SUBSTANCE EVALUATION CONCLUSION
as required by REACH Article 48
and
EVALUATION REPORT

for

Dichloromethane
EC No 200-838-9
CAS No 75-09-2

Evaluating Member State: Italy

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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2016

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Dichloromethane was originally selected for substance evaluation in order to clarify concerns about:

- Carcinogenic
- Suspected mutagenic
- Suspected reprotoxic
- Suspected sensitiser
- Potential endocrine disruptor
- High (aggregated) tonnage

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Dichloromethane is a restricted substance in the Annex XVII of the REACH Regulation (Entry 59).

Up-to-date information on the activities planned, ongoing or completed on dichloromethane under REACH and CLP regulation can be found here:

https://echa.europa.eu/it/dichloromethane

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

The eMSCA considers that reproductive toxicity and endocrine disrupting endpoints may need to be further investigated in case the harmonised classification proposal (CLH proposal) for Carc 1B is not agreed by the Committee for Risk Assessment (RAC).
This is to ensure that potential further appropriate administrative risk management measures could be taken based on properties potentially emerging from these endpoints.

4. FOLLOW-UP AT EU LEVEL

On the basis of the available information, a revision of the harmonized classification of the substance is envisaged by eMSCA, as a follow-up at EU level by adding the following hazard category: Carc 1B H315 and Muta cat 2.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

The eMSCA has the intention to prepare an Annex XV dossier with a proposal for harmonized classification and labelling tentatively in 2021.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Annex XV dossier for Classification	2021	Italy

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Dichloromethane was selected for substance evaluation in order to clarify concerns about:

- Carcinogenic
- Suspected mutagenic
- Suspected reprotoxic
- Suspected sensitiser
- Potential endocrine disruptor
- High (aggregated) tonnage

Table 3

EVALUATED ENDPOINTS **Endpoint evaluated**	**Outcome/conclusion**
Endpoint 1 Carcinogenicity	Concern confirmed. A revision of the harmonised classification and labelling is proposed as Carc 1B.
Endpoint 2 Suspected mutagenicity	Concern confirmed. An update of the classification and labelling as muta cat 2 will be performed.
Endpoint 3 Suspected reprotoxic	Concern unresolved. The eMSCA reserves the possibility to further investigate on this endpoint in case the CLH proposal for Carc 1B will not be accepted.
Endpoint 4 Suspected sensitiser	Concern not substantiated. No further action.
Endpoint 5 Potential endocrine disruptor	Concern unresolved. The eMSCA reserves the possibility to further investigate on this endpoint in case the CLH proposal for Carc 1B will not be accepted.

7.2. Procedure

The Substance evaluation has started on March 2016.

The substance was listed in the CoRAP for the following reasons:

- The OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) study has been provided concluding that at concentrations as high as 1500 ppm (ca. 5300 mg/m^3) dichloromethane did not affect any of the reproductive parameters examined. However, the study does not cover several important parameters, such as organ weights, sperm parameters, estrous cyclicity, implantation sites and histopathology.

  Additionally, in a supporting study for carcinogenicity endpoints (mechanistic study) it was found that during a two-week exposure period at 3500 ppm, dichloromethane

caused a statistically significant ($p<0.05$) increase in the length of the oestrus cycle and elevated serum prolactin concentrations in female Sprague-Dawley rats.

Therefore due to the lack of important parameters for reproductive toxicity and the possible effect of the substance to the oestrus cycle the toxicity of dichloromethane to reproduction is not clear.

- In a developmental toxicity study similar to OECD TG 414 examining the effects of maternally inhalated methylene chloride on embryonal and fetal development in rats and mice, foetal skeletal variations were observed which may have been caused by hypoxia as increased carboxyhaemoglobin levels were seen in the dams and hypoxia is known to affect the developing foetus. As a result the level of 4300 mg/m^3 (ca. 1250 ppm) was established to be a LOAEC for developmental toxicity (mild foetotoxicity) and for slight maternal toxicity. However, it should be noted, that LOAEC value in this study does not correlate with the findings in the reprotoxicity study (2-gen).

  Dichloromethane is thought to readily transfer across the blood-brain barrier by passive diffusion, as evidenced by the detection of radioactivity in brain tissue 48 hours after exposures of rats to radiolabeled dichloromethane at concentrations of 50, 500, or 1500 ppm for 6 hours. It can be transferred across the placenta, and small amounts can be excreted in urine or in milk. Historically it is demonstrated that dichloromethane has transient sedative and anesthetic properties in humans. Due to this it is not possible to conclude that the skeletal variations were caused by hypoxia and maternal toxicity. Therefore possible developmental toxicity of the substance cannot be excluded.

- Dichloromethane was found to be genotoxic *in vitro*. A reliable *in vivo* study conducted according to OECD TG 474 is available and showed negative results for mutagenicity. There are no reliable studies that would examine DNA breakages based on which it would be possible to conclude on the genotoxic properties of the substance. However, in the endpoint summary for genetic toxicity it is mentioned that DNA damage was detected in the liver and lung using the alkaline single cell gel electrophoresis (SCG) assay.

  Additionally classifications as Muta. 1A and 2 have been notified in the C&L inventory.

- There was some evidence of carcinogenicity of dichloromethane for male F344/N rats and clear evidence of carcinogenicity of dichloromethane for female F344/N rats as shown by increased incidences of benign neoplasms of the mammary gland. Additionally, marginally increased incidences in exposed groups of rats included adrenal gland pheochromocytomas and interstitial cell tumors of the testis in males and pituitary gland adenomas/carcinomas in both sexes. However these effects were not dose-related and incidences were not considered compound related. Tumors´ types show a possible relationship with disturbed endocrine function and raise the possibility of a hormonal mechanism.

- There is a single case referred in the dossier(s) that dichloromethane may have produced asthma or reactive airways dysfunction syndrome in a worker. However the Registrant(s) has concluded that in view of the solvent's extensive, widespread and long-standing use, and the scarcity of published evidence in the area of skin or respiratory sensitization indicates that dichloromethane does not possess any significant sensitising potential.

  Additionally one study on rabbits showed allergic reactions after inhalation. However, the experimental protocol of this study is questionable and the result has not been confirmed. Based on the above mentioned substance may have respiratory sensitising properties.

After evaluating the information available, the eMSCA considers that the available data on carcinogenicity and the genotoxicity/mutagenicity on diclorometha‌ne are sufficient to

trigger a proposal for the revision of the existing Harmonized Classification and Labelling (see IARC monograph 110; 2016).

The ED concern based on reproductive/developmental effects should be clarified also considering a read-across with dichloroethane (EC no. 203-458-1, CAS No 107-06-2), in which the latter has been linked to an altered signalling pathway (i.e. the CREM/CREB signaling pathway) leading to altered hormones status (i.e. testosterone, gonadotropin-releasing hormone, luteinizing hormone/LH) in the testes as well as to malformation of spermatozoa, reduced sperm concentration, and pathological impairment of the testes.

The eMSCA concluded the evaluation without any further need to ask more information from the Registrant(s) under Article 46(1) decision.

However, since the concern for reprotoxicity and ED properties still exists, the eMSCA reserves the possibility to further investigate these endpoints in case the CLH proposal for Carc 1B will not be accepted. This is to ensure that potential further appropriate administrative risk management measures could be taken based on properties potentially emerging from these endpoints.

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	dichlorometane
EC number:	200-838-9
CAS number:	75-09-2
Index number in Annex VI of the CLP Regulation:	602-004-00-3
Molecular formula:	CH2Cl2
Molecular weight range:	
Synonyms:	Methylene chloride

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:

7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	liquid
Vapour pressure	58 400 Pa at 25°C
Water solubility	13 200 mg/L at 25°C pH 7
Partition coefficient n-octanol/water (Log Kow)	log Pow 1.25 at 20°C pH 7
Flammability	--
Explosive properties	--
Oxidising properties	--
Granulometry	--
Stability in organic solvents and identity of relevant degradation products	--
Dissociation constant	--

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☒ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of 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.

Table 7

USES	
	Use(s)
Uses as intermediate	See below.
Formulation	This substance is used in the following products: adhesives and sealants and coating products. Release to the environment of this substance can occur from industrial use: formulation of mixtures.

