg/m^3)

Inhalation: whole-body

Exposure:6 h/day, 5 days/wk, for 102 wk</td><td>Males
<table><tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr><tr><th>0</th><th>1000</th><th>2000</th><th>4000</th></tr><tr><td>Mammary gland adenoma or fibroadenoma (combined) (%)</td><td>0/50*</td><td>0/50</td><td>2/50 (4%)</td><td>5/50 (10%)**</td></tr><tr><td>Subcutis, fibroma or sarcoma (combined):</td><td>1/50 (2%)***</td><td>61/50 (2%)</td><td>2/50 (4%)</td><td>5/50 (10%)</td></tr></table>*P < 0.001 (trend)c
**P = 0.023
***P = 0.026 (trend)

Females:
<table><tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr><tr><th>0</th><th>1000</th><th>2000</th><th>4000</th></tr><tr><td>Mammary gland adenoma or fibroadenoma (combined) (%)</td><td>5/50 (10%)</td><td>11/50 (22)</td><td>13/50 (26%)</td><td>23/50 (26%)**</td></tr></table>P < 0.001 (trend), Incidental tumour test
P < 0.001 (high dose)
P < 0.05 (mid-dose)
P < 0.05 (low dose)

Survival: the survival of exposed male rats was comparable to that of the chamber controls, however a reduction in all doses in males (32, 32, 34 and 18) and at the higher dose in females (60%, 44%, 44% and 30%) was reported at the termination of the study.</td><td>NTP, 1986; Mennear, 1988</td></tr>
<tr><td>Carcinogenicity study in rats (Sprague-Dawley)

Male/female

Age:

-13 weeks (breeders)

-12 day of gestation (embryos)

54 rats/group

Equivalent or similar to OECD TG</td><td>dichloromethane

75-09-2

200-838-9

Purity 99%

Inhalation: 0, 60 ppm (0, 208 mg/m^3) for embryos; and 0, 100 ppm (0, 347 mg/m^3) for breaders

Inhalation: whole-body

Exposure: (breeders) 7 hours per day, 5 days per week for 7 weeks,</td><td>Breaders (F)

No significant differences in tumour incidence between control and treated rats.

Embryos (F and M)

No significant differences in tumour incidence between control and treated rats.

Survival:

No excess in mortality was found in all the exposed groups.

Comments:

Low exposure concentration and inadequate reporting of data.</td><td>Maltoni, 1988</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>451</td><td>then 7 h/day, 5 days/wk, for 97 wk
Start at age 13 wk

(embryos M/F) 4 h/day,5 days/wk, for 7 wk, then 7 h/day, 5 days/wk, for 97 wk; or 7 h/day, 5 days/wk, for 8 wk;

The breeders and a first group of offspring were exposed for 104 weeks, and a second group of offspring was exposed for 15 weeks only.

Control groups were composed of 60 female rats (untreated breeders controls), and 158 males and 149 females (untreated offspring controls). The rats were observed for their lifespan.</td><td></td><td></td></tr>
<tr><td>Carcinogenicity study in rats (Sprague-
Dawley)
Male/female
Age: unspecified.
90rats/group</td><td>dichloromethane
75-09-2
200-838-9
Purity 99.5%
Inhalation: 0, 50, 200, 500 ppm;
Fifth group (F): 500 ppm for 12 mo, then to 0 ppm for 12 mo (30 rats/group);
Sixth group (F): 0 ppm for 12 mo, then to 500 ppm for</td><td>Males:
No significant differences in tumour incidence between control and treated rats.

Females
<table><tr><th>Concentration (ppm)</th><th>Mammary gland adenoma or fibroadenoma (combined) (%)</th></tr><tr><td>0</td><td>52/70 (74%)</td></tr><tr><td>50</td><td>58/70 (82%)</td></tr><tr><td>200</td><td>61/70 (71%)*</td></tr><tr><td>500</td><td>55/70 (78%)</td></tr><tr><td>500 fifth group</td><td>23/30 (77)</td></tr><tr><td>500 sixth group</td><td>23/30 (77)</td></tr></table>*P<0.05</td><td>Nitschke, 1988</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Equivalent or similar to OECD TG 451</td><td>12 mo (30 rats/group).
Inhalation: whole-body
Exposure: (M) 6 h/day, 5 days/wk; for 20 months;
(F) 6 h/day, 5 days/wk for 0, 50 and 200 ppm for 24 months
(F) fifth group: see above;
(F) sixth group: see above.</td><td>Fisher exact test

Survival:
No exposure-related adverse effect on body weight or mortality was observed both in males and females.</td><td></td></tr>
<tr><td>Carcinogenicity study in rats (F344/DuCrj)
Male/female
Age:
50 rats/group
24 months

Equivalent or similar to OECD TG 451</td><td>dichloromethane
75-09-2
200-838-9
Purity 99.9%
Inhalation whole body: 0, 1000, 2000, 4000 ppm (0, 3470, 6940, or 13 900 mg/m^3)
Exposure: 6 h/day, 5 days/wk, for 104 wk</td><td>Males:
<table><tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr><tr><th>0</th><th>1000</th><th>2000</th><th>3500</th></tr><tr><td>Subcutis fibroma</td><td>1/50 (2%)</td><td>4/50 (8%)</td><td>7/50 (14%) *</td><td>12/50 (24%)**</td></tr><tr><td>Mammary gland fibroadenoma</td><td>1/50 (2%)</td><td>2/50 (4%)</td><td>3/50 (6%)</td><td>8/50 (16%)*</td></tr><tr><td>Peritoneal mesothelioma</td><td>3/50 (6%)</td><td>1/50 (2%)</td><td>0/50</td><td>7/50 (14%)</td></tr></table>Subcutis fibroma:
P<0.001 (trend); P<0.001 (high dose), P<0.05 (mid dose) with Peto-test and Fisher exact test.
Mammary gland fibroadenoma:
P<0.001 (trend); P<0.001 (high dose) with Fisher exact test.
Peritoneal mesothelioma:
P<0.005 (trend) with Peto-test and Fisher exact test.

Females:
<table><tr><th rowspan="2">Type of tumour</th><th colspan="4">Concentration (ppm)</th></tr><tr><th>0</th><th>1000</th><th>2000</th><th>3500</th></tr><tr><td>Mammary gland fibroadenoma</td><td>7/50 (14%)</td><td>7/50 (14%)</td><td>9/50 (18%)</td><td>14/50 (28%)</td></tr></table>P<0.001 (trend); with Peto-test and Fisher exact test.

Survival:
The survival in males was 64%, 86%, 76% and 56% respectively at 0, 1000, 2000, 4000 ppm.
The survival in females was 90%, 80%, 86% and 60% respectively at 0, 1000, 2000, 4000 ppm. The survival of females exposed to 4000 ppm was decreased compared with the controls (no statistical analysis reported).</td><td>JBRC, 2000b;
Aiso, 2014</td></tr>
<tr><td>Carcinogenicity study in hamster Syrian golden (Ela:Eng)</td><td>dichloromethane
75-09-2
200-838-9</td><td>Males:
No significant differences in tumour incidence between control and treated hamsters.</td><td>EPA, 1985
Burek, 1984</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Male/female

Age: 8 weeks

95 hamsters/group

24 months

Equivalent or similar to OECD TG 451</td>
<td>Purity 99.9%

Inhalation: 0, 500, 1500, 3500 ppm

Exposure:6 h/day, 5 days/wk, for 104 wk</td>
<td>Females:

A statistically significant increase in the total number of benign tumours was observed in females exposed to 3500 ppm, but this was considered to be secondary to the increased survival of this group.

<table>
<tr><th></th><th>Concentration (ppm)</th><th>Males cumulative totals</th><th>Females cumulative totals</th></tr>
<tr><td>Total number of hamster during this period</td><td>0
500
1500
3500</td><td>107
104
103
107</td><td>106
102
101
105</td></tr>
<tr><td>Total number of hamster with a tumor</td><td>0
500
1500
3500</td><td>25
29
27
29</td><td>19
21
19
32 *</td></tr>
<tr><td>Total number of hamster with a benign tumor</td><td>0
500
1500
3500</td><td>20
19
13
19</td><td>13
9
13
26*</td></tr>
<tr><td>Total number of hamster with a malignant tumor</td><td>0
500
1500
3500</td><td>7
13
15
10</td><td>8
13
7
10</td></tr>
</table>
*significantly different from controls when analysed by Fisher’s exact test , p<0.05

Lymphosarcoma [malignant lymphoma] in female hamster: 1/91 (1%), 6/92 (6%), 3/91 (3%), 7/91 (8%)*
*P < 0.05 (Fisher exact test)

Survival:

At the end of the study the numbers of hamsters surviving were 16, 20, 11, and 14 in males, and 0, 4, 10, and 9 in females, respectively at 0, 500, 1500, 3500 ppm.

Historical control not available.

Note: the data reported in the table are extracted from EPA, 1985. The Working Group of IARC monograph 110 (IARC, 2017) noted that the higher survival in treated hamsters may have contributed to this non-dose-dependent result.</td>
<td></td>
</tr>
</table>

Table 16: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Occupational cohort study on cancer	Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1954–76 Exposure: to DCM based on a combination of personal and area samples, median exposure levels (8-hour TWA) in 1977 were reported to be 140, 280, and 475 ppm [486, 971, 1650 mg/m3] in three main work areas, but no dose–response analysis was performed. The workers had been also exposed to **acetone and methanol.**	Subjects analysed: 1271 (551 men and 720 women). Location and follow-up period: USA; 1954-1990. Results based on mortality records; adjusted for age, sex, race, and calendar period.	**SMRs** (Standardized Mortality Ratios) were elevated for cancer of the liver and biliary tract (SMR, 2.98; 95% CI, 0.81–7.63; 4 cases). Each of the deaths due to cancers of the **liver** and **biliary tract** occurred among employees with ≥ 10 years of employment and ≥ 20 years since first employment (SMR, 5.83; 95% CI, 1.59–14.92). Three out of these four deaths were attributed to cancer of the biliary tract, with durations of exposure to DCM of < 1 to 28 years. These four cases were also observed in the initial analysis by Lanes *et al*. (1990) with an SMR of 5.75 (95% CI, 1.82–13.8) for cancers of the liver and biliary tract combined; the SMR estimated for cancer of the biliary tract alone was 20 (95% CI, 5.2–56) compared with a national referent population. Results for other cancers were unremarkable; no results were reported for non-Hodgkin lymphoma (NHL). Note of IARC, 2017: Although some of the subjects were also exposed to acetone and methanol, the Working Group considered these to be unlikely explanations for the observed risks because they were not known to be linked to cancer of the liver.	Lanes, 1993
Cohort study on cancer	Exposure: to DCM, Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 months in 1970–81. The workers had been also exposed **to acetone and methanol.**	Subjects analysed: 3211 white workers (2187 men and 1024 women) Location and follow-up period: USA, 1970–1989 Results based on mortality records; adjusted for age, sex, race, and calendar period.	The risk of mortality from cancers of **liver** and **biliary tract** was not increased. Except for cancer of the prostate, for which there was a non-significant excess, SMRs for other cancers were < 1.0 for all exposure categories among men. The SMRs for women were based on very small numbers and were unstable. No data were reported for NHL	Gibbs, 1996
Cohort study on cancer	Exposure: to DCM, Workers from a plant producing cellulose triacetate film, engaged for ≥ 1 yr in one of three areas in which dichloroethane was used (roll coating,	Subjects analysed: 1311 male white workers Location and follow-up period: USA,1964–1994	Malignant neoplasms with elevated SMRs were cancer of brain and central nervous system (SMR, 2.16; 95% CI, 0.79–4.69; 6 cases), leukaemia (SMR, 2.04; 95% CI, 0.88–4.03; 8 cases), and Hodgkin disease (SMR, 1.82; 95% CI, 0.20–6.57; 2 cases). Mortality from leukaemia increased with cumulative exposure among four exposure categories: for the group with the highest	Hearne and Pifer, 1999

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	doping, distilling) in 1946–70. Exposure to dichloromethane (8-hour TWA) was: 0–520 ppm [0–1800 mg/m^3] in 1946–1965, 0–300 ppm [0–1040 mg/m^3] in 1966–1985, and 0–100 ppm [0–347 mg/m^3] in 1986–1994. Workers may have also been exposed to methanol, 1,2-dichloropropane, 1,2-dichloroethane, acetone, and benzene, but exposure levels were not reported for these agents.	Referent population (mortality) from New York, excluding New York City.	cumulative exposure, the SMR for leukaemia was 5.89 (95% CI, [1.89–13.6]; 5 cases). Three of the eight cases of leukaemia had also been exposed to benzene in the past. **SMRs for cancer of the liver and NHL were less than unity**, based on very small numbers (one and two cases, respectively). **Limits of the study:** the small numbers of exposed cases, which hampers analysis of exposure–response patterns.	
Cohort study on cancer	Exposure levels: Workers were exposed to numerous chemicals. Exposure was assessed quantitatively for trichloroethylene, and qualitatively (ever/never) to other agents including dichloromethane. Co-exposures: several organic solvents, in particular trichloroethylene, and other occupational exposures.	Subjects analysed: 1222 workers of a military-aircraft maintenance facility. Location and follow-up period: USA, 1952–2000. Covariates: Age, race. Internal comparison of deaths.	Exposure to dichloromethane was associated with increased risks (hazard ratio, HR) of **NHL** (HR, 2.02; 95% CI, 0.76–5.42; 8 exposed cases) and multiple myeloma (HR, 2.58; 95% CI, 0.86–7.72; 7 exposed cases) for male workers, and cancer of the breast (HR, 2.35; 95% CI, 0.98–5.65; 6 exposed cases) for female workers. Results for other cancer sites in relation to DCM exposure were not reported. The strengths of this study: included a large number of the subjects and a long follow-up period. **Limits**: because the primary analysis was for trichloroethylene, the exposure assessment and analysis for DCM were limited.	Radican, 2008
Cohort study on cancer	Exposure to DCM; levels were estimated from area	Subjects analysed: 1785 male Location and follow-	No cancers of the **liver** were observed among exposed or unexposed workers (expected, 3.3 cases), and there was a	Tomenson, 2011

