ECHA Scientific report

for evaluation of limit values for 1,2-dichloropropane at the workplace

Prepared by the European Chemicals Agency

19 October 2022

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Preamble

The Commission, in view of the preparation of the proposals for amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens, mutagens or reprotoxic substances at work (CMRD) and in line with the 2017 Commission Communication '*Safer and Healthier Work for All' - Modernisation of the EU Occupational Safety and Health Legislation and Policy*[1], asked the advice of RAC to assess the scientific relevance of occupational exposure limits

Therefore, the Commission made a request on 23 February 2022 to ECHA in accordance with the Service Level Agreement (SLA) (Ares (2022)711149), to evaluate, in accordance with Directive 2004/37/EC, the following substance: 1,2-Dichloropropane (EC number: 201-152-2).

In support of the Commission's request, ECHA has prepared a scientific report concerning occupational limit values at the workplace. This scientific report is made available at: Occupational exposure limits-Consultations on OEL recommendation on **19 October 2022** and interested parties were invited to submit comments by **19 December 2022**.

In the preparatory phase of making this report, a call for evidence was started on **02 May 2022** to invite interested parties to submit comments and evidence by **01 August 2022**.

The Committee for Risk Assessment (RAC) will develop its opinion on the basis of the scientific report submitted by ECHA.

[1] http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes

Table of Contents

Figures

Tables

List of abbreviations

Abbreviation	Definition
AID	Activation-Induced Cytidine Deaminase
ATSDR	The Agency for Toxic Substances and Disease Registry, Atlanta, Georgia
BAR	Biological reference value Background level of a substance present concurrently, at a particular time, in a reference population of persons of working age who are not occupationally exposed to this substance.
BAT	Biological tolerance value (for occupational exposure)
BER	Base Excision Repair
BGV	Biological Guidance Value
BiIIN	Biliary Intraepithelial Neoplasia
BLV	Biological Limit Value
CAD	Chemical Agents Directive
CI	Confidence Interval
CLP	Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures (CLP Regulation)
CMD/CMRD	Carcinogens and Mutagens Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. The amendment of the CMD, Directive 2022/431/EU also brought reprotoxic substances within the scope of the directive, changing the original title on the protection of workers from the risks related to exposure to carcinogens or mutagens at work to the protection of workers from the risks related to exposure to carcinogens, mutagens or reprotoxic substances at work (CMRD).
CMR	Carcinogens, Mutagens or substances toxic to Reproduction
CNS	Central nervous system
CSR	Chemical safety report
1,2-DCP	1,2-dichloropropane
DFG	Deutsche Forschungsgemeinschaft (“German Research Foundation”)
DCM	Dichloromethane
EC	European Commission
ECHA	European Chemicals Agency
EPA	U.S. Environmental Protection Agency
EU	European Union
GESTIS Substance Database	GEfahrSToffInformationsSystem (German information system for the safe handling of hazardous substances and other chemical substances at work)
GLP	Good Laboratory Practice
Hct	Haematocrit
IARC	International Agency for Research on Cancer (World Health Organization)
IPNB	Intraductal Papillary Neoplasms of the Bile duct
JHIA	Japan Health Insurance Association
JNIOSH	Japanese National Institute of Occupational Safety and Health

Abbreviation	Definition
JSOH	Japan Society for Occupational Health
LOQ	Limit of quantification
LLNA	Local Lymph Node Assay
MAK Commission	The Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area The Senate Commission is the only independent, science-driven body in Germany that evaluates the health-related effects of substances in the work area.
MHLW	Ministry of Health, Labour and Welfare of Japan
MN	Micronucleated
MoA	Mode of Action
NEC	Normochromatic Erythrocyte
NHANES	National Health and Nutrition Examination Survey in the United States
NIOSH	National Institute for Occupational Safety and Health (USA)
NOCCA	Nordic Occupational Cancer Study
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OECD TG	OECD Guidelines for the Testing of Chemicals
OEL(s)	Occupational exposure limit(s)
OR	Odds ratio
OSHA	Occupational Safety and Health Administration (USA)
RAC	Committee for Risk Assessment
RBC	Red Blood Cell
REACH	Regulation (EC) No 1907/2006 of the European Union concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
RET	Reticulocytes
ROS	Reactive Oxygen Species
SCE	Sister Chromatid Exchange
SCOEL	Scientific Committee on Occupational Exposure Limits (former committee of the European Commission)
SIR	Standardised Incidence Ratio
SLA	Service Level Agreement
SMART	Somatic Mutation And Recombination Test
SMR	Standardised mortality ratio
SNV	Single Nucleotide Variant
SPRR	Standardised prevalence ratio
STEL	Short term exposure limit
TCE	Trichloroethane
TC-NER	Transcription-coupled Nucleotide Excision Repair
TWA	Time-Weighted-Average
U.S./USA	United States of America

Abbreviation	Definition
VOC	Volatile organic compound
WHO	World Health Organization

Scope of the task and literature search

ECHA has been tasked by the European Commission to evaluate the exposure to 1,2-Dichloropropane (1,2-DCP) to assess the option of an airborne occupational exposure limit, other limit values (BLV/BGV) and notations.

This report is based on international assessments such as (ATSDR, 2021, EPA, 2016, Hartwig and MAK Commission, 2021, IARC, 2017, OECD SIDS, 2005). This has been complemented by a literature search of published papers from the last ten years.

ECHA evaluation and recommendation

1,2-Dichloropropane is a non-threshold carcinogen. Consequently, no health-based OEL can be identified and an exposure-risk relationship (ERR) expressing the excess risk for cancer (bronchoalveolar adenomas/carcinomas) in function of air concentration is derived.

The tables below present the outcome of the scientific evaluation to derive limit values for 1,2-DCP.

Derived Limit Values

OEL as 8-hour TWA:	No proposal
STEL:	No proposal
BLV:	No proposal
BGV:	No proposal

Notations

Notations:	Skin

Cancer Exposure-Risk Relationship*

1,2-DCP concentration in air (mg/m^3)	Excess life-time cancer risk (Cases per 100 000 exposed)
0.007	1
0.028	4
0.07	10
0.28	40
0.7	100
2.8	400

* Assuming exposure 8 hours per day and 5 days per week over a 40-year working life period.

1. Chemical Agent Identification and Physico-Chemical Properties

As explained in Ullmann's Encyclopaedia of Industrial Chemistry[2], "*1,2-Dichloropropane is a colorless, flammable liquid with a chloroform like odor*". "*It is miscible with most organic solvents, such as alcohols, esters, and ketones, as well as with aromatic, aliphatic, and chlorinated hydrocarbons. 1,2-Dichloropropane is stable at room temperature but is dehydrochlorinated by thermal or catalytic cracking to allyl chloride and 1-chloro-1-propene. It is incompatible with strong oxidizers, strong acids, and active metals. It is dehydrochlorinated by NaOH to give mainly 1-chloro-1-propene (45% cis and 55% trans isomer)*."

Table 1: Chemical Identifications

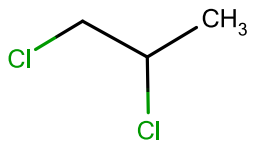
Identifier	
IUPAC Name	1,2-dichloropropane
Synonyms	-
EC/ List No	201-152-2
CAS RN	78-87-5
Chemical structure	
Chemical formula	C3H6Cl2
Molecular weight	112.99 g/mol

Table 2: Physico-chemical properties[3]

Property	
Appearance	Liquid (at 20°C and 1013 hPa)
Boiling point	96.4 °C (at 101 325 Pa)
Density	1.155 (at 20°C)
Vapour pressure	66.32 hPa (at 25 °C)
Partition coefficient (log Pow)	2 (at 25 °C)
Water solubility	2800 mg/L (at 20 °C)
Viscosity	0.757 mm²/s (static) (at 20 °C)
Conversion factor	1 ppm = 4.70 mg/m³ (at 20 °C)[4] 1 mg/m³ = 0.21 ppm (at 20 °C)

[2] Ullmann's Encyclopaedia of Industrial Chemistry 2022 - Chloropropanes, Chlorobutanes, and Chlorobutenes

[3] Values obtained from registration data published on www.echa.europa.eu

[4] $concentration\left[\frac{mg}{m^3}\right] = 112.99\ \frac{g}{mol} \cdot \frac{1.013 \cdot 10^5 Pa \cdot 1m^3}{8.314 \cdot \frac{Pa \cdot m^3}{mol \cdot K} 293.15K} \cdot 10^{-3} \cdot concentration[ppm]$

2. EU Harmonised Classification and Labelling - CLP (EC) 1272/2008

Table 3: EU classification: Summary of existing classification

Index No	International chemical ID	EC No	CAS RN	Annex VI of CLP hazard class and category	Hazard statement code
602-020-00-0	1,2-dichloropropane; propylene dichloride	201-152-2	78-87-5	Flam. Liq. 2 Acute Tox. 4 Acute Tox. 4 Carc. 1B	H225 H302 H332 H350

Regulation (EU) 2016/1179 (9th adaptation to technical and scientific progress) of 11 July 2016 modified the classification of 1,2-DCP from Category 2 carcinogen to Category 1B carcinogen (date of effect: 1 March 2018).

3. Chemical Agent and Scope of Legislation - Regulated uses in the EU

3.1 Directive 98/24/EC (CAD) and Directive 2004/37/EC (CMRD)

There is currently no binding or indicative occupational exposure limit value for 1,2-DCP under CAD or CMRD.

3.2 REACH Registrations

Table 4: REACH Registrations and tonnage

Substance		Tonnage (tonnes/annum)	
Name	EC number	Full registration	Intermediate use
1,2-dichloropropane	201-152-2	>1000 (3 registrants)	used as an intermediate to produce perchloroethylene and several other related chlorinated chemicals

3.3 Authorised uses under Annex XIV of REACH

1,2-DCP is not currently listed in Annex XIV of REACH ("Authorisation List").

3.4 Restricted uses under Annex XVII of REACH

1,2-DCP is not currently listed in Annex XVII of REACH.

3.5 Plant Protection Products Regulation (EC) 1107/2009

1,2-DCP is "not approved" under Directive 91/414/EEC[5] and Regulation (EC) No 1107/2009[6]. Regulation (EC) No 2076/2002[7] lists 1,2-DCP as an active substance not included in Annex I to Directive 91/414/EEC.

[5] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A01991L0414-20110601
[6] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02009R1107-20210327
[7] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02002R2076-20140715

3.6 Human and Veterinary Medicinal Products Directives 2001/83/EC and 2004/28/EC respectively

1,2-DCP is not listed among authorised medicines contained in the Article 57 of Regulation (EC) No 726/2004[8], and is also not subject to maximum residue levels (MRLs). It is therefore not included in Annex II of Council Regulation (EEC) No 2377/90[9], in accordance with Directive 2004/28/EC.

3.7 Biocidal Products Regulation (EU) 528/2012 and Biocidal Products Directive 98/8/EC

There are no biocidal products authorised on the EU/EEA market which are based on or include 1,2-DCP.
1,2-DCP is not listed as an active substance under Regulation (EC) No 528/2012[10] or Directive 98/8/EC[11].

3.8 Other legislations

1,2-DCP is included on the list of substances regulated under the European VOC (Volatile organic compounds) Solvent Emission Directive 1999/13/EC[12].

4. Existing Occupational Exposure Limits

Several EU Member States have established OEL values for 1,2-DCP. Some Member States have additionally established short-term limit values (STEL). Table 5 presents these values along with those established in Australia, Canada, China, Japan, New Zealand, Norway, Singapore, South Korea, Switzerland and the USA. No BLV and BGV has been found.

The list should not be considered as exhaustive.

Table 5: Existing Occupational Exposure Limits (OELs) indicated as 8-h Time-Weighted Average (TWA) for 1,2-DCP

Country	TWA (8 h) ppm	TWA (8 h) mg/m³	STEL (15 min) ppm	STEL (15 min) mg/m³	Remarks
EU countries					
Austria	75 (1)	350 (1)	375 (1) (2)	1750 (1) (2)	(1) TRK[1] based on technical feasibility (2) 30 minutes average value
Belgium	10	47			
Denmark	75	350	150	700	
Finland	10	46	20 (1)	92 (1)	(1) 15 minutes average value
France	75	350			
Hungary		50		50	
Ireland	10	46			
Poland		50			
Romania	22	100	44 (1)	200 (1)	(1) 15 minutes average value
Spain	10	47			

[8] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32004R0726
[9] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A01990R2377-20080816
[10] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32012R0528
[11] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31998L0008
[12] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31999L0013

Country	TWA (8 h) ppm	TWA (8 h) mg/m³	STEL (15 min) ppm	STEL (15 min) mg/m³	Remarks
Non-EU countries					
Australia	75	347	110	508	
Canada-Ontario	10				
Canada-Québec	75	347	110	508	
China		350		500 (1)	(1) 15 minutes average value
Japan (MHLW)[2]	1				
Japan (JSOH)[3]	1	4,6			
New Zealand	5	23			
Norway	40	185			
Singapore	75	347	110	508	
South Korea	75	350	110	510	
Switzerland	75	350			
USA-OSHA[4]	75	350			

Source: GESTIS - International limit values for chemical agents (Occupational exposure limits, OELs); https://www.dguv.de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index-2.jsp (accessed June 2022; searched for "1,2-dichloropropane")

Notes: [1] TRK: Technical Guidance Concentrations; [2] MHLW: Ministry of Health, Labour and Welfare of Japan; [3] JSOH: Japan Society for Occupational Health; [4] OSHA: Occupational Safety and Health Administration

5. Occurrence, Use and Occupational Exposure

5.1 Occurrence

Industrial activities are probably responsible for all releases of 1,2-DCP into the environment. Most of these releases finally end up in the air or groundwater.
1,2-DCP is an organic chlorocarbon compound, obtained as a by-product from the chlorohydrin process.

5.2 Production and Use Information

1,2-DCP is a by-product, produced in significant quantities, during the manufacturing process of propylene oxide by the chlorohydrin process (Rossberg et al., 2006) method to produce epichlorohydrin, in three steps:

1. Chlorination of propylene to obtain allyl chloride,
2. Reaction of the allyl chloride with hypochlorous acid to produce glycerol dichlorohydrins,
3. Reaction of the glycerol dichlorohydrin isomers with sodium (or calcium) hydroxide to produce epichlorohydrin.

Hydrochloric acid, sodium (or calcium) chloride, and water are produced along with by-products including 1,2-DCP, and 1,2,3-trichloropropane. 1,2-DCP is separated by distillation from the reaction mass and then further purified by distillation (in three distillation columns). The pure 1,2-DCP is sent to storage facilities via dedicated pipelines. All the manufacturing stages are performed in closed systems.

1,2-DCP was historically used as a soil fumigant, chemical intermediate, as well as an industrial solvent. It was found in paint strippers, varnishes, and furniture finish removers.

Some of these uses have been discontinued in the EU: no longer used as a soil fumigant since 2003. Currently its main use is as an intermediate in the manufacture of perchloroethylene and other chlorinated chemicals.

The registration data indicates that approximately 10,000 tonnes/year are manufactured in the EU. Most of the manufactured tonnage (about 80% w/w) is exported outside the EU, where it is used as an intermediate in the manufacturing of many other compounds. The remainder of the produced volume (about 20% w/w) is transported to EU formulation sites by truck-tanks, where it is mixed with other components to produce mainly industrial and professional preparations to be used (i) as degreasers/ cleaning products, (ii) solvents/thinners for painting products/inks and, (iii) to a lesser extent, solvents for glues and adhesives, stain removers for fabric and paint removers. The content of 1,2-DCP can vary in the final product, from about 1-2% w/w, when it is used as denaturing additive for other solvents (e.g. alcohols, white spirit), to about 90% w/w, when its main function is as solvent (e.g. in paint removers). The typical concentration in the formulated products is about 40% w/w.

There are five registrations for the substance under REACH:

- two are submitted under Article 18 (transported isolated intermediates used under strictly controlled conditions; indicative of rigorously contained conditions, by technical means during the whole lifecycle);
- one registration concerns the monomer of an imported polymer (so no exposure from its direct use in the EU).
- two are submitted under Article 10 (full registrations): while the manufacturing and industrial formulations steps are in closed or mainly closed systems (opened for sampling etc.), the use of the mixtures is not closed. Industrial and professional workers use the mixture in activities where there is significant potential for exposure (spraying, dipping and pouring, roller application or brushing, and hand-mixing with intimate contact and only personal protective equipment available).
  One of these Article 10 registrations also describes consumer uses as degreasers, solvent/thinner for painting products, in glues and adhesives, as stain removers for fabric, and paint removers.
  Note: as the substance has a carcinogenicity category 1B harmonised classification under CLP, its use must be restricted in consumers products, when its concentration in the product is equal to or greater than 0.1% w/w (Entry 28, Annex XVII of the REACH Regulation)[13].