Uses at industrial sites	This substance is used in 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 in printing and recorded media reproduction. This substance is used for the manufacture of chemicals, textile, leather or fur, rubber products, plastic products, mineral products (e.g. plasters, cement), machinery and vehicles and furniture. Release to the environment of this substance can occur from industrial use in processing aids at industrial sites, as an intermediate step in further manufacturing of another substance (use of intermediates) and of substances in closed systems with minimal release.
Uses by professional workers	This substance is used in coating products, washing & cleaning products, adhesives and sealants, biocides (e.g. disinfectants, pest control products) and plant protection products. This substance is used in scientific research and development and agriculture, forestry and fishing. Other release to the environment of this substance is likely to occur from indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners) and outdoor use as processing aid.
Consumer Uses	This substance is used in adhesives and sealants, plant protection products, washing & cleaning products, biocides (e.g. disinfectants, pest control products) and coating products. Other release to the environment of this substance is likely to occur from indoor use as processing aid and outdoor use as processing aid.
Uses advised against	<u>Uses at industrial sites</u> Use as paint strippers in concentrations equal to or greater than 0,1 % by weight in industrial installations if conditions listed in annexe XVII point 4 are not fulfilled. <u>Uses by professional workers</u> Paintstripper >0.1% dichloromethane (DCM), use as paint strippers in concentrations equal to or greater than 0,1 % by weight. Hairspray (Cosmetics, personal care products) <u>Consumer uses</u> Paintstripper >0.1% DCM, use as paint strippers in concentrations equal to or greater than 0,1 % by weight. According to Commission regulaiton 2019/831 the use of dichlormethane in cosmetics is banned.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

The substance is currently listed on Annex VI of CLP Regulation ((EC) No 1272/2008).

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
602-004-00-3	dichloromethane methylene chloride	200-838-9	75-09-2	Carc. Cat.2	H351		

7.6.2. Self-classification

- In the registration(s):

Skin Irrit. 2 H315
Eye Irrit. 2 H319
STOT SE 3 H336

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Acute Tox. 4 H302
Acute Tox. 1 H300
Acute Tox. 1 H310
Acute Tox. 1 H330
Asp. Tox. 1 H304
Skin Corr. 1A H314
Skin Sens. 1 H317
Eye Dam. 1 H318
Eye Irrit. 2B H320
Resp. Sens. 1 H334
Carc. 2 H351 (oral)
Carc. 2 H351 (Inhalation)
Muta. 1A H340 (Oral)
Muta. 2 H341
Repr. 1A H360 (test) (Oral)
Lact. H362
STOT SE 1 H370 (CNS)
STOT SE 1 H370 (other:test) (Oral)
STOT SE 3 H335 (respiratory tract) (Inhalation)
STOT SE 3 H335 (Blood, skin and.lung.)
STOT SE 3 H336 (central nervous, respiratory tract, brain) (Inhalation)
STOT SE 3 H336 (Affected Organs)
STOT SE 3 H336 (Central nervous system) (Oral)
STOT SE 3 H336 (Narcotic effect)
STOT RE 1 H372 (other:test) (Oral)
STOT RE 1 H372 (Central nervous system)
STOT RE 2 H373 (CNS, blood, skin, liver, kidney, resp.tract) (Inhalation)
STOT RE 2 H373 (liver) (Oral)
Ozone 1 EUH059
Expl. 1.1 H200
Flam. Gas 1 H220
Aerosol 1 H222, H229
Flam. Liq. 1 H224

Flam. Sol. 1 H228
Org. Perox. A H240
Self-react. A H241
Pyr. Sol. 1 H250
Pyr. Liq. 1 H250
Self-heat. 1 H251
Water-react. 1 H260
Ox. Gas 1 H270
Ox. Sol. 1 H271
Ox. Liq. 1 H271
Press. Gas (Comp.) H280
Met. Corr. 1 H290
Aquatic Chronic 1 H400
Aquatic Acute 2 H401
Aquatic Chronic 2 H411
Aquatic Chronic 3 H412

7.7. Environmental fate properties

Not evaluated.

7.8. Environmental hazard assessment

Not evaluated.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Due to its lipophilic properties and to low relative molecular mass, DCM can readly 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 (gluthatione S-transferase T1), 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, Angelo *et al*. (1986b) 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.

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

In humans, once absorbed DCM enter in circulation and is rapidly distributed to tissues. Due to the lipophilic properties of DCM, the highest concentrations are aspected in adipose tissues and or other fatty tissue (Engstrom & Bjurstrom, 1977).

Even in animals DCM is 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 *et al*., 1986a)

One pathway for metabolism of DCM is a reductive dehalogenation catalysed by cytochrome P450 2E1 (CYP2E1) (Guengerich *et al*., 1997). 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 gluthatione (GSH) to generate formylglutathione that rearranges to form CO_2. In this pathway CO (producted only by this pathway), that has a great affinity for hemoglobin, by replacing oxygen, forms carboxyhemoglobin (COHb).

Another pathway is via conjugation with GSH. The first product of the reaction is S-chloromethyl GSH: this conjugation is catalysed by glutathione S-transferase enzymes from which the most active is the isoform *theta-1* (GSTT1). *S*-Chloromethyl GSH is believed

to be one of the dichloromethane metabolites responsible for DNA binding and mutagenicity (Graves & Green, 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 CO2. Both metabolic pathways of DCM involve polymorphic and variously distributed enzymes in human tissues. The different distribution of these enzymes, particulary GSTT1, plays an important role in the definition of the susceptible populations.

Oxidative metabolism of dichloromethane (*via* CYP2E1) was first demonstrated in occupationally exposed humans. 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 & Kaplan (1981). Mainwaring *et al*. (1996) determined mRNA and protein expression of GSTT1 in cells from human liver and lung, both of which are target organs for dichloromethane 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 dichloromethane was measured in three samples of lung: it was about one order of magnitude less than that in human liver. Even the subcellular localisation of GSTT1 is an important question to take into account in evaluating the differences in the effects between human and experimental animals. GSTT1 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 *et al*., 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.

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

7.9.3.1 Respiratory sensitisation

The Registrant(s) provide a data waiving on this endpoint, with a justification based on the fact that no hypersensitivity reactions associated with exposure to dichloromethane were reported in humans. Based on the epidemiological data and direct observation reliable reported in the dossier, there is no more indication of concern for the effects on humans with regard to respiratory sensitization.

Moreover, in the Recommendation from the Scientific Committee on Occupational Exposure Limits for methylene chloride (dichloromethane) (SCOEL/SUM/130 June 2009) it is reported that the absence of any case reports suggests that methylene chloride is unlikely to cause respiratory (or dermal) sensitisation in humans.

In conclusion, based on the respiratory sensitization data and taking into account that no structural alert has been highlighted with OECD QSAR Toolbox 3.3.5 "Respiratory Sensitization" profiler (https://www.qsartoolbox.org/), eMSCA agrees with data waiving.

Therefore eMSCA agrees with the conclusion reported in the registration dossier that the substance does not cause respiratory sensitisation in human.

7.9.4. Repeated dose toxicity

The eMSCA reports, for sake of completeness, the following information presented in the CSR taken into account by Registrant(s) for risk assessment.

Several chronic and subchronic oral repeated dose studies were performed in rats and mice, as well as one subacute study in rats. The 2-year NOAEL for oral toxicity was 6 mg/kg bw/day in rats, based on increased incidence of foci/areas of cellular alteration and fatty changes in the liver (Serota D.G. *et al*., 1986). The study is considered by eMSCA as the key study and the resulting NOAEL was considered as point of departure for the derivation of oral and dermal DNELs both for workers and consumers.

7.9.5. Mutagenicity

Several studies are available for genotoxicity both *in vitro* and *in vivo*. The evaluation took into account all the available data.

Genotoxicity *in vitro*

Dichloromethane was mutagenic in Styphimurium 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 E. *et al*., 1981).

Gene mutation were 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 HPGRT assay after one hour exposure to 0.5 -5% (v/v) dichloromethane without metabolic activation. The reliability of this study was limited by a short exposure time and the lack of metabolic activation (Jongen *et al*., 1981). Dichloromethane was mutagenic in Chinese hamster ovary 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 *et al*., 1990). It is noted that DNA sequence analysis of the Hprt mutants of Chinese hamster ovary cells treated with dichloromethane indicated that most mutations were GC→AT transitions (4 out of 8), with two 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 dichloromethane 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 *et al*., 1996). The only gene mutation study available in mouse lymphoma L5178Y cells showed ambiguous results (Myhr *et al*., 1990).

Table 9. Summary of gene mutation studies in mammalian and bacterial cells.