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	samples according to time period and work group. TWA exposures were estimated to range from 2 to 20 ppm [7–69 mg/m^3] before 1960, 6 to 127 ppm [21–441 mg/m^3] during the 1960s, 10 to 165 ppm [35–573 mg/m^3] during the 1970s, and 7 to 88 ppm [24–305 mg/m^3] during the 1980s Tomenson et al. (1997). The workers had been also exposed to acetone and methanol.	up period: England, 1946–2006. Covariates: Age, calendar period.	significant deficit of cancer of the lung. Data for **NHL** were not reported. Analysis of cumulative exposure for four cancer sites, including brain, did not show any significant trends with the level of exposure to DCM. **Limits of the study:** small number of deaths, which limited the ability to conduct exposure–response analysis.	
Prospective cohort	Exposure: DCM The air toxic concentration was obtained from NATA (database created by the US EPA of modelled air toxic concentrations).	Study location and period: USA, 2003-2009 Subject analysed: 50,884 women from across the US who were ages 35–74 at enrolment. Participants were recruited from 2003 to 2009. Women were eligible for the Sister Study if they had a sister who had been diagnosed with breast cancer, but no prior breast cancer themselves. At baseline, women completed a computer-assisted telephone interview and written questionnaires to assess demographics,	Type of tumours: **breast cancer.** Aim of the study: To examine the association between breast cancer incidence and 29 non-metallic hazardous air pollutants. Results: Over follow-up (average = 8.4 years), 2975 women were newly diagnosed with breast cancer (invasive or ductal carcinoma in situ). Several air toxics, including methylene chloride, polycyclic organic matter, propylene dichloride, and styrene, were associated with increased risk. Of these, methylene chloride was most consistently associated with risk across multiple analyses. It was associated with overall ($HR_{quintile4vs1}$ = 1.21 (95%CI = 1.07–1.38)) and estrogen receptor positive (ER+) invasive breast cancer ($HR_{quintile4vs1}$ = 1.28 (95%CI = 1.08–1.52)) in individual pollutant models, although no dose-response was observed. Associations were stronger among overweight/obese (vs. non-overweight/obese) women ($p < 0.05$) for six air toxics. The classification tree identified combinations of age, methylene	Niehoff, 2019

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		lifestyle factors, medical and family history, and residential history. Participants complete annual health updates and triennial follow-up questionnaires to assess changes in health and risk factor information.	chloride, BMI, and four other toxics (propylene dichloride, ethylene dibromide, ethylidene dichloride, styrene) related to overall breast cancer. In conclusion some non-metallic air toxics, particularly DCM, were associated with the hazard for overall and Endocrine Receptor positive (ER+) breast cancer. Overweight/obese women may be particularly susceptible to air toxics.	
Case- control study	Exposure: DCM Information including occupational history and risk factors for cancer of the brain was obtained by interview of next-of-kin and exposure estimates were assigned using a job-exposure matrix. Co-exposure: organic solvents, carbon tetrachloride, methyl chloroform, tetrachloroethylene, trichloroethylene	Study location and period: Louisiana, New Jersey, and Philadelphia, USA, 1979–81 Covariates: Age, study area Subjects analysed: 300 men who died from astrocytic cancer of the brain in Louisiana and Pennsylvania, USA, and 320 men who died from other causes not associated with occupational exposure to chlorinated hydrocarbons.	Tumours: **Brain and CNS** Aim of the study: to examine the associations between astrocytic cancer of the brain and exposure to six chlorinated solvents including. Results: After adjusting for age at death and study area, significant trends in risk were observed with increasing probability and intensity of exposure, as well as with increasing exposure duration and cumulative exposure when the probability of exposure was high. Limits: the exposure assessment was based on the data obtained from the next of kin.	Heineman, 1994
case- control study	Exposure: DCM Probability and intensity of exposure were assigned using occupation and industry titles from subjects' death certificates and a job-exposure matrix. Co-exposure: electromagnetic fields, solvents, chlorinated aliphatic hydrocarbons, benzene, lead, nitrosamines,	Study location and period: 24 states in USA, 1984-92; Covariates: state and race. Subjects analysed: Cases were 12.980 women who died due to cancer of central nervous system in 24 states of the USA. Controls were 51.920 randomly selected women who died from non-malignant diseases, excluding	Tumours: Brain and others CNS. Aim of the study: to examine associations between mortality from the cancer of the brain and other parts of central nervous system and exposure to 11 factors including DCM. Results: After adjusting for age at death, marital status, and socioeconomic status, the odds ratio for the association of exposure to DCM and all cancer of the central nervous system was 1.2 (95% CI, 1.1–1.3). Odds ratios were generally similar for all categories of probability and intensity of exposure. Limits: this study, like others using similar methods, assessed exposure from occupational information from death certificates, the specificity for DCM was	Cocco, 1999

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	polyaromatic hydrocarbons, insecticides and fungicides, herbicides, contact with the public.	neurological disorders.	poor.	
case- control study	Exposure: DCM Self-reported exposure by parents and review by industrial hygienists.	Study location and period: USA and Canada, 1 May 1992- 30 April 1994; Covariates: child's age, maternal race, maternal age, and maternal education. Subject analysed: 405 case fathers and 302 control fathers. Control: Population controls from random-digit dialling.	Tumours: Neuroblastoma. Aim of the study: to identify paternal occupational exposures associated with an increased risk of cancer of the brain in children. Results: Maternal exposures to most chemicals were not associated with neuroblastoma. When considering paternal exposure to DCM as assessed by an industrial hygienist, the odds ratio for neuroblastoma was 0.70 (95% CI, 0.2–2.8; 4 exposed cases; adjusted by age, maternal race, maternal age, and maternal education). Paternal exposures to hydrocarbons such as diesel fuel (odds ratio (OR) = 1.5; 95% confidence interval (CI): 0.8, 2.6), lacquer thinner (OR = 3.5; 95% CI: 1.6, 7.8), and turpentine (OR = 10.4; 95% CI: 2.4, 44.8) were associated with an increased incidence of neuroblastoma, as were exposures to wood dust (OR = 1.5; 95% CI: 0.8, 2.8) and solders (OR = 2.6; 95% CI: 0.9, 7.1).	De Roos, 2001
Case-control study	Exposure levels: DCM; Information about occupational history and other potential risk factors was obtained by in-person interview, and probability and intensity of occupational exposure to individual chemicals and chemical classes were assigned by expert assessment. Co-exposures: benzene,	Subjects analysed: study included 1428 cases of **NHL** (including 285 with small lymphocytic lymphoma, 308 with diffuse lymphoma, 100 with follicular lymphoma, and 315 with other lymphomas), and 1530 controls. Location and follow-up period: Italy, 1991–1993 Covariates: Sex, age, education and area Control: population.	**Type of tumours: NHL** Aim of the study: to evaluate the association between risk of lymphoma and exposure to DCM and nine other organic solvents Results: Odds ratios were adjusted by area, sex, age, and education, excluding subjects with low probability of exposure. The OR for NHL in the category for combined medium- and high-intensity exposure to DCM was 1.7 (95% CI, 0.7–4.3; 13 cases; P for trend, 0.46). Among the NHL subtypes, an odds ratio for DCM was reported only for small lymphocytic NHL: for medium or high exposure, the odds ratio was 3.2 (95% CI, 1.0–10.1). The study also included cases of Hodgkin lymphoma, but odds ratios for exposure to DCM were not reported	Miligi, 2006

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane OR not reported for follicular NHL, diffuse NHL, and other NHL.			
Case-control study	Exposure: DCM: In-person interview obtained occupational history, medical history, and lifestyle. Co-exposure: trichloroethene, tetrachloroethylene, carbon tetrachlorine, benzene, toluene, xylene and styrene.	Subjects analysed: Malignant lymphoma, 710 cases; Controls, 710 Location and follow-up period: Germany, 1999–2003 Covariates: Smoking and alcohol Control: population.	**Type of tumours:** malignant lymphoma Aim of the study: to examine the relationship between malignant lymphoma and exposure to eight organic solvents including DCM. Results: ORs were adjusted for smoking and alcohol consumption. The OR for high cumulative exposure to DCM was 2.2 (95% CI, 0.4–11.6; P for trend, 0.40) for all lymphomas, and 2.7 (95% CI, 0.5–14.5; P for trend, 0.29) for B-cell NHL.	Seidler, 2007
case- control study	Exposure: DCM; Exposure was assessed by expert rating to assign metrics of probability and intensity of exposure to several solvents. Subjects with a low probability of exposure were excluded from the analysis. Co-exposures: benzene, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane.	Subjects analysed: 586 cases of leukaemia and 1278 controls from seven areas in Italy. Location and follow-up period: Italy, 1991-1993. Covariates: Sex, age, education and area Control: population.	Type of tumours: **Leukaemia** Aim of the study: to evaluate the risks associated with exposure to ten organic solvents including DCM. Results: No associations between acute leukaemia or myeloma and DCM were seen. Four cases of chronic lymphocytic leukaemia (now classified as a type of NHL) were observed, with a non-significant odds ratio of < 1 for very low/low exposure, and an odds ratio of 1.6 (95% CI, 0.3–8.6) for medium/ high exposure.	Costantini, 2008
case- control study	Exposure: DCM. Information about occupational history and other potential risk factors was obtained by in-	Subjects analysed: 601 female cases, and 717 controls, matched for age, collected from the general population in Connecticut, USA.	Type of tumour: **NHL** Aim of the study: to examine the association between NHL and exposure to nine organic solvents including DCM. Results: ORs were adjusted by race, age, family history of haematopoietic cancer,	Wang, 2009

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	person interview and probability and intensity of exposure to solvents were assigned using a previously developed job-exposure matrix. Co-exposures: benzene, formaldehyde, chloroform, carbon tetrachloride, dichloroethane, trichloroethylene.	Location and follow-up period: USA 1996-2000. Covariates: Age, family history of haematopoietic cancer, alcohol consumption, and race. Control: population	and alcohol consumption. Subjects ever-exposed to DCM had an increased risk of NHL (OR, 1.5; 95% CI, 1.0–2.3). Analyses by intensity and probability of exposure indicated elevated ORs, but trends were not statistically significant.	
case- control study	Exposure: DCM; In-person interviews obtained occupational history and additional job-specific modules were applied when solvent exposure was likely. Exposure metrics of probability, frequency, intensity, confidence, and cumulative exposure were assigned using a job-exposure matrix. In secondary analyses, jobs assessed with low confidence are considered unexposed.	Subjects analysed: Multiple myeloma, 180 cases, 481 controls were collected from the general population in the same areas/ population Location and follow-up period USA, 2000–2002 Covariates: Age, race, study site, and years of education. Control: population.	Type of tumour: multiple myeloma Aim of the study: to evaluate the associations between risk of multiple myeloma and exposure to DCM and other chlorinated solvents. Results: ORs were adjusted by area, race, sex, age, and education. Overexposure to DCM entailed elevated risk of multiple myeloma (OR, 1.5; 95% CI, 0.9–2.3). Significant trends with exposure duration were observed when occupations that had low confidence scores were included in the unexposed category: the odds ratio for ever exposure was 2.0 (95% CI, 1.2–3.2) and odds ratios of 2.7 (95% CI, 1.1–6.5), and 2.1 (95% CI, 0.9–5.2), were observed for workers employed for 12–29 years and 30–51 years, respectively (P for trend, 0.01). No such trend was seen for cumulative exposure.	Gold, 2011
case- control study	Exposure: DCM. Information about occupational history and other potential risk factors was obtained by in-person interview. Co-exposures:	Subjects analysed: Women from the study by Wang et al. (2009) who provided a blood or buccal cell sample for genotyping; adjusted for age and race.	Type of tumour: **NHL** Aim of the study: to evaluate whether genetic variation in four genes involved in metabolism (CYP2E1, EPHX1, NQO1, MPO) modifies associations between exposure to organic solvents and risk of NHL or five major histological subtypes of NHLL (diffuse large B-cell lymphoma, follicular lymphoma, chronic lymphocytic	Barry, 2011

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	benzene, formaldehyde, chloroform, carbon tetrachloride, dichloroethane, trichloroethylene.	Location and follow-up period: USA, 1996–2000 Covariates: Age, family history of haematopoietic cancer, alcohol consumption, and race. Control: population	leukaemia/small lymphocytic lymphoma, marginal zone lymphoma, and T-cell lymphoma). Results: Everexposure to DCM entailed elevated risk of NHL (OR, 1.69; 95% CI, 1.06–2.69). The risk associated with ever-exposure to DCM was higher (OR, 4.42; 95% CI, 2.03–9.62) among women with the TT genotype for CYP2E1 rs2070673. In contrast, no effects with DCM was observed among women with the TA or AA genotype (OR, 0.80; 95% CI, 0.36–1.75). Similar patterns were observed for diffuse large B-cell lymphoma and follicular lymphoma. No interactions with other single-nucleotide polymorphisms (SNPs) in the studied genes, including CYP2E1, EPHX1 NQO1, or MPO, were statistically significant. (The IARC Working Group noted that the functional role of the CYP2E1 polymorphism is unclear).	
Case- control study	Exposure: DCM Exposure to solvents was assessed by an industrial hygienist based on detailed occupational histories collected by interview.	Hospital based case-control study. Study location and period: Arizona, Massachusetts and Pennsylvania, USA, 1994–98 Covariates: Age group, race sex, hospital site and proximity of residence to hospital. Subject analysed: Cases were 484 patients with glioma and 197 patients with meningioma diagnosed in Massachusetts, Pennsylvania, and Arizona, USA. Controls were 797 patients admitted to the same hospitals for non-malignant conditions and were frequency-matched to cases by sex, age,	Type of tumour: Glioma or other neuroephitheliomatous neoplasm and meningioma. Aim of the study: to examine associations between glioma and meningioma and exposure to six chlorinated solvents including DCM. Results: Odds ratios adjusted for the matching factors did not show any association between glioma or meningioma and overall exposure to DCM or other metrics, including duration, intensity, and cumulative exposure. No consistent evidence for increased brain tumour risk related to chlorinated solvents was found.	Neta, 2012

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		race, hospital, and proximity to the hospital.		
Case- control study	Occupational exposures were derived using a combination of subject-reported job history and expert assessment. We examined the associations between **two chemical families** and six chlorinated solvents with 11 sites of cancer.	Subjects analysed: 3730 cancer cases and 533 population controls. Location and follow-up period: Canada, 1979–85.	Type of tumour: 11 cancer sites Aim of the study: to evaluate the association between exposure to chlorinated solvents and cancer. Results: The majority of the associations examined were null, although many were based on small numbers. We found two significantly elevated ORs, one between perchloroethylene and prostate cancer (OR = 4.3; 95% CI: 1.4 to 13) and another between trichloroethylene and melanoma (OR = 3.2; 95% CI: 1.0 to 9.9).	Christensen, 2013
Case- control study	Exposure: DCM Lifetime occupational histories were obtained by interview and several exposure metrics were assigned by an industrial hygienist.	Study location and period: Iowa, Michigan, Minnesota, Wisconsin, USA, 1995–97 Covariates: Age, education, sex. Subject analysed: Cases were 798 patients with intracranial glioma in Iowa, Michigan, Minnesota, and Wisconsin, USA, and controls were 1175 residents selected from the same area.	Tumours: **Glioma** Aim of the study: to examine associations between glioma and exposure to six chlorinated solvents including DCM. Results: Odds ratios adjusted for the frequency matching variables (age group and sex), and for age and education. There were no associations between glioma and overall exposure to DCM, or exposure probability and cumulative exposure.	Ruder, 2013
Multicentre case–control study of meningioma	Exposure: no subjects classified as exposed to DCM after assessment of lifetime occupational histories using a modified version of the Finnish national job-exposure matrix	Study location and period: multicentre population of Australia, Canada, France, Germany, Israel, New Zealand and the UK; 2000-2004; Covariates: Demographic factors, and lifestyle factors.	Tumours: **meningioma.** Aim of the study: to examine associations between occupational exposure to selected organic solvents and meningioma. No association was observed between any of the organic solvents and meningioma, in either men or women, and no dose–response relationships were observed in internal analyses using either exposure duration or cumulative exposure.	McLean, 2014