5.3 Occupational exposure

Occupational exposure to 1,2-DCP may occur during its production, its use in chemical reactions, its use as an industrial solvent, or the disposal of processing wastes. Workers involved in cleaning hazardous waste or spill sites that contain 1,2-DCP may potentially be exposed.

(IARC, 2017) reports some studies from Italy:

- From 8 car-paintings workshops, only one reported measurements of 1,2-DCP above the level of detection, and personal and stationary measurements of 5.3 mg/m^3 were recorded during 5.5 hours of monitoring;
- In another study, measurements of 1,2-DCP in the breathing zone and the urine were reported for workers in plastic-product, paint-, and chemical-manufacturing industries, with most of the air concentrations being between 10 and 150 mg/m^3 (two were > 400 mg/m^3). Urinary concentrations (in µg/L) correlated very closely with the air concentrations.

For the registered uses, registrants have modelled the exposure based on ECETOC TRA v 3.1: for the intermediate uses the exposure estimates are very low; for the industrial workers the exposures are relatively well controlled (due to mainly closed systems).

[13] https://echa.europa.eu/documents/10162/0645e093-576f-c279-ceb9-4f2d1ec3e3bd

However, for the professional workers the formulation step and mixture uses are not so well-controlled (as expected). The spraying activity in particular by professional workers indicated a relatively high level of exposure in the registrants' chemical safety reports (CSRs). The CSRs are confidential but the indicated exposure levels are in the same range of levels indicated for the Italian car-painting workshops mentioned above.

5.4 Routes of exposure and uptake

5.4.1 Worker exposure

Occupational exposure is primarily by inhalation (main intake pathway is via the respiratory tract), and also dermal contact where the substance is manufactured or used. For industrial workers, exposure should be limited because the substance is mainly used in closed systems, whereas professional workers (and consumers) who use mixtures during manual tasks face a significant potential for exposure.

5.4.2 General population

Most of the 1,2-DCP released into the environment ends up in the air or groundwater. The greatest potential for the general population to be exposed to 1,2-DCP is through inhalation of contaminated ambient air and consumption of contaminated drinking water. The general population may also be exposed while using consumer products containing 1,2-DCP via dermal contact.

People living in the vicinity of landfills containing 1,2-DCP and hazardous waste sites may be exposed to 1,2-DCP present in off-gases. Not enough information is available to estimate what the level of exposure from this source might be.

Very little information is available on exposure of the general population to 1,2-DCP, anywhere in the world. In the United States National Health and Nutrition Examination Survey (NHANES) in 2003–2004, 1,2-DCP was not detected in any of 1364 blood samples.

One REACH registration covers also consumer uses. The modelled exposure estimates indicate that the risks are high for all uses, and especially for use as paint removers (where the risk characterisation ration (RCR) in the CSR is almost 1.0). The potential for exposure is similar to professional workers, with the manual tasks involved, but consumers are not expected to have any risk management measures in place.

6. Monitoring Exposure

6.1 External exposure

We found one official validated method (NIOSH, 1994). However, the limit of quantification indicated in the report may not be low enough for the OEL derived later. Thus, also peer review articles have been considered to assess the possibilities of measuring low concentrations of 1,2-DCP in workplaces.

The principle of the methods is as follows: air sampling is performed by passing air actively through a sorbent tube or by using diffusive sampling with a sorbent tube. The retained 1,2-DCP is then extracted for analysis by either thermal desorption or desorption on CS2 (depending on the sorbent tube used) followed by analysis via gas chromatography with different detectors. Table 6 shows some of the available methods for measurement of 1,2-DCP in air, in the range of µg/m^3/ppb.

Table 6: Methods for measurement of 1,2-DCP in air

Sampling methods/desorption	Analytical technique	LOQ, flowrate, sampling volume and time	Comments	Reference
Petroleum charcoal (active) acetone/cyclohexane desorption	GC with Hall electrolytic conductivity detector	0.05 ppm (0.25 mg/m^3) Flow rate: 0.01 to 0.2 l/min 2L (10 min to 3 hours)	Breakthrough volume at lower concentrations for the recommended flowrate range is 20L Sampling volume could be increased to ≈13L LOQ≈0.04 mg/m^3	NIOSH 1013 (NIOSH, 1994)
Tenax TA tubes (passive) Thermal desorption	GC/MS	0.001 mg/m^3 Passive sampling		(Jia and Fu, 2017)

6.2 Biomonitoring of exposure (internal exposure)

Up to now, available parameters for a biomonitoring of exposure to 1,2-DCP are urinary levels of 1,2-DCP levels or of N-Acetyl-S-(2-hydroxypropyl)-L-cysteine (2-HPMA). No relevant human data on internal exposure are currently available to indicate that other metabolites can be measured in urine.

2-HPMA is a non-specific parameter for 1,2-DCP exposure, since it is also a metabolite of several other compounds such as 1,2-epoxypropane (propylene oxide), propylene, and other halogenated propanes (Eckert et al., 2021).

6.2.1 Background levels

1,2-Dichloropropane is not known to occur naturally (IARC, 2017). The main sources of exposure for the general population are through inhalation of contaminated ambient air and consumption of contaminated drinking water. The general population may also be exposed while using consumer products containing 1,2-DCP via dermal contact.

Background concentrations of 1,2-DCP biomarkers have been reported in different publications.

(Bader et al., 2016) reports background concentrations of 2-HPMA in urine in the general population. Based on studies from (Schettgen et al., 2008) and (Eckert et al., 2011), (Bader et al., 2016) reports a biological reference value (BAR) of 25 µg 2-HPMA/g for 1,2-epoxypropane (as explained above 2-HPMA in urine is not a specific indicator for 1,2-DCP). However, (Eckert et al., 2021) did not establish a BAR for 1,2-DCP based on these studies because there are no human studies available that would show clear evidence that 1,2-DCP is metabolised to 2-HPMA in human.

(Kawai et al., 2015) and (Park et al., 2020) used un-metabolised 1,2-DCP as a biomarker of exposure.

Kawai et al. (2015) included 5 non-exposed male controls. 1,2-DCP levels was below the limit of quantification for the controls. The limit of quantification was 10 µg/L urine.

(Park et al., 2020), also included non-exposed workers (office workers) in the study. The result for non-exposed workers was also below the limit of detection (0.3 µg/L urine).

6.2.2 Occupational exposure

Metabolites of 1,2-DCP have not been determined in human urine of occupationally exposed individuals (Eckert et al., 2021).

(Kawai et al., 2015) studied the correlation between air and urine concentrations of 1,2-DCP in the printing industry. Urine samples were taken after the end of the shift. The study

showed a high correlation between the internal and external exposures and low background (close to zero). Three correlations were calculated: no correction, creatinine correction and correction for urine specific density. The correlation without corrections showed the highest correlation. The authors found conceivable that this was due to the mechanism of transfer of un-metabolised 1,2-DCP into urine. The mechanism is thought to be simple diffusion with no relation to creatinine metabolism or metabolism of specific gravity-affecting substances in urine.

The study by (Park et al., 2020) covered several processes in printing and manufacturing industry where urine samples were taken at the end of the work shifts. In agreement with the findings of (Kawai et al., 2015), this study also showed that a positive correlation and a dose-response relationship exists between the 1,2-DCP concentration in air and urine and a higher correlation for concentration of 1,2-DCP in urine. In this study, the period of urine sample analysis was divided into "within 2 weeks" and "after 4 weeks": the results of analysis within 2 weeks showed that the explanatory power for exposure-urinary concentration was significantly high.

Table 7 shows the correlations between concentrations in air and urine, reported for uncorrected 1,2-DCP.

Table 7: Overview of the correlations for uncorrected 1,2-DCP in urine

Reference	N	Air (ppm)	Urine (µg/L)	Regression line parameters		
				Intercept	Slope	Corr. Coef.
(Kawai et al., 2015)	33	GM=7.1 GSD= 2.44	GM= 77 GSD=1.90	7.568	9.022	0.909
(Park et al., 2020)	29 (All) 16*	GM=17.44 GSD=3.92	GM= 231 GSD=4.05	80.065 −40.400	13.672 22.300	0.517 0.801

* Samples analysed within two weeks of sampling

One of the main limitations of the studies is the lack of human data on the elimination of 1,2-DCP. The substance half-life in humans has not been tested.

Based on animal studies, the half-life of the substance is estimated to be around 3 hours.

6.2.3 Biomonitoring analytical methods

Table 8 gives an overview of the methods available to measure the urinary biomarkers for 1,2 DCP, detailed in the sections above.

Table 8: Analytical methods for different biomarkers of 1,2 DCP

Method/reference	Biomarker (in urine)	Analytical technique	LOQ
(Schettgen et al., 2008)	2-HPMA	HPLC-MS/MS High performance liquid chromatography (HPLC) and detected using tandem mass spectrometry.	1.0 µg 2-HPMA per litre urine
(Kawai et al., 2015)	1,2-DCP	HS-GS-FID Head space/ Gas chromatography	10 µg/L urine
(Park et al., 2020)	1,2-DCP	GC/MS Gas chromatography /mass spectrometry.	1 µg/L urine

7. Health Effects

7.1 Toxicokinetics (Absorption, distribution, metabolism and excretion - ADME)

7.1.1 Human data

The main routes of exposure to 1,2-DCP at the workplace are the respiratory tract and the skin. Systemic toxic effects after ingestion in humans show that absorption of 1,2-DCP also occurs through the gastrointestinal tract (IARC, 2017). Systemic toxicity in a human case report following prolonged dermal exposure (~5 hours) to a commercial fixative containing 30–40% 1,2-DCP and 33–38% toluene (Fiaccadori et al. 2003) may also be attributable, at least partly to dermal absorption of 1,2-DCP. A human skin permeability constant of 0.01 cm/hour and a permeability coefficient of 0.206 cm/hour were calculated by EPA (1992). Additionally, Fiserova-Bergerova et al. (1990) estimated that 1,2-DCP had a significant dermal absorption potential based on a dermal penetration rate (flux) predicted from physical properties (as reported in (ATSDR, 2021)).

In 22 workers exposed to 1,2-DCP in the plastics and paint industries or in shoe factories, the air concentration and urine content of 1,2-DCP were in correlation (Ghittori et al., 1987).

Also, exposure to 7.1 ml of 1,2-DCP/m^3 (time-weighted average value – TWA, geometric mean, maximum 23.1 ml/m^3) led to a mean urine content of 77 µg 1,2-DCP/l of urine in 30 exposed printing workers. No 1,2-DCP was found in the urine of 5 unexposed control persons (Kawai et al., 2015).

7.1.2 Animal data

7.1.2.1 Absorption

Several studies assessed the toxicokinetics of 1,2-DCP in animals exposed orally or via inhalation concluding that the substance is readily absorbed from the lungs and from the gastrointestinal tract.

In an OECD compliant study, four F344 rats per group per sex were dosed orally with single dose of 1 or 100 mg/kg bw of radiolabelled 1,2-DCP followed by 1 mg/kg bw of non-radiolabelled substance for 7 days, and by a final radiolabelled dose of 1 mg/kg bw on day 8. Within 24h of dosing, an average of 74–95% of the [^{14}C]-1,2-DCP dose was excreted in the urine or in expired air. The radioactive 1,2-DCP was still detected in the body 48 h after oral administration (Timchalk et al., 1991, OECD SIDS, 2005, IARC, 2017).

Thirty-six F344/DuCrlCrlj male rats per group were exposed by gavage to 62 or 125 mg/kg bw of 1,2-DCP in corn oil. In both groups, the concentration in blood, lungs, liver, kidneys and abdominal fat increased until 1h after dosing, to gradually decrease thereafter, in a time-related manner. At the high dose, the concentration in blood reached its maximum 3 h after dosing, with 1,2-DCP remaining in blood and tissues for a prolonged period post administration (still detectable 24 h after oral dosing) (Take et al., 2017, Hartwig and MAK Commission, 2021).

Forty-two male F344/DuCrlCrlj rats were exposed via inhalation (whole body) to 80 or 500 ppm (ml/m^3) for 6h to 1,2-DCP (purity 99.5%). Blood samples and necropsy were scheduled at 0, 1, 3 or 6 h during the inhalation exposure and at 1, 3 or 18 h after the end of exposure. Blood concentrations in both groups increased in a time-related manner after the start of inhalation, indicating that steady-state was not reached, with concentrations being dictated by the blood-to-gas partition coefficient. At the end of exposure, concentrations in the blood decreased in a time-dependent fashion. The area under the curve ($AUC_{0\text{-}19h}$) in blood and tissues exposed to 500 ppm was at least 13 times higher than in the 80-ppm group (Take et al., 2014).

No data on dermal adsorption are available as such, but skin absorption can be inferred based on the systemic toxicity reported in a 1969 dermal study in rabbits (IARC, 2017).

7.1.2.2 Distribution

In their gavage study, (Take et al., 2017) measured the time-course changes in tissue concentrations of 1,2-DCP in F344/DuCrlCrlj male rats in blood, liver, kidneys, lung and abdominal fat. In all organs or tissues, 1,2-DCP concentrations peaked at 60 minutes after exposure and slowly decreased thereafter. In the lower dose group 1,2-DCP was still detectable in the liver, kidney and lung after at 9h and in the abdominal fat up to 24h after dosing, while in the 125 mg/kg bw dose group, 1,2-DCP was detectable in all tissues examined after 24h. At each time point, the concentration in the abdominal fat was greater than in the other organs or tissues in accordance with the lipophilic nature of the substance. The half-lives ($t_{1/2}$) were 193 and 315 minutes in the blood, 144 and 187 minutes in the liver, 144 and 193 minutes in the kidneys, 114 and 165 minutes in the lungs, and 257 and 330 minutes in the abdominal fat, in the 62 and 125 mg/kg bw dose groups, respectively. After 2 days, small amount of 1,2-DCP were found in the blood and all other examined tissues in the high dose group, suggesting that DCP remains in the tissues for a prolonged period of time after administration (Take et al., 2017, Hartwig and MAK Commission, 2021). In the ^{14}C-labeled 1,2-DCP Timchalk oral study the distribution of radioactivity in the tissues of rats was similar following inhalation and oral exposure with the exception of the lungs (low radioactivity after oral exposure). Male Wistar rats (n=5/dose) were dosed with 55 or 110 mg/kg bw 1,2-DCP orally. 1,2-DCP blood levels reached C_{max} after 30 minutes, with $t_{1/2}$ of 3.1 and 5.0 hours, for the two dose groups, respectively. When administered with 120 or 440 mg/kg bw, the maximum blood level was reached later (1 to 2 hours), and the half-lives were 4.3 and 13.6 hours, respectively (Di Nucci et al. 1988; Greim 1993, as reported in (Hartwig and MAK Commission, 2021)).

In their inhalation study, (Take et al., 2014) measured the time-course changes in tissue concentrations of 1,2-DCP in F344/DuCrlCrlj male rats in blood, liver, kidneys, lung and abdominal fat. The $t_{1/2}$ in the 80 and 500 ppm groups were 182 and 168 minutes in the blood, 39 and 61 minutes in the lungs, 57 and 125 minutes in the liver, 59 and 127 minutes in the kidneys and 154 and 186 minutes in abdominal fat, respectively, where accumulation also occurred. In the 80-ppm exposure group, C_{max} in the lungs, liver and kidneys was reached after 1 hour and remained constant until the end of the exposure period (6h). In the abdominal fat, the 1,2-DCP concentrations increased in a time-related manner throughout the exposure period and were higher than in the other tissues, at each time pint. The authors postulated that high concentration in the abdominal fat is due to the high lipid solubility of 1,2-DCP. After exposure to the high dose, steady state was not reached in any tissue, therefore the metabolic saturation point was exceeded at 500 ppm. The authors were able to measure 1,2-DCP concentration in blood up to 18h after dosing in both groups, while 1,2-DCP was present in all examined tissues of high dosed rats (Take et al., 2014, Hartwig and MAK Commission, 2021).