Study	Results	Note	Reference
Bacterial reverse mutation Assay	Positive +/- S9 in TA 98 and TA100	Test concentrations: 125, 250, 500, and 750 µl in a 9 litre desiccator	Gocke E, King M-T, Eckhardt K, Wild D; 1981
Bacterial reverse mutation Assay in TA 100	Positive +/- S9 in TA 100	Mutagenic activity enhanced with rat liver microsomes (CYP metabolism) or cytosolic fraction (GST metabolism).	Jongen *et al*. 1981
Bacterial reverse mutation Assay in TA 100	Positive +/- S9 in TA 100	The mutagenic activity was enhanced only when rat liver post-mitochondrial S9 fraction (glutathione conjugation of DCM) was added and not rat liver microsomes.	Green, 1983
Bacterial reverse mutation Assay in TA 100 GSH wt and TA 100 GSH-deficient strain (NG54)	Positive +/- S9 in TA 100	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	Dillon *et al*., 1992
Bacterial reverse mutation Assay in Salmonella TA1535 strain that had been modified by the cloning of the rat gene for GSTT1 into its genome	Positive in TA 1535 stain –S9	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 GC→AT transitions in the TA100 strain, a homologue strain that lacks the rat GSTT1 gene.	De Marini *et al*., 1997

Gene mutation, Chinese hamster ovary cells, Hprt locus	Negative -S9	short exposure and lack of metabolic activation are the limits of this study	Jongen *et al*., 1981
Gene mutation, Chinese hamster ovary cells (Hprt locus)	Positive +S9	Tested both + and –S9	Graves & Green, 1996
Gene mutation, Chinese hamster lung V79 cells, Hprt locus	Negative –S9	Tested only –S9	Jongen *et al*., 1981
Gene mutation, mouse lymphoma L5178Y cells, Tk locus	Inconclusive +/- S9		Myhr *et al*., 1990

Chromosomal aberrations were observed in Chinese hamster ovary cells in the presence and absence of an exogenous metabolic system in the study of Thilagar & Kumaroo, 1983, while negative results were reported in the Anderson *et al*., 1990 study, see table 10.

Micronuclei induced by dichloromethane were prevalently kinetochore-positive (which is an indication of aneuploidy) in a study by Doherty *et al*. (1996). On the contrary, a prevalence of kinetochore-negative micronuclei 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 cDNAs encoding cytochrome P450 isoenzymes expressed in human can metabolize halogenated hydrocarbons, such as dichloromethane, to genotoxic species.

Table 10. Summary of clastogenic/aneugenic effects.

Study	Results	Note	Reference
Chromosomal aberrations, Chinese hamster ovary CHO cells	Positive +/- S9	Maximum concentration tested 6500 µg/mL	Thilagar & Kumaroo (1983)
Chromosomal aberrations, Chinese hamster ovary CHO cells	Negative +/– S9	Maximum concentration tested 5000 µg/mL	Anderson *et al*. (1990)
Micronucleus test, human MCL-5 and h2E1 lymphoblastoid cells	Positive - S9 Induction of kinetochore-positive and -negative micronuclei	Maximum concentration tested 200 µg/mL Positive in MCL-5, h2E1 cell lines, increasing with increasing concentrations from 2 to 10 mM	Doherty *et al*. (1996)
Micronucleus test, human AHH-1 lymphoblastoid cells	Negative - S9	Maximum concentration tested 850 µg/mL	Casanova *et a.l* 1997

Genotoxicity *in vivo*

Dichloromethane did not induce micronucleus formation *in vivo* in the bone marrow of mice treated by gavage or intraperitoneal injection (Gocke *et al*., 1981; Sheldon *et al*., 1987; Morita *et al*., 1997). Mice treated with dichloromethane by 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 *et al*., 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,

dichloromethane 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 *et al*., 2014).

Table 11. Micronucleus *in vivo* studies (clastogenic/aneugenic effects).

Study	Results	Note	Reference
Micronucleus test, NMRI mouse bone marrow	Negative	1700 mg/kg, ip × 2	Gocke *et al*. (1981)
Micronucleus test, C57BL/6J/Alpk mouse bone marrow	Negative	4000 mg/kg, po × 1	Sheldon *et al*. (1987)
Micronucleus test, CD-1 mouse bone marrow	Negative	1720 mg/kg, ip × 1	Morita et al. (1997)
Micronucleus test, B6C3F1 mouse erythrocytes	Negative in lung cells at this dose; Positive in erythrocytes after exposure to 8000 ppm for 6 hours per day [10 000 mg/kg bw], 5 days per week, for 2 weeks	2000 ppm, inh., 6 h/day, 5 days/wk, 12 wk	Allen *et al*. (1990)
Micronucleus test, male B6C3F1 mouse reticulocytes and normochromatic erythrocytes	Negative	1600 ppm, inh., 6 h/days, 5 days/wk, 6 wk	Suzuki *et al.* (2014)

Dichloromethane did not cause chromosomal aberration *in vivo* in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins *et al*., 1990; Allen *et al*., 1990). A small increase in the frequency of chromosomal aberration in mouse bone marrow and lung cells was reported after exposure to dichloromethane at 8000 ppm by inhalation for 6 hours per day, 5 days per week, for 2 weeks (Allen *et al*., 1990). Negative results were also reported in an assay for chromosomal aberration in rat bone marrow (Burek *et al*.,1984).

Table 12. Chromosomal aberrations.

Study	Results	Note	Reference
Chromosomal aberrations, B6C3F1 mouse bone marrow	Negative	5000 µg/mL sc × 1	Allen *et al*., 1990
Chromosomal aberrations, C57BL/6J mouse bone marrow	Negative	1500 mg/kg ip × 1	Westbrook-Collins *et al*., 1990
Chromosomal aberrations, B6C3F1 mouse bone marrow	Weakly positive	8000 ppm, inh., 6 h/day, 5 days/ wk, 2 wk	Allen *et al*. (1990)
Chromosomal aberrations, Sprague-Dawley rat bone marrow	Negative	3500 ppm, inh., 6 h/day, 5 days/ wk, 2 yr	Burek *et al*., 1984
Chromosomal aberrations, B6C3F1 mouse lung cells	Weakly positive	8000 ppm, inh., 6 h/day, 5 days/ wk, 2 wk	Allen *et al*., 1990

No gene mutations were observed in the following two experiments after inhalation exposure to dichloromethane: a Pig-a assay in the erythrocytes of peripheral blood of male B6C3F1 mice exposed to dichloromethane 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 dichloromethane at 800 ppm (Suzuki *et al*., 2014) where liver cells were analysed.

Dichloromethane 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).

Table 13. Gene mutation and DNA repair *in vivo.*

Study	Results	Note	Reference
Gene mutation, Pig-a assay, male B6C3F1 mouse, erythrocytes	Negative	1600 ppm, inh., 6 h/day, 5 days/wk, 6 wk	Suzuki *et al*., 2014
Gene mutation, transgenic rodent, male Gpt Delta C57BL/6J mouse liver	Negative	800 ppm, inh., 6 h/day, 5 days/wk, 4 wk	Suzuki *et al*., 2014
Unscheduled DNA synthesis, F344 rat hepatocytes	Negative	1000 µg/mL, po × 1	Trueman & Ashby, 1987
Unscheduled DNA synthesis, F344 rat hepatocytes	Negative	4000 ppm, inh., 6 h	Trueman & Ashby, 1987
Unscheduled DNA synthesis, B6C3F1 mouse liver	Negative	4000 ppm, inh., 6 h	Trueman & Ashby, 1987

Mechanistic studies *in vitro* and *in vivo*

Two major metabolic pathways for the metabolism of dichloromethane have been characterized in humans and experimental animals (as also reported in the section 7.9.1). One pathway is CYP2E1-mediated reductive dehalogenation, which ultimately generates CO and CO2 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 dichloromethane, resulting in the formation of reactive metabolites, including formaldehyde and S-chloromethyl GSH.

The relationship between the metabolism (CYP and GST patways) of dichloromethane and mutagenicity has been examined in several studies with various assays for bacterial mutation as also reported in the IARC monograph 110. In summary, the observed *in vitro* mutagenicity of DCM cannot be univocally attributed to a specific metabolic pathway (Jongen *et al*.,1982; Green, 1983; Dillon *et al*., 1992; De Marini *et al*., 1997, see table 14).

Table 14. Role of GST pathway in bacteria.