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		Subject analysed: 1906 cases and 5565 controls, in seven countries.		
case- control study	Exposure: DCM data on air releases of DCM, in pounds per year, were obtained from the TRI database.	Study location and period: California, USA, 1988 to 2012 Covariates: age and gestational ages. Subject analysed: We frequency matched by birth year approximately 20 cancer-free controls identified from birth records to all childhood cancers ages 0–5 in the California Cancer Registry diagnosed from 1988 to 2012; i.e. 13,636 cases and a total of 270,673 controls.	Type of tumour: **germ cells and AML** (acute myeloid leukemia) Aim of the study: to investigate the association between childhood cancers and exposures to DCM releases from industrial plants, as reported to the EPA's Toxics Release Inventory, near (≤3 km) residences of pregnant women and infants living in California. Results: elevated risks for germ cell tumours [Odds Ratio (OR): 1.52, 95% Confidence Interval (CI) 1.11, 2.08], particularly teratomas (OR: 2.08, 95% CI 1.38–3.13), and possible increased risk for AML (OR: 1.64, 95% CI 1.15–2.32 in the quadratic decay model) were reported. Risk estimates were similar in magnitude whether releases occurred in pregnancy or the child's first year of life. Some possible excess risks, based on very small numbers, were observed when analysing childhood CLL within a 3 km buffer between residences and emitting facilities, though not supported by statistical significance. Conclusion: The exposure to industrial DCM releases may be a risk factor for childhood germ cell tumours, teratomas, and possibly AML. NHL	Park, 2017
case- control study	Exposure: DCM exposure to selected solvents was estimated by using the NOCCA job-exposure matrix (NOCCA-JEM).	Study location and period: Finland, Iceland, Norway, and Sweden, census in 1960, 1970, 1980/1981, and 1990. Subject analysed: 20,615 CLL cases diagnosed in 1961–2005 in Finland, Iceland, Norway, and Sweden, and	Type of tumours: **adult chronic lymphocytic leukemia (CLL).** Aim of study: to assess the effect of occupational solvent exposure on the risk of adult chronic lymphocytic leukemia (CLL). Results: Non significant CLL risk elevations were observed for DCM, perchloroethylene, and1,1,1-trichloroethane. Compared to unexposed, significantly increased risks were observed for cumulative perchloroethylene exposure ≤ 13.3 ppm-years (OR 1.85, 95% CI 1.16–2.96) and average life-time perchloroethylene	Talibov, 2017

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		103,075 population-based controls matched by year of birth, sex, and country were included.	exposure ≤ 2.5 ppm (1.61, 95% CI 1.01–2.56) among women, and cumulative DCM exposure ≤12.5 ppm-years (OR 1.19, 95% CI 1.01–1.41) and 12.5–74.8 ppm-years (OR 1.23, 95% CI 1.01–1.51) among men in an analysis with 5 years lag-time, though without dose–response pattern. Decreased CLL risk was observed for aliphatic and alicyclic hydrocarbon solvents and toluene. Conclusion: This study did not support associations for solvent exposure and CLL. Observed weak associations for DCM, perchloroethylene, 1,1,1-trichloroethane exposures, aliphatic and alicyclic hydrocarbons and toluene were not consistent across sexes, and showed no gradient with amount of exposure.	
A retrospective comparative population study	Exposure: DCM The plant systematically used DCM in its operations starting around 1983 until 2009 (closure of the plant). DCM measurements at the stack of the plant were taken in 2005 and 2006 by the Department of Labour Inspection.	Study location and period: Latsia municipality, Nicosia, Cyprus; 1983-2009 Subject analysed: a group of 82 cancer cases were included in the study. The control was the cancer incidence rate (1998–2008) for the study area.	Type of tumours: Brain and CNS Aim of the study: Results: Mean stack emissions of DCM of 88 mg/Nm^3 and flow rates of 850 g/h exceeded the permissible DCM limits established for industrial zones. Brain and central nervous system (CNS) cancer incidence rates showed significant ($P < 0.001$) increase in the study area around the plant when compared with those observed in other areas of Cyprus. Calculated standardized incidence ratios for brain/CNS after adjusting for the age at diagnosis ranged from 11.3–25.7 [mean 6.5 (3.02 : 12.3)] for the study area. An association between chronic, unintentional DCM exposures and brain/CNS cancer cases for the general population located in a residential area being in close proximity with a plant historically emitting DCM was observed.	Makris, 2018
Review of retrospective cohort and case-control studies	Exposure: DCM and other solvents.	Details on study design: Papers for review were identified through Medline (National Library of Medicine) and were limited to epidemiology studies. Studies were classified using three categories. Primary studies focused on the association	Objective: To critically review and summarize the epidemiological evidence published to date on the carcinogenicity of methylene chloride to humans. Conclusions: No strong or consistent finding for any site of cancer was apparent despite several studies of large occupational cohorts of workers potentially exposed to high concentrations of methylene chloride. Sporadic and weak associations were reported for cancers of the pancreas, liver	Dell, 1999

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		between methylene chloride and cancer among occupational cohorts primarily exposed to methylene chloride. Secondary studies identified methylene chloride a priori as a potential exposure of interest, and the investigators either characterized the methylene chloride exposure or described results for the methylene chloride-exposed workers separately. Tertiary studies evaluated cohorts either minimally exposed to methylene chloride or presumed exposed but for which no exposure estimation or separate classification was made.	and biliary passages, breast and brain. Although these studies collectively cannot rule out the possibility of any cancer risk associated with methylene chloride exposure, they do support a conclusion of no substantive cancer risk. Continued follow-up of the established cohorts may elucidate the few and inconsistent relationships reported to date; however, it appears likely that risks associated with methylene chloride exposure, if any, are small and limited to rare cancers. The usefulness of additional cohort studies for the evaluation of cancer risks associated with methylene chloride exposure will depend largely on whether the relevant exposure period has passed and whether exposure characterization (e.g. peak or intermittent exposure or intensity) can be improved.	
Comprehensive Review	Exposure: DCM	A review paper that integrates the animal toxicity data and the occupational epidemiology data in DCM-exposed workers into an overall weight-of-evidence assessment of the available data and existing uncertainties.	Objective: critical review of all epidemiologic, carcinogenicity and mechanistic data available. Conclusion: dose-dependent toxicokinetics of DCM suggest that DCM is a threshold carcinogen in mice, initiating carcinogenicity via the low affinity/high capacity GSTT1 pathway; a biotransformation pathway that becomes relevant only at high exposure concentrations. Rats and hamsters have very low activities of this DCM-metabolizing GST and humans have even lower activities of this enzyme. Based on the induction of specific tumours selectively in the mouse, the dose- and species-specific toxicokinetics in this species, and the absence of a malignant tumour response by DCM in rats and hamsters having a closer relationship to DCM toxicokinetics in humans and thus being a more relevant animal model, the current harmonised classification of DCM	De Kant, 2021

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			as human carcinogen cat. 2 remains appropriate. In particular, as to human data, the authors conclude that the new available information on the occupational exposure in cohort studies, not assessed in the last IARC-evaluation, confirms that exposure to 1,2-DCP and not to DCM is the basis for the positive associations between alleged DCM-exposures and biliary tract cancer, and that an association of NHL with DCM exposure remains doubtful.	

SMR, Standardized mortality ratios; NHL, non-Hodgkin lymphoma; OR, odds ratio; CNS, central nervous system; CLL, Chronic lymphocytic leukemia; NOCCA, Nordic Occupational Cancer Studies; AML, Acute myeloid leukemia

Table 17: Summary table of other human studies relevant for carcinogenicity of DCM and 1,2-DCP in relation to cholangiocarcinoma

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Case series	DCM and 1,2-dichloropropane (1,2-DCP) Concentrations of 1,2-DCP and DCM estimated by simulation and mathematical modelling The estimated airborne concentrations in the proofprinting room (51 workers) were 100–670 ppm [462–3090 mg/m3] for 1,2-DCP and 80–540 ppm [278–1870 mg/m3] for DCM. In the front room (11 workers), the airborne concentrations were estimated to be 70–110 ppm [323–508 mg/m3] for 1,2-DCP and 50–130 ppm [173–451 mg/m3] for DCM.	Study location and period: Osaka, Japan 1991-2006 Subject analysed: 51 men who had worked in the proof-printing room, and 11 men who had worked in the front room for at least 1 year between 1991 and 2006. Overall, 11 cholangiocarcinoma patients	Type of tumour: cholangiocarcinoma Aim if the study: The study was conducted to investigate the relationship between occupational chemical exposure and incidence of cholangiocarcinoma among workers in the offset colour proof-printing section of a small printing company in Osaka, Japan. Results: Workers used 1,2-DCP from approximately 1985 to 2006, and DCM from approximately 1985 to 1997/1998. Exposure concentrations were estimated to be 100-670 ppm for 1,2-DCP and 80-540 ppm for DCM among the proof-printing workers. All 11 patients were pathologically diagnosed with cholangiocarcinoma from 1991 to 2011. Ages at diagnosis were 25-45 years, and ages at death were 27-46 years among the six deceased individuals. The primary cancer site was the intrahepatic bile duct for five patients, and the extrahepatic bile ducts for six. All patients were exposed to 1,2-DCP for 7-17 years and diagnosed with cholangiocarcinoma 7-20 years after their first exposure. Ten patients were also exposed to DCM for 1-13 years. The SMR for cholangiocarcinoma was 2900 (expected deaths: 0.00204, 95% CI 1100 to 6400) for all workers combined. Conclusions: These findings suggest that 1,2-DCP and/or DCM may cause cholangiocarcinoma in humans.	Kumagai, 2013
Case series	two cholan-giocarcinoma patients exposed to 1,2-DCP or DCM in different offset printing companies. Case 1 in Fukuoka was exposed to white gasoline and 1,2-DCP for 13 years and 12 years, respectively.	Study location and period: One case in Fukuoka, Japan (also described by Yamada, 2014) One case in Aichi Prefecture, Japan. 1988-2013. Subject analysed: Two additional cases of cholangiocarcinoma in	Type of tumour: cholangiocarcinoma Aim if the study: The study describes two cholangiocarcinoma patients exposed to 1,2-DCP or DCM in different offset printing companies. Results Case 1 was a man born in 1950. He worked in the printing section in a proof-printing company for 26 years. He was diagnosed as cholangiocarcinoma in 1998 and died in 2000. In proof-printing operations, he used gasoline for 14 years and 1,2-DCP for 11 years to remove ink from a rubber transcription roller	Kumagai, 2014a

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	Case 2 from Aichi was exposed to both DCM and 1,1,1-TCE for 11 years, but not to 1,2-dichloropropane IARC Working Group members confirmed that the case exposed to DCM only was the same case without exposure to 1,2-DCP reported by MHLW, 2013a from the Aichi Prefecture.	addition to Kumagai 2013. One case was exposed to DCM e 1,1,1-TCE and one only to 1,2-DCP.	(blanket). The exposure concentration of 1,2-DCP was estimated to be between 72 and 5,200 ppm. Case 2 was a man born in 1963. He worked in the printing section in a general offset printing company for 11 years. He was diagnosed with cholangiocarcinoma in 2007. In printing operations, he used both kerosene and a mixture of 50% DCM and 50% 1,1,1-trichloroethane (1,1,1-TCE) for 11 years to remove ink from a blanket. The exposure concentration of DCM was estimated to be between 240 and 6,100 ppm. He was simultaneously exposed to similar levels of 1,1,1-TCE. **Conclusions:** Because the offset printing process may cause cholangiocarcinoma, occupational history should be examined for patients with this cancer.	
Case series	DCM and 1,2-DCP Concentrations of 1,2-DCP and DCM estimated by simulation and mathematical modelling workers in offset colour at a proof-printing company for 6−19 years (mean, 12 years). They were exposed to 1,2-DCP for 6−17 years (mean, 10 years) and kerosene for 6−19 years (mean, 12 years). Five patients were also exposed to DCM for 2−8 years (mean, 5 years), and three patients were additionally exposed to 1,1,1-trichloroethane for 3−4 years (mean, 3 years).	Study location and period: Proof-printing company, Osaka, Japan - 1988-2013. Subject analysed: 17 cases of cholangiocarcinoma. 13 cases with company records available, and 10 cases gave consent to participate in this study. Health examination records during employ-ment and after retirement, and blood parameters for 10 chol-angiocarcinoma patients	Type of tumour: **cholangiocarcinoma** Objectives: to evaluate blood parameters in cholangiocarcinoma cases among proof-printing workers during and after exposure. Results: All study patients were exposed to 1,2-DCP for 6-17 years. Red blood cells, hemoglobin, hematocrit, total cholesterol, triglycerides, and fasting plasma glucose were within the standard ranges for almost all patients, but the γ-glutamyl transpeptidase (γ-GTP) levels exceeded the standard range during 1,2-DCP exposure for six patients. Two of the six patients were diagnosed with cholangiocarcinoma during 1,2-DCP exposure, and the other four patients were diagnosed 1-9 years after termination of exposure. The remaining four patients had γ-GTP levels within the standard range during 1,2-DCP exposure, but had increased γ-GTP levels thereafter, and were diagnosed with cholangiocarcinoma 4-10 years after termination of exposure. Aspartate aminotransferase and alanine aminotransferase levels started to increase following the increase in γ-GTP levels. Conclusions: Even small increases in γ-GTP levels should be considered a signal of early development of cholangiocarcinoma.	Kumagai, 2014b

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Retrospective study	Exposure: dichloromethane and 1,2-dichloropropane. The period of exposure to chlorinated organic solvent ranged from 6 years, 1 month to 16 years, 1 month (median: 9 years, 7 months).	Study location and period: Japan, 1996 to 2013 from 13 hospitals. 17 cases of cholangiocarcinoma among 111 men who were former or current workers at an offset colour proof-printing department at at the same Osaka printing company as in Kumagai, 2013. Based on data from a subsequent government investigation (MHLW, 2013b) and clinical records, with description of the clinico-pathological characteristics of cancers.	Type of tumours: cholangiocarcinoma Aim of the study: to clarify the characteristics of the patients with cholangiocarcinoma. Results: The cholangiocarcinoma was diagnosed at 25–45 years old (mean 36 years). They were exposed to chemicals, including DCM and 1,2-DCP. The serum γ-glutamyl transpeptidase activity was elevated in all patients. Dilated intrahepatic bile ducts without tumour-induced obstruction were observed in five patients. The cholangiocarcinomas arose from the large bile ducts. The precancerous or early cancerous lesions, such as biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile ducts, as well as non-specific bile duct injuries, such as fibrosis, were observed in various sites of the bile ducts in all eight patients for whom operative specimens were available. In conclusion, the results showed that cholangiocarcinomas occurred at a high incidence in relatively young workers of a printing company, who were exposed to chemicals including chlorinated organic solvents.	Kubo, 2014
Retrospective cohort study	1,2-DCP and DCM	Study location and period: Osaka, Japan January 1, 1985, and December 31, 2012 Subject analysed: 116 workers (94 men and 22 women) who had worked in the offset colour proof printing section at the printing company in Osaka between 1985 and 2012.	Type of tumour: bile duct Objective: to examine the risk of bile duct cancer among current and former workers in the offset colour proof printing department at a printing company in Osaka, Japan. Results: Among 106 workers with a total of 1,452.4 person-years of exposure, 17 bile duct cancer cases were observed, resulting in an estimated overall Standardized Incidence Ratios (SIR) of 1,132.5 (95% confidence interval (CI): 659.7-1,813.2). The SIR was 1,319.9 (95% CI: 658.9-2,361.7) for those who were exposed to both DCM and 1,2-DCP, and it was 1,002.8 (95% CI: 368.0-2,182.8) for those exposed to 1,2-DCP only. SIRs tended to increase according to years of exposure to 1,2-DCP but not DCM when a 5-year lag time was assumed. The SIRs were higher for the cohorts in which observation started in 1993-2000, particularly in cohorts in which it started in 1996-1999, compared with those in which it started before or	Sobue, 2015