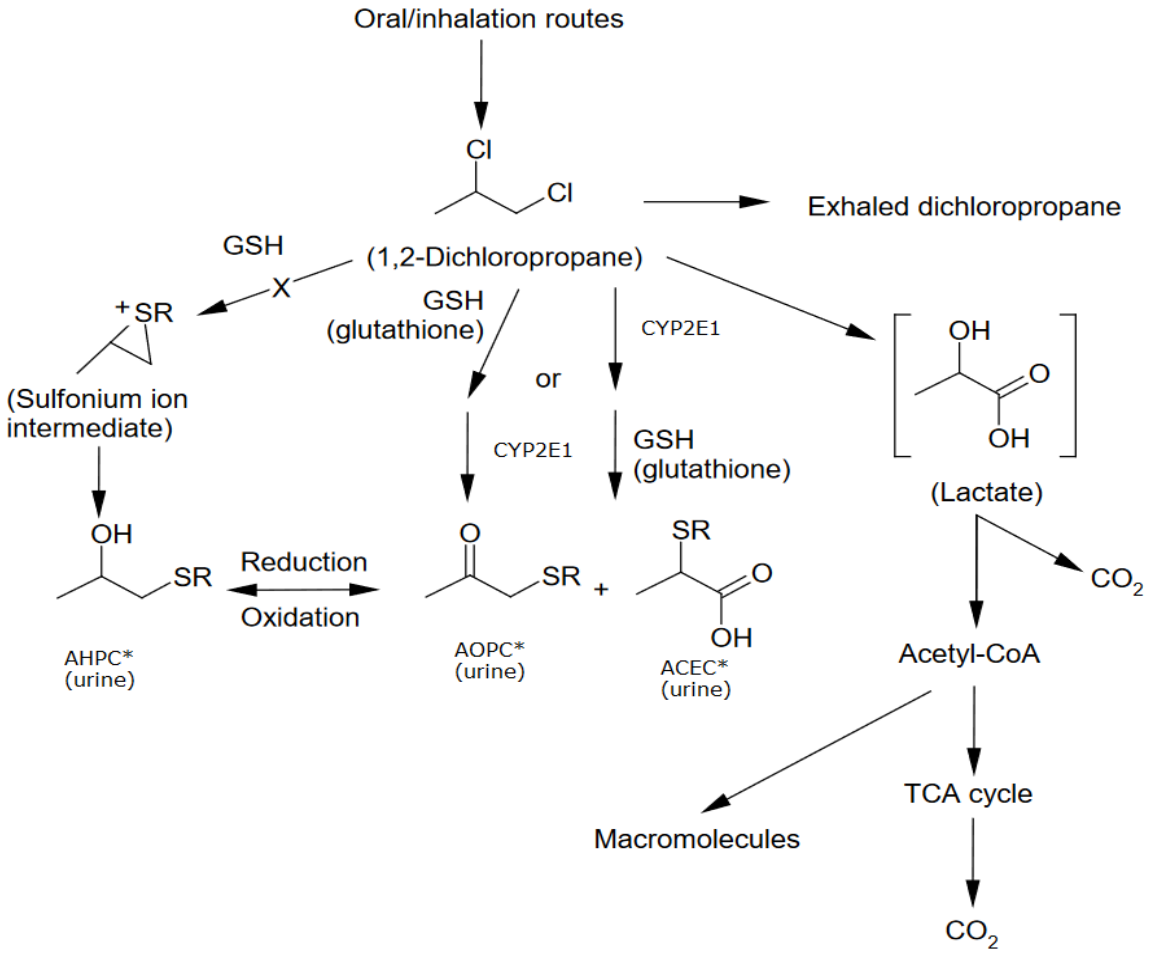
7.1.2.3 Metabolism

1,2-DCP is metabolised primarily in the liver via two main mechanisms: CYP450-mediated oxidation and glutathione conjugation by glutathione S-transferase (GST) T1-1 as first step. The proposed metabolic pathway, based on rat studies is shown in Figure 1.

The major urinary metabolite in Sprague-Dawley and F344 rats dosed with 100 mg of 1,2-DCP orally was identified as *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine (AHPC) (25-30% and 10.2% in Sprague-Dawley and F344 rats, respectively). In F344 rats, two other metabolites were characterised in the urine: *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine (AOPC, 14.5%) and *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine (ACEC, 1.8%). AHPC and AOPC can interconvert via a redox reaction (Greim, 1993 as described in (Hartwig and MAK Commission, 2021)).

Bartels and Timchalk demonstrated that the main metabolisation involves the CYP2E1-GST pathway (GST glutathione-S-transferase) (Bartels and Timchalk, 1990 as described in (Hartwig and MAK Commission, 2021)).

A single dose of 500 mg/kg bw 1,2-DCP in olive oil was administered orally to 57BL/6J mice (number not specified), FVB/NJcJ (number not specified) and SD rats (n=9). Four hours after dosing, 1,2-DCP was not detected in the bile, where 9 metabolites were identified (Toyoda et al. 2016 as reported in (Hartwig and MAK Commission, 2021)).



GSH: glutathione; R: *N*-acetylcysteine; *: detected *in vivo*; AHPC: *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; AOPC: *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine; ACEC: *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine; TCA cycle: tricarboxylic acid cycle

Figure 1: Proposed metabolic pathways for 1,2-DCP in the rat (adapted from (ATSDR, 2021, Hartwig and MAK Commission, 2021)

7.1.2.4 Excretion

Take et al. determined that 1,2-DCP excretion took place via the urine or by exhalation in rat exposed by inhalation (Take et al., 2014, Hartwig and MAK Commission, 2021).

In an OECD compliant study, four F344 rats per sex were exposed orally to radiolabelled 1,2-DCP vapours for 6 h with target concentrations of 5, 50 and 100 ppm:

- within the first 24h, 71-88% of the radiolabel was recovered from the fecies, with 55-65% in the urine and 13-23% in the expired air;
- within 48h, excretion via the faeces was about 10% (Timchalk et al., 1991, OECD SIDS, 2005, IARC, 2017).

Male Wistar rats (n=5/dose) were dosed with of 55 or 110 mg/kg bw 1,2-DCP orally. 1,2-DCP was excreted in the urine (>50%) as a glutathione conjugate. After administration of radiolabelled 1,2-DCP, the radioactivity in the expired air was identified as carbon dioxide (~20%) and 1,2-DCP (~20%), and additional 5-8% radioactivity was recovered from the faeces (Di Nucci et al. 1988; Greim 1993, as reported in (Hartwig and MAK Commission, 2021)).

7.1.3 *In vitro* data

Guengerich et al. tested the metabolisation of 1,2-DCP *in vitro* using human CYP2E1 and GHS and found a rate of conversion to AOPC of 1.1 nmol/min/nmol CYP2E1. The conversion rate decreased if CYP2E1 inhibitors were added to the system (Guengerich et al., 1991, as reported in (Hartwig and MAK Commission, 2021)).

The rate of 1,2-DCP metabolism was also tested in an *in vitro* reaction system containing mice liver homogenate from wild type and cyp2e1-null mice. The rates of conversion were 21.86 and 0.22 nmol/min/mg protein, from the two strains, respectively, suggesting that the first step in the metabolism of 1,2-DCP is exclusively catalysed by CYP2E1 in mouse liver. The results correlated with slower elimination of 1,2-DCP from the blood of CYP2E1-deficient mice after a single i.p. injection of 300 mg/kg bw 1,2-DCP, even if the difference was not statistically significant with the wild type mice (Yanagiba et al. 2016, as reported in (Hartwig and MAK Commission, 2021)).

7.1.4 Summary

A limited number of studies have assessed the toxicokinetic of 1,2-DCP in exposed animals. The main findings are summarised below.

- Rapid and near complete adsorption after oral or inhalation exposure in rats. No data are available for the dermal routes. However, based on the adverse effects observed in a dermal study, the adsorption is assumed.
- Once absorbed, 1,2-DCP is distributed to the whole body.
- 1,2-DCP is rapidly and extensively metabolised plausibly via CYP2E1 oxidation and glutathione conjugation. It was postulated it may also conjugate with lactate yielding acetyl CoA and carbon dioxide.
- 1,2-DCP and its metabolites are excreted via urine (37-65%), exhaled air (18-40%), extensively within 1 day from a single exposure. After oral exposure, a small amount was detected in the faeces.

7.2 Acute toxicity

7.2.1 Human data

7.2.1.1 Acute oral toxicity

Larcan et al. (1977) described a fatal acute poisoning in a 46-year-old man who had ingested 50 ml of a cleansing substance that was identified as containing 1,2-DCP. Deep coma with mydriasis and hypertension developed within two hours. Recovery from consciousness occurred after 24 hours of artificial hyperventilation and osmotic diuresis. However, at 36 hours from the ingestion, acute delirium with tremor developed and the man died with a picture of irreversible shock with cardiac failure, lactic acidosis and hepatic cytolysis.

Pozzi et al. (1985) described fatal acute poisoning in a 28-year-old Italian man who had accidentally ingested of a stain remover with a high concentration of 1,2-DCP (the concentration of 1,2-DCP in this type of commercial products, Trielina, was reported to be 70-98%). On hospital admission a few hours after the ingestion, haematocrit, renal and liver function, and coagulation test results were normal. Two days later he developed renal failure and liver damage. Slight disseminated intravascular coagulation was also found.

Forced diuresis was carried out and renal function improved but liver damage persisted and haemolytic anaemia. On the seventh day the patient died of septic shock. The ingested amount was not estimated.

Di Nucci et al. (1990) described a fatal acute poisoning of a 71-year-old man who had ingested 180 ml of a dry-cleaning product containing 90% 1,2-DCP and 10% 1,1,1-trichloroethane. Eight hours after ingestion, liver dysfunction appeared. The man was comatose and developed progressively acute liver and kidney failure, severe blood coagulation disorders, metabolic acidosis, disseminated intravascular coagulation, shock and myocardial failure and died 48 hours after admission.

ATSDR (2021) referred to other case reports of acute poisoning following ingestion of 1,2-DCP published in languages other than English ((Chiappino and Secchi, 1968), (Perbellini et al., 1985))

Hartwig and MAK Commission (2021) referred to a study reporting a case series of 56 Italian patients with effects on the central nervous system, liver, kidney and heart following oral ingestion of an unknown amount of the cleaning agent Trielina containing varying amounts of trichloroethane and 1,2-DCP (Imberti et al., 1987). Mortality was 6%.

7.2.1.2 Acute dermal toxicity

Fiaccadori et al. (2003) described a 46-year-old Italian man who developed oliguric acute, renal failure, acute hepatocellular necrosis, rhabdomyolysis and severe disseminated intravascular coagulation shortly after he had been painting for 6 h outdoors with a commercial paint fixative containing 1,2-DCP (35–40%) and toluene (33–38%). The chemical accidentally spilled from its container onto his upper trunk and abdomen. He waited 5 hours before removing his clothes and washing himself and reported only transient skin reddening on the cutaneous areas involved. Because the man had been working outdoors in a well-ventilated environment, without reporting signs or symptoms of upper respiratory tract irritation during or immediately after painting, the authors suspected that the poisoning was due to the extensive and prolonged skin contact rather than via inhalation exposure. The amount of 1,2-DCP absorbed was not estimated. The renal and hepatic function recovered in two weeks.

7.2.1.3 Acute inhalation toxicity

Conner et al. (1962) reported an incident of release of 3000 gallons of DOW 421 (4 parts o-dichlorobenzene: 2 parts propylene dichloride:1 part ethylene dichloride) in a US railway tank car accident resulting in an explosion. Forty-five men were exposed in a narrow courtyard. In addition to three fatalities in the blast, four other men died within 24 hours in hospital due to injuries or pulmonary disorders. Altogether 39 firemen or policemen were hospitalised due to possible pulmonary, ocular, or cutaneous injuries or with acute psychiatric problems. Six of them developed severe respiratory tract injuries (destruction of upper or lower respiratory tract epithelium) and three of them died. Air concentrations of 1,2-DCP or other chemicals were not measured.

Rubin (1988) described respiratory effects in humans resulting from exposure to an accidental spill of 2,000 gallons of 1,2-DCP from a truck in the US. The exposure resulted in chest discomfort, dyspnoea, and cough in some of the patients, indicating that 1,2-DCP is a respiratory tract irritant. Altogether, 129 persons were treated at a nearby emergency department, 15 of these being admitted to hospital. The persons exposed included truck drivers, highway patrol officers, firefighters, and a number of hospital employees who were secondarily exposed as a result of contact with the victims' contaminated clothing. Air concentrations of 1,2-DCP were not measured.

(Pozzi et al., 1985) described a 20-year-old Italian girl who developed renal failure, acute liver damage, haemolytic anaemia and disseminated intravascular coagulation after sniffing of a stain remover with 70-98% of 1,2-DCP. Renal biopsy findings were consistent with acute tubular necrosis. Three weeks after hospital admission the patient was

discharged with complete recovery of her renal and liver function and normal. The girl had a one-month episode of sniffing the same product 8 months earlier and was then hospitalized due to vomiting, abdominal pain, widespread ecchymoses, haematuria, and metrorrhagia. However, the above-described second episode occurred when she restarted sniffing and repeated the operation four times during the course of one night: the symptoms appeared the next morning. No inhalation exposure estimates were presented.

(Pozzi et al., 1985) also described a 55-year-old Italian woman suffering from membranoproliferative glomerulonephritis and being on home haemodialysis treatment. She developed severe liver failure, haemolytic anaemia and slight disseminated intravascular after having spent six hours cleaning her flat using two litres of solvent, during which time she noticed no untoward effects, only a nasty smell. The solvent apparently contained 70-98% of 1,2-DCP. During the following three days she developed anorexia, abdominal pain, and nocturnal sweating, and was admitted to hospital. No inhalation exposure estimates were presented.

In another case report, abdominal pain and vomiting were observed in a 73-year-old woman who after cleaning some garment fell asleep for 2 hours in close proximity to a glass filled with a stain remover containing 1,2-DCP and developed an acute liver disease and haemolytic anaemia (Lucantoni et al., 1992). The exposure intensity was not further quantified.

Kubo et al. (2015) described a severe acute hepatitis developed in a male worker in an offset colour proof-printing department in Japan. The worker was exposed to various solvents, including 1,2-DCP, dichloromethane (DCM), and 1,1,1-trichloroethane (TCE). His serum AST, ALT and LDH levels were remarkably elevated at the time of admission to the hospital and improved rapidly after admission (stopping exposure) and treatment. He did not have any known cause of severe acute hepatitis, such as viral hepatitis, autoimmune hepatitis, alcoholic liver disease, viral infection, or biliary tract disease and the histology of the liver biopsy was not compatible with such hepatitis but rather by that caused by exposure to chlorinated organic solvents. The liver function recovered within a few weeks after cease of exposure. When preparing printing plates, the worker used high-purity TCE to remove stains from glass plates for about one year just before developing severe acute hepatitis. The amount of TCE he used per day was estimated to be 1-2 litre. No respiratory protection was used. In addition to TCE, the worker was exposed during the past 5 months to pure 1,2-DCP (98%) used by proof-printing workers and before that to a mixture of DCP (40−50%), DCM (40−50%) and petroleum hydrocarbons (1−10%). No industrial hygiene measurement results were reported.

Kwak et al. (2018) described a 41-year-old male worker of an automotive accessory manufacturing plant in Korea. The worker visited hospital with dizziness, headache, severe nausea, vomiting, diplopia, dizziness, and ataxia. Brain magnetic resonance imaging revealed bilateral abnormal findings in the thalami and after excluding other types of encephalopathy the authors concluded that the worker suffered from an acute toxic encephalopathy induced by exposure to 1,2-DCP. Before the symptoms, the worker was exposed over the course of 7 working days while removing rust from inside cleaning trays of an ultra sonicator that used 1,2-DCP as a detergent to clean automotive parts. He had already been using 1,2-DCP as a detergent for 5 months before the acute symptoms and before that methylene chloride had been used. During the first 4 months, cleaning was, however, performed in a closed environment without problems but then rust began to appear daily, and the worker started to open the door of the ultrasonic cleaning machine and removed the rust without using protective equipment. Industrial hygiene measurements for 1,2-DCP were performed only retrospectively: one month after the acute symptoms, the 8-hour TWA was 8.4 ppm and three months after 8-hour TWA was 27 and 42 ppm and short-term exposure was 50 and 77 ppm when re-enacting rust removal over 15 minutes. After recovery, the man returned to work and was re-assigned to a job where he was no longer exposed to detergents or organic solvents, and the central nervous symptoms (CNS) symptoms did not recur.