Study	Results	Note	Reference
Bacterial reverse mutation Assay in TA 100	Positive +/- S9 in TA 100	The mutagenic activity was enhanced only when rat liver post-mitochondrial S9 fraction (glutathione conjugation of DCM) was added and not rat liver microsomes.	Green (1983)
Bacterial reverse mutation Assay in TA 100 GSH wt	Positive +/- S9 in TA 100	The NG54 strain was slightly less responsive to dichloromethane exposure, addition of rat liver	Dillon *et al*., 1992

and TA 100 GSH-deficient strain (NG54)		cytosol marginally increased the mutagenic response to dichloromethane, but addition of GSH had little effect	
Bacterial reverse mutation Assay in Salmonella TA1535 strain that had been modified by the cloning of the rat gene for GSTT1 into its genome	Positive in TA 1535 stain –S9	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 GC→AT transitions in the TA100 strain, a homologue strain that lacks the rat GSTT1 gene.	De Marini *et al.,* 1997

Dichloromethane was also tested for its ability to induce DNA damage measured by comet assay *in vitro* (see table 15). The frequency of DNA single-strand breaks was increased in mice B6C3F1 hepatocytes without metabolic activation (Graves *et al.,* 1994) and in Chinese hamster ovary cells (CHO) cultured with dichloromethane in the presence, but not in the absence, of an exogenous metabolic activation system (Graves *et al*., 1994). In the Graves and Green (1996) study the effect were stronger with metabolic activation. Conversely, DNA single-strand breaks were not induced in Syrian hamster hepatocytes (Graves *et al*., 1995).

Dichloromethane 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 *et al*., 1997). DNA–protein cross-links were also induced in Chinese hamster ovary cells exposed to dichloromethane with or without exogenous metabolic activation, with DNA damage being greater in the presence of metabolic activation (Graves & Green, 1996). Hu *et al*., (2006) performed the 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. Dichloromethane induced DNA damage in both cell types. However, the study showed the presence of dichloromethane-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.

Genotoxicity data are also available in human cells. Dichloromethane did not induce DNA single strand breaks (SSB) in human primary hepatocytes (Graves *et al*., 1995); no induction of DNA–protein cross-links *in vitro* was observed in human hepatocytes with functional GSTT1 genes (Casanova *et al*., 1997) after treatment with dichloromethane.

The induction of Sister chromatid exchange (SCEs) was investigated by Landi *et al*., 2003 in human peripheral blood lymphocyte cultures, showing a role of GSTT1.

Table 15. DNA damage in *in vitro* studies.

Study	Results	Note	Reference
DNA SSB (single strand breaks) in B6C3F1 mouse hepatocytes	Positive –S9	Maximum concentration tested 34 µg/mL	Graves *et al*., 1994
DNA SSB (single strand breaks) in Chinese hamster ovary cells	Positive + S9, negative –S9	Maximum concentration tested 5100 µg/mL	Graves *et al*., 1994
DNA SSB (single strand breaks) in Chinese hamster ovary cells	Positive +/- S9 Stronger effects +S9	Maximum concentration tested 3975 µg/mL	Graves and Green 1996
DNA–protein cross-links, B6C3F1 mouse hepatocytes	Positive - S9	Maximum concentration tested 43 µg/mL	Casanova *et al*., 1997
DNA–protein cross-links, F344 rat hepatocytes	Negative - S9	Maximum concentration tested 425 µg/mL	Casanova *et al*., 1997

DNA–protein cross-links, Syrian hamster hepatocytes	Negative - S9	Maximum concentration tested 425 µg/mL	Casanova *et al*., 1997
DNA–protein cross-links, Chinese hamster ovary cells	Positive +/- S9 Stronger effects +S9	Maximum concentration tested 3975 µg/mL	Graves and Green 1996
DNA–protein crosslinks, V79 cells	Negative - S9	Maximum concentration tested 850 µg/mL	Hu *et al*. , 2006
DNA–protein cross-link, murine GSTT1 transfected V79 cells	Positive - S9 after treatment with proteinase K	Maximum concentration tested 212 µg/mL	Hu *et al*., 2006
Single-strand breaks, human primary hepatocytes	Negative - S9	Maximum concentration tested 5100 µg/mL	Graves *et al*. 1995
DNA–protein cross-link, human hepathocytes (expressing GSTT1)	Negative - S9	Maximum concentration tested 425 µg/mL	Casanova *et al* 1997
DNA damage by comet assay Primary human lung epithelial cells	Weak trend, independent of GST activity (GST enzymatic activity not present in the cultured cells)	10, 100, 1,000 µM	Landi *et al*., 2003

In addition, several studies that detected DNA damage also *in vivo* are available for dichloromethane.

DNA–protein cross-links were induced *in vivo* in the liver, but not the lung of B6C3F1/CrlBR mice exposed through inhalation to dichloromethane (Casanova *et al*., 1992). No DNA–protein cross-links were detected in Syrian hamster liver or lung after inhalation of dichloromethane (Casanova *et al*., 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 dichloromethane by inhalation (Casanova *et al*., 1996).

In a study *in vivo*, mice treated with dichloromethane at 2000 ppm [6940 mg/m3] for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of sister-chromatid exchange in lung cells (Allen *et al*., 1990). Exposure to higher concentrations (8000 ppm [27 800 mg/m3] for 2 weeks) also induced an increase in the frequency of sister-chromatid exchange in peripheral blood erythrocytes. Dichloromethane did not induce sister-chromatid exchange in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins *et al*., 1990; Allen *et al*., 1990).

Table 16. DNA damage in *in vivo* studies.

Study	Results	Note	Reference
DNA single strand breaks, B6C3F1 mouse liver	Positive	4831 ppm, inh., 6h	Graves *et al*., 1994
DNA single strand breaks, AP rat liver	Negative	4257 ppm, inh., 6h	Graves *et al*., 1994
DNA single strand breaks, CD rat liver	Positive	1275 µg/mL, poX1	Kitchin & Brown, 1994
DNA single strand breaks, B6C3F1 mouse liver	Positive Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage	4000 ppm, inh., 6h	Graves *et al*., 1995
DNA single strand breaks, B6C3F1 mouse lung	Positive Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a	2000 ppm, inh., 3h	Graves *et al*., 1995

	decrease in DNA damage		
DNA single strand breaks, AP rat lung	Negative	4000 ppm, inh., 3h	Graves *et al*., 1995
DNA damage, male B6C3F1 mouse liver comet assay	Negative	1600 ppm, inh., 6h/day, 5 days/wk, 6wk	Suzuki *et al*., 2014
DNA–protein cross-links, B6C3F1/CrlBR mouse Liver and lung	Positive in liver Negative in mouse lung	4000 ppm, inh., 6 h/day, 2 days	Casanova *et al*., 1992
DNA–protein cross-links, Syrian hamster, liver and lung	Negative	4000 ppm, inh., 6 h/day, 2 days	Casanova *et al*., 1992
DNA–protein cross-links, male B6C3F1/CrlBR mouse liver	Positive	498 ppm, inh., 6 h/day, 2 days	Casanova *et al*., 1996
DNA–protein cross-links, Syrian golden hamster, liver	Negative	3923 ppm, inh., 6 h/d, 2 days	Casanova *et al*., 1996
Sister-chromatid exchange, B6C3F1 mouse lung cells	Positive 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	2000 ppm, inh., 6 h/day, 5 days/ wk 12wk	Allen *et al*., 1990
Sister-chromatid exchange, B6C3F1 mouse bone marrow	Negative	5000 µg/mL, sc × 1	Allen *et al*., 1990
Sister-chromatid exchange, C57BL/6J mouse bone marrow	Negative	1500 µg/mL, ip × 1	Westbrook-Collins *et al*., 1990

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 methylene chloride-induced tumours. The role of GST-mediated metabolism is further confirmed by studies demonstrating increases in damage with the addition of GSTT1 to the test system and decreases in damage by addition of a GSH depletory. The eMSCA notes that the GSTT1 metabolic pathway has been measured in human tissues although this activity is generally lower than in rodents. In addition, human cells exhibited genotoxicity without exogenous addition of GSTT1 (Doherty *et al.*, 1996 and U.S. EPA, 2020).

When comparing metabolism of methylene chloride by the GST pathway in liver and lung tissues among species, mice are more active than rats, followed by humans and then hamster (U.S. EPA, 2020). Similarly, Thier *et al*. in 1998 cited by U.S. EPA (2011) found species specific liver GSTT1 isozyme activity after methylene chloride exposure to be ordered as follows (from highest to lowest): mice, rats, human high and low conjugators, hamsters and human non-conjugators. Thier *et al*. (1998) also reported that high and low human conjugators exhibited GSTT1 activities in erythrocytes approximately 11 and 16 times higher, respectively, than the human liver activities of high and low conjugators. Furthermore, the human high conjugator GSTT1 activity in erythrocytes was the same as male mouse liver activity and 61% of the female mouse liver activity. Increased GSTT1 activity in some human tissues may be partly responsible for the observed associations between increased methylene chloride exposure and cancer incidence in certain epidemiological studies.