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			after 1993-2000. **Conclusions:** an extraordinarily high risk of bile duct cancer among the offset colour proof printing workers was observed. Elevated risk may be related to cumulative exposure to 1,2-DCP, but there remains some possibility that a portion of the risk is due to other unidentified substances.	
Case study	Exposure: 1,2-DCP, DCM. chemical exposure concentrations was obtained from the Ministry of Health, Labour and Welfare, Japan.	Study location and period: Osaka, Japan, 2012 Subject analysed: the subjects included five printing workers who were employed at small-scale printing plants (those with fewer than 50 employees), and two printing workers who were employed at middle-scale plants (those with 50−299 employees). All subjects were diagnosed with cholangiocarcinoma and were recognized as having developed an occupational disease by the MHLW.	Type of tumour: cholangiocarcinoma. Objective: This study aimed to identify the chemicals used by seven printing workers who developed cholangiocarcinoma, as well as to estimate the levels of chemical exposure among them. Results: Four of the seven printing workers were exposed to both 1,2-DCP and DCM. The estimated maximum exposure concentrations for each of the four workers were 230 to 420 ppm for 1,2-DCP and 58 to 720 ppm for DCM, and the estimated shift average exposure concentrations were 0 to 210 ppm for 1,2-DCP and 15 to 270 ppm for DCM. The remaining three workers were exposed to DCM but not 1,2-DCP. The estimated maximum exposure concentrations of DCM for each of the three workers were 600 to 1,300 ppm, and the estimated shift average exposure concentrations were 84 to 440 ppm. **Conclusions:** The findings suggest that DCM may contribute to the development of cholangiocarcinoma in humans.	Yamada, 2015 a
Case study	Exposure: 1,2-DCP, DCM. chemical exposure concentrations was obtained from the Ministry of Health, Labour and Welfare, Japan.	Study location and period: Osaka, Japan, 2012 Subject analysed: the subjects included four printing workers and one coating worker who were employed at small-scale plants (fewer than 50 employees) and one printing worker who was employed at a middle-scale plant (50−299 employees).	Type of tumour: cholangiocarcinoma. Objective: This study aimed to identify the chemicals used by five printing workers and one coating worker who developed cholangiocarcinoma and estimate the workers' levels of chemical exposure. Results: All five printing workers were exposed to both 1,2-DCP and DCM. The estimated maximum exposure concentrations for each of the five workers were 190 to 560 ppm for 1,2-DCP and 300 to 980 ppm for DCM, and the estimated shift average exposure concentrations were 0 to 230 ppm for 1,2-DCP and 20 to 470 ppm for DCM. The coating worker was exposed to 1,2-	Yamada, 2015 b

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			DCP, but not DCM. He did not use ink, and thus was subjected to different conditions than the printing workers. The estimated maximum exposure concentration of 1,2-DCP was 150 ppm, and the estimated shift time-weighted average exposure concentration was 5 to 19 ppm. Conclusions: Our findings support the notion that 1,2-DCP contributes to the development of cholangiocarcinoma in humans and the notion that DCM may also be a contributing factor. The finding that the coating worker was exposed to 1,2-DCP at a lower exposure concentration is important for determining the occupational exposure limit. Furthermore, the subject did not use ink, which suggests that ink did not contribute to the development of cholangiocarcinoma.	
Cohort study	Exposure: 1,2-DCP Exposure concentrations for printing workers were measured.	Study location and period: Osaka, Japan. 1987 and 2006. Subject analysed: 95 workers of a printing company (78 men and 17 women) who had been exposed to 1,2-DCP.	Aim of the study: to evaluate the relationship between cumulative exposure to 1,2-DCP and incidence risk of cholangiocarcinoma among workers in the offset proof-printing section of a small printing company in Osaka, Japan. Results: Cumulative exposures to 1,2-DCP ranged from 32 to 3433 ppm-years (mean, 851 ppm-years) and the SIR was 1171 (95% CI 682 to 1875). In the analysis of the four exposure categories, SIRs increased significantly in the three highest exposure categories, but not in the lowest category. Adjusted rate ratios (RRs) in the middle and high exposure categories were 14.9 (95% CI 4.1 to 54.3) and 17.1 (95% CI 3.8 to 76.2), respectively, in the analysis without lag time, and were 11.4 (95% CI 3.3 to 39.6) and 32.4 (95% CI 6.4 to 163.9), respectively, in the analysis with a 5-year lag. The trend analysis revealed a significant increase in RR in association with increasing cumulative exposure to 1,2-DCP. DCM exposure was not significantly associated with the development of cholangiocarcinoma. In conclusions the study demonstrated an exposure–response relationship between exposure to 1,2-DCP and the development of cholangiocarcinoma.	Kumagai, 2016

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

All the available information on human studies, animal studies and mechanistic data were taken into account for the hazard evaluation. Most of these data were reported in the IARC monograph 110 (IARC, 2017) and in the Chemical Safety Report (CSR) provided by the Registrant(s). The most recent publications were also taken into consideration.

Human data

The IARC monograph 110 classified as limited the evidence in humans for the carcinogenicity of DCM based on a positive association between DCM exposure and cancer of biliary tract and non-Hodgkin lymphoma (NHL) (IARC, 2017).

Most evidence derives from epidemiological studies conducted in relation to occupational exposure. They include cohort studies of workers producing cellulose triacetate fibres and films, cohort studies of aircraft workers exposed to multiple solvents including DCM; case-control studies of several different cancers and occupational exposure to solvents; case series, case-control and cohort studies of workers employed in the printing industry in Japan, who were exposed to DCM, 1,2-DCP, and other solvents. Few studies refer to residential exposure to DCM (see tables 16 and 17).

Cancers of liver and biliary tract

The occupational cohort mortality study of Lanes in the USA (Lanes, 1993) included 1271 workers followed-up from 1954 to 1990, employed in the production of cellulose triacetate fibre that entailed exposure to DCM, but not to 1,2-DCP. The authors reported a positive association for cancer of the liver and of biliary tract (SMR, 2.98; 95% CI, 0.81–7.63 – 4 obs). All 4 deaths occurred among employees with ≥ 10 years of employment and ≥ 20 years since first employment (SMR, 5.83; 95% CI, 1.59–14.92), and three out of these four deaths were due to cancer of the biliary tract. The SMR estimated for the three cancer cases of the biliary tract alone was very high (SMR, 20; 95% CI, 5.2–56) as reported in the previous analyses (Lanes, 1990). Although some of the subjects were also exposed to acetone and methanol, the IARC Working Group considered this an unlike explanation for the observed risks because they were not known to be linked to cancer of the liver (IARC, 2017).

An occupational cohort mortality study of another facility in the USA (Gibbs, 1996) similar to that reported by Lanes (Lanes, 1993), followed up 3211 workers from 1970 to 1989. This study did not show an association between DCM exposure and cancers of the liver and biliary tract (SMR, 0.78; 95% CI, 0.09–2.81, 2 deaths). Both deaths were actually cancers of the biliary tract. The IARC working group noted that though the authors did not report an SMR specific for cancer of the biliary tract, if the value were to be computed, it might be higher than that reported for liver and biliary tract combined. To this regard an other study (Dell, 1999) reported that biliary passage cancers are much rarer than liver cancers, suggesting that the relative risk for biliary cancers would be much higher.

A cohort mortality study of workers at a plant producing cellulose triacetate film base, in England (Tomenson, 2011) extending earlier analyses (Tomenson, 1997), included 1785 male workers who had been employed at the site at any time between 1946 and 1988, and followed until 2006, of whom 1473 had been employed in jobs with potential exposure to DCM. The workers had been also exposed to acetone and methanol. Four exposure categories were identified based on cumulative exposure, but 30% of the exposed could not be classified because employment histories were insufficiently precise. No cancers of the liver were observed among exposed or unexposed workers. Among the limits of the study there was a small number of deaths, which limited the ability to conduct exposure–response analysis.

Another cohort mortality study carried out in USA (Hearne and Pifer, 1999), updating previous analyses, followed-up 1311 workers from 1964 to 1994 employed in the production of cellulose triacetate fibre. Workers were exposed to DCM with measured exposure concentrations, and also to

methanol, 1,2-DCP,1,2-dichloroethane, acetone, and benzene, but exposure levels were not reported for these agents. The study showed SMRs for cancer of the liver less than unity, based on one death.

A series of studies investigated the occurrence of cancer of the liver and biliary tract in workers of a printing company in Osaka, Japan in relation to the exposure to DCM, 1,2-DCP, and other solvents. In a study (Kumagai, 2013), an exceptionally high incidence of cholangiocarcinomas and exposure to DCM and 1,2-DCP was observed in former and current workers; all 11 observed cases were exposed to 1,2-DCP, and 10 of them were also exposed to DCM. The SMR for cholangiocarcinoma was 2900 (expected deaths: 0.00204, 95% CI 1100 to 6400) for all workers combined. Later, two additional cases of cholangiocarcinoma were described in workers employed in two different printing shops in Japan. One of the two had been co-exposed to DCM and to 1,1,1-trichloroethane (1,1,1-TCE), the other had been exposed to 1,2-DCP and to white gasoline (Kumagai, 2014a).

An retrospective study (Kubo, 2014) reported overall 17 cholangiocarcinoma patients identified in 13 hospitals, starting from 111 former or current workers employed at the printing company in Osaka, as of June 2013. The 17 cholangiocarcinomas were diagnosed at early age (25–45 years old - mean 36 years). All 17 patients were men exposed to 1,2-DCP, 11 patients were also exposed to DCM, and 8 were also exposed to 1,1,1-TCE. Many other chemicals, however, had been used in the printing department (dichlorofluoroethane, 2-butanol, 2-methylpen-tane, 3-methylpentane, n-hexane, cyclohexane, isopropyl alcohol, ethanol, diethylene glycol, monobutyl ether, propylene glycol, monomethyl ether, 2-methyl-2,4-pentadiol, 3-methyl-3-methoxybutanol, solvent naphtha, xylene, mineral oil, hydrocarbons, aromatic hydrocarbons and inks), but these chemicals were ruled out as possible causative agents because of their low amount used and/or short period of exposure. At the time of diagnosis, the serum γ-glutamyl transpeptidase (γ-GTP) activity was elevated in all patients.

Another study from Kumagai (Kumagai, 2014b) concerned 10 out of 17 cholangiocarcinoma patients identified among workers employed at the Osaka printing company. These subjects gave consent to access their blood and health examination records during employment and after retirement. Patients resulted without any known risk factors of cholangiocarcinoma including primary sclerosing cholangitis, liver fluke infestation, biliary stones, fibropolycystic liver disease, viral hepatitis, exposure to thorotrast, and heavy drinking and smoking. All patients had worked at the company for 6−19 years. Cases were all exposed to 1,2-DCP for 6−17 years and kerosene for 6−19 years, with five of them also exposed to DCM for 2−8 years. Moreover, 3 patients were exposed to 1,1,1-trichloroethane, and 3 to glycol ethers, alcohols and/or cycloaliphatic hydrocarbons. The γ -GTP levels exceeded the standard range 4−11 years after the first exposure to 1,2-DCP, and patients were diagnosed with cancer 2−10 years after the increase in γ -GTP levels. 5 of these patients were also exposed to DCM. The study highlighted that even small increases in γ-GTP levels should be considered a signal of early development of cholangiocarcinoma.

Yamada, 2014 aimed to identify chemicals used by printing workers with cholangiocarcinoma, as well as the levels of exposure to the chemicals in printing companies like the Osaka plant. He identified six printing workers employed at three plants (Miyagi, Fukuoka, and Hokkaido). All six workers had been exposed to 1,2-DCP for 10−16 years. The estimated working environment concentrations of 1,2-DCP in the printing rooms were 17−180 ppm and estimated exposure concentrations during the ink removal operation were 150−620 ppm. Shift TWA (Time Weighted Averages) values were estimated to be 62−240 ppm. Four of the six workers had also been exposed to DCM at estimated working environment concentrations of 0−98 ppm and estimated exposure concentrations during the ink removal operation of 0−560 ppm. Shift TWA values were estimated to be 0−180 ppm. Other chlorinated organic solvents (1,1,1-trichloroethane, 1,1-dichloro-1-fluoroethane) and petroleum solvents (gasoline, naphtha, mineral spirit, mineral oil, kerosene) were also used in the ink removal operation.

The most recent studies, published since last IARC monograph on DCM (IARC, 2017) continue to suggest a possible role of DCM exposure in the development of cholangiocarcinoma in humans (Yamada 2015a and Yamada 2015b, Sobue 2015, Kumagai, 2016).

In his second report, chemical exposure levels in 7 printing workers with cholangiocarcinoma not included in the previous report were assessed (Yamada, 2015a). Four of the seven printing workers with intrahepatic or extrahepatic bile duct cancer (cholangiocarcinoma) were exposed to both 1,2-DCP and DCM. The estimated maximum exposure concentrations for each of the four workers were 230 to 420 ppm for 1,2-DCP and 58 to 720 ppm for DCM, and the estimated shift average exposure concentrations were 0 to 210 ppm for 1,2-DCP and 15 to 270 ppm for DCM. The remaining three workers were exposed to DCM but not to 1,2-DCP. The estimated maximum exposure concentrations of DCM for each of the three workers were 600 to 1,300 ppm, and the estimated shift average exposure concentrations were 84 to 440 ppm. The authors suggest that DCM may contribute to the development of cholangiocarcinoma in humans.

In the third report (Yamada, 2015b), chemical exposure levels in further 5 printing and 1 coating workers with cholangiocarcinoma were analysed. All five printing workers were exposed to both 1,2-DCP and DCM. The estimated maximum exposure concentrations for each of the five workers were 190 to 560 ppm for 1,2-DCP and 300 to 980 ppm for DCM, and the estimated shift average exposure concentrations were 0 to 230 ppm for 1,2-DCP and 20 to 470 ppm for DCM. The coating worker was exposed to 1,2-DCP, but not to DCM. He did not use ink, and thus was subjected to different conditions than the printing workers. The estimated maximum exposure concentration of 1,2-DCP was 150 ppm, and the estimated shift TWA exposure concentration was 5 to 19 ppm. The authors concluded that their findings support the notion that 1,2-DCP contributes to the development of cholangiocarcinoma in humans and the notion that DCM may also be a contributing factor.