7.2.2 Animal data

7.2.2.1 Acute oral toxicity

1,2-DCP showed low oral toxicity: the reported LD_{50} values are between 487 and 2200 mg/kg bw for rats, 860 to 1000 mg/kg bw for mice, and between 2000 and 4000 for guinea pigs (WHO, 2003, Fan and Alexeeff, 1999, OECD SIDS, 2005, ATSDR, 2021).

Rats (5/sex/dose) were exposed via gavage to pure 1,2-DCP at doses of 1470, 2150, 3160, 4680, 6810, or 10000 mg/kg bw. Mortalities were observed in all groups (2, 3, 8, 10, 10, 10, 10, respectively) and occurred mainly within 24 h. The authors derived the following LD_{50}: 1100, 1800 and 1600 mg/kg bw for male, female and combined, respectively (EPA, 2016).

Wistar rats (6, sex, group) were exposed via gavage to undiluted 1,2- DCP at doses of 145, 230, 366, 582, 926, or 1472 mg/kg bw. Mortalities were recorded from 582 mg/kg bw (0, 0, 0, 8, 8, 12, respectively), consequently an LD50 of 487 mg/kg bw was derived (EPA, 2016).

Male ddY mice were dosed with 1,2-DCP in olive oil and an LD_{50} of 960 mg/kg bw was determined. No details on the number of animal per dose or the doses are available (EPA, 2016).

In an old study, as reported in (Fan and Alexeeff, 1999), no deaths were observed after dosing dogs with a single 1,2-DCP oral dose (230-5800 mg/kg bw). However, the adverse effects were observed on the CNS (marked incoordination, loss of balance, unsteady gait), in the liver (congestion, haemorrhage, cloudy swelling, fatty and parenchymatous degeneration), and in the kidneys (congestion of the cortex, fatty infiltration, gross discoloration).

7.2.2.2 Acute dermal toxicity

In rabbits, an LD_{50} value of 10100 mg/kg bw (24 h, occluded, ~8.75 mL/kg bw, ca 10.2 g/kg bw) was reported (OECD SIDS, 2005), with the animals being followed up for 14 days. No additional information is available on the studies.

Wistar rats (6/sex/group) were dosed with 2340 mg/kg bw 1,2-DCP undiluted, under occlusive condition for 24 h. No deaths were recorded up to 14 days after exposure, thus the LC_{50} was estimated at >2340 mg/kg bw (EPA, 2016).

7.2.2.3 Acute inhalation toxicity

The LC_{50} for rats after acute inhalation for 8 h was 2000 mg/L 1,2-DCP. In addition, CNS depression and irritation of eyes and respiratory tract were reported (Fan and Alexeeff, 1999). In another review (OECD SIDS, 2005), rats LC_{50} values for 4 h were reported as 2000 ppm and 9.4 mg/L, and as >2200 ppm (or >10.3 mg/L) for a 7 h exposure (OECD SIDS, 2005).

In another study, all rats exposed for 4 h to 1000 ppm 1,2-DCP died (ATSDR, 2021).

Mice (10-30/group) were exposed for 10 h to concentration of 300, 380, 390, 700, 715, or 1625 ppm 1,2-DCP. Recorded mortalities were: 2/10, 11/20, 7/10, 30/30, and 10/10, thus an LC_{50} of 480 ppm (or 1850 mg/m^3) was calculated (Fan and Alexeeff, 1999, ATSDR, 2021, EPA, 2016).

In mice (no other information available), a 10 h inhalation LC_{50} value of 480 ppm was reported; all mice (22–26 animals) died after a single exposure of 4 h to 1000 or 1500 ppm of 1,2-DCP, while 3/10 mice died after a single 2 h exposure to 1500 ppm (Dow Chemical Co. 1968, as reported in (ATSDR, 2021)).

All mice died after a 7 h exposure to ≥1000 ppm (Heppel et al. (1946), as reported in (ATSDR, 2021)). In another study, 100% mortality was observed in mice within 24 h of a

6 h exposure to 1500 ppm; at 500 ppm the mice became lethargic and 2/5 mice died within 3 days of exposure (Nitschke and Johnson 1983, as reported in (ATSDR, 2021)).

Groups of rats, guinea pigs, and rabbits (12, 6 or 2/group, respectively) were exposed to 0 or 1600 ppm (7400 mg/m^3) for 7 h. In rats, incoordination at the end of the exposure period and 3 deaths were reported; no toxicity or death were observed in guinea pigs and rabbit (EPA, 2016).

7.2.3 Summary

Acute poisonings have been observed after oral, dermal and inhalation exposure to 1,2-DCP in occupational, accidental and domestic exposures to 1,2-DCP containing solvents and also after sniffing such solvents. In some cases, concomitant exposure to other solvents occurred. The toxic effects have included liver and kidney damage, intravascular coagulation, haemolytic anaemia and various central nervous system symptoms. Fatal cases have been described following oral and inhalation exposure. In nearly all cases, the air concentrations and oral or dermal doses are poorly characterised. In one occupational non-fatal case retrospective industrial hygiene measurements indicated 8-hour TWA levels of 8-42 ppm and 15-minute levels of 50-77 ppm, based on three and two measurements, respectively.

All available acute toxicity animal studies on 1,2-DCP pre-date modern guidelines and GLP. Nonetheless they indicate a low acute toxicity on all routes of exposure.

7.3 Specific target organ toxicity/Repeated dose toxicity

7.3.1 Human data

(Kwak et al., 2018) described a case of acute toxic encephalopathy. The worker has also earlier longer-term exposure to 1,2-DCP but as the disease seemed to be linked to an acute high exposure it is described in section 7.2.1.3.

7.3.2 Animal data

7.3.2.1 Oral

Male B6C3F1 mice were dosed with 1,2-DCP via gavage at 0 or 500 mg/kg bw/d for 3 days or at 0, 125 or 250 mg/kg bw/d in corn oil for four weeks. In the 3 days experiment, one mouse died before receiving the 3rd dose, food consumption and body weight were decreased for the treated animals. Extensive centrilobular hepatocellular necrosis and mild liver fatty change were observed in all animals (including the deceased one). In the 4-week experiment, a dose-dependent, significant increase of absolute and relative liver weights was observed in the dosed animals. Histopathology revealed mild fatty change in the liver of all treated animals, but no necrosis. Significant increase of total cholesterol, glycerol and albumin was reported at the high dose. The authors measured the following significant changes in mRNA: increase of CYP2A4, CYP4A14 (≥125 mg/kg bw/d) and CYP1A1 (250 mg/kg bw/d), decrease of CYP2C9, CYP3CA11 and GST-T1 (≥125 mg/kg bw/d) (Gi et al., 2015a, EPA, 2016, ATSDR, 2021).

Male Syrian hamsters (5/group) dosed with 1,2-DCP via gavage at 0 or 500 mg/kg bw/d for 3 days or at 0, 125 or 250 mg/kg bw/d in corn oil for four weeks. The 500 mg/kg bw/d was reduced to 250 mg/kg bw/d after the death of one animal, and the morbidity observed after the first dose. In the 3-day study, significant increased incidence of fatty acid change and centrilobular necrosis were detected. In the 4-week study, mortalities (1 animal at 125 mg/kg bw/d in week 1, 3 animals at 250 mg/kg bw/d in weeks 1, 2 and 3), statistically increased relative liver weight at the high dose and significant increase incidence of fatty change in the liver in both exposed groups were recorded (Gi et al., 2015a, ATSDR, 2021).

In a dose-range developmental study, SD female rats (10/group) received 0, 50, 125, 250, or 500 mg/kg bw/d 1,2-DCP via gavage in corn oil, from gestation days (GDs) 6 to 15. A detailed observation battery was performed for approximately 60 minutes after

dosing on GD6, 7 and 15. Clinical signs of toxicity (lethargy, salivation, and/or perineal staining) were observed on GD6-8 in 5/10 and 10/10 dams at 250 and 500 mg/kg bw/d, respectively. Significant increases in the signs of CNS depression on GD6 in all dose groups within an hour of administration of 1,2-DCP, included decreased respiration, movement, muscle tone, and extensor thrust reflex and increased salivation and lacrimation. Perineal urine staining was also observed on GD6 in some animals from 125 mg/kg bw/d. These effects were observed with less frequency on GD7, and only from 250 mg/kg bw/d. The only significant observations on GD15 were an increased incidence of salivation and perineal urine staining, at the highest dose (Dow Chemical, 1989 as reported in (EPA, 2016)).

In the following developmental study, SD female rats (30, group) received 0, 10, 30, or 125 mg/kg bw/d 1,2-DCP via gavage in corn oil from GD6 to 15. In the high dose group, clinical signs of toxicity were observed on GD6, with individual signs (decreased movement, muscle tone, and extensor thrust reflex and increased salivation and lacrimation) occurring in 6 to 23 dams (0 to 1 in control). These signs were less frequent (1 to 3 dams) on GD7, and not observed in the other treated groups. Decreased body weight and body weight gain were reported for high dose dams, however no information is available on corrected body weight. In this group, food consumption was significantly decreased by 25% during GD6-9, however, it was comparable to control after GD9. Water consumption was significantly increased by ~25% from GD9-15. There were no significant differences in organ weight between treated animals and controls (Kirk et al., 1995 as reported in (EPA, 2016)).

Gi et al. studied the potential effect of 1,2-DCP on N-nitrosobis(2-oxopropyl)amine (BOP)-induced cholangiocarcinogenesis, and on the promotion of neoplastic lesions in the pancreas, lung, or kidneys in male hamsters. Animals were divided in 5 groups, groups 1 to 3 (24/group) received a subcutaneous injection of BOP (10 mg/kg bw) four times (day 1, 3, 5 and 7), while groups 4 and 5 were analogously dosed with the vehicle (saline). On day 14, groups 1 to 3 received 1,2-DCP by gavage in corn oil (0, 62.5 or 125 mg/kg bw/d) for 15 weeks (17 weeks treatment total, 9 animals/group) or for 17 weeks (19 weeks treatment total, 15 animals/group). The nine animals in group 4 received 125 mg/kg bw/d of 1,2-DCP for 17 weeks, and the six animals in group 5 were dosed with corn oil vehicle for 17 weeks. One death was reported in group 2 treated for 17 weeks (62.5 mg/kg bw/d 1,2-DCP+N-nitrosobis(2-oxopropyl)amine). Body weight was statistically and/or biologically significantly decreased in group 3 (125 mg/kg bw/d 1,2-DCP + N-nitrosobis[2-oxopropyl]amine) by 13% and 8.8% at the end of 17 and 19 weeks, respectively. No significant effects were observed on absolute or relative liver weight. The study authors reported no significant histopathological findings in the liver, pancreas, lung, or kidneys. There were also no significant effects on the expression of CYP2E1, GST-T1, and Ki-67. Overall, the study authors concluded that 1,2-DCP had no effect on BOP-induced pre-neoplastic or neoplastic lesions in any of the tissues examined (Gi et al., 2015b).

Male Sprague-Dawley rats were exposed to 1,2-DCP in corn oil at 0, 100, 250, 500, or 1000 mg/kg bw/d for 5 or 10 days. In animals dosed with 100 mg/kg bw/d for 10 days, liver effects were reported as increased levels cytochrome P450, and nucleolar enlargement (already present after 5 days exposure at all doses); increased liver injuries were reported at higher doses, such as toxic hepatitis, periportal vacuolization, changes in liver enzyme concentrations, and haemolytic anaemia. Nuclear enlargements in hepatocytes were observed at all dose levels at 5 and 10 days. In addition, hemosiderin accumulation and hyperplasia of the hematopoietic elements was observed in the spleen of animal exposed to 500 mg/kg bw/d and above for 5 or 10 days. CNS depression, decreased body weight gain, increased renal non-protein sulfhydryl levels were seen from 250 mg/kg bw/d. Rats demonstrated an adaptive resistance to 1,2-DCP over 10 the days of exposure, resulting in hepatic lesions being less severe at 10 days than at 5 days (Bruckner et al., 1989 as reported in (WHO, 2003, Fan and Alexeeff, 1999)).

Female NZW rabbits (n=2/dose) were administered for 13 days 1,2-DCP by gavage at 0, 250, 500 or 1000 mg/kg bw/d. Five treated animals died during the exposure period or were submitted to necropsy in a moribund condition. General signs of toxicity included lethargy and slight-to-moderate ataxia. Animals dosed with 500 or 1000 mg/kg bw/d had hepatic necrosis with minor hepatocellular alterations in the remaining viable hepatocytes. Minor hepatic lesions, analogous to those observed at the mid and high doses, but no necrosis, were seen also in one rabbit at the low dose. In addition, signs of renal toxicity (pale kidneys, dilation of the renal collecting ducts or the entire tubular system) were reported for some treated animals (Dow Chemical Co., 1988b as reported in (Fan and Alexeeff, 1999)).

Fischer 344/N rats and B6C3F1 mice (n=5/sex/group) were administered 1,2-DCP by gavage for 14 days (0, 125, 250, 500, 1000 or 2000 mg/kg bw/d). All rats in the highest group died during the study, while decreased body weight (14-15%) was reported for the animals at 1000 mg/kg bw/d. High mortality was observed in animals dosed from 500 mg/kg bw/d (3/0, 5/4, 5/5 m/f at 500, 1000 or 2000 mg/kg bw/d, respectively). Mean body weights of surviving mice were not affected. At necropsy, redness of the renal medullae was observed in both rats and mice in the higher dose groups. Histopathology was not performed (NTP, 1986, as reported in (Fan and Alexeeff, 1999)).

Fischer rats (n=10/sex/dose) were dosed by gavage for 14 days to 0, 300, or 500 mg/kg bw/d 1,2-DCP. Transient clinical effects (tearing, blinking, and lethargy) and significant decreases in body weight were observed in all treated animals. A dose-related increase in liver and kidney weights was observed in both sexes. Histopathologic changes (prominent nucleoli of hepatocytes, degeneration and necrosis of liver cells) were found in all animals, while no microscopic effects were noted in kidneys (Dow Chemical Co., 1989 as reported in (Fan and Alexeeff, 1999)).

Male Sprague-Dawley rats (n=15-16/group) were exposed by gavage to 1,2-DCP in corn oil at 0, 100, 250, 500, or 750 mg/kg bw/d for 10 consecutive days or 13 weeks (5 d/week). More than half of the high dose animals died within 10 days and within the 13 weeks exposure at 500 mg/kg bw/d. No animals died in the 100 or 250 mg/kg bw/d groups. The authors reported a significant dose-dependent decrease in body weight gain in all groups. At the two highest doses, pronounced CNS depression with substantially lower water and food intake were observed in these two dose group animals. Histopathology on the high dosed animals revealed effects in the liver (mild hepatitis), in the spleen (haemosiderosis), in the adrenal glands (medullary vacuolization and cortical lipidosis), as well as effects in the testis and epididymis (see 7.3.2.2). On the animals dosed with 500 mg/kg bw/d, histopathology revealed effects on the liver (periportal vacuolization, active fibrosis, increased absolute and relative liver weight), on the spleen (hyperplasia of the erythropoietic elements and increased relative weight) on testis and epididymis (see 7.3.2.2). The increased spleen and liver weights was observed also at 250 mg/kg bw/d but not on the lowest dose (100 mg/kg bw/d). At the lowest dose of 100 mg/kg bw/d, haemosiderosis and splenic hyperplasia were still present. Overall, manifestations of haemolytic anaemia (increased bilirubin, decreased haematocrit and Hg, hemosiderosis and hyperplasia of erythropoietic elements of the spleen, renal tubular cell hemosiderosis and hepatic Kupffer cell hemosiderosis) were found to varying degrees in all dosed animals. Morphologic changes in spleen were dose-dependent ranging from slight to moderately severe. Effects observed at 100 and 250 mg/kg bw/d largely disappeared during the one-week recovery period following the 13-weeks of exposure (Bruckner et al., 1989, as reported in the abstract and in (WHO, 2003, ATSDR, 2021, Fan and Alexeeff, 1999)).

Fischer 344/N rats (10, sex, dose) were dosed by gavage with 0, 60, 125, 250, 500, or 1000 mg/kg bw/d of 1,2-DCP in corn oil for 13 weeks (5 d/week). Mortality (5/10, 10/10 at 500 and 1000 mg/kg bw/d, respectively), decreased body weight at termination (16/8% in m/f, respectively at 500 mg/kg bw/d), and liver effects (congestion in m/f, necrosis and

fatty changes in females only at the highest dose) were reported (NTP, 1986 as reported in (Fan and Alexeeff, 1999)(WHO, 2003)).