Conclusion

Dichloromethane has been assessed for genotoxicity in a variety of assays *in vitro* (bacteria and mammalian cells). In general dichloromethane induces gene mutations in bacteria,

but not in mammalian cells *in vitro*, whereas evidence of clastogenicity *in vitro* was reported, preferentially linked to GST-mediated metabolism, although a role of P450 mediated metabolism cannot be excluded (Casanova *et al*. 1997). In human cell lines or isolated cells, dichloromethane induced micronucleus formation and sister-chromatid exchange (Hallier *et al*., 1993; Doherty *et al*., 1996; Olvera-Bello *et al*., 2010), while studies of DNA–protein cross-links, DNA single-strand binding proteins (SSBs), and unscheduled DNA synthesis largely gave negative results (Jongen *et al*., 1981; Graves *et al*., 1995; Casanova *et al*., 1997). In one study, the extent of sister-chromatid exchange was greater in cells from individuals without GST activity (Hallier *et al*., 1993). In another study, by contrast, the extent of sister-chromatid exchange was greater in cells from individuals with high GSTT1 activity (Olvera-Bello *et al*., 2010).

Dichloromethane was also tested in several *in vivo* studies. Dichloromethane was not able to induce micronucleus (MN) *in vivo* in bone marrow. Positive results were reported at high concentrations in erythrocytes and lung cells, after treatment *via* several routes of exposure (oral, inhalation) (Allen *et al*., 1990). However it is important to note that, as reported by Crebelli *et al*. (1999), the halogenated hydrocarbons (such as dichloromethane) 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 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 methylene chloride-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.

All together, 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. On this basis eMSCA suggests a revision of the harmonized classification by adding the hazard class mutagen category 2.

7.9.6. Carcinogenicity

Non-human information

Carcinogenicity: oral

The results of studies on carcinogenicity after oral administration are summarised in the following table:

Table 17. Carcinogenicity studies after oral administration.

Method	Results	Reference

rat (Fischer 344) male/female (oral: drinking water) Doses / Concentrations: 0, 5, 50, 125, 250, 250 (recovery, 18 months exposure) Basis: nominal 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 Vehicle: water Exposure: 104 weeks (daily) according to EU Method B.32 (Carcinogenicity Test)	Males: No treatment-related tumours were found up to the highest level of 250 mg/kg bw Females: hepatocellular adenoma or hepatocellular adenocarcinoma significantly increased, but in the range of historical controls	Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RG, Morgareidge K 1986
mouse (B6C3F1) male/female (oral: drinking water) Doses / Concentrations: 0 , 60, 125, 185, 250 mg/kg/bw/day 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 Vehicle: water Exposure: 104 weeks (daily) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	Males: Increased incidence of hepatocellular carcinoma at the highest dose compared with the first control group. A dose-related increase in the incidence of hepatocellular adenoma or carcinoma (combined) was also observed. Females: No treatment-related tumours were found up to the highest dose tested of 250 mg/kg bw/day.	Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RG, Morgareidge K 1986
Mouse (Swiss) Male/female Oral: gavage Doses/concentration: 100 or 500 mg/kg bw in olive oil by gavage once per day, for 4 or 5 days per week, for 64 weeks Equivalent to carcinogenicity test (lifetime)	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%) Females: No treatment-related tumours	Maltoni *et al*., 1988
Rat (Sprague-Dawley) Male/female Oral: gavage Doses/concentration: 0 (untreated control), 0 (olive oil), 100, 500 mg/kg bw by gavage in olive oil, 4–5 days/wk, for 64 wk 20 or 50 rats for groups. Equivalent to carcinogenicity test (lifetime)	Males: No significant differences in tumour incidence between control and treated rats. Females: No significant differences in tumour incidence between control and treated rats.	Maltoni *et al*., 1988

*statistically significant

Carcinogenicity: inhalation

The results of studies on carcinogenicity after inhalation exposure are summarised in the following table:

Table 18. Carcinogenicity studies after inhalation exposure.

Method	Results	Reference
rat (Fischer 344) male/female inhalation: vapour (whole body) Doses / Concentrations: 0, 1000, 2000 and 4000 ppm Doses / Concentrations: 0, 1004, 2009, and 3982 ppm Vehicle: unchanged (no vehicle) Exposure: 102 weeks (6 h/d, 5 d/w) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Males:** Mammary gland adenoma or fibroadenoma (combined): 0/50*, 0/50, 2/50 (4%), 5/50 (10%)** Subcutis, fibroma or sarcoma (combined): 1/50 (2%)***, 1/50 (2%), 2/50 (4%), 5/50 (10%) *P < 0.001 (trend)c **P = 0.023 ***P = 0.026 (trend) c=incidental tumour test NOAEC: 2000 ppm (male) increased incidence of beningn tumors of the mammary gland or subcutaneous tissue **Females:** Mammary gland adenoma or fibroadenoma (combined): 5/50 (10%), 11/50 (22%), 13/50 (26%), 23/50 (26%) P < 0.001 (trend)c P < 0.001 (high dose) P < 0.05 (mid-dose) P < 0.05 (low dose) LOAEC: 1000 ppm (female) increased incidences of benign tumors of the mammary gland	NTP 1986 Mennear JH, McConnell EE, Huff JE, Renne RA, Giddens E 1988
mouse (B6C3F1) male/female inhalation: vapour (whole body) Doses / Concentrations: 0, 2000, and 4000 ppm Doses / Concentrations: 0, 2009, and 3982 ppm (analytical conc.) Vehicle: unchanged (no vehicle) Exposure: 102 weeks (6 h/d, 5 d/w) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Males:** Bronchiolo-alveolar adenoma: 3/50 (6%)*, 19/50 (38%)**, 24/50 (48%)*** Bronchiolo-alveolar carcinoma: 2/50 (4%)*, 10/50 (20%)***, 28/50 (56%)** Hepatocellular adenoma: 10/50 (20%), 14/49 (29%), 14/49 (29%) Hepatocellular carcinoma: 13/50 (26%), 15/49 (31%), 26/49 (53%)*** Hepatocellular adenoma or carcinoma (combined): 22/50 (44%)*, 24/49 (49%), 33/49 (67%)*** *P < 0.001 (trend)[a] **P < 0.001 ***P < 0.05 [a]Incidental tumour test **Females:** Bronchiolo alveolar adenoma: 2/50 (4%)*, 23/48 (48%)**, 28/48 (58%)** Bronchiolo alveolar carcinoma: 1/50 (2%)*, 13/48 (26%)**, 29/48 (58%)** Hepatocellular adenoma: 2/50 (4%)*, 6/48 (13%), 22/48 (46%)** Hepatocellular carcinoma: 1/50 (2%)*, 11/48 (23%)***, 32/48 (67%)** Hepatocellular adenoma or carcinoma (combined): 3/50 (6%)*, 16/48 (33%)***,	NTP 1986 Mennear JH, McConnell EE, Huff JE, Renne RA, & Giddens E 1988