A retrospective cohort study was carried out to examine the risk of bile duct cancer among current and former workers in the offset colour proof printing department at a printing company in Osaka, between 1985 and 2012 (Sobue, 2015). Among 106 workers, a total of 1,452.4 person-years and 17 bile duct cancer cases were observed (11 cases exposed to both DCM and 1,2-DCP, and 6 exposed to 1,2-DCP only). Age at diagnosis was between 20−29 years for 2 cases, 30−39 years for 11 cases and 40−49 years for 4 cases. DCM and 1,2-DCP were used to remove ink from the ink rollers. Both chemicals were used between April 1991 and February 1996, and subsequently only 1,2-DCP was used until October 2006. Those who had worked during the period 1996−1999 had higher risks, which implies that some substances or conditions present in this period have some role in increasing the risk of bile duct cancer. The study highlighted a very high risk from cholangiosarcomas among all workers, resulting in an estimated overall SIR of 1,132.5 (95% confidence interval (CI): 659.7−1,813.2). The risk of cholangiosarcomas was always higher in workers exposed to both 1.2-DCP and DCM than among workers exposed only to 1,2-DCP. At lag 0 year, the SIR was 1,319.9 for those who were exposed to both DCM and 1,2-DCP, and 1,002.8 for those exposed to 1,2-DCP only. At lag 3 year, the SIR was 1,372.4 for workers exposed to both chemicals, and equal to 1,150.5 for those exposed to 1,2-DCP only. The same pattern was also evident at lag 5 year: SIR=1,422.9 in workers exposed to both solvents, and 1,319.8 for those exposed to 1,2-DCP only.

Although no clear association with years of exposure appeared when assuming a 0- or 3-year lag time, the SIRs tended to increase with years of exposure to 1,2-DCP but not DCM when a 5-year lag time was assumed. In terms of ability to explore associations with length of exposure, DCM was used over a period of 5 years (1991-1996) while 1,2-DCP was used for about 16 years (from 1991 to 2006). The authors concluded that elevated risk may be related to cumulative exposure to 1,2-DCP, but also that there remains some possibility that a portion of the risk is due to other unidentified substances.

A further study (Kumagai, 2016) evaluated for the first time the relationship between cumulative exposure (ppm-years) to 1,2-DCP and risk of cholangiocarcinoma among 95 workers of the Osaka printing plant who had been exposed to 1,2-DCP between 1987 and 2006. Cumulative exposures to 1,2-DCP ranged from 32 to 3433 ppm-years (mean, 851 ppm-years) and the SIR was 1,171 (95% CI 682 to 1,875 – 17 cases).
Workers were mainly exposed to both 1,2-DCP and DCM solvents (62 subjects), about a third were exposed only to 1,2-DCP (33 subjects), while no one was exposed to DCM alone. The SIR was higher among DCP/DCM workers, compared to DCP workers: 1275 (95% CI 636 to 2280) and 1019 (95% CI 374 to 2218), respectively, but the 95% CIs for these estimates overlapped each other, the difference in SIR was not conclusive.
Adjusted RRs in the middle and high exposure categories were 14.9 (95% CI 4.1 to 54.3) and 17.1 (95% CI 3.8 to 76.2), respectively, in the analysis without lag time, and 11.4 (95% CI 3.3 to 39.6) and 32.4 (95% CI 6.4 to 163.9), respectively, in the analysis with a 5-year lag. However, the 95% CIs of the RR estimates in the middle and high exposure categories always overlapped each other, again making the differences among exposure classes not conclusive.
The Poisson regression and trend analysis revealed a significant increase in RR in association with classes of increasing cumulative exposure to 1,2-DCP, while the presence/absence of DCM exposure was not significantly associated with the development of cholangiocarcinoma. The authors concluded that they could not determine whether DCM contributed to the development of cholangiocarcinoma. However, the results of Poisson regression analyses (supplementary material in the web appendix of the paper) showed that the incidence rate of cholangiocarcinoma, in a single regression model, significantly increases with increasing levels of cumulative exposure to DCM (continuous variable). Moreover, this increase (β coefficient =0.0023- IC95%: 0.0012 – 0.0033) was higher than that observed for 1,2-DCP (β coefficient = 0.0014, IC95%: 0.0010 – 0.0018). In the supplementary material, the multiple regression model including both 1,2-DCP and DCM cumulative exposures, reveal only the β coefficient related to 1,2-DCP remains significant, and this might reflect, according to the authors, the positive correlation between the cumulative exposure to these two solvents (Pearson's correlation coefficient = 0.6).

Some aspects have to be mentioned in relation to the evidence in humans of a carcinogenic effects of DCM exposure in the risk of cholangiocarcinoma. The cancer of biliary tract is rare, while its incidence was enormously high, in particular among workers of the printing plants in Japan, and characterised by an early age at diagnosis. Exposure to DCM occurred some decades ago (in the early 90s), and among workers principally exposed to 1,2-DCP, but also to several other chemicals, making it very complex to retrospectively attribute the observed excess of risks to specific agents.
An aspect that deserves some attention is that cancers of the biliary tract were also diagnosed among workers exposed to DCM but not to 1,2-DCP, and this was seen both among those employed in the production of cellulose triacetate fibre (Lanes 1990, 1993), and among subject working in the printing companies in Japan (Kumagai, 2014a; Yamada, 2015a). The cohort from Lanes (1990, 1993) enrolled workers in activities (preparation and extrusion areas) that entailed exposure to the highest concentrations of DCM, estimated to be substantially greater than for the cohort of photographic film manufacturers (Hearne, 1987). The 3 deaths from cancer of the biliary passages (2 among females) occurred after ten or more years of employment and at least 20 years since first employment, with a risk 20 times higher than in the general population (SMR =20, 95 % CI 5.2-56).
The case of cholangiocarcinoma identified by Kumagai (Kumagai, 2014a) diagnosed at age 41 years, was employed at a general offset printing company in Nagoya, Japan, and engaged in printing operations. He was exposed to DCM for 11 years to a concentration estimated to be between 240 and 6,100 ppm, and was not exposed also to other risk factors for cholangiocarcinoma (liver fluke infection, primary sclerosing cholangitis, biliary malformation, biliary stone, viral hepatitis, heavy drinking and smoking, and exposure to chemicals such as thorotrast). Other three workers with cholangiocarcinoma, employed in printing activities in Japan, were exposed to DCM but not to 1,2-

DCP. The estimated maximum exposure concentrations of DCM for each of the three workers were 600 to 1,300 ppm, and the estimated shift average exposure concentrations were 84 to 440 ppm (Yamada, 2015a).
A further point of interest about a possible carcinogenic role of DCM in the development of cholangiosarcoma is that, in almost all available studies, when assessed, the risk of biliary tract cancer was higher in subjects exposed to both 1,2-DCP and DCM than in workers exposed to 1,2-DCP but not to DCM, though the 95% CIs often overlapped each other, making the differences in risk not conclusive.
The data reported above show that there is an evidence about the association between cumulative exposure to 1,2-DCP and increased incidence of cholangiocarcinoma (Kumagai, 2016), which suggests that an exposure–response relationship exists for this solvent. Because the primary objective of this study was to assess the relationship between cholangiocarcinoma and exposure to 1,2-DCP, the analyses for DCM were limited. This same study did not show a significant effect of exposure to DCM on incidence risk when it is analysed as dichotomous variable (presence/absence), while revealed a statistically significant positive association of cholangiocarcinoma risk with cumulative exposure to DCM (continuous variable) in Poisson regression analysis.

Non-Hodgkin lymphoma (NHL)
In the IARC monograph 110 (IARC, 2017) two cohort studies (Hearne and Pifer, 1999; Radican, 2008) and three case–control studies (Miligi, 2006; Seidler, 2007; Wang, 2009) have been analysed concerning the risk for non-Hodgkin lymphoma (NHL) in relation to occupational exposure to DCM, and all except one cohort study reported increased risks among exposed workers.
The cohort study (Hearne and Pifer, 1999), analysed mortality data of workers at a plant producing cellulose triacetate film base, in the USA. It included 1013 male workers who had been employed in the roll-coating department at any time between 1964 and 1970 and were followed until 1994. The SMR for NHL was less than unity, based on two cases. Workers may have also been exposed to methanol, 1,2-dichloropropane ,1,2-dichloroethane, acetone, and benzene, but exposure levels were not reported for these agents. The small numbers of exposed cases hamper the analysis of exposure–response patterns, and was reported by IARC as an important limitation of this study.
The cohort mortality study from Radican (Radican, 2008) included workers at a military-aircraft maintenance facility in the USA, updating earlier studies (Spirtas, 1991; Blair, 1998). The cohort consisted of civilian employees employed between 1952 and 1956 and followed until 2000. Workers were exposed to numerous chemicals. Exposure was assessed quantitatively for trichloroethylene, and qualitatively (ever/never) to other agents including DCM. The number of workers exposed to DCM was 1222. Exposure to DCM was associated with increased risks (hazard ratio, HR) of NHL (HR, 2.02; 95% CI, 0.76–5.42; 8 exposed cases).
In three case-control studies (Miligi 2006; Seidler, 2007; Wang, 2009) a positive association between DCM exposure and occurrence of NHL was reported.
In a case–control study conducted in Italy (Miligi, 1996) to evaluate the association between risk of lymphoma and exposure to DCM and nine other organic solvents. The study included 1428 cases of NHL and 1530 controls. Probability and intensity of occupational exposure to individual chemicals and chemical classes were assigned by expert assessment. Odds ratios were adjusted by area, sex, age, and education, excluding subjects with low probability of exposure. The odds ratio (OR) for NHL in the category for combined medium- and high-intensity exposure to DCM was 1.7 (95% CI, 0.7–4.3; 13 cases; P for trend, 0.46). Among the NHL subtypes, an odds ratio for DCM was reported only for small lymphocytic NHL: for medium or high exposure, the odds ratio was 3.2 (95% CI, 1.0–10.1).
A case–control study to examine the relationship between malignant lymphoma and exposure to eight organic solvents including DCM was conducted (Seidler, 2007). The study included 710 LNH and 710 general-population controls matched for area, sex, and age collected from six areas in Germany. Exposure was assessed for several chlorinated solvents, with metrics of intensity, frequency, and

confidence assigned by an industrial hygienist, and cumulative exposure was calculated. Odds ratios, adjusted for smoking and alcohol consumption, for high cumulative exposure to DCM were 2.2 (95% CI, 0.4–11.6; P for trend, 0.40) for all lymphomas, and 2.7 (95% CI, 0.5–14.5; P for trend, 0.29) for B-cell NHL. A third case-control study examined the association between NHL and exposure to nine organic solvents including DCM, among 601 female cases, and 717 general-population controls, matched for age, in Connecticut, USA (Wang, 2009). Probability and intensity of exposure to solvents were assigned using a previously developed job-exposure matrix. Odds ratios, adjusted by race, age, family history of haematopoietic cancer, and alcohol consumption, showed that subjects ever-exposed to DCM had an increased risk of NHL (OR, 1.5; 95% CI, 1.0–2.3). The working group of IARC (IARC, 2017) observed that analyses by intensity and probability of exposure indicated elevated ORs, but trends were not statistically significant.
A further study carried out in a subset of the population studied by Wang (Wang, 2009) evaluated whether genetic variation in four genes involved in metabolism (CYP2E1,EPHX1, NQO1, MPO) modifies associations between exposure to organic solvents and risk of NHL (Barry, 2011). Ever-exposure to DCM entailed elevated risk of NHL (OR, 1.69; 95% CI, 1.06–2.69), and it was higher among ever-exposed women with the TT genotype for CYP2E1 rs2070673 (OR, 4.42; 95% CI, 2.03–9.62), while no effects of DCM was observed among women with the TA or AA genotype (OR, 0.80; 95% CI, 0.36–1.75). Similar patterns were observed for diffuse large B-cell lymphoma and follicular lymphoma. The IARC Working Group (IARC, 2017) noted that the functional role of the CYP2E1 polymorphism is unclear.

The IARC (IARC, 2017) in its summary of human carcinogenicity data stated that while positive associations for NHL were consistent among studies using different designs, and in several countries, most subjects were exposed to several solvents (some of which have been previously associated with NHL) and the risk estimates were based on small numbers. This association was also suggested in two studies (Talibov, 2017 and Park, 2017) not included in the evaluation of IARC (IARC, 2017). In the first sudy (Talibov, 2017) was observed a significantly increased risk for cumulative DCM exposure ≤12.5 ppm-years (OR 1.19, 95% CI 1.01–1.41) and 12.5–74.8 ppm-years (OR 1.23, 95% CI 1.01–1.51) among men in an analysis with 5 years lag-time, though without dose–response pattern.

In the other study (Park, 2017) some possible excess risks, based on very small numbers, when analysing childhood CLL and DCM within a 3 km buffer between residences and emitting facilities were observed, though not supported by statistical significance.

Other Cancer types

Cancers of brain and central nervous system

An association between astrocytic cancer of the brain and exposure to six chlorinated solvents was found but the reliability of the exposure assessment was judged to be relatively low because occupational information was obtained from the next of kin (Heinmann, 1994).
In an other case-control study (Cocco, 1999) the association between mortality from the cancer of the brain and other parts of central nervous system and exposure to 11 factors including DCM was studied. After adjusting for age at death, marital status, and socioeconomic status, the odds ratio for the association of exposure to DCM and all cancer of the central nervous system (CNS) was 1.2 (95% CI, 1.1–1.3). Odds ratios were generally similar for all categories of probability and intensity of exposure. Then, no evidence of a strong contribution of 11 occupational hazards to the etiology of CNS cancer was reported in the study.
In an other study the effects of parental occupational chemical exposures on incidence of neuroblastoma in offspring was evaluated (De Roos, 2001). Maternal exposures to most chemicals were not associated with neuroblastoma. Paternal exposures to hydrocarbons such as diesel fuel (odds ratio (OR) = 1.5; 95% confidence interval (CI): 0.8, 2.6), lacquer thinner (OR = 3.5; 95% CI: 1.6, 7.8), and turpentine (OR = 10.4; 95% CI: 2.4, 44.8) were associated with an increased incidence of

neuroblastoma, as were exposures to wood dust (OR = 1.5; 95% CI: 0.8, 2.8) and solders (OR = 2.6; 95% CI: 0.9, 7.1). The increased incidence of neuroblastoma is not specifically related to DCM exposure.
In a hospital-based case-control study to examine associations between glioma and meningioma and exposure to six chlorinated solvents including DCM was conducted (Neta, 2012). Odds ratios adjusted for the matching factors did not show any association between glioma or meningioma and overall exposure to DCM or other metrics, including duration, intensity, and cumulative exposure.
A population-based case-control study to examine associations between glioma and exposure to six chlorinated solvents including DCM was conducted (Ruder, 2013). There were no associations between glioma and overall exposure to DCM, or exposure probability and cumulative exposure.
Among the studies not included in the IARC monograph 110 (IARC, 2017) there is a study that carried out a retrospective comparative population study in an area around a plant using DCM in the shoe soles production in Cyprus from 1983 until 2009 (Makris, 2018). Mean stack emissions of DCM of 88 mg/Nm3 and flow rates of 850 g/h exceeded the permissible DCM limits established for industrial zones by the EU Directive. Brain and CNS cancer incidence rates were much higher in the study area around the plant when compared with those observed in other areas of Cyprus. Among people living or working in the area within a radius of 500 meters from the plant, standardized incidence ratios for brain/CNS cancer, after adjusting for the age at diagnosis, was 6.5 (95% CI 3.02 : 12.3), based on 8 observed cases *versus* 1,2 expected.