B6C3F1 mice (10, sex, dose) were dosed by gavage with 0, 30, 60, 125, 250, or 500 mg/kg bw/d of 1,2-DCP in corn oil for 13 weeks (5 d/week). The only reported effect was a marginal body weight depression at 500 mg/kg bw/d (NTP, 1986, as reported in (Fan and Alexeeff, 1999, WHO, 2003)).

In a neurotoxicity study, F344 rats received by gavage 0, 20, 65, or 200 mg/kg bw/d of 1,2-DCP for 13 weeks (5 d/week). No changes were found in the results of the monthly neurological tests (functional observation battery, hindlimb grip strength, motor activity). After 13 weeks, extensive neurohistopathology as well as histopathology of liver, kidneys, and spleen were performed on 4 rats per group. The remaining 11 rats per group were observed for another 9 weeks, after which 5 rats per group were subjected to gross pathological examination. In male rats reduced body weight was observed the highest dose, still evident at the end of the recovery period (Johnson & Gorzinski, 1988, as reported in (WHO, 2003, ATSDR, 2021)).

In a carcinogenicity study (see 7.7.2), B6C3F 1 mice (n=50/sex/dose) were dosed with 1,2-DCP (purity, > 99%) in corn oil by gavage at a dose of 0, 125, or 250 mg/kg bw/day, 5 d/week, for 103 weeks. Mortality was increased in females at the highest dose. However, findings are confounded by evidence of infection in 60% of all females that died. Non-tumorous liver lesions were seen with an increased incidence in males at both dose levels, and included hepatomegaly, focal hepatocellular necrosis, and centrilobular necrosis (NTP, 1986).

In a carcinogenicity study (see 7.7.2), F344/N rats (n=50/sex/dose) received 1,2-DCP (purity, >99%) in corn oil by gavage at a dose of 0, 62, or 125 mg/kg bw bw/day, 5 d/week, for 103 weeks. Decreased body weight on animals at the high dose and survival of females at the high dose were reported (NTP, 1986).

7.3.2.2 Inhalation

Male mice (C57BL/6J) were exposed for 2 days (6 h on D1, 3 h on D2, whole body) to 0, 100, 200 or 400 ppm 1,2-DCP. No effects were reported ((Toyooka et al., 2017) as reported in (EPA, 2016)).

Liver effects (fat-droplets) were observed in another F344 rat study at 3000 ppm. Three animals were exposed for 7 d, 8 h/d (whole body) to 0, 300, 1000, 3000 ppm 1,2-DCP (EPA, 2016).

F344 rats (n=5/sex/group) were exposed from 2 weeks (4-5 d/week, 6 h/d, whole body) to 1,2-DCP at concentrations of 0, 100, 300 or 1000 ppm. Increased liver weight (M/F) and hepatocellular hypertrophy (F) were reported at the high dose, while olfactory mucosal degeneration was observed at all doses (Nitschke and Johnson, 1983 as reported in (EPA, 2016)).

B6C3F1 mice (n=5/sex/group) were exposed from 2 weeks (4-5 d/week, 6 h/d, whole body) to 1,2-DCP at concentrations of 0, 30, 100 or 300 ppm. Increased liver weight, hepatocellular hypertrophy, vacuolisation, olfactory mucosal degeneration, and decreased thymus weight and lymphoid cells were reported at the high dose (Nitschke and Johnson, 1983 as reported in (EPA, 2016)).

Rats, mice, and rabbits were exposed for 13 weeks to 1,2-DCP (15, 50, and 150 ppm for rats and mice and 150, 500, or 1000 ppm in rabbits) in a GLP compliant study. In rats, nasal respiratory changes and slight reductions in body weight were reported from 50 ppm. No effects were observed in mice. In rabbits, slight changes in the nasal tissue at the high dose, and in the red blood cell parameters, indicative of a macrocytic normochromic, regenerative anaemia (from 150 or 500 ppm in males and females, respectively) (Nitschke et al., 1988 as reported in (OECD SIDS, 2005)).

F344 rats (n=10/sex/group) were exposed from 13 weeks (5 d/week, 6 h/d) to 1,2-DCP at concentrations of 0, 15, 50, or 150 ppm. The body weight of high dose male was significantly reduced (10%) at termination. The study reported lesions in the respiratory tract of all exposed animals; hyperplasia (observed mainly in the anterior region if the nasal cavity), incidence and severity were dose-dependent and statistically significant from 50 ppm. Olfactory mucosa degeneration was also significantly increased from 50 ppm in all animals with increased severity at the highest dose. Submucosal inflammation was significantly increased in the larynx of high dose males only (Dow Chemical, 1988a as reported in (EPA, 2016)).

F344/DuCrj (SPF) rats (n=10/sex/group) were exposed from 13 weeks (5 d/week, 6 h/d) to 1,2-DCP at concentration of 0, 125, 250, 500, 1000, or 2000 ppm. One female on the high group died during the 12th week of exposure. Body weight reductions were statistically significant in all exposed males (5-27%), and in females from 500 ppm (5-18%). Changes in the blood parameters included an increase in platelets, statistically significant in males and females from 1000 or 2000 ppm, respectively; decreased erythrocyte count in males and females from 500 ppm (4-19%, statistically significant); decreased haemoglobin (Hb) (3-10%) in males (≥500 ppm) and in females (≥1000 ppm), and haematocrit (Hct) in all animals from 1000 ppm (4-5%). A significant increase of 2 to 6 folds in percentage of reticulocytes in males (≥1000 ppm) and females (≥500 ppm) was also reported. The authors concluded that these finding are consistent with haemolytic anaemia. The significant clinical chemistry alterations reported were increase in total serum bilirubin and GGT in males at 2000 ppm and females from 1000 ppm (25−56%, ~2-3 fold activity, respectively). Histopathological lesions were observed in the nasal cavity, spleen, bone marrow, liver, and adrenal glands. A statistically significant incidence of respiratory epithelium hyperplasia (mainly in the anterior nasal cavity) with a dose-dependent increase in severity in males was reported in 50/50 males and in 45/49 females. Atrophy of the olfactory epithelium was observed in all exposed animals with a dose-dependent increase in severity for both males and females. Increase of inflammation of the respiratory epithelium in the nasal cavity was reported in males (from 250 ppm, statistically significant from 1000 ppm), and in females (from 1000 ppm). In the spleen, a statistically significant increase in extramedullary haematopoiesis from 1000 ppm (all males, 8/10 and 9/9 females, respectively), and increased deposition of haemosiderin from 1000 ppm in males and in females from 500 ppm (all exposed animals) were reported. From 1000 ppm, all animals showed a significant increase in haematopoiesis in the bone marrow, while centrilobular liver swelling was significant at the highest dose (9/10 males, 6/9 females). In the adrenal glands, fatty changes were significantly increase only in females at the highest dose (9/9 affected) ((Umeda et al., 2010) (EPA, 2016, ATSDR, 2021)).

B6D2F1/Crlj (SPF) mice (n=10/sex/group) were exposed for 13 weeks (5 d/week, 6 h/d) to 1,2-DCP at concentrations of 0, 50, 100, 200, 300, or 400 ppm. Mortalities were reported in males (2/10 and 6/10 at 300 and 400 ppm, respectively) and in females (1/10 at 400 ppm). Decreased body weight was reported only in males exposed at and above 200 ppm (statistically significant 9-18%). In all animals, a significant increase in liver weight (absolute and relative, 14-66%) was observed from 300 ppm, while relative spleen weight was significantly increased only at 400 ppm (21-38%). Hyperplasia of the stomach was observed in both males and females from 200 ppm (statistically significant at 400 /from 300 ppm in M/F respectively). Other histopathological changes were observed in the nasal cavity (metaplasia, atrophy and necrosis of the olfactory epithelium significant from 300 ppm and significant desquamation in males at 400 ppm), in the liver (fatty changes and central necrosis in males from 300 ppm, vacuolic changes and mineralisation in all animals at the highest dose, swelling in all animals from 300 ppm). In the bone marrow, increased erythropoiesis was reported from 300 ppm (both sexes), while congestion only in males from 300 ppm. In the spleen, atrophy was reported in males from 300 ppm, increased extramedullary haematopoiesis and megakaryocyte were observed from 300 ppm, haemosiderin deposition at 400 ppm and increased extramedullary

haematopoiesis from 300 ppm in both sexes. Haematological changes were reported in both sexes, with decrease in red blood cell (RBC) count, Hb, Hct significant from 50 and 300 ppm in males and females respectively, increase in MCV (significant in males from 50 ppm and in females from 200 ppm), increase in platelet (significant from 300/400 ppm in m/f, respectively). Changes in clinical chemistry included increased T-bilirubin (significant at 400 ppm), phospholipid (significant from 300 ppm), increased AST and LDH (significant at 400 ppm), ALT (significant at 400 ppm in males), and ALP (significant from 300/400 ppm in males) (Matsumoto et al., 2013).

B6C3F1 mice (n=10/sex/group) were exposed for 13 weeks (5 d/week, 6 h/d) to 1,2-DCP at concentrations of 0, 15, 50 or 150 ppm. The only changes observed were significant decreases in RBC counts, Hb, and packed cell volume at 15 and 150 ppm in male mice. These changes were considered by the authors as not biologically relevant because of the low severity (<10% of control) and their absence in females (Dow Chemical, 1988a as reported in (EPA, 2016)).

New Zeeland White (NZW) rabbits (n=7/sex/group) were exposed for 13 weeks (5 d/week, 6 h/d) to 1,2-DCP at concentrations of 0, 150, 500, or 1000 ppm. Haematological changes started to be significant on week 11 and they included 10-25% reduction in erythrocyte count, Hb, packed cell volume from 500 ppm; reduction in erythrocyte was already significant in males at 150 ppm. In addition, at the end of the study, a 2-4-fold increase in percent reticulocytes from 500 ppm (statistically significant), and fourfold increase in nucleated erythrocytes at 1000 ppm in males. A significant increase in absolute and relative liver weight in males from 500 ppm was reported. Histopathological changes were observed in the nasal cavity (very slight-to-slight marginally significant increase in olfactory epithelium degeneration in males at 1000 ppm) and in the bone marrow (slight to moderate hyperplasia from 500/1000 ppm in M/F, respectively, and a non-significant increase of hemosiderin-laden macrophages at 1000 ppm) (Dow Chemical, 1988a as reported in (ATSDR, 2021, EPA, 2016).

F344/DuCrj (SPF) rats (n=50/sex/group) were exposed from 2 years (5 d/week, 6 h/d) to 1,2-DCP at concentration of 0, 80, 200 or 500 mL/m^3. There was a slight dose-dependent decrease in body weight on male rats, and an anaemic tendency was observed in high dosed females (Umeda et al., 2010).

In a carcinogenicity study, B6D2F1 mice (n=50/sex/dose) were dosed with 0, 32, 80 or 200 mL/m^3 1,2-DCP by inhalation, for 2 years (5 d/week, 6 h/d), according to OECD TG 451. Increases in spleen and kidney weights were recorded in the high dosed animals. Haemoglobin concentration was lower in males from the mid dose and in females at the high dose, while mean corpuscula volume was increased in females at the high dose. No other haematological or biochemical parameters were affected (Matsumoto et al., 2013).

7.3.3 *In vitro* data

No data available.

7.3.4 Summary

There are no human data on longer-term 1,2-DCP exposure and non-cancer chronic disease.

Repeated dose toxicity of 1,2-DCP in animals has been examined in oral and inhalation studies of duration from a few days to 2 years. Effects were observed in the liver (increased weight, hypertrophy, fatty changes, central necrosis, vacuolisation), in the respiratory tract (respiratory epithelium hyperplasia, olfactory mucosa degeneration or atrophy, inflammation of the respiratory epithelium in the nasal cavity, larynx) in the majority of the studies, whereas effects in the spleen (increased extramedullary haematopoiesis, megakaryocyte, haemosiderin deposition, atrophy), in the adrenal glands (fatty changes), in the bone marrow (increased erythropoiesis), and depression of the CNS were reported in some but not all studies.

Lastly, in some studies haematological and clinical chemistry alterations were also observed.

7.4 Irritancy and corrosivity

7.4.1 Human data

Case reports (Rubin (1988) and Conner et al (1962), see section 7.2.1.3) on chemical accidents suggest that inhalation exposure to 1,2-DCP causes respiratory irritation in humans, following acute exposure. The exposure levels were presumably high but not further quantified.

7.4.2 Animal data

Observations from GLP-compliant skin and eyes irritation studies (skin: minimal redness and slight oedema, eyes: marked redness, oedema and slight opacity 24 h after instillation, fully reversed after 8 days) indicated that 1,2-DCP is slightly irritating to skin and eyes, as reported by (OECD SIDS, 2005).

Redness and inflamed skin were reported in rats exposed to 2.34 mg/kg bw 1,2-DCP for 24 h in occluded conditions (ATSDR, 2021).

On a 24 h Draize occlusive patch test, a significantly different reaction was observed between male and female rabbits: the exposure to 1.16 g/mL in males caused mild skin irritation, while in females an extreme skin irritation (chemical burns, superficial necrosis) was recorded. The effects were still evident in both sexes 21 days after exposure, including hardening and lifting of skin in female rabbits. No reason is known for this difference (ATSDR, 2021).

In another study, initial pain, redness, iridial irritation, and corneal ulceration were observed following direct ocular instillation of undiluted 1,2-DCP into rabbit eyes, all effects were reversible withing 14 days. In addition, conjunctivitis was reported in guinea pigs exposed to 2200 ppm of 1,2-DCP for 7 h (ATSDR, 2021).

7.4.3 Summary

Human data on irritant effects of 1,2-DCP are limited to few accident case reports indicating irritation effects at presumably high, but not further characterised exposure levels.

Based on the results of the available animal studies, 1,2-DCP is considered slightly irritant to skin and eyes.

7.5 Sensitisation

7.5.1 Human data

7.5.1.1 Respiratory sensitisation

No case reports or epidemiological studies were identified for respiratory sensitisation. The study of Choi et al. (2009) described in section 7.5.1.2 also included a comparison of asthma and non-asthma cases. No difference in 1,2-DCP exposure was observed, based on residential VOC levels measured at one time point only.

7.5.1.2 Skin sensitisation

Grzywa and Rudzki (1981) described two cases of dermatitis in workers exposed to 1,2-DCP. A 47-year-old woman had been exposed for 6 years in production of various plastic products of polypropylene, polystyrene, metaplex and bakelite and with daily spraying application of Siliform AR-1, AR-2 and AR-3 containing of 7-13% of 1,2-DCP, 4-9 % of methylsilicone oils and about 85% of Freons 11 and 12 in 1;1 ratio. Patch tests were strongly positive for 1,2-DCP (1% in petrolatum) and AR-1 and slightly positive for a

number of other substances. A 55-year-old woman had been exposed for 4 years in production of bakelite parts for cars and with daily exposure to almost exclusively Siliform AR-1. Patch tests were positive for chromate and 1,2-DCP but negative for AR-1. Grzywa and Rudzki (1981) also tested 12 unexposed controls, and none was skin prick positive for 1,2-DCP or AR-1. The first case had 21 colleagues performing similar tasks and none developed dermatitis while the second case had 39 colleagues performing similar tasks and two had skin problems but had not been seen or tested by the authors.

Baruffini et al. (1989) described a series of 10 painters and metalworkers from engineering industry that had been diagnosed with a dermatitis caused by 1,2-DCP in 1985-1988. All had been in contact with solvent mixtures where a 10-40% concentration of 1,2-DCP had been confirmed with gas chromatography. Patch tests had been performed for the European standard series (Hermal-Trolab), 1,2-DCP in different concentrations in petrolatum as well for other substances used at work (resins, solvents, mineral oils, perchloroethylene, trichlororethylene). All 10 were positive for 1,2-DCP and one worker also for methyl acrylate, but the latter was not due to current contact with the substance and the dermatitis was unrelated to that exposure. All 120 unexposed control subjects tested for 1,2-DCP were negative.

Choi et al. (2009) did not find a significant difference in indoor and outdoor residential air levels of 1,2-DCP between individuals with atopic dermatitis (n=50) or asthma (n=36) and control subjects (n=28) without atopy or asthma. Altogether 49 VOCs (11 alkanes, 13 aromatics, 7 chlorinated hydrocarbons, 3 terpenes and 15 aldehydes) were measured in the study and the comparison of exposure was limited to one point in time.

7.5.2 Animal data

7.5.2.1 Respiratory sensitisation

No data.

7.5.2.2 Skin sensitisation

In an unpublished Local Lymph Node Assay (LLNA) study (GLP, OECD TG 429, Woolhiser et al., 2003 as reported in (OECD SIDS, 2005)), mice were exposed with up to 80% 1,2-DCP. No proliferation was observed thus the substance is considered as not a sensitizer.