	40/48 (83%)** *P < 0.001 (trend)[a] **P < 0.001 ***P < 0.004 [a]Incidental tumour test LOAEC: 2000 ppm (male/female) increased incidences of lung and liver tumours	
rat (Fischer 344/DuCrj) male/female inhalation: vapour (whole body) 0, 1000, 2000, 4000ppm Vehicle: air Exposure: 104 weeks (6 h/day, 5d/wk) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Males:** Subcutis fibroma: 1/50 (2%), 4/50 (8%), 7/50 (14%), 12/50 (24%) P< 0.001 (trend), P < 0.001 (high dose) P < 0.05 (mid-dose)[d] [d]Peto test', Fisher exact test **Females:** Mammary gland fibroadenoma: 1/50 (2%), 2/50 (4%), 3/50 (6%), 8/50 (16%) Peritoneal mesothelioma: 3/50 (6%), 1/50 (2%), 0/50, 7/50 (14%) P < 0.001 (trend), P < 0.05 (high dose)b P < 0.05 (high dose)d [b]Fisher exact test [d]Peto test', Fisher exact test NOAEC: 1000 ppm (male) based on: (test mat.) increased benign tumor incidences in the mammary gland of males exposed to 4000 ppm and in subcutis fiibromas in the mammary chain area of males exposed to 2000 or 4000 ppm	Aiso S, Take M, Kasai T, Senoh H, Umeda Y, Matsumoto M, Fukushima S 2014 JRBC 2000
mouse (Crj: BDF1) male/female inhalation: vapour (whole body) 0, 1000, 2000, 4000ppm Vehicle: air Exposure: 104 weeks (6 h/day, 5 d/wk) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	**Males:** Bronchiolo alveolar adenoma: 7/50 (14%)*, 3/50 (6%), 4/50 (8%), 14/50 (28%) The incidence of haemangioma (all organs) in males at the highest dose did not exceed the upper limit of the historical controls of the laboratory. Bronchiolo-alveolar carcinoma: 1/50 (2%)*, 14/50 (28%)**, 22/50 (44%)**, 39/50 (78%)** Bronchiolo-alveolar adenoma or carcinoma (combined): 8/50 (16%)*, 17/50 (34%)***, 26/50 (52%)**, 42/50 (84%)** Hepatocellular adenoma: 10/50 (20%)*, 13/50 (26%), 14/50 (28%), 15/50 (30%) Hepatocellular carcinoma: 10/50 (20%)*, 9/50 (18%), 14/50 (28%), 20/50 (40%)*** Hepatocellular adenoma or carcinoma or hepatoblastoma (combined): 15/50 (30%)*, 20/50 (40%), 25/50 (50%)***, 29/50(58%)*** Liver haemangioma: 0/50, 4/50 (8%), 3/50 (6%), 5/50 (10%)*** Adrenal gland pheochromocytoma: 1/50 (2%)****, 0/50, 1/50 ((2%), 3/50 (6%) Haemangioma (all organs): 1/50 (2%)****, 5/50 (10%), 6/50 (12%), 7/50 (14%)*** *P < 0.001 (trend)[c] **P < 0.001 ***P < 0.05 ****P < 0.05 (trend) [c]Peto test', Fisher exact test	Aiso S, Take M, Kasai T, Senoh H, Umeda Y, M,Fukushima S 2014 JBRC 2000

	Femeles: Bronchiolo-alveolar adenoma: 2/50 (4%), 4/50 (8%), 5/49 (10%), 12/50 (24%)** Bronchiolo-alveolar carcinoma: 3/50 (6%)*,1/50 (2%), 8/49 (16%), 20/50 (40%)** Bronchiolo-alveolar adenoma or carcinoma (combined): 5/50 (10%)*, 5/50 (12%), 12/49 (24%)***, 30/50 (60%)** Hepatocellular adenoma: 1/50 (2%)*, 7/49 (9%)***, 4/49 (8%), 16/50 (32%)** Hepatocellular carcinoma: 1/50 (2%)*, 1/49 (2%), 5/49 (10%), 19/50 (38%)** Hepatocellular adenoma or carcinoma (combined): 2/50 (4%)*, 8/49 (16%)***, 9/49 (18%)***, 30/50 (60%)** Liver haemangioma or haemangiosarcoma (combined): 3/50 (6%)****, 2/49 (4%), 0/49, 7/50 (14%) *P < 0.001(trend)[c] **P < 0.001 ***P < 0.05 ****P < 0.01(trend) [c]Peto test', Fisher exact test NOAEC: <1000 ppm (male) based on: concentration related increase of bronchiolar-alveolar carcinomas at all concentration levels; increased incidence of hepatocellular carcinomas at 4000 ppm NOAEC: 2000 ppm (female) based on: increased incidence of bronchiolar-alveolar adenomas and carcinomas, and of hepatocellular adenomas and carcinomas at 4000 ppm	
rat (Sprague-Dawley) male/female inhalation: vapour (whole body) Doses / Concentrations: 50, 200 and 500 ppm Doses / Concentrations: 50 +/- 3, 199 +/- 5 and 499 +/- 10 ppm Basis: analytical conc. Vehicle: air Exposure: 20 months in males and 2 years in females (6 h/day, 5 d/week) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity /Carcinogenicity Studies)	**Males:** No treatment-related tumours were found up to the highest level tested of 500 ppm. **Females:** Mammary gland adenoma or fibroadenoma: 52/70 (74%), 58/70 (82%), 61/70 (71%)*, 55/70 (78%), 23/30 (77%), 23/30 (77%) *P < 0.05[b] [b] Fisher exact test	Nitschke KD, Burek JD, Bell TJ, Kociba, RJ, Rampy LW, McKenna MJ 1988
rat (Sprague-Dawley) male/female inhalation: vapour (whole body) Doses / Concentrations: 3430 ppm (3158-3627 ppm) Exposure: 6 hours/day for males: 5 consecutive days females: 15-19 consecutive days (two weeks + one	Mechanistic study	Dow Chemical Company 1986

additional oestrus cycle) (daily) investigate study, no guideline exists The purpose of this study was to investigate the effect of DCM upon serum prolactin levels in rats, to evaluate hyperprolactinaemia as a potential mode of action underlying mammary gland tumour formation.		

Carcinogenicity: dermal

No relevant information available.

Human information

The exposure-related observations in humans are summarised in the following table:

Table 19: Exposure-related observations on carcinogenicity in humans.

Method	Results	Reference
Study type: Cohort study on cancer	**Subjects** analysed: 1271 (551 men and 720 women) Location and follow-up period: USA; 1954-1990. Exposure: workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1954–76. Exposure: 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. Results: Standardized mortality ratios (SMRs) 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 dichloromethane 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 2016: 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 *et al*., 1993
Study type: Cohort study on cancer	**Subjects** analysed: 3211 white workers (2187 men and 1024 women) Location and follow-up period: USA, 1970–1989 Exposure: Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1970–81. The workers had been also exposed to acetone and methanol Results: 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,	Gibbs *et al*., 1996

	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	
Study type: Cohort study on cancer	**Subjects** analysed: 1311 male white workers Location and follow-up period: USA,1964–1994 Exposure: Workers from a plant producing cellulose triacetate film, engaged for ≥ 1 yr in one of three areas in which dichloroethane was used (roll coating, doping, distilling) in 1946–70. Exposure to dichloromethane (8-hour time-weighted average, TWA) was: 0–520 ppm [0–1800 mg/m3] in 1946–1965, 0–300 ppm [0–1040 mg/m3] in 1966–1985, and 0–100 ppm [0–347 mg/m3] 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. Results: 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 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.	Hearne & Pifer (1999)
Study type: Cohort study on cancer	**Subjects** analysed: 1785 male Location and follow-up period: England, 1946–2006 Exposure levels were estimated from area samples according to time period and work group. TWA exposures were estimated to range from 2 to 20 ppm [7–69 mg/m3] before 1960, 6 to 127 ppm [21–441 mg/m3] during the 1960s, 10 to 165 ppm [35–573 mg/m3] during the 1970s, and 7 to 88 ppm [24–305 mg/m3] during the 1980s (Tomenson *et al*. (1997)). The workers had been also exposed to acetone and methanol. Results: Only for cancer of the brain and central nervous system (SMR, 1.83; 95% CI, 0.79–3.60, among exposed workers) was the number of deaths more than 1.2 times that expected. No cancers of the liver were observed among exposed or unexposed workers (expected, 3.3 cases), and there was a significant deficit of cancer of the lung. Data for NHL were reported. Analysis of cumulative exposure for four cancer sites, including brain, did not show any significant trends with the level of exposure to dichloromethane. Limits of the study:small number of deaths, which limited the ability to conduct exposure–response analysis.	Tomenson, 2011
Study type: Cohort study on cancer	**Subjects** analysed: 1222 workers of a military-aircraft maintenance facility Location and follow-up period: USA, 1952–2000 Exposure levels: Workers were exposed to numerous chemicals. Exposure was assessed quantitatively for trichloroethylene, and qualitatively (ever/never) to other agents including dichloromethane. Results: Exposure to dichloromethane was associated with increased risks (hazard ratio, HR) of NHL (HR, 2.02;	Radican *et al*., 2008

	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 dichloromethane 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 dichloromethane were limited.	
Study type: review of retrospective cohort and case-control studies. 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 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. Endpoint addressed: carcinogenicity	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 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.	Dell LD, Mundt KA, McDonald M, Tritschler JP, Mundt DJ 1999 Reported only in the CSR (critical review)