Breast cancer
The IARC classified as inadequate the evidence on the association between DCM exposure and the risk of cancer types different form the ones discussed above (IARC, 2017).
Among those, breast cancer entails some specific concern as this neoplasm has been observed to be at risk among rats exposed to DCM. One of the early studies concerned occupational exposures and female breast cancer mortality in USA. A suggestive association for probability and level of exposure were found for DCM. The cohort mortality study in USA (Radican, 2008) included civilian employees at the military-aircraft maintenance facility exposed to numerous chemicals, including DCM. DCM exposure was assessed qualitatively (ever/never) and the results showed a possible excess risk of female breast cancer (HR, 2.35; 95% CI, 0.98–5.65, based on 6 exposed cases).
A recent prospective cohort (Niehoff, 2019) based on 49,718 women from the Sister Study, identified 2975 women with newly diagnosed breast cancer, and the incidence was examined in relation to 29 non-metallic hazardous air pollutants previously found to be mammary gland carcinogens in animal models and part of the 2005 National Air Toxics Assessment (NATA). Several air toxics were associated with increased risk, and among these, DCM was most consistently associated with risk across multiple analyses. It was associated with overall (HR quintile 4vs1 = 1.21 (95%CI = 1.07–1.38)) and estrogen receptor positive (ER+) invasive breast cancer (HR quintile 4vs1 = 1.28 (95%CI = 1.08–1.52)) in individual pollutant models, although no dose-response was observed.

Conclusion on Human data

Overall, the DS, based on the assessment of the currently available pertinent epidemiological studies, supports the previous evaluation of the IARC that “there is limited evidence in humans for the carcinogenicity of dichloromethane” (IARC, 2017). The DS conclusion is mainly based on the confirmed positive association between DCM exposure and cancer of biliary tract, and, at less extent, on evidence concerning non-Hodgkin lymphoma.

Animal data:
There were six studies of carcinogenicity with DCM in mice: in two studies DCM was administered orally to both males and females (one in drinking-water, and one by gavage), in three studies by inhalation (two in males and females, one in females), and in one study DCM was injected

intraperitoneally in males. Moreover, there were seven carcinogenicity studies with DCM in rats: two oral administration studies (one drinking-water study in males and females and one gavage study in males and females), five inhalation studies (four in males and females, one in pregnant females and their male and female offspring). Only one study is available in hamster following DCM exposure by inhalation (both in males and females).

Studies in Mice

In the oral study in mice (Serota, 1986a) two control groups were used. At the highest dose in male mice the incidence of hepatocellular carcinomas showed a statistically significant increase only when compared with the first control group. Treatment-related toxic effects were observed in the liver of both male and female B6C3F mice following administration of DCM in drinking water. A statistically significant increase in hepatocellular carcinoma was reported at the highest dose in males, but the value was within the range of historical controls. A slight increase in proliferative hepatocellular lesions was noted in the treated male groups but was not dose related and was within historical control ranges. Then no induction of a treatment-related carcinogenic response was reported in B6C3F mice in the experimental conditions of this study. In the other oral study (Maltoni, 1988) the incidence of pulmonary adenoma or adenocarcinoma (combined) was significantly increased in male mice only at the highest dose. No increase was observed in female. The validity of the study is limited because, due to an excess of mortality, at the highest dose the time of exposure was only 64 weeks, and the study was interrupted at 78 weeks (instead of 104).

In a inhalation study in mice (NTP, 1986a) a concentration-related increases in the incidence of bronchiolar-alveolar adenoma, carcinoma, and combined adenoma and carcinoma were reported in both male and female B6C3F1 mice. In addition, concentration-related increases in the incidence of hepatocellular adenoma, carcinoma, and combined adenoma and carcinoma were seen in both males and females.

In an other study in mice (Kari, 1993) only the lung and liver were evaluated histopathologically. The aim of the study was to assess the progressive development of liver and lung neoplasia. Additionally, a series of stop-exposure treatments (26, 52 or 78 weeks) was conducted to evaluate the role of different DCM exposure durations on the induction of hepatic and pulmonary neoplasia in female mice. The incidences of bronchiolo-alveolar adenoma, bronchiolo-alveolar carcinoma, and adenoma or carcinoma (combined), and the incidences of hepatocellular adenoma, hepatocellular carcinoma, and adenoma or carcinoma (combined) were significantly increased in all groups in which exposure was begun during the first 26 weeks of the study. The study was performed only in female mice. A reduced tumour latency was reported in the study both for lung and liver tumours.

The observed tumours in the NTP study (NTP, 1986a) were confirmed in a different mouse strain (Aiso, 2014).

A DCM-concentration related increases in the incidences of lung and liver adenomas and carcinomas were observed. In males, a concentration-related increase in bronchiolar-alveolar carcinomas was seen, while in females a statistically significant increase only occurred at highest dose (4000 ppm). A statistically significant increase in hepatocellular carcinomas was seen in males and females exposed to 4000 ppm; and an increased incidence of hepatocellular adenoma in females exposed to 4000 ppm. The incidence of liver haemangioma was significantly increased in males at the highest dose. The incidence of liver haemangioma or haemangiosarcoma (combined) showed a statistically significant dose-response trend in females ($p<0.01$). Moreover, hyperplasia in the terminal bronchiole (this lesion may be classified as a preneoplastic lesion capable of developing into bronchiolo-alveolar adenoma and carcinoma) and peripheral vacuolar change in the liver were increased in males and females at the highest dose (NTP, 1986 and Aiso, 2014). Inhalation of DCM resulted in increased incidences of bronchiolar–alveolar adenomas and carcinomas in the lung and hepatocellular adenomas and carcinomas in male and female mice.

A very old study performed by intraperitoneal injection is also available (Theiss, 1977). No significant increase was found in the multiplicity of bronchiolo-alveolar adenoma in exposed male mice.

Studies in rats

Two oral studies in rats are available. In the first study (Serota, 1986b) Fischer 344 rats were exposed to DCM 0, 5, 50, 125 and 250 mg/kg bw/day in drinking water over 104 weeks. An additional group received a level of 250 mg/kg bw/day for 78 weeks followed by a 26-week recovery period during which only deionized water was presented. The increased incidence of hepatic tumours observed in females treated at 50 and 250 mg/kg bw/day was within the range of historical control incidences. In view of an unusually low incidence of similar tumours in the concurrent control groups and of the absence of an increased incidence of hepatic tumours in the group treated at 125 mg/kg bw/day, the effect seen at 50 and 250 mg/kg bw/day was not considered to be attributable to DCM treatment (Serota, 1986b). Non-neoplastic lesions in Sprague Dawley rats treated by gavage were reported also in the other oral study (Maltoni, 1988).

Five inhalation studies in rats are also available. In one study (Burek, 1984) there was no significant increase in the incidence of benign or malignant tumours of the mammary gland; however, the total number of benign tumours of the mammary gland (type not specified) showed a small dose-related increase in males and a dose-related increase in females (statistics not reported). Exposure to 3500 ppm resulted in increased mortality in female rats during the last 6 months of exposure, compared to control values. The mortality in the female rats at the highest dose was probably caused by the numerous benign mammary tumours in this group. Toxic effects on the liver were also reported both in male and female rats. The incidence of sarcoma located around the salivary glands was increased in males at the highest dose. However, it should be noted that an infection of rats with sialodacryoadenitis virus was reported in the study.

In an other inhalation study (NTP 1986b) a significantly increased incidence of benign tumours of the mammary gland (all fibroadenoma, except for one adenoma in the group at the highest dose) was observed in treated females (5/50, 11/50, 13/50, 23/50). In males there was a positive trend in the incidences of adenoma or fibroadenoma (combined) of the mammary gland, and of fibroma or sarcoma (combined) of the subcutis. There was no difference in the distribution of other types of tumours in the control and treated groups.

No significant differences in tumours incidence between control and treated rats were observed in an other study (Maltoni, 1988). However, in this study the rats were exposed to a very low level of DCM (60 or 100 ppm).

Other data showed, no significant increase in the incidence of any tumour type was reported in males, while a significant increase in the incidences of benign tumours of the mammary gland (adenomas and fibroadenomas, combined) was observed only at intermediate dose in female rats (Nietschke, 1988).

Some evidence of carcinogenic activity of DCM in male and female rats, based on the increased incidences of fibromas of the subcutis, mammary gland fibroadenomas (at the highest dose) and peritoneal mesotheliomas (positive trend) in males and mammary gland fibroadenomas (positive trend) in females was also reported (Aiso, 2014).

Hamster

There was only one study of carcinogenicity in hamsters treated with DCM by inhalation (Burek, 1984 reported also in EPA, 1985). An increased number of female hamsters with a benign tumour was observed in the 3500-ppm exposure group. Moreover, a statistical significant increase of malignant lymphoma (lymphosarcoma) was reported in the highest dose group of female hamster. The authors of the study considered the increased tumour observed independent from DCM exposure

and related to the higher survival. In fact, the positive results at the highest dose in females (for both benign and malignant tumours) when corrected for the survival became not significant.

Conclusion of animal studies

In conclusion, DCM increased the incidence of hepatocellular carcinoma in two inhalation studies in male mice (NTP, 1986a and Aiso, 2014), and in three studies of inhalation in female mice (NTP, 1986a; Aiso, 2014 and Kari, 1993). DCM increased the incidence of hepatocellular adenoma or carcinoma (combined) in two inhalation studies in male mice and three inhalation studies in female mice. Increased incidence of bronchiolo-alveolar carcinoma following DCM treatment was reported in two inhalation studies in male mice and three inhalation studies in female mice, and bronchiolo-alveolar adenoma or carcinoma (combined) in three inhalation studies in male mice and three inhalation studies in female mice. DCM increased the incidences of haemangioma of the liver and of all organs (including the liver) in one inhalation study in male mice, while incidence of liver haemangioma or haemangiosarcoma (combined) showed a statistically significant dose-response trend in females ($p<0.01$) (Aiso, 2014; JBRC, 2000a).

DCM increased the incidence of fibroma of the subcutis in two inhalation studies in male rats and fibroma or fibrosarcoma of the subcutis in one inhalation study in male rats. DCM caused salivary gland sarcomas in one inhalation study in male rats (however the sialodacryoadenitis virus was detected in these rats; the effect of this virus on carcinogenesis is unknown).

DCM increased the incidence of mammary gland adenoma or fibroadenoma (combined) in two inhalation studies in female rats and one inhalation study in male rats. The incidence of mammary gland adenoma was also increased in another inhalation study in males and another one in females.

There was one inhalation study on DCM in male and female Syrian hamsters in which there was an increase in the incidence of benign tumours only in females at highest dose, but this was considered secondary to the increased survival of this group.

Table 18: Summary of studies showing evidence of carcinogenic effect *in vivo*

Type of tumours	Mice (inhalation)		Type of tumours	Rats (inhalation)	
	Male	Female		Male	Female
Hepatocellular carcinoma	Aiso, 2014 NTP, 1986b	Aiso, 2014 NTP, 1986b Kari, 1993	fibroma of the subcutis	NTP, 1986a Aiso, 2014	
hepatocellular adenoma or carcinoma (combined)	Aiso, 2014 NTP, 1986b	Aiso, 2014 NTP, 1986b Kari, 1993	fibroma or fibrosarcoma of the subcutis	NTP, 1986a	
bronchiolo-alveolar carcinoma	Aiso, 2014 NTP, 1986b	Aiso, 2014 NTP, 1986b Kari, 1993	salivary gland sarcomas	Burek, 1984	
bronchiolo-alveolar adenoma or carcinoma (combined)	Aiso, 2014 NTP, 1986b Maltoni, 1988	Aiso, 2014 NTP, 1986b Kari, 1993	mammary gland adenoma or fibroadenoma (combined)	NTP, 1986a	NTP, 1986a Aiso, 2014
haemangioma of the liver and of all organs (including the liver)	Aiso, 2014		Total number of benign mammary tumours	Burek, 1984	Burek, 1984
haemangioma or haemangiosarcoma (combined) in the liver		Aiso, 2014*			

*the increase was whithin the historical control values

Mechanistic information:
DCM is a volatile lipophilic compound that is readily absorbed after oral, inhalation or dermal exposure and distributed systemically. Two important metabolic pathways for the metabolism of DCM have been characterized in humans and experimental animals. One pathway is CYP2E1-mediated, which ultimately generates carbon monoxide (CO) and carbon dioxide (CO_2) as stable end products. One of the intermediates, formyl chloride, is reactive with nucleophiles. Glutathione conjugation, catalysed primarily by glutathione S-transferase theta-1 (GSTT1), is the other important metabolic pathway, and results in the formation of reactive metabolites, including formaldehyde and S-chloromethyl glutathione. CYP2E1-mediated metabolism is predominant at lower concentrations, but can be easily saturated, with glutathione S-transferase-mediated metabolism eventually predominating at higher concentrations. P450 and glutathione S-transferase (GST)-mediated metabolism of DCM are qualitatively similar between humans and rodents, but quantitative differences exist across species, tissues, and cell types, and among individuals. Differences in GSTT1 expression and localization may be important determinants of site-specific carcinogenicity caused by DCM. In human cells, DCM induces micronucleus formation and SCEs, but not DNA–protein cross-links and DNA damage. In experimental animals, DCM-induced genotoxicity is associated with the GST pathway. Studies in bacterial systems *in vitro* showed evidence of mutagenicity, particularly in the presence of GST activity. Evidence for the role of GSTT1 in genotoxicity in humans is mixed. Overall, the genotoxicity of DCM appears to be strongly associated with GST-mediated metabolism, consistently with the formation of reactive metabolites through this pathway. However, a role of P450 in genotoxicity cannot be ruled out.
There is little evidence for non-genotoxic mechanisms of carcinogenesis with DCM. No studies with DCM in humans have investigated whether GSTT1 polymorphisms are associated with cancer.