7.5.3 In vitro data

No groups associated with sensitisation potential are present in 1,2-DCP (OECD SIDS, 2005).

7.5.4 Summary

There are occasional case reports on skin sensitisation effects of 1,2-DCP. However, the exposure concerned usually multiple chemicals. There is no human data on respiratory sensitisation. 1,2-DCP is considered non-sensitiser based on a negative LLNA study and absence of structural alerts.

7.6 Genotoxicity

7.6.1 Human data

A number of studies addressing genotoxicity endpoints in clinical samples from occupationally exposed printing workers in Japan, presenting with cholangiocarcinoma have been reported and are summarised in Table 9. In an attempt to elucidate the aetiology and pathogenesis of the high incidence of unusually early-onset intrahepatic cholangiocarcinoma among workers exposed to high concentrations of 1,2 DCP and/or dichloromethane, whole-exome analysis was performed on four occupational cholangiocarcinoma cases in male workers (Mimaki et al., 2016).

One patient (case 1) was exposed to both 1,2-DCP and DCM, while the other three (cases 2–4) used 1,2-DCP for 1–11 years (Kubo et al., 2014b). (Kumagai et al., 2013) has previously estimated the concentration of volatile solvent in a proof-printing room to be 100–670 ppm for 1,2-DCP.

Resected tumour and matched normal tissue samples were processed, along with common late-onset intrahepatic cholangiocarcinoma (n=4) and early-onset bile duct carcinomas (n=3) as additional controls. Genomic DNA was retrieved from tissues, whole-exome sequencing libraries prepared, and the captured exomes were subsequently subjected to sequencing. The occupational cholangiocarcinomas presented as high mutation burden tumours. A significantly higher number of somatic single-nucleotide variants (SNVs) and an approximately 30-fold increase in mutation rate (mutations/Mb) was observed in the exomes of the four cases investigated (average number of SNVs: 1451±1089; average mutation rate: 44.6±33.5/Mb) compared to the late-onset (44.8±11.9; 1.4±0.4/Mb) and early-onset (50.0±23.4; 1.5±0.7/Mb) common tumour control samples. Notably, a significantly smaller number of insertions and deletions (INDELs) were detected in the occupational tumours (6.8±5.0 (0.2±0.27/Mb), unlike other hypermutated solid tumours such as microsatellite-instable colorectal cancers. Among the somatic SNVs, C:G to T:A transitions were predominant (45-60% of total SNVs), displaying a substantial strand bias specific to the four occupational cases, followed by C:G to A:T transversions. Such 'strand bias' can derive from DNA transcription or replication or through stand-specific DNA repair processing such as transcription-coupled nucleotide excision repair (TC-NER) of DNA-distorting adducts. However, "strand bias" can also be caused by a disruption in DNA replication processes or by the defective activity of cytidine deaminase enzymes such as AID (2021 MAK report, references therein). Analysis of the flanking 5' and 3' sites of each mutated base substitution, revealed that the most characteristic trinucleotide mutational pattern, shared by all four printing workers' cases was GpCpY to GpTpY (Y=pyrimidine base) followed by NpCpY to NpTpY or NpApY (N=any base). This mutational signature was characteristic and unique for the occupational cholangiocarcinomas, not encountered in the common tumour control samples, nor previously reported for other primary cancers. Further analysis in select genes, frequently mutated in bile tract carcinoma, demonstrated that all occupational cholangiocarcinoma genomes harboured amino acid-altering mutations in 2-6 of these genes. Mutations in *ARID1A*, *BRAF*, *CDKN2A* and *MLL3* detected in case 1, were further confirmed by Sanger sequencing. Collectively, the above characteristic mutational profile (i.e. high somatic mutation burden, substantial strand bias in C:G to T:A mutations and unique trinucleotide changes) shared by all of the investigated occupational cases, suggests exposure to a common strong mutagen and therefore a contribution of 1,2-DCP to mutagenesis in genes potentially involved in cholangiocarcinoma carcinogenesis. The mutagenic profile observed in the human clinical samples was only partially recapitulated in *in vitro* assays in the *Salmonella typhimurium* strain TA100 but not in human epithelial cell-derived cell lines after either single or repeated exposure (Mimaki et al., 2016).

In a follow-up study on the same occupational patients and control samples, (Mimaki et al., 2020) performed whole-exome analysis of further lesions including invasive carcinoma and precancerous lesions, precancerous. The occupational lesions presented again with a significantly higher number/mutation rate (mean values of 76.3/Mb and 71.8/Mb in invasive carcinomas and precancerous lesions, respectively) compared to the control tumours' exomes (mean 1.6/Mb), confirming the previous observations, while no significant difference was observed in the number of somatic mutations between precancerous and invasive carcinoma lesions. Upon further analysis, the limited overlap of the detected somatic mutations in 11/16 investigated lesions in the occupational cholangiocarcinomas suggested that they arose from different clones. However they all shared the previously reported unique trinucleotide mutational signature of GpCpY to GpTpY suggesting that the entire bile ducts of the workers had been exposed to one or several common strong mutagens and cancer-related genes mutations of different clonal origins induced multifocal carcinogenesis.

Amino acid-altering mutations in 'Mut-driver genes' (i.e. genes that contain driver gene mutations and increase the selective growth advantage of tumour cells, as defined by (Vogelstein et al., 2013) were detected in both precancerous and invasive lesions. Of these genes, *ARID1A*, *ARID2*, *MLL2*, and *SETBP1* were mutated at a higher rate in invasive than in the precancerous lesions. In another study aiming to characterise the pathology of occupational cholangiocarcinoma, (Kinoshita et al., 2016) examined operative or autopsy liver specimen from 16 patients.

These former and contemporary workers at printing companies in Japan were exposed to various types of chlorinated organic solvents, including 1,2-DCP, DCM, and 1,1,1-trichloroethane (TCE). A spectrum of pathological changes spanning from chronic bile duct injury, early neoplastic and pre-invasive lesions (Biliary intraepithelial neoplasia (BiIIN) and intraductal papillary neoplasms of the bile duct (IPNB)) to invasive cholangiocarcinoma were observed in all patients. In three of the patients, DNA damage was evaluated by assessing the levels of γ-H2AX via immunohistochemistry. Highly positive results for γ-H2AX were noted in invasive carcinoma, BilIN, and IPNB, whereas positive results were noted in peribiliary glands and in non-neoplastic biliary epithelium, in all evaluable samples from the three patients. The detection of γ-H2AX presumably induced by 1,2-DCP or its metabolites, in almost all of the large bile ducts, in both pre-cancerous and cancerous lesions renders the chemical-related DNA injury and associated chronic bile duct injury a key factor in the carcinogenic process of occupational cholangiocarcinoma (Kinoshita et al., 2016). (Sato et al., 2014) had also previously reported a semiquantitative analysis of the expression of γ-H2AX in occupational cholangiocarcinoma cases in printing workers in Japan. They showed that the expression of γ-H2AX was significantly increased in foci of non-neoplastic biliary epithelial cells of the large bile duct, BilIN, IPNB and invasive carcinoma in the printing company cases when compared with that of control groups of cholangiocarcinoma with hepatolithiasis and BilIN, and conventional IPNB. Mutations of KRAS and GNAS have been reported in the occupational cases by (Sato et al., 2014) and (Mimaki et al., 2020) (Table 9).

Table 9: Summary of genotoxicity findings in humans

Endpoint studied/Method/No samples (pathology)	Results	Remarks	References
Mutagenicity/Whole-exome sequencing/n=4 (occupational cholangiocarcinoma)	**positive** significant increase in SNVs; 30-fold increase in mutation rate (mutations/Mb) in occupational over control tumour samples High mutation burden, C:G to T:A transitions with substantial strand bias; unique trinucleotide mutational changes of GpCpY to GpTpY and NpCpY to NpTpY or NpApY	Whole-exome sequencing of a single invasive lesion in each case	(Mimaki et al., 2016)
Mutagenicity/Whole-exome sequencing of multiple lesions/n=4 (occupational cholangiocarcinoma); identical to above	**positive** significantly higher mutation burden was observed in both the invasive carcinomas (mean 76.3/Mb) and precancerous lesions	Whole-exome analysis of 12 lesions in addition to the 4 above, including invasive carcinoma lesions,	(Mimaki et al., 2020)

Endpoint studied/Method/No samples (pathology)	Results	Remarks	References
	(mean 71.8/Mb) in 3/4 occupational cases vs non-occupational cholangiocarcinomas (n=7)(mean 1.6/Mb) Most somatic mutations identified in 11/16 lesions did not overlap with each other a unique trinucleotide mutational signature of GpCpY to GpTpY was shared among lesions	precancerous lesions; same control non-occupational tumour samples as above	
DNA damage/γ-H2AX (immunohistochemistry)/n=8 (occupational cholangiocarcinoma)	**positive** γ-H2AX detected in **occupational cases:** 7/8 in the foci of invasive carcinoma; 6/8 in non-neoplastic tissue 6/8 in BiIIN 4/4 in IPNB **tumour control cases:** 7/16 3/16 in BiIIN **conventional control IPNB**: 6/19 Negative in non-neoplastic biliary epithelial cells of the large bile duct and peribiliary glands		(Sato et al., 2014)
DNA damage/γ-H2AX (immunohistochemistry)/n=3 (occupational cholangiocarcinoma)	**positive** ++: 3/3 cases of invasive carcinoma, BiIIN-2/3, IPNB and 1/3 peribiliary gland + in 2/2 evaluable cases of non-neoplastic bile duct epithelium	Staining evaluation: ++: >20% of examined cells staining positive +: ≤20% of examined cells staining positive	(Kinoshita et al., 2016)
DNA damage/γ-H2AX (immunohistochemistry)/single case of occupational cholangiocarcinoma (exposed to 1,2-DCP and/or DCM, developed 18 years later)	**positive** γ-H2AX detected in all portions of the invasive carcinoma, BilIN, and IPNB; also detected within the non-neoplastic biliary epithelium		(Kinoshita et al., 2019)
Mutagenicity/KRAS and GNAS genes PCR amplified and	**positive** KRAS mutation was	one focus of non-neoplastic biliary	(Sato et al., 2014)

Endpoint studied/Method/No samples (pathology)	Results	Remarks	References
sequenced)/n=3 (occupational cholangiosarcoma)	detected in one focus of BilIN (GGC to GGT at codon 13); GNAS mutation was detected in one focus of BilIN, (same case that had KRAS mutation but from the different focus; CGT to CGA at codon 201)- The other foci examined were wild type for both KRAS and GNAS.	epithelial cells of the large bile duct, 3 foci of BilIN, 4 foci of IPNB and 3 foci of invasive carcinoma were selected and analysed	
Mutagenicity KRAS, Mut-driver genes, TP53/n=4 (occupational cholangiocarcinoma)	**positive** KRAS: 3/7 precancerous lesions; 2/9 invasive carcinoma "Mut-driver" genes frequently mutated in invasive carcinoma, at higher rate compared to precancerous lesions (ARID1A 66.7% vs 28.6%; ARID2 66.7% vs 14.3%); ARID1A and ARID2 in invasive carcinoma lesions were "damaging" mutations		(Mimaki et al., 2020)

7.6.2 Animal data (*in vivo*)

Available evidence from animal studies (summarised in Table 10) indicates a lack of marked *in vivo* mutagenicity of 1,2-DCP in exposed rodents. 1,2-DCP failed to induce any significant increases, compared to controls, in the mutation frequencies of the *Pig-a* gene in erythrocytes of mice repeatedly exposed by inhalation to up to 600 ppm 1,2-DCP for 6 weeks. A 32% increase -albeit non-significant - in *gpt* mutation frequencies in the liver of transgenic mice exposed to a single dose of 300 ppm 1,2-DCP, was further enhanced and reached statistical significance in the group co-exposed to dichloromethane (Suzuki et al., 2014). In transgenic mutation studies in rats however, no effect on the *gpt* gene, or the $Sp1^-$ phenotype was elicited in the liver of rats exposed to the chemical by oral administration (Hirata et al., 2017). Earlier studies assessing sex-linked recessive lethal mutations in *Drosophila Melanogaster* exposed by injection or inhalation for up to two weeks or dominant lethal mutations in rats exposed to doses up to 162 mg/kg bw via drinking water for 14 weeks were also all negative ((ATSDR, 2021); references therein). 1,2-DCP however yielded significantly positive results in a somatic mutation and recombination test (SMART) in *Drosophila melanogaster*, when delivered by inhalation for 48 h, at LC_{50} concentrations (Chroust et al., 2007). In trials conducted by NTP, assessing sister chromatid exchange and chromosomal aberrations in the bone marrow of mice receiving single intraperitoneal injection of up to 450 mg/kg bw, 1,2-DCP produced negative results (Table 10). Although 1,2-DCP did not display any clastogenic/aneugenic activity in mouse reticulocytes or erythrocytes following inhalation exposure, DNA damage was consistently observed in the form of DNA strand breaks (including double-strand breaks) and the induction of H2AX phosphorylation in the liver of mice exposed to ≥100 ppm 1,2-DCP (Table 10). The observed damage is likely to result from direct interaction with DNA, as no significant increase in the levels of 8-OHdG adducts was reported in the

livers of mice or hamsters, exposed to gavage doses of up to 250 mg/kg bw for up to four weeks (Table 10).

Collectively, 1,2-DCP displayed a genotoxic potential in the liver tissue of mice exposed by inhalation, reflecting the species and site specificity of carcinogenesis (see section 7.7.2).

Table 10: Summary of *in vivo* genotoxicity studies

Assay/Species, strain, sex; No/group	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
Dominant lethal assay/Sprague-Dawley rats (M)	(0.24% w/v) in drinking water equivalent to a time-weighted daily dosage of 162 mg/kg bw	negative Statistically significant increase was observed after 1 wk of breeding in preimplantation losses and resorption rate at 0.024% and 0.24% treated groups compared to controls, however data from the second week showed no significant treatment-related difference	Continuous 14 wk treatment	Hanley et al, EPA (1989), as reported in (IARC, 2017)
Pig-a/B6C3F$_1$ mice (M; n=8-10)	(600 ppm)	negative no increase in mutant frequency of erythrocytes in mice exposed to 1,2-DCP alone or in co-exposure with DCM	Inhalation of 150, 300 and 600 ppm 1,2-DCP, alone or in combination with dichloromethane (DCM), 6 h per day, 5 d/wk for a total of 6 wk; mice euthanised 18 h after last exposure; blood collected in wk 3 and 6 h post inhalation **No mutagenic potential in hematopoietic stem cells**	(Suzuki et al., 2014)
gpt mutation/*gpt* Delta C57BL/6J mice (M; n=5/group)	(300 ppm)	negative non-significant increase (32%) of gpt mutant frequencies in the liver over controls in single treatments **positive** with co-exposure to 800 ppm DCM	inhalation of 300 ppm 1,2-DCP; 6 h/day, 5 d/wk for a total of 4 wk; mice euthanised 7 d after final inhalation; more than 1,500,000 colonies derived from the rescued phages/liver/mo	(Suzuki et al., 2014)

Assay/Species, strain, sex; No/group	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
		(significant 2.6-fold increase over controls)	use were analysed; single concentrations used in co-exposures **1,2-DCP may have genotoxic potential in liver, potentiated by DCM**	
gpt mutation/*Spi*$^-$ assay in liver; CYP2E1 and GSTT1 expression levels/F344 *gpt* delta rats (M; n=7/group)	(200 mg/kg bd wt)	negative no increase in *gpt* and *Spi*- mutation frequencies; no change in CYP2E1 and GSTT1 gene/protein expression	Oral administration by gavage, daily for 4 wk; no treatment-related histopathological changes observed; CYP2E1 and GSTT1 gene and protein expression levels quantified by qPCR and immunoblotting, respectively **Lack of *in vivo* genotoxicity in the liver of rats correlated to CYP2E1 and GSTT1 expression** (CYP pathway not saturated and GST pathway did not begin to become predominant; GSTT1 levels in rats>mice)	(Hirata et al., 2017)
In vivo micronucleus assay/ B6C3F$_1$ mice (M; n=8-10)	(600 ppm)	negative	Treatment doses as above; frequencies of micronucleated reticulocytes (MN-RETs) and micronucleated normochromatic erythrocytes (MN-NCEs) were determined in blood specimens	(Suzuki et al., 2014)