Case-control study	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 Aim of the study: to evaluate the association between risk of lymphoma and exposure to dichloromethane and nine other organic solvents Exposure levels: 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. Results: 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 dichloromethane was 1.7 (95% CI, 0.7–4.3; 13 cases; P for trend, 0.46). Among the NHL subtypes, an odds ratio for dichloromethane 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 dichloromethane were not reported	Miligi *et al*., 2006
Study type: case-control studies	Subjects analysed: Malignant lymphoma, 710 cases; Controls, 710 Location and follow-up period: Germany, 1999–2003 Aim of the study: to examine the relationship between malignant lymphoma and exposure to eight organic solvents including dichloromethane Exposure: In-person interview obtained occupational history, medical history, and lifestyle. Exposure was assessed for several chlorinated solvents, with metrics of intensity, frequency, and confidence assigned by an industrial hygienist, and cumulative exposure was calculated. Results: Odds ratios were adjusted for smoking and alcohol consumption. The odds ratio for high cumulative exposure to dichloromethane 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 *et al*., 2007
Study type: case-control studies	Subjects analysed: 586 cases of leukaemia and 1278 controls from seven areas in Italy. Location and follow-up period: Italy, 1991-1993. Aim of the study: to evaluate the risks associated with exposure to ten organic solvents including dichloromethane Exposure: 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 and odds ratios were adjusted by area, sex, age, and education. Results: No associations between acute leukaemia or myeloma and dichloromethane 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 *et al*., 2008
	Subjects analysed: Multiple myeloma, 180 cases, 481 controls were collected from the general population in the same areas/ population	Gold *et al*., 2011

	Location and follow-up period USA, 2000–2002 Aim of the study: to evaluate the associations between risk of multiple myeloma and exposure to dichloromethane and other chlorinated solvents. Exposure: 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. Results: Odds ratios were adjusted by area, race, sex, age, and education. Overexposure to dichloromethane 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.	
Case-control study	Subjects analysed: 601 female cases, and 717 controls, matched for age, collected from the general population in Connecticut, USA. Location and follow-up period: USA Aim of the study: to examine the association between NHL and exposure to nine organic solvents including dichloromethane. Exposure: Information about occupational history and other potential risk factors was obtained by in-person interview and probability and intensity of exposure to solvents were assigned using a previously developed job-exposure matrix. Results: Odds ratios were adjusted by race, age, family history of haematopoietic cancer, and alcohol consumption. Subjects ever-exposed to dichloromethane 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.	Wang *et al*., 2009
Case-control study	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. Exposure: 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. Aim of the study: to examine the associations between astrocytic cancer of the brain and exposure to six chlorinated solvents including dichloromethane. 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	Heineman *et al*., 1994

	when the probability of exposure was high.	
Case-control study	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 neurological disorders. Exposure: Probability and intensity of exposure were assigned using occupation and industry titles from subjects' death certificates and a job-exposure matrix. 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 dichloromethane. Results: After adjusting for age at death, marital status, and socioeconomic status, the odds ratio for the association of exposure to dichloromethane 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 dichloromethane was poor.	Cocco *et al.*, 1999
Case-control study	Subject analysed: 405 case fathers and 302 control fathers. Aim of the study: to identify paternal occupational exposures associated with an increased risk of cancer of the brain in children. Results: When considering paternal exposure to dichloromethane 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).	De Roos *et al.*, 2001
Case-control study	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, race, hospital, and proximity to the hospital. Aim of the study: to examine associations between glioma and meningioma and exposure to six chlorinated solvents including dichloromethane. Exposure: Exposure to solvents was assessed by an industrial hygienist based on detailed occupational histories collected by interview. Results: Odds ratios adjusted for the matching factors did not show any association between glioma or meningioma and overall exposure to dichloromethane or other metrics, including duration, intensity, and cumulative exposure.	Neta *et al.*, 2012
Case-control study	Subject analysed: Cases were 798 patients with	Ruder *et al.*,

	intracranial glioma in Iowa, Michigan, Minnesota, and Wisconsin, USA, and controls were 1175 residents selected from the same area. Aim of the study: to examine associations between glioma and exposure to six chlorinated solvents including dichloromethane. Exposure: Lifetime occupational histories were obtained by interview and several exposure metrics were assigned by an industrial hygienist. 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 dichloromethane, or exposure probability and cumulative exposure.	2013
Multicentre case–control study of meningioma	Subject analysed: 1906 cases and 5565 controls, in seven countries. Exposure: no subjects classified as exposed to dichloromethane after assessment of lifetime occupational histories using a modified version of the Finnish national job-exposure matrix	McLean *et al.*, 2014

Summary and conclusion of 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 also reported in the IARC monograph 110 (2016) and in the CSR provided by the Registrant(s).

Human data:

As reported in the IARC monograph 110, two cohort studies of workers exposed to dichloromethane (as well as acetone and methanol, but not 1,2-dichloropropane) in the USA reported findings for cancers of the liver and biliary tract, based on small numbers. One of the studies reported a positive association for cancer of the liver and biliary tract, while the other did not. Cancer of the biliary tract constituted three of the four liver cancers in the study with a positive association, and both of the liver cancers in the other one. Given that cancer of the biliary tract normally represents a small proportion of cancers of liver and biliary tract combined, these proportions are very high.

In a case series of cancer of the biliary tract (histologically identified as cholangiocarcinoma) among printing workers in Japan, most of the cases were exposed to dichloromethane, and all except one of these were also exposed to 1,2 dichloropropane. The high risk of this rare cancer in one cohort study of workers without exposures to other likely risk factors and among exposed printing workers in Japan is consistent with a causal association, but the number of exposed cases was small and the printing workers had other potentially confounding exposures, notably to 1,2 dichloropropane.

Two cohort studies and three case–control studies in several countries evaluated non-Hodgkin lymphoma (NHL), and all except one cohort study reported increased risks among workers exposed to dichloromethane. 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. There were several studies that assessed other cancer sites, but these data were regarded as inadequate by the working group of IARC in the monograph 110.

Overall, positive associations have been observed between exposure to dichloromethane and cancer of the biliary tract and non-Hodgkin lymphoma. However, these studies represent a limited evidence of dichloromethane carcinogenicity in humans due to the low numbers of cases and to the co-exposures to other chemicals.

Animal data:

There were six studies of carcinogenicity with dichloromethane 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. Dichloromethane increased the incidence of hepatocellular carcinoma in three studies in male mice (two by inhalation, one in drinking-water), and in three studies of inhalation in female mice. Dichloromethane increased the incidence of hepatocellular adenoma or carcinoma (combined) in two inhalation studies in male mice and three inhalation studies in female mice. Dichloromethane increased the 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. Dichloromethane increased the incidences of haemangioma of the liver and of all organs (including the liver) in one inhalation study in male mice, while the incidence of haemangioma or haemangiosarcoma (combined) in the liver in one inhalation study in female mice was statistically increased but this increase was whithin the historical control values.

There were seven carcinogenicity studies with dichloromethane 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). Dichloromethane 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. Dichloromethane caused salivary gland sarcomas in one inhalation study in male rats (the sialodacryoadenitis virus was detected in these rats; the effect of this virus on carcinogenesis is unknown). Dichloromethane 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 dichloromethane in male and female Syrian hamsters in which there was an increase in the incidence of malignant lymphoma in females.

Mechanistic information:

Dichloromethane 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 dichloromethane 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 dichloromethane 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 dichloromethane. In human cells, dichloromethane induces micronucleus formation and sister-chromatid exchange, but not DNA–protein cross-links and DNA damage. In experimental animals, dichloromethane-induced genotoxicity is associated with the GST pathway. Studies in non-mammalian systems *in vitr*o showed evidence of mutagenicity, particularly in systems with GST activity. Evidence for the role of GSTT1 in genotoxicity in humans is mixed. Overall, the genotoxicity of dichloromethane 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.

Hepatic, neurological, renal, splenic, reproductive, and developmental toxicity have also been reported in humans or experimental animals.

There is little evidence for non-genotoxic mechanisms of carcinogenesis with dichloromethane. No studies with dichloromethane in humans have investigated whether GSTT1 polymorphisms are associated with cancer. One study has reported an association between a CYP2E1 polymorphism and non-Hodgkin lymphoma in dichloromethane-exposed individuals; however, the functional significance of this polymorphism is unknown.

Overall, given the extensive evidence for genotoxicity, in association with metabolic pathways that are operative in humans, IARC concluded that the mode of action of the carcinogenesis reported in animals is relevant for human. The e-MSCA support this conclusion.