10.9.2 Comparison with the CLP criteria

Table 19: Results of human and carcinogenicity studies in comparison to the CLP criteria

Human and Toxicological results	CLP criteria
The assessment of the currently available pertinent epidemiological studies performed by DS supports the evaluation of the IARC (IARC, 2017) that "there is limited evidence in humans for the carcinogenicity of dichloromethane". The DS conclusion is mainly based on the confirmed positive association between DCM exposure and cancer of biliary tract, and, at less extent, on evidence concerning non-Hodgkin lymphoma. Thus a classification as Carc. 1A is not appropriate for DCM.	***Category 1A*** *(known human carcinogen), known to have carcinogenic potential for humans, classification is largely based on human evidence … The classification in Category 1A … is based on strength of evidence together with additional considerations … 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)* (EC, 2008). *The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:* *— sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;* *— limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or*

	confounding could not be ruled out with reasonable confidence.
The classification in the category 1B is based on limited evidence in human studies and sufficient evidence in animal studies with a MoA relevant to humans. DS supports the evaluation of IARC (IARC, 2017) that "there is limited evidence in humans for the carcinogenicity of dichloromethane". These evidences are mainly based on two types of tumours: cancer of biliary tract, and, at less extent, on evidence concerning non-Hodgkin lymphoma. Sufficient evidence in animal studies is based on the following factors: **Strenght of evidence:** A clear carcinogenic effect was reported in two inhalation studies in mice both in males and females. Carcinogenic effects were reported also in male and female rats (benign and malignant tumours) following DCM exposure by inhalation. Thus, carcinogenic effect, was clearly reported in two species in both sexes in several inhalation studies. **Tumour type and background incidence:** **Mice** An increased incidence of hepatocellular carcinoma or adenoma (combined) was reported in two inhalation studies in male mice and in three inhalation studies in female mice. An increased incidence of bronchiolo-alveolar carcinoma in two inhalation studies in male mice and three inhalation studies in female mice, and bronchiolo-alveolar adenoma or carcinoma (combined) in three inhalation studies in male mice and three inhalation studies in female mice were also reported. DCM increased the incidences of haemangioma of the liver and of all organs (including the liver) in one inhalation study in male mice, while incidence of liver haemangioma or haemangiosarcoma (combined) showed a statistically significant dose-response trend in females ($p<0.01$). **Rats** DCM increased the incidence of fibroma of the subcutis in two inhalation studies in male rats and fibroma or fibrosarcoma of the subcutis in one inhalation study in male rats. DCM caused salivary gland sarcomas in one inhalation study in male rats (however the sialodacryoadenitis virus was detected in these rats; the effect of this virus on carcinogenesis is unknown). DCM increased the incidence of mammary gland adenoma or fibroadenoma (combined) in two inhalation	***Category 1B*** *(presumed human carcinogen), presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. … The classification in Category … 1B is based on strength of evidence together with additional considerations … Such evidence may be derived from … animal experiments for which there is sufficient 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* (EC, 2008). *…. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:* *— sufficient evidence of carcinogenicity: 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: 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.* *Some important factors which may be taken into consideration, when assessing the overall level of concern are:* *(a) tumour type and background incidence;* *(b) multi-site responses;* *(c) progression of lesions to malignancy;* *(d) reduced tumour latency;*

studies in female rats and one inhalation study in male rats. The incidence of mammary gland adenoma was also increased in another inhalation study in males and another one in females. **Hamster** Only one inhalation study in male and female Syrian hamsters is available. Although the limited reported on the study, an increased incidence of malignant lymphoma in females was reported. **Multi-site responses:** DCM induces tumours in various tissues: liver, lung, in mice; mammary gland, salivary gland in rats. **Progression of lesions to malignancy:** Related benign and malignant tumours were observed in mouse (hepatocellular adenoma and carcinoma, hepatic haemangioma and haemangiosarcoma, bronchiolo-alveolar adenoma and carcinoma) and in rat (fibroma and fibrosarcoma of the subcutis). **Reduced tumour latency :** In Kari (1993), a reduced latency for lung and liver tumour in mice was reported. The first observation of tumour occurrence in lung was at 52 weeks compared to 75 weeks reported in the control; also in the liver, the first tumour appeared at 52 weeks compared to 83 weeks reported in the control mice. **Whether responses are in single or both sexes:** Tumours were reported in both sexes in mice and rats. **Whether responses are in a single species or several species:** Tumours occurred both in mice and in rats. **Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:** None. **Routes of exposure:** The available carcinogenicity studies were performed by oral and inhalation route. Negative results were reported in all the oral studies. The inhalation route showed clear carcinogenic effects in mice and rats although in different organs (liver and lung in mice; salivary gland, mammary glad and subcutis sarcoma in rats). **Comparison of absorption, distribution, metabolism and excretion between test animals and humans:** The ADME of DCM was extensively studied in mice and rats.	*(e) whether responses are in single or both sexes;* *(f) whether responses are in a single species or several species;* *(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;* *(h) routes of exposure;* *(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;* *(j) the possibility of a confounding effect of excessive toxicity at test doses;* *(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.* *Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.*

DCM is readily absorbed after oral, inhalation, or dermal exposure, and distributed systemically. Two important metabolic pathways for the metabolism of DCM have been characterized in humans and experimental animals. One pathway is CYP2E1-mediated, the other important metabolic pathway is the Glutathione conjugation by glutathione S-transferase theta-1 (GSTT1). CYP2E1-mediated metabolism is predominant at lower concentrations, but can be easily saturated, with glutathione S-transferase-mediated metabolism eventually predominating at higher concentrations. P450 and glutathione S-transferase (GST)-mediated metabolism of DCM are **qualitatively** similar between humans and rodents, but quantitative differences exist across species, tissues, and cell types, and among individuals. **The possibility of a confounding effect of excessive toxicity at test doses:** All the carcinogenic effects were reported below the MTD. **Mode of action and its relevance for humans:** So far, the mode of action of the carcinogenicity is not fully clarified. A link between genotoxicity and carcinogenicity is expected, considering the results obtained in genotoxicity studies. Differences in GSTT1 expression and localization may be important determinants of site-specific carcinogenicity caused by DCM. In human cells, DCM induces micronucleus formation and SCEs, but not DNA–protein cross-links and DNA damage. In experimental animals, DCM-induced genotoxicity is associated with the GST pathway. Studies in bacterial systems *in vitro* showed evidence of mutagenicity, particularly in the presence of GST activity. Evidence for the role of GSTT1 in genotoxicity in humans is mixed. Overall, the genotoxicity of DCM appears to be strongly associated with GST-mediated metabolism, consistent with the formation of reactive metabolites through this pathway. However, a role of P450 in genotoxicity cannot be ruled out. No evidence of a non-genotoxic MoA of DCM carcinogenicity is available. **Consideration of mutagenicity:** There is sufficient evidence for the *in vivo* mutagenicity of DCM. Therefore, a genotoxic MoA for the observed DCM carcinogenicity is plausible. **Conclusions** All the tumours observed in the animal studies are of human relevance for classification.	

Based on these results, there is sufficiently convincing evidence to propose a classification for DCM as Category 1B.	
Based on the data reported above, category 2 is not appropriated.	*Category 2 (suspected human carcinogen): 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 from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies* (EC, 2008).

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the results of the carcinogenicity studies available, there are limited evidence in human studies and sufficient evidence of DCM carcinogenicity in mice and rats. There is extensive evidence for genotoxicity, in association with metabolic pathways that are operative in humans, considering that the metabolic differences between species, organs, tissues and cells are quantitative but not qualitative. Overall, the available experimental evidence suggests that the mode of action of the carcinogenesis reported in animals is relevant for human.
Based on the overall information the DS concludes that a classification as Carc 1B, H350 is warranted.

10.10 Reproductive toxicity

Not evaluated.

10.11 Specific target organ toxicity-single exposure

Not evaluated.

10.12 Aspiration hazard

Not evaluated.

11 ENDOCRINE DISRUPTION FOR HUMAN HEALTH

Not evaluated.

12 EVALUATION OF AQUATIC HAZARDS UNDER CLP ANNEX I, 4.1

Not evaluated.

13 PERSISTENT, BIOACCUMULATIVE AND TOXIC (PBT) OR VERY PERSISTENT, VERY BIOACCUMULATIVE (VPVB) PROPERTIES UNDER CLP ANNEX I, 4.3

Not evaluated.

14 PERSISTENT, MOBILE AND TOXIC (PMT) OR VERY PERSISTENT, VERY MOBILE (VPVM) PROPERTIES UNDER CLP ANNEX I, 4.4

Not evaluated.

15 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated.

16 ADDITIONAL LABELLING

Not evaluated.

17 REFERENCES

Aiso S, Take M, Kasai T, Senoh H, Umeda Y, Matsumoto M, et al. (2014). Inhalation carcinogenicity of dichloromethane in rats and mice. Inhal Toxicol, 26(8):435–51.

Allen J, Kligerman A, Campbell J, Westbrook-Collins B, Erexson G, Kari F, et al. (1990). Cytogenetic analyses of mice exposed to dichloromethane. Environ Mol Mutagen, 15(4):221–8.

Andersen ME, Clewell HJ 3rd, Gargas ML, Smith FA, Reitz RH (1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol, 87(2):185–205. doi:10.1016/0041-008X(87)90281-X PMID:3824380

Anderson BE, Zeiger E, Shelby MD, Resnick MA, Gulati DK, Ivett JL, et al. (1990). Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environ Mol Mutagen, 16(Suppl 18):55–137.

Angelo MJ, Pritchard AB, Hawkins DR, Waller AR, Roberts A (1986a). The pharmacokinetics of dichloromethane. I. Disposition in B6C3F1 mice following intravenous and oral administration. Food Chem Toxicol, 24(9):965–74. doi:10.1016/0278-6915(86)90325-X PMID:3096853

Angelo MJ, Pritchard AB, Hawkins DR, Waller AR, Roberts A (1986b). The pharmacokinetics of dichloromethane. II. Disposition in Fischer 344 rats following intravenous and oral administration. Food Chem Toxicol, 24(9):975–80. doi:10.1016/0278- 6915(86)90326-1 PMID:3096854

Barry KH, Zhang Y, Lan Q, Zahm SH, Holford TR, Leaderer B, et al. (2011). Genetic variation in metabolic genes, occupational solvent exposure, and risk of non-hodgkin lymphoma. Am J Epidemiol, 173(4):404– 13.

Benbrahim-Tallaa, L., Lauby-Secretan, B., Loomis, D., Guyton, K.Z., Grosse, Y., El Ghissassi, F., Bouvard, V., Guha, N., Mattock, H., Straif, K., International Agency for Research on Cancer Monograph Working, G., 2014. Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone. Lancet Oncol. 15, 924–925.

Blair A, Hartge P, Stewart PA, McAdams M, Lubin J (1998). Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow up. Occup Environ Med, 55(3):161–71.

Bos PMJ, Zeilmaker MJ, van Eijkeren JCH (2006). Application of physiologically based pharmacokinetic modeling in setting acute exposure guideline levels for methylene chloride. Toxicol Sci, 91(2):576–85. doi:10.1093/toxsci/kfj176

Burek JD, Nitschke KD, Bell TJ, Wackerle DL, Childs RC, Beyer JE, et al. (1984). Methylene chloride: a two-year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam Appl Toxicol, 4(1):30–47.

Casanova M, Deyo DF, Heck HD (1992). Dichloromethane (methylene chloride): metabolism to formaldehyde and formation of DNA-protein cross-links in B6C3F1 mice and Syrian golden hamsters. Toxicol Appl Pharmacol, 114(1):162–5.

Casanova M, Conolly RB, Heck HD (1996). DNA-protein cross-links (DPX) and cell proliferation in B6C3F1 mice but not Syrian golden hamsters exposed to dichloromethane: pharmacokinetics and risk assessment with DPX as dosimeter. Fundam Appl Toxicol, 31(1):103–16.

Casanova M, Bell DA, Heck HD (1997). Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes. Fundam Appl Toxicol, 37(2):168–80.

Christensen KY, Vizcaya D, Richardson H, Lavoué J, Aronson K, Siemiatycki J (2013). Risk of selected cancers due to occupational exposure to chlorinated solvents in a case-control study in Montreal. J Occup Environ Med, 55(2):198–208.

Clewell HJ 3rd (1995). Incorporating biological information in quantitative risk assessment: an example with methylene chloride. Toxicology, 102(1-2):83–94. doi:10.1016/0300-483X(95)03038-H

Crebelli R, Carere A, Leopardi P, Conti L, Fassio F, Raiteri F, Barone D, Ciliutti P, Cinelli S and Vericat J.A. Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test. Mutagenesis vol.14 no.2 pp.207–215, 1999

Cocco P, Heineman EF, Dosemeci M (1999). Occupational risk factors for cancer of the central nervous system (CNS) among US women. Am J Ind Med, 36(1):70–4.

Costantini AS, Benvenuti A, Vineis P, Kriebel D, Tumino R, Ramazzotti V, et al. (2008). Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian Multicenter Case-control study. Am J Ind Med, 51(11):803–11.

De Roos AJ, Olshan AF, Teschke K, Poole C, Savitz DA, Blatt J, et al. (2001). Parental occupational exposures to chemicals and incidence of neuroblastoma in offspring. Am J Epidemiol, 154(2):106–14.

Dell LD, Mundt KA, McDonald M, Tritschler JP, Mundt DJ (1999): Critical Review of the epidemiology literature on the potential cancer risks of methylene chloride (publication), Environ Health 72, 429-442.

DeMarini DM, Shelton ML, Warren SH, Ross TM, Shim J-Y, Richard AM, et al. (1997). Glutathione S-transferase-mediated induction of GC–>AT transitions by halomethanes in Salmonella. Environ Mol Mutagen, 30(4):440–7.

Dekant W, Jean Paul, Arts Josje. Evaluation of the carcinogenicity of dichloromethane in rats, mice, hamsters and humans. Regul Toxicol Pharmacol. (2021) Mar;120:104858.

Dillon D, Edwards I, Combes R, McConville M, Zeiger E (1992). The role of glutathione in the bacterial mutagenicity of vapour phase dichloromethane. Environ Mol Mutagen, 20(3):211–7.

DiVincenzo GD, Kaplan CJ (1981). Uptake, metabolism, and elimination of methylene chloride vapor by humans. Toxicol Appl Pharmacol, 59(1):130–40.doi:10.1016/0041-008X(81)90460-9

Doherty AT, Ellard S, Parry EM, Parry JM (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells. Mutagenesis, 11(3):247– 74.

ECHA (2023) Substance Information - ECHA (europa.eu)

EPA (1985). Health assessment document for dichloromethane (methylene chloride). Final report (EPA/600/8-82/004F). Washington (DC): Office of Health and Environmental Assessment, United States.

Gargas ML, Clewell HJ 3rd, Andersen ME (1986). Metabolism of inhaled dihalomethanes in vivo: differentiation of kinetic constants for two independent pathways. Toxicol Appl Pharmacol, 82(2):211–23. doi:10.1016/0041-008X(86)90196-1

Gibbs GW, Amsel J, Soden K (1996). A cohort mortality study of cellulose triacetate-fiber workers exposed to methylene chloride. J Occup Environ Med, 38(7):693–7.

Gocke E, King MT, Eckhardt K, Wild D (1981). Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat Res, 90(2):91–109.

Gold LS, Stewart PA, Milliken K, Purdue M, Severson R, Seixas N, et al. (2011). The relationship between multiple myeloma and occupational exposure to six chlorinated solvents. Occup Environ Med, 68(6):391–9.

Graves RJ, Callander RD, Green T (1994a). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride. Mutat Res, 320(3):235–43.

Graves RJ, Coutts C, Eyton-Jones H, Green T (1994b). Relationship between hepatic DNA damage and methylene chloride-induced hepatocarcinogenicity in B6C3F1 mice. Carcinogenesis, 15(5):991–6.

Graves RJ, Coutts C, Green T (1995). Methylene chloride-induced DNA damage: an interspecies comparison. Carcinogenesis, 16(8):1919–26.

Graves RJ, Green T (1996). Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay. Mutat Res, 367(3):143–50.

Green T (1983). The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using Salmonella typhimurium. Mutat Res, 118(4):277–88.

Guengerich P, Kim DH and Iwasaki M (1991) Role of Human Cytochrome P-450 IIE1 in the Oxidation of Many Low Molecular Weight Cancer Suspects - Chem. Res. Toxicol. 4, 168-179

Hallier E, Langhof T, Dannappel D, Leutbecher M, Schröder K, Goergens HW, et al. (1993). Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. Arch Toxicol, 67(3):173–8. doi:10.1007/BF01973304.