Assay/Species, strain, sex; No/group	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
			collected in wk 6 **No clastogenicity/ aneugenicity or adverse effects on hematopoiesis in bone marrow cells**	
SCE bone marrow/B6C3F$_1$ mice (M; n=4/dose)	(450 mg/kg bw)	negative	single i.p. injection; sampling time 23 h and 42 h (2 trials)	NTP Study ID: 568899_SCE
Chromosomal Aberration in bone marrow/ B6C3F1 mice (M; n=8/dose)	(450 mg/kg bw)	negative	single i.p. injection, sampling time 17 h and 36 h (3 trials)	NTP study ID: 568899_CA
Alkaline Comet assay (liver)/ B6C3F$_1$ mice (M; n=8-10)	300 ppm/600 ppm	**positive** dose-dependent, statistically significant (at 300 and 800 ppm) increases in Tail intensity over controls, further increased, at lower 1,2-DCP doses when in combination with DCM	Treatment conditions as above; 100 cells tested per experimental point **1,2-DCP may induce DNA damage in the liver which is enhanced by DCM**	(Suzuki et al., 2014)
Alkaline Comet assay (liver)/*cyp2e1*$^{+/+}$ /*cyp2e1*$^{-/-}$ B6C3F$_1$ mice (M; n=8-10)	(300 mg/kg bw)	negative no increase in Tail intensity in either genotype	i.p. dosing, evaluated 16 h post treatment DNA damage might have been repaired at this time point	(Yanagiba et al., 2016)
Oxidative DNA damage in liver (HPLC)/ B6C3F$_1$ mice (M; n=5/group)	(250 mg/kg bw)	negative no significant increase in 8-OHdG formation in the liver of exposed groups, compared to controls	Gavage, 4 wk treatments; all animals in the high dose group (250 mg/kg bw) survived	(Gi et al., 2015a)
Oxidative DNA damage in liver (HPLC)/Syrian hamsters (M; n=5/group)	(250 mg/kg bw)	negative As above	Gavage, 4 wk treatments; 2/5 animals survived in the high dose group (250 mg/kg bw)	(Gi et al., 2015a)
Generation of γ-H2AX in liver (immunoblotting) /C57BL/6J mice	100 ppm/400 ppm	**positive** increased γ-H2AX levels as detected by immunoblotting,	Inhalation of 100, 200, and 400 ppm; for 6 h on the first day,	(Toyooka et al., 2017)

Assay/Species, strain, sex; No/group	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
(M)		compared to untreated controls	followed by 3 h on the second day; mice were sacrificed 2 h after the termination of inhalation exposure;	
DNA double-strand breaks (biased sinusoidal field gel electrophoresis (BSFGE) in liver/C57BL/6J mice (M)	100 ppm/400 ppm	**positive** Generation of DNA double strand breaks in the liver tissue of mice exposed to 100, 200 and 400 ppm	Experimental conditions as above **Inhalation exposure generates DNA double-strand breaks measured directly or indirectly via induction of γ-H2AX**	(Toyooka et al., 2017)

7.6.3 *In vitro* data

Relevant *in vitro* genotoxicity studies in bacterial test systems are tabulated in Table 11. Results of early mutagenicity studies on *Salmonella typhimurium* TA100 and TA1535, in the presence and absence of metabolic activation were positive-albeit at high doses-by the plate incorporation method (De Lorenzo et al., 1977, Principe et al., 1981), while marginally positive results were reported with the same strains, only in the absence of metabolic activation by (NTP, 1986). Negative results were consistently yielded in the above-mentioned studies, with strains TA1537 and TA98, regardless of S9 supplementation. Early positive results in strains TA98, TA100, TA1535 and TA1537 reported by (Haworth et al., 1983), were later deemed negative upon re-evaluation applying more stringent criteria (Prival and Dunkel, 1989). Dose-dependent mutagenicity, independent of GSTT1, was however reported in *S. typhimurium strain* TA100 and human GSTT1-expressing TA100, at vapor concentrations ranging from 600 to 3,000 ppm without metabolic activation using a closed plate system (Akiba et al., 2017, Mimaki et al., 2016). Mutation spectrum analysis revealed that the C:G to T:A transitions in the *hisG* gene reversions were the predominant mutagenic events, mirroring the findings in human cholangiocarcinomas cell lines (Table 12) and in clinical cholangiocarcinoma samples' genomic findings (Table 9).

Table 11: Summary of bacterial genotoxicity studies

Assay/Species, strain	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
Bacterial reverse mutation assay/ *S. typhimurium* TA1535, TA100, TA1978 (+TA1537, 1598)	-/+S9: TA1535, TA100: 10 mg/50 mg -/+S9: TA1978: 50 mg	**positive** TA1535: 22-fold (-S9) and 15-fold (+S9) increases in number of revertants/plate compared to controls, at 50 mg TA100: 10-fold (-/+ S9) increase at 50 mg negative	In the same study the mutagenicity of 100 mg-10 mg Telone (containing 20% 1,2-DCP) and 500 mg-25 mg D.D. soil fumigant (40% 1,2-DCP) was also assessed. Mutagenic activity of both was evident in strains TA1978, TA1535 and TA100 (-/+ S9). Strains TA1537 and TA98 were negative.	(De Lorenzo et al., 1977)
Bacterial reverse mutation assay/ *S. typhimurium* TA100	-/+ S9 TA100: (11 mg/plate)	negative	complete inhibition of bacterial growth at highest (toxic) dose; no activity at 1 or 10 mmoles/plate=0.11/1.1 mg/plate	(Stolzenberg and Hine, 1980)
Bacterial reverse mutation assay/*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100; Forward mutation-induction of resistance/ *Streptomyces coelicolor*	-/+S9: TA1535: 1 ml/plate = 1.16 mg/plate/10 ml/plate=11.6 mg/plate) TA100: 5 ml/plate/10 ml/plate=11.6 mg/plate -/+ S9: TA1537, TA1538, TA98 (10 ml/plate=1.1 mg/plate) *Streptomyces coelicolor* (100 ml/plate)	Weakly **positive** (significant); TA1535: 4.2-fold (-S9) and 2.3-fold (+S9) increase in number of revertants at the highest dose, compared to solvent TA100: ~2-fold (-/+S9) increase at the highest dose negative negative in spot and plate test experiments	responses partially suppressed in the presence of microsomal fractions	(Principe et al., 1981)
Bacterial reverse mutation assay/*S. typhimurium* TA100, TA98, TA1537, and TA1535	- S9 TA100: 1 mg/plate/2 mg/plate TA1535: 1 mg/plate/2 mg/plate TA1537, TA98	Dose-related response, **marginally positive** at the highest doses (1-2 mg/plate)	Performed with a preincubation modification of the Ames protocol; Potential for impurities to have caused these marginal responses	(National Toxicology, 1986)

Assay/Species, strain	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
	+S9 TA100, TA1535, TA98, TA1537 (2 mg/plate)	negative negative	**"No clearly positive responses"**	
Ames II (microfluctuation test procedure)/TAMix ; S. typhimurium TA98	-/+ S9 (4400 mg/ml)	negative	6 histidine mutant *Salmonella* tester strains, TA7001-TA7006, each with a different base pair substitution mutation mixed in equal proportions (TAMix) in liquid cultures; concentration range tested -/+S9: 4.44-4400 mg/ml	(Kamber et al., 2009)
Bacterial reverse mutation assay/S. typhimurium TA100	<1000 ppm/3000 ppm	**positive** dose-dependent induction of revertants/plate (>600/plate at 3000 ppm)	-S9; standard plate incorporation method with slight modification for testing volatile chemicals; after 2 h vapour exposure in plastic bags, plated were removed and incubated for a further 48 h	(Mimaki et al., 2016)
Bacterial reverse mutation assay /huGSTTT1-expressing *S. typhimurium* TA100 (TA100-GST)	600 ppm/3000 ppm	**positive** dose-dependent mutagenic activity; 11.3 ±1.7 revertants/ 100 pm in TA100-pCTC, comparable to TA100-GST; no increase in number of revertants in TA100-GSTT1 over mock control (TA100-pCTC)	-S9; empty vector TA00 (TA100-pCTC) was used as mock control; after 2 h vapour exposure in plastic bags, plated were removed and incubated for a further 48 h	(Akiba et al., 2017)
SOS chromotest/*E. coli* PQ37		negative	-/+S9; SOS response as measured by b-galactosidase activity in a simple colorimetric assay	(von der Hude et al., 1988)
DNA damage, *umu* test/ *S. typhimurium* TA1535/pSK1002	-/+ S9 (5000 mg/ml)	negative only slight induction of the *umuDC-LacZ* gene expression; maximum ratio (unit treated/units control) at 2000 ug/ml (-S9)	The umu test measures the expression of the *umuDC-lacZ* by b-galactosidase activity, colorimetrically	(Yasunaga et al., 2004)

Assay/Species, strain	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
Whole-genome sequencing of *S. typhimurium* TA100 (from above)	3000 ppm/6000 ppm	**positive** Significant increases of mutagenic events in bacterial DNA versus untreated controls; mutation rates (0.2/Mb and 06/Mb in clones exposed to 3000 and 6000 ppm) were 1.4 and 5.1-fold higher vs controls Predominantly C:G to T:A transitions in TA100 exposed to 3000 and 6000 ppm, respectively TA100 clones exposed to 1,2-DCP harboured NpCpC to NpTpC changes, more prominent at 6000 ppm	Exclusion of revertant mutations occurring within the *hisG* gene Mutagenic activity especially at C:G residues	(Mimaki et al., 2016)
Mutation spectrum analysis of *his* (*hisG46* site) reversions/GSTTT 1-expressing *S. typhimurium* TA100 (TA100-GST)	3000 ppm	**positive** increases of C:G to T:A transitions over background spontaneous mutation spectrum, predominant in both TA100-pCTC and TA100-GSTT1; trinucleotide pattern CCC to CTC dramatically increased in TA100-pCTC; in TA100-GST slight increase in CCC to TCC	-S9; GSTT1 has little involvement in DCP mutagenicity	(Akiba et al., 2017)

In non-mammalian eukaryotic organisms, 1,2-DCP tested positive in *Aspergillus nidulans* eliciting a 4-fold increase in 8-azaguanine resistant mutants, compared to solvent controls at the highest concentration (Principe et al., 1981). In contrast, when used at limit dose (154 mM), 1,2-DCP was ineffective in inducing somatic segregation-related events such as mitotic non-disjunction, mitotic crossing-over and haploidisation in the same organism (Crebelli et al., 1984). 1,2-DCP failed to induce DNA repair processes in bacterial systems (von der Hude et al., 1988, Yasunaga et al., 2004).

In mammalian cells (Table 12), 1,2-DCP did not show any mutagenic activity in the *Hprt* locus in chinese hamster ovary (CHO) cells but induced mutations in the thymidine kinase (*tk*) locus in L5178Y mouse lymphoma cells with exogenous metabolic activation (Myhr and Caspary, 1991). In cholangiocarcinoma cells, neither single not repeated exposure to 1-2 DCP induced significant mutagenesis (Toyooka et al., 2017). Despite its limited mutagenic activity, 1,2-DCP consistently exhibited DNA damaging effects (i.e., induction of γ-H2AX, DNA strand breaks) and the potential for chromosomal alterations (sister chromatid exchange, aberrations) in mammalian cells, including human hepatocytes and cholangiocytes. In the latter case, dose- and time-dependent induction of γ-H2AX was reported in cells exposed to 1-10 mM 1,2-DCP, as a result of DNA double-strand breaks, which were detected in the hepatocytes, triggering the activation of the ATM signalling pathway. The DNA damage response was mediated to a different extent by CYP2E1 and ROS as γ-H2AX formation was attenuated by both specific and unspecific CYP inhibitors and an antioxidant. DNA damage was enhanced when cholangiocytes were co-cultured with human macrophages and was attributed to proinflammatory signalling from the exposed macrophages (Zong et al., 2018).

Table 12: Summary of *in vitro* genotoxicity studies in mammalian cells

Assay/Cells	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
Unscheduled DNA synthesis ([^{3}H]TdR uptake)/human lymphocytes	-/+S9 (0.01 M)	negative	-/+ S9; 4 h exposures, 1-2-DCP was not cytotoxic as assessed by trypan blue staining	(Perocco et al., 1983)
Mammalian cell gene mutation test (*Hprt* locus)/CHO cells		negative	4 h exposures	Myrh et al – 1988 Abstract (to insert manually)
Mammalian cell gene mutation test (*Tk* locus)/L5178Y mouse lymphoma cells	-S9 (800 nl/ml) +S9 10 nl/ml/80 nl/ml (2nd trial)	negative **positive** 1st trial: 2.3-fold increase at 50 nl/ml 2nd trial: dose-related increase in mutation frequency; 10-fold increase at the highly toxic dose of 80 nl/ml	+S9 two trials	(Myhr and Caspary, 1991)
Whole exome sequencing/cholangiocarcinoma cell lines NCC-CC1 and HEK293	NCC0CC1: (250 µL/plate) for single exposure; 1x (180 µL/plate) and 4x (120 µL/plate) for multiple exposure HEK293: (50 µL/plate) for single exposure	negative Only limited mutagenic activity mutation rate of two NCC-CC1 clones after a single exposure to 1,2-DCP at a cytotoxic dose was 0.1-0.5/Mb and 0.5-1.3/Mb	After 2 and 4h of vapor exposure for NCC-CC1 and HEK293, respectively, the cells were reseeded and cultured for 4 to 6 wk to isolate individual clones. For multiple exposures, NCC-CC1 cells were exposed to 1,2-DCP subsequently after recovery from	(Toyooka et al., 2017)

Assay/Cells	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
		after repeated exposure C:G to T:A transitions and C:G to A:T transversions were predominant (≈60% of total SNVs) in both cell lines	cytotoxicity, and this procedure was repeated up to five times **Nether single nor repeated exposure induced significant mutagenesis**	
SCE/CHO cells	-/+ S9: 376 mg/ml /1127 mg/ml	**positive** increased numbers of SCE/cell; top dose response (2.4 to 3.6-fold increase com-pared to DMSO controls) comparable to positive controls (Mitomycin C and Cyclophosph-amide)	Total test/control compound exposure time: 26 h (-S9) or 2 h (+S9) 50 cells scored/dose	(National Toxicology, 1986)
SCE/CHO	-S9: 113 mg/ml /1130 mg/ml[a] +S9: 376 mg/ml/ 1130 mg/ml[a]	**positive** -/+S9 the lowest effective dose produced a 20% increase in SCEs	Treatment periods were approximately 25 hr without S9 and 2 hr with S9; harvest was after 28.5 to 37.3 hr in BrdUrd; colcemid present during the final 2-3 hr	(Galloway et al., 1987)
SCE/Chinese hamster V79 cells	-/+S9: 1 mM/10 mM	**positive** -/+S9: Dose-dependent, significant increases in SCE/cell at 3.3 and 10 mM; at the high dose (10 mM), the increase was 2.7-fold and 1.8-fold, -S9 and +S9, respectively	Two independent experiments with a total of 50 analysed meta-phases per experimental point; cells exposed for 3 hr with S9 and 28 hr without S9 Mix; a shor-tened incubation without S9 for 3 h resulted in slight reduction in SCE/cell, which became more substantial upon addition metabolic activation	(von der Hude et al., 1987)
Chromosome aberrations/CH O	-/+ S9: 1370 mg/ml /1580 mg/ml	**positive** Increases in both the numbers of aberrations/100 cells and in % of cells with aberrations; At the highest concentration:	Cells incubated with test compound/solvent for 8-10 h (-S9) or 2 h (+S9); 100 cells scored/dose	(National Toxicology, 1986)

Assay/Cells	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
		8.5-fold (-S9) and 6.5-fold (+S9) increase in % cells with aberrations compared to DMSO controls		
Chromosome aberrations/ CHO	-S9: (1580 mg/ml)[a] +S9: 660 mg/ml / 950 mg/ml	**positive** -S9: positive for aberration induction when the harvests were delayed and done between 20 and 24 hr and negative or only weakly responding at 10-12 hr +S9 the lowest effective dose produced a statistically significant increase in aberrations	harvest time was 19.5-26 h from beginning of treatment	(Galloway et al., 1987)
Alkaline comet assay/MMNK-1 (human cholangiocytes) cells and co-cultures of MMNK-1/THP-1 cells (differentiated macrophages)	50 mM	**positive** increased tail DNA% (significantly) and tail moment in exposed MMNK-1; enhanced (significant for both parameters) response in co-cultures (synergistic interaction)	0 or 50 mM 1,2-DCP, 24 h exposure,	(Zong et al., 2018)
Alkaline comet assay/co-cultures of MMNK-1/THP-1 cells (differentiated macrophages)	50 mM	**positive** significant increase in tail DNA% in monocultures and both tail DNA% and tail moment in co-cultures; synergistic interaction between 1,2-DCP exposure and 'co-culture'	24 h exposure to 1-5000 mM 1,2-DCP did not reduce viability of MMNK1 cells or THP-1 cells **DNA damage induced by exposure to 1,2-DCP was enhanced in MMNK-1 cholangiocytes co-cultured with macrophages**	(Zong et al., 2019)

Assay/Cells	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
		for both parameters		
Alkaline comet assay/co-cultures of MMNK-1/THP-1 cells (differentiated macrophages)		**positive** significant increases in Tail DNA%, Tail moment and Tail olive moment in exposed co-cultures, over controls	24 h exposure at a concentration range (0.1-0.8 mM) matching the occupational exposure level in ppm (100-670 ppm)	(Ekuban et al., 2021)
Generation of γ-H2AX (immunoblotting, immunofluorescence, FACS)/ WRL-68 (normal human hepatocytes) and MMNK-1 (normal human cholangiocytes) cell lines	1 mM/10 mM	**positive** time- and dose-dependent increase of γ-H2AX levels detectable at 1 mM and within 1 h or treatment; dose-dependent increase in number and intensity of γ-H2AX foci detected in both cell lines; dose-dependent attenuation of γ-H2AX formation by non-specific CYP and CYP2E1 inhibitors in both cell lines; only partly attenuated by the antioxidant N-acetylcystein (NAC)	1mM corresponds to approximately 110 ppm in the air of workplaces Cells were treated with 5 mM 1,2-DCP (corresponding to 550 ppm) for a time-course experiment and subsequently with (1–10 mM) for a time course experiment (1–24 h) **CYP2E1 plays a critical role in γ-H2AX generation by 1,2-DCP** **Concentration of 1,2-DCP deemed "too high, relative to the estimated exposure levels of affected factory workers" in subsequent studies**	(Toyooka et al., 2017)
Generation of γ-H2AX (immunofluorescence)/MMNK-1 (normal human cholangiocytes) and co-cultures of MMNK-1/THP-1 cells (differentiated macrophages)	(500 μM) 100 μM/500 μM	negative No increase in Number of γ-H2AX foci/cell in MMNK-1 cells over controls **positive** Dose-dependent, significant increases in γ-H2AX in co-cultures at 100 (median Number of foci/nuclei: 12) and 500 μM (15) over controls (9)	-S9, 24 h exposure did not affect viability in MMNK-1 monocultures or co-cultures with macrophages, at up to 500 μM **Macrophages play a critical role in 1,2-DCP-induced DNA double strand break in MMNK-1 cells** **exposure to 1,2-DCP induces inflammatory responses and increases the levels of inflammatory cytokines, which**	(Takizawa et al., 2021)

Assay/Cells	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
		Treatment of co-cultures with TNF-α and IL-6, dose-dependently, significantly increased the number/distribution of γ-H2AX	**increased the expression of γ-H2AX.**	
Generation of g-H2AX (immunofluorescence)/ MMNK-1 (human cholangiocytes) cells and co-cultures of MMNK-1/THP-1 cells (differentiated macrophages)	(0.8 mM) 0.1 mM/0.8 mM	negative in MMNK-1 monocultures **positive** In MMNK-1+THP-1 co-cultures; significant increases in number of g-H2AX foci/nucleus at 0.4 and 0.8 mM	Experimental conditions as above; 1,2-DCP increased proliferation and cell viability over controls in in MMNK-1 monocultures but not in co-cultures; 1,2-DCP exerted cytotoxicity in co-cultures and not monocultured cells **1,2-DCP causes DNA damage in co-cultured cells (MMNK-1+macrophrages) but not monocultured cells**	(Ekuban et al., 2021)
ROS production (DCFH-DA, FACS)/WRL-68 cells	10 mM/10 mM	**positive** Increase in intracellular levels of ROS; attenuated by CYP/CYP2E1 inhibitors and NAC	WRL-68 cells treated for 4 h with a single dose of 1,2-DCP **ROS produced via the CYP2E1 metabolic process of 1,2-DCP is a major causal factor for γ-H2AX generation by 1,2-DCP**	(Toyooka et al., 2017)
ROS production (DCFH-DA)/ MMNK-1 (human cholangiocytes) cells and co-cultures of MMNK-1/THP-1 cells (differentiated macrophages)	(0.8 mM) 0.1 mM/0.8 mM	negative in MMNK-1 monocultures or THP-1 cells **positive** significantly increased ROS production in MMNK-1/THP-1 co-cultures at all dose levels	experimental conditions as above 1,2-DCP enhances ROS production in co-cultured cells but not monocultured cells	(Ekuban et al., 2021)
DNA double-strand breaks (53BP1 foci, biased sinusoidal field gel electrophoresis (BSFGE)/WRL-68 cells	1 mM/10 mM	**positive** 1,2-DCP treatment produced 53BP1 foci co-localised with g-H2AX (4 h; WRL-68); DNA double-strand breaks directly detected	WRL-68 cells treated with 5 mM 1,2-DCP for 4 h (for foci detection) and with 1-10 mM 1,2-DCP for 4 h (for double-strand breaks detection by BSFGE) **γ-H2AX formation after treatment with 1,2-DCP occurs via a DSB-**	(Toyooka et al., 2017)

Assay/Cells	Lowest effective/ highest (ineffective) dose	Findings	Remarks	Reference
		by BSFGE; g-H2AX generation attenuated by ATM inhibitor	**dependent activation of the ATM signaling pathway**	
DNA repair (transcriptomic analysis)/co-cultures of MMNK-1 (human cholangiocytes) cells with THP-1 cells (differentiated macrophages)	0.1 mM/0.4 mM	**positive** Dose-dependent upregulation of differential expression of genes involved in Base Excision Repair and other processes (e.g., LIG1, PARP4, POLD1, OGG1) and homologous recombination (HR) (e.g., NBN, RPA1) No apparent involvement of the non-homologous end joining (NHEJ) pathway	24 h exposures at 0.1 or 0.4 mM 1,2-DCP **Concentration-dependent upregulation of genes involved predominantly in BER; involvement of other mechanisms (DNA replication, cell death, other DNA repair processes)** **No significant involvement of NHEJ**	(Ekuban et al., 2022)

[a] Precipitate or immiscibility

7.6.4 Summary

In a number of genomic studies on patients' samples of exposed printing workers in Japan, occupational cholangiocarcinoma presented as heavy mutation burden tumours, with somatic mutations dominated by C:G to T:A transitions, and a characteristic unique trinucleotide mutational signature shared by all examined cases. Mutations in a number of genes, including KRAS and γ-H2AX expression were detected (Table 9).

The results of bacterial mutagenicity testing in Salmonella typhimurium TA100 and TA1535 in the presence and absence of metabolic activation were positive in early studies, while marginally positive results were later reported with the same strains, only in the absence of metabolic activation by. All other strains examined tested negative. More recent studies on TA100 have demonstrated significant increases in the number of revertants over solvent controls, without exogenous activation and without the involvement of GSTT1, and have confirmed the base substitutions as predominantly C:G to T:A transitions. DNA repair processes were not detected by relevant assays in either Salmonella strain TA1535 or E. coli (Table 11).

In mammalian cells, 1,2-DCP induced mutations in the *tk* locus in mouse lymphoma cells but not in the hprt locus in CHO cells. No significant mutagenesis was shown by whole-exome sequencing in human cholangiocarcinoma cell lines. In contrast, 1,2-DCP consistently yielded positive results in assays detecting DNA strand breaks directly or indirectly (i.e. Alkaline comet assay, γ-H2AX formation, DNA double-strand breaks assessed by electrophoresis) in human hepatocytes and chloangiocytes (Table 12). Although increased SCE rates and chromosomal aberrations were detected in vitro in chinese hamster ovary (CHO) and V79 cells, relevant tests produced negative results in the bone marrow of B6C3F1 mice, receiving 1,2-DCP by single i.p. injections, in NTP-conducted studies. No mutations were found in the *Pig-a* locus in erythrocytes of mice

exposed to 1,2-DCP by inhalation and similarly no mutations in the *gpt* locus in the liver, unless the mice were concomitantly exposed to 1,2-DCP and DCM.

The lack of *in vivo* mutagenicity was also observed in the liver of rats orally administered 1,2-DCP. Inhalation exposure generated DNA damage (including DNA double-strand breaks; detected by the same assays as in the in vitro experiments) in the liver of B6C3F1 and C57BL/6J mice (Table 10).

7.7 Carcinogenicity

IARC (2017) concluded that 1,2-DCP is *carcinogenic to humans (Group 1)*. This conclusion was based on *sufficient evidence* in humans and *sufficient evidence* in experimental animals for the carcinogenicity of 1,2-DCP.

7.7.1 Human data

The human data evaluated by IARC (2017) concerned a series of case reports and retrospective cohort studies of Japanese printing companies' employees indicating that exposure to 1,2-DCP (and/or other chlorinated solvents) increases the risk of developing cholangiocarcinoma (CCA). In addition to classifying 1,2-DCP as Group 1 Carcinogen, IARC (2017) further concluded, based on human data, that 1,2-DCP caused a rare type of bile duct /biliary tract cancer (also known as cholangiocarcinoma (CCA)).

IARC (2017) noted that the interpretation of these studies was challenging because the populations were small and workers were exposed not only to 1,2-DCP, but also to more than 20 other chemicals, including other chlorinated solvents (such as dichloromethane and 1,1,1-trichloroethane), gasoline, kerosene and printing inks. Exposure estimated in those studies were mainly based on indirect information, like amount of solvent use, instead of direct industrial hygiene measurements.

More specifically, IARC concluded: *The major challenge in evaluating the occurrence of cancer in the Japanese printing plants was to determine whether the observed excess of cholangiocarcinoma could be attributed to a specific agent, measured or unmeasured. Workers were exposed to numerous chemicals, but 1,2-dichloropropane was known to be common to all except one of the 24 cases of cholangiocarcinoma. Moreover, 6 of the cases had no exposure to dichloromethane and the Working Group's estimate of the relative risk for these cases was extremely high. Based on this evidence, the majority of the Working Group concluded that 1,2-dichloropropane is the causative agent responsible for the large excess of cholangiocarcinoma among the workers exposed to 1,2-dichloropropane, but not dichloromethane. However, a minority of the Working Group concluded that the association between 1,2-dichloropropane and cancer of the biliary tract was credible, but the role of exposure to other agents, principally dichloromethane, could not be separated with complete confidence, and noted that most of the evidence came from studies in a single plant.*

The working Group of IARC (2017) also estimated the standardized incidence ratio (SIR) for exposure to 1,2-DCP (SIR= 1053, 95%CI=386–2291) and to both 1,2-DCP and dichloromethane (SIR=1487, 95%CI=742–2660,) and concluded that "*the relative risk for 1,2-dichloropropane only was extremely high, and it was not possible to determine which agent was responsible for the relative risk in the group exposed to both 1,2-dichloropropane and dichloromethane*".

More recently ATSDR (2021) reviewed the human studies on cholangiocarcinoma risk following exposure to 1,2-DCP. In comparison to IARC (2017), eight additional publications of Japanese printing workers published since 2014. ATSDR (2021) concluded that '*Hepatic effects are a presumed health effect for humans*' as one of their hazard identification conclusions.

Japanese studies have been reviewed and adapted from (ATSDR, 2021), no more recent studies were identified. The studies have been divided into studies with an epidemiologic design including a comparison group (Table 14) and case series reports (Table 13). Some of these Japanese studies appear to be overlapping. The studies indicate that 1,2-DCP is associated with CCA. However, the interpretation of these studies is difficult because the populations were small and workers were exposed not only to 1,2-DCP, but also to more than 20 other chemicals, and exposure measurement data is lacking.

Table 13: Summary of case reports/case series on bile duct cancer (cholangiocarcinoma (CCA)) risk in Japanese printing industry workers exposed to 1,2-DCP (adapted in parts from (ATSDR, 2021))

Reference	Description	Exposure assessment	Exposure level of 1,2-DCP	Co-exposures and levels	Comments
Printing companies based in Osaka					
(Kubo et al., 2014b)	Case-series report of 17 former or current workers male print shop workers diagnosed with CCA between 1996-2012. CCA prevalence from 1981 to 2012 was 17/111 (15%). Employment duration: 6–19 years (mean: 11 years).	Exposure to 1,2-DCP, DCM, and 1,1,1-TCE determined based on job history: 1,2-DCP (used from 1991 to 2006), DCM (used from 1991 to 1996), 1,1,1-TCE (used from 1991 to 1992). No quantitative exposure assessed.	17/17 cases exposed to 1,2-DCP.	11/17 cases exposed to DCM 8/17 cases exposed to 1,1,1-TCE	All printers were employed at the printing company described by (Kumagai et al., 2013, Kumagai, 2014)
(Kumagai, 2014)	Case-series report of two CCA cases from small printing companies. Employment duration: Case 1 (26 years), Case 2 (11 years)	Exposure to 1,2-DCP and DCM determined based on installed ventilator specifications and amount of chemicals used.	Case 1: 72-5200 ppm (exposed for 11 years) Case 2: not exposed	Case 1: gasoline (exposed for 14 years) Case 2: kerosene, mixture of DCM and 1,1,1-TCE (exposed for 11 years, exposure to DCM estimated 240-6100 ppm)	Unclear whether these cases are a part of (Kumagai et al., 2013)
(Kinoshita et al., 2019)	Case report of a 41 year old patient with an advanced stage CCA previously worked at a printing company. Employment duration: 6 years	Exposure to high concentrations of 1,2-DCP and DCM. No quantitative exposure assessed.	Not assessed	Exposure to DCM not assessed	Employed at a printing company where the initial CCA cluster occurred (Kumagai et al., 2013)
Printing companies in other Japanese cities					
(Kubo et al., 2014a)	Case-series of nine printers diagnosed with CCA between 1988-2011 from 11 print shops in Japan (Osaka, Miyagi, Fukuoka, Hokkaido, Aomori, Saitama, Aichi): Employment duration: 3–19 years (mean 13 years)	Exposure to 1,2-DCP, DCM, and 1,1,1-TCE determined based on job history. No quantitative exposure assessed.	7/9 cases exposed to 1,2-DCP (during 3–16 years)	9/9 cases exposed to DCM (during 3–19 years) 4/9 cases exposed to 1,1,1-TCE (duration of exposure not reported)	Not all cases exposed to all three solvents. Two cases without 1,2-DCP exposure exposed to both DCM and 1,1,1-TCE

Reference	Description	Exposure assessment	Exposure level of 1,2-DCP	Co-exposures and levels	Comments
(Yamada et al., 2014)	Case-series report of six male printers diagnosed with CCA between 1998-2013 from three print shops (Miyagi, Fukuoka, Hokkaido) Employment duration: 10–16 years	Exposure estimates based on amounts of the chemicals reportedly used	6/6 cases exposed to 1,2-DCP (ppm): Shop 1: 80–170; Shop 2: 62–200; Shop 3: 110–240	4/6 cases exposed to DCM (ppm): Shop 1: <1; Shop 2: 0–180; Shop 3: 0–180 4/6 cases exposed to TCE: Shops 1 and 3: used (no exposure estimates) 2/6 cases exposed to DCFE: used in shop 2 (no exposure estimates)	
(Yamada et al., 2015a)	Case-series report of seven male printers diagnosed with CCA between 2002-2011 from eight print shops from five cities (Osaka, Aichi, Shizuoka, Saitama, Aomori) Employment duration: 4–19 years	Exposure estimates were based on amounts of the chemicals reportedly used	4/7 cases exposed to 1,2-DCP, shift TWA in ppm: Shop 1: 92–100; Shop 2: 16–29; Shop 4: 7–17; Shop 5: 58–210; no exposure in Shops 3, 6, 7, 8	7/7 cases exposed to DCM, shift TWA in ppm: Shop 1: 15–18; Shop 2: 25–55; Shop 3: 68–94; Shop 4: 20; Shop 5: 31–270; Shop 6: 84–90; Shop 7: 440; Shop 8: 77–110 3/7 cases exposed to 1,1,1-TCE, used in shops 5, 6, and 7 (no exposure estimates) 1/7 cases exposed to DCFE, used in shop 5 (no exposure estimates)	One printer worked in both Shop 2 and 3
(Yamada et al., 2015b)	Case-