In conclusion, although the evidence in humans for the carcinogenicity of dichloromethane is limited, eMSCA considered the available data on carcinogenicity of dichloromethane in experimental animals and the mechanistic information sufficient to support a revision of the classification of dichloromethane as carcinogen category 1B.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

In the Justification Document for listing the substance in the CORAP the following information was available:

The OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) study has been provided concluding that at concentrations as high as 1500 ppm (ca. 5300 mg/m^3) dichloromethane did not affect any of the reproductive parameters examined. However, the study does not cover several important parameters, such as organ weights, sperm parameters, estrous cyclicity, implantation sites and histopathology.

Additionally, in a supporting study for carcinogenicity endpoint (mechanistic study) it was found that during a two-week exposure period at 3500 ppm, dichloromethane caused a statistically significant ($p<0.05$) increase in the length of the oestrus cycle and elevated serum prolactin concentrations in femal e Sprague-Dawley rats.

Therefore due to the lack of important parameters for reproductive toxicity and the possible effect of the substance to the oestrus cycle the toxicity of dichloromethane to reproduction is not clear.

In addition, read across with dichloroethane (EC no. 203-458-1, CAS No 107-06-2) should be eventually taken into account, since the latter has been linked to an altered signalling pathway leading to altered hormones status (i.e. testosterone, gonadotropin-releasing hormone, luteinizing hormone/LH) in the testes as well as to malformation of spermatozoa, reduced sperm concentration, and pathological impairment of the testes.

In a developmental toxicity study similar to OECD Guideline 414 examining the effects of maternally inhaled methylene chloride on embryonal and fetal development in rats and mice, foetal skeletal variations were observed which may have been caused by hypoxia as increased carboxyhaemoglobin levels were seen in the dams and hypoxia is known to affect the developing foetus. As a result the level of 4300 mg/m^3 (ca. 1250 ppm) was established to be a LOAEC for developmental toxicity (mild foetotoxicity) and for slight maternal toxicity. However, it should be noted, that LOAEC value in this study does not correlate with the findings in the reprotoxicity study (2-generation).

Dichloromethane is thought to readily transfer across the blood-brain barrier by passive diffusion, as evidenced by the detection of radioactivity in brain tissue 48 hours after exposures of rats to radiolabeled dichloromethane at concentrations of 50, 500, or 1500 ppm for 6 hours. It can be transferred across the placenta, and small amounts can be excreted in urine or in milk. Historically it is demonstrated that dichloromethane has

transient sedative and anesthetic properties in humans. Due to this it is not possible to conclude that the skeletal variations were caused by hypoxia and maternal toxicity. Therefore possible developmental toxicity of the substance cannot be excluded.

Conclusion:

The reprotoxicity concern based on reproductive/developmental effects remains to be clarified. Looking at dichlorethane in a weight of evidence approach would support to address the concern for reproductive toxicity. Hence, the eMSCA is of the opinion that a testing strategy would be necessary to address such a lack of information.The eMSCA reserves the possibility to further investigate this concern in case the CLH proposal for Carc 1B will not be accepted. This is to ensure that potential further appropriate administrative risk management measures could be taken based on potentially emerging reproductive/developmental properties.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Table 20

CRITICAL DNELS/DMELS					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s)** **(e.g. NOAEL, NOAEC)**	**DNEL**	**Justification/ Remarks**
Inhalation Workers	Systemic effects – Long-term	SCOEL assessment (2009)	MAK value of 50 ppm	DNEL = 176 mg/m³	
Dermal Workers	Systemic effects – Long-term	Repeated dose toxicity (Oral)	NOAEL dermal 582 mg/kg bw/day NOAEL oral 6 mg/kg bw/day *(97/1) = corrected NOAEL dermal 582 mg/kg bw/day (REACH Guidance)	DNEL = 12 mg/kg bw/day	AF for interspecies differences (allometric scaling): 4 AF for other interspecies differences: 2.5 AF for intraspecies differences: 5 Overall Assessment Factor: 50
Inhalation Consumers	Systemic effects – Long-term	repeated dose toxicity (By inhalation)	MAK value of 50 ppm	DNEL = 44 mg/m³	The SCOEL values has been used to set inhalation DNELs for the general population using the assessment factor 10 for

					intraspecies variation instead of 5, and considering differences in exposure and activity (10 m^3 in 8 h for workers, 20 m^3 in 24 h for the general population (176 mg/m^3 x 5/10 x 10/20 = 44 mg/m^3) The SCOEL values has been used to set inhalation DNELs for the general population using the assessment factor 10 for intraspecies variation instead of 5, and considering differences in exposure and activity (10 m^3 in 8 h for workers, 20 m^3 in 24 h for the general population (353 mg/m^3 x 5/10 x 10/20 = 88.3 mg/m^3)
				DNEL for infrequent uses = 88 mg/m^3	
Dermal Consumers	Systemic effects – Long-term	Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RG (1986a) Repeated dose toxicity (Oral)	NOAEL dermal 582 mg/kg bw/day NOAEL oral 6 mg/kg bw/day *(97/1) = corrected NOAEL dermal 582 mg/kg bw/day (REACH Guidance)	DNEL = 5.82 mg/kg bw/day	AF for interspecies differences (allometric scaling): 4 AF for other interspecies differences: 2.5 AF for intraspecies differences: 10 Overall Assessment Factor: 100

Oral Consumers	Systemic effects – Long-term	Serota DG, Thakur AK, Ulland BM, Kirschman JC, Brown NM, Coots RG (1986a) Repeated dose toxicity (Oral)	NOAEL oral 6 mg/kg bw/day	DNEL = 0.06 mg/kg bw/day	AF for interspecies differences (allometric scaling): 4 AF for other interspecies differences: 2.5 AF for intraspecies differences: 10 (general population) Overall Assessment Factor: 100

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

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 category: Carc category 1B and Muta category 2.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Not evaluated.

7.10.2. Endocrine disruption - Human health

No Endocrine Disruptor (ED)-like activity has been so far detected neither reported in the main existing ED- or SVHC-databases (as in the SIN http://chemsec.org/business-tool/sin-list/ e TEDX http://www.endocrinedisruption.org/endocrine-disruption/tedx-list-of-potential-endocrine-disruptors/overview lists of chemicals of concern).

Despite this, literature data concerning DCM reproductive/developmental effects points out directly to a potential ED-like mode of action. In particular, taking into account both: i) the CYP-mediated mechanism(s) supporting both hepatic and biliary tract carcinogenesis alert at low dichloromethane doses, and ii) the CYP2E1-mediated mechanism supporting the reproductive/developmental alert in the male germ line, a concern on the possibility that dichloromethane could act via an ED-like mechanism exists. A study (Mennear JH *et al.*, 1988) on dichloromethane-mediated carcinogenesis in F344/N rats indicated that a set of endocrine-regulated tissues (mammary glands, testis, adrenals) were responding, in a non linear dose-dependent manner, to the treatment with dichloromethane, suggesting a possible relationship with disturbed endocrine function and raising the possibility of a hormonal-mediated mechanism(s) at a realistic scenario of exposure to humans.

7.10.3. Conclusion on endocrine disrupting properties (combined/separate)

The concern for ED properties still exists and is supported on the basis of a read-across with dichloroethane highlighting at least further concerns about reproductive toxicity potentially linked to ED-related pathways. For this reason, the eMSCA reserves the possibility to further investigate on this endpoint in case the CLH proposal for Carc. 1B will not be accepted. This is to ensure that potential further appropriate administrative risk management measures could be taken based on potentially emerging ED properties.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

7.12.1. Human health

The eMSCA is of the opinion that the exposure assessment provided by the Registrant(s) both for workers and consumers is acceptable.

7.13. Risk characterisation

No further action.

7.14. References

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7.15. Abbreviations

AF	Assessment Factor
eMSCA	Evaluating Membre State Competent Authority
CYP	Cytochrome P450
CO	Carbon monoxide
CO_2	Carbon dioxide

COHb	Carboxyhemoglobin
DCM	Dichloromethane
DMEL	Derived Minimum Effect Level w
DNEL	Derived No Effect Level
GHS	Gluthatione
GSTT1	Gluthatione S-transferase T1
LOAEC	Lowest Observed Adverse Effect Concentration
NOEC	No observable adverse effect concentration
NOAEL	No observed adverse effect level
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation No. 1907/2006)
PACT	Public activities coordination tool
PBT	Persistent, Bioaccumulative and Toxic
ppm	parts per million
SCOEL	Scientific Committee on Occupational Exposure Limits
SCG	Alkaline Single Cell Gel Electrophoresis
vPvB	Very Persistent Very Bioaccumulative
t	tons