Hearne FI, Grose F, Pifer JW, Friedlander BR, Raleigh RL. (1987) Methylene chloride mortality study: dose response characterization and animal comparison. J Occup Med, 29:217-28.

Hearne FT, Pifer JW (1999). Mortality study of two overlapping cohorts of photographic film base manufacturing employees exposed to methylene chloride. J Occup Environ Med, 41(12):1154–69.

Heineman EF, Cocco P, Gómez MR, Dosemeci M, Stewart PA, Hayes RB, et al. (1994). Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Am J Ind Med, 26(2):155–69.

Hu Y, Kabler SL, Tennant AH, Townsend AJ, Kligerman AD (2006). Induction of DNA-protein crosslinks by dichloromethane in a V79 cell line transfected with the murine glutathione-S-transferase theta 1 gene. Mutat Res, 607(2):231–9.

Hughes, NJ; Tracey, JA. (1993). A case of methylene chloride (nitromors) poisoning, effects on carboxyhaemoglobin levels. Hum Exp Toxicol 12: 159-160.

IARC (1999). Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC Monogr Eval Carcinog Risks Hum, 71:1–315.

IARC (2017). Dichloromethane, Some Chemicals Used as Solvents and in Polymer Manufacture. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans International Agency for Research on Cancer, Lyon, France, 110 : 177–255

IRIS, 2011-TOXICOLOGICAL REVIEW OF DICHLOROMETHANE (METHYLENE CHLORIDE) (CAS No. 75-09-2) In Support of Summary Information on the Integrated Risk Information System (IRIS) November 2011 U.S. Environmental Protection Agency,Washington

Kitchin KT, Brown JL (1994). Dose-response relationship for rat liver DNA damage caused by 49 rodent carcinogens. Toxicology, 88(1-3):31–49.Kumagai S, Kurumatani N, Arimoto A, Ichihara G

(2013). Cholangiocarcinoma among offset colour proofprinting workers exposed to 1,2-dichloropropane and/or dichloromethane. Occup Environ Med, 70(7):508–10.

Kubo, S., Nakanuma, Y., Takemura, S., Sakata, C., Urata, Y., Nozawa, A., Nishioka, T., Kinoshita, M., Hamano, G., Terajima, H., Tachiyama, G., Matsumura, Y., Yamada, T., Tanaka, H., Nakamori, S., Arimoto, A., Kawada, N., Fujikawa, M., Fujishima, H., Sugawara, Y., Tanaka, S., Toyokawa, H., Kuwae, Y., Ohsawa, M., Uehara, S., Sato, K. K., Hayashi, T., Endo, G., (2014). Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan. J Hepatobiliary Pancreat Sci 21, 479–488.

Kumagai S, Kurumatani N, Arimoto A, Ichihara G (2013). Cholangiocarcinoma among offset colour proofprinting workers exposed to 1,2-dichloropropane and/or dichloromethane. Occup Environ Med, 70(7):508–10.

Kumagai S (2014a). Two offset printing workers with cholangiocarcinoma. J Occup Health, 56(2):164–8.

Kumagai S., Kurumatani N., Arimoto A. and Ichihara G. (2014b).Time Course of Blood Parameters in Printing Workers with Cholangiocarcinoma. J Occup Health 56: 279–284

Kumagai, S., Sobue, T., Makiuchi, T., Kubo, S., Uehara, S., Hayashi, T., Sato, K.K., Endo, G., (2016). Relationship between cumulative exposure to 1,2-dichloropropane and incidence risk of cholangiocarcinoma among offset printing workers. Occup. Environ. Med. 73, 545–552.

JBRC (, 2000a).. Summary of inhalation carcinogenicity study of dichloromethane in BDF1 mice. Hadano:Japan Bioassay Research Center, Japan Industrial Safety and Health Association. Study No. 0279.

JBRC (, 2000b).. Summary of inhalation carcinogenicity study of dichloromethane in F344 rats. Hadano: Japan Bioassay Research Center, Japan Industrial Safety and Health Association.

Jongen WM, Lohman PH, Kottenhagen MJ, Alink GM, Berends F, Koeman JH (1981). Mutagenicity testing of dichloromethane in short-term mammalian tests systems. Mutat Res, 81(2):203–13.

Jongen WM, Harmsen EG, Alink GM, Koeman JH (1982). The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium. Mutat Res, 95(2-3):183–9.

Lanes SF, Cohen A, Rothman KJ, Dreyer NA, Soden KJ (1990). Mortality of cellulose fiber production workers. Scand J Work Environ Health, 16(4):247–51.

Lanes SF, Rothman KJ, Dreyer NA, Soden KJ (1993). Mortality update of cellulose fiber production workers. Scand J Work Environ Health, 19(6):426–8.

Landi, S; Naccarati, A; Ross, MK; Hanley, NM; Dailey, L; Devlin, RB; Vasquez, M; Pegram, RA; DeMarini, DM. (2003). Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells. Mutat Res Genet Toxicol Environ Mutagen 538: 41-50.

Mainwaring GW, Williams SM, Foster JR, Tugwood J, Green T (1996). The distribution of theta-class glutathione S-transferases in the liver and lung of mouse, rat and human. Biochem J, 318(Pt 1):297–303. doi:10.1042/bj3180297

Makris, K.C., Voniatis, M., (2018). Brain cancer cluster investigation around a factory emitting dichloromethane. Eur. J. Publ. Health 28, 338–343.

Makisimov GG, Mamleyeva NK 1977: An assessment of the hazard presented by methylene chloride entering the organism percutaneously (in Russian) (publication), Absorbtion of industrial poisons through the skin, and prevention thereof, 83-88

Maltoni C, Cotti G, Perino G (1988). Long-term carcinogenicity bioassays on methylene chloride administered by ingestion to Sprague-Dawley rats and Swiss mice and by inhalation to Sprague-Dawley rats. Ann N Y Acad Sci, 534:1. Living in a C: 352–66.

MAK, 2015-Deutsche forschungsgemeinschaft, commission for the investigation of health hazards of chemical compounds in the work area: dichloromethane. In: MAK (Ed.), 2015. Supplement 2015; Series: MAK Value Documentation. The MAK Collection for Occupational Health and Safety.

McLean D, Fleming S, Turner MC, Kincl L, Richardson L, Benke G, et al. (2014). Occupational solvent exposure and risk of meningioma: results from the INTEROCC multicentre case-control study. Occup Environ Med, 71(4):253–8.

McKenna MJ, Zempel JA (1981). The dose-dependent metabolism of [14C]methylene chloride following oral administration to rats. Food Cosmet Toxicol, 19(1):73–8. doi:10.1016/0015-6264(81)90306-0

Mennear JH, McConnell EE, Huff JE, Renne RA, Giddens E (1988). Inhalation toxicity and carcinogenesis studies of methylene chloride (dichloromethane) in F344/N rats and B6C3F1 mice. Ann N Y Acad Sci, 534:1. Living in a C: 343–51.

Miligi L, Costantini AS, Benvenuti A, Kriebel D, Bolejack V, Tumino R, et al. (2006). Occupational exposure to solvents and the risk of lymphomas. Epidemiology, 17(5):552–61.

Myhr B, McGregor D, Bowers L, Riach C, Brown AG, Edwards I, et al. (1990). L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environ Mol Mutagen, 16(Suppl 18):138–67.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, et al.; Collaborative study of the micronucleus group test. Mammalian Mutagenicity Study Group (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Mutat Res, 389(1):3–122.

Neta G, Stewart PA, Rajaraman P, Hein MJ, Waters MA, Purdue MP, et al. (2012). Occupational exposure to chlorinated solvents and risks of glioma and meningioma in adults. Occup Environ Med, 69(11):793–801.

Niehoff, N.M., Gammon, M.D., Keil, A.P., Nichols, H.B., Engel, L.S., Sandler, D.P., White, A.J., 2019. Airborne mammary carcinogens and breast cancer risk in the Sister Study. Environ. Int. 130, 104897.

Nitschke KD, Burek JD, Bell TJ, Kociba RJ, Rampy LW, McKenna MJ (1988). Methylene chloride: a 2-year inhalation toxicity and oncogenicity study in rats. Fundam Appl Toxicol, 11(1):48–59.

NTP (1986). NTP toxicology and carcinogenesis studies of dichloromethane (methylene chloride) (CAS No. 75-09-2) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser, 306:1–208

Olvera-Bello AE, Estrada-Muñiz E, Elizondo G, Vega L (2010). Susceptibility to the cytogenetic effects of dichloromethane is related to the glutathione S-transferase theta phenotype. Toxicol Lett, 199(3):218–24. doi:10.1016/j.toxlet.2010.09.002

Park, A.S., Ritz, B., Ling, C., Cockburn, M., Heck, J.E., (2017). Exposure to ambient dichloromethane in pregnancy and infancy from industrial sources and childhood cancers in California. Int. J. Hyg Environ. Health 220, 1133–1140.

Radican L, Blair A, Stewart P, Wartenberg D (2008). Mortality of aircraft maintenance workers exposed to trichloroethylene and other hydrocarbons and chemicals: extended follow-up. J Occup Environ Med, 50(11):1306–19.

Ruder AM, Yiin JH, Waters MA, Carreón T, Hein MJ, Butler MA, et al.; Brain Cancer Collaborative Study Group (2013). The Upper Midwest Health Study: gliomas and occupational exposure to chlorinated solvents. Occup Environ Med, 70(2):73–80.

Seidler A, Möhner M, Berger J, Mester B, Deeg E, Elsner G, et al. (2007). Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. J Occup Med Toxicol, 2(1):2.

Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RH, et al. (1986a). A two-year drinking- water study of dichloromethane in rodents. II. Mice. Food Chem Toxicol, 24(9):959–63.

Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RH, et al. (1986b). A two-year drinking- water study of dichloromethane in rodents. I. Rats. Food Chem Toxicol, 24(9):951–8.

Sheldon T, Richardson CR, Elliott BM (1987). Inactivity of methylene chloride in the mouse bone marrow micronucleus assay. Mutagenesis, 2(1):57–9.

Sherratt PJ, Pulford DJ, Harrison DJ, Green T, Hayes JD (1997). Evidence that human class theta glutathione S-transferase T1–1 can catalyse the activation of dichloromethane, a liver and lung carcinogen in the mouse. Comparison of the tissue distribution of GST T1–1 with that of classes Alpha, Mu and Pi GST in human. Biochem J, 326(Pt 3):837–46. doi:10.1042/bj3260837

Sherratt PJ, Williams S, Foster J, Kernohan N, Green T, Hayes JD (2002). Direct comparison of the nature of mouse and human GST T1–1 and the implications on dichloromethane carcinogenicity. Toxicol Appl Pharmacol, 179(2):89–97. doi:10.1006/taap.2002.9348

Spirtas R, Stewart PA, Lee JS, Marano DE, Forbes CD, Grauman DJ, et al. (1991). Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. Br J Ind Med, 48(8):515–30.

Sobue, T., Utada, M., Makiuchi, T., Ohno, Y., Uehara, S., Hayashi, T., Sato, K.K., Endo, G., (2015). Risk of bile duct cancer among printing workers exposed to 1,2-dichloropropane and/or dichloromethane. J. Occup. Health 57, 230–236.

Suzuki T, Yanagiba Y, Suda M, Wang RS (2014). Assessment of the genotoxicity of 1,2-dichloropropane and dichloromethane after individual and co-exposure by inhalation in mice. J Occup Health, 56(3):205–14.

Thilagar AK, Kumaroo V (1983). Induction of chromosome damage by methylene chloride in CHO cells. Mutat Res, 116(3-4):361–7.

Talibov, M., Auvinen, A., Weiderpass, E., Hansen, J., Martinsen, J.I., Kjaerheim, K., Tryggvadottir, L., Pukkala, E., (2017). Occupational solvent exposure and adult chronic lymphocytic leukemia: No risk in a population-based case-control study in four Nordic countries. Int. J. Canc. 141, 1140–1147.

Theiss JC, Stoner GD, Shimkin MB, Weisburger EK (1977). Test for carcinogenicity of organic contaminants of United States drinking waters by pulmonary tumor response in strain A mice. Cancer Res, 37(8 Pt 1):2717–20.

Tomenson JA, Bonner SM, Heijne CG, Farrar DG, Cummings TF (1997). Mortality of workers exposed to methylene chloride employed at a plant producing cellulose triacetate film base. Occup Environ Med, 54(7):470–6.

Tomenson JA (2011). Update of a cohort mortality study of workers exposed to methylene chloride employed at a plant producing cellulose triacetate film base. Int Arch Occup Environ Health, 84(8):889–97.

Trueman RW, Ashby J (1987). Lack of UDS activity in the livers of mice and rats exposed to dichloromethane. Environ Mol Mutagen, 10(2):189–95.

Yamada K, Kumagai S, Nagoya T, Endo G (2014). Chemical exposure levels in printing workers with cholangiocarcinoma. Tokyo: Occupational Health Research and Development Center, Japan Industrial Safety and Health Association.

Yamada, K., Kumagai, S., Endo, G., (2015a). Chemical exposure levels in printing workers with cholangiocarcinoma (second report). J. Occup. Health 57, 245–252.

Yamada, K., Kumagai, S., Kubo, S., Endo, G., (2015b). Chemical exposure levels in printing and coating workers with cholangiocarcinoma (third report). J. Occup. Health 57, 565–571.

Ursin C, Hansen CM, Van Dyk JW, Jensen PO, Christensen IJ, Ebbehoej J (1995). Permeability of commercial solvents through living human skin. Am Ind Hyg Assoc J, 56(7):651–60. doi:10.1080/15428119591016665

US EPA (2011). Toxicological Review of Dichloromethane (Methylene Chloride) In Support of Summary Information on the Integrated Risk Information System (IRIS).EPA/635/R-10/003F. http://www.epa.gov/iris/toxreviews/0070tr.pdf.

Vetro J, Koutsogiannis Z, Jones DA, Canestra J (2012). A case of methylene chloride poisoning due to ingestion of home-distilled alcohol and potential new treatment with ethanol infusion. Crit Care Resusc, 14(1):60–3.

Wang R, Zhang Y, Lan Q, Holford TR, Leaderer B, Zahm SH, et al. (2009). Occupational exposure to solvents and risk of non-Hodgkin lymphoma in Connecticut women. Am J Epidemiol, 169(2):176–85.

Westbrook-Collins B, Allen JW, Sharief Y, Campbell J (1990). Further evidence that dichloromethane does not induce chromosome damage. J Appl Toxicol, 10(2):79–81.

Watanabe Kengo, Guengerich F Peter (2006). Limited reactivity of formyl chloride with glutathione and relevance to metabolism and toxicity of dichloromethane. Chem Res Toxicol Aug;19(8):1091-6. doi: 10.1021/tx060087n.

Wu Z, Zhang X, Shen L, Xiong Y, Wu X, et al. (2013) A Systematically Combined Genotype and Functional Combination Analysis of CYP2E1, CYP2D6, CYP2C9, CYP2C19 in Different Geographic Areas of Mainland China – A Basis for Personalized Therapy. PLoS ONE 8(10): e71934. doi:10.1371/journal.pone.0071934

18 ANNEXES

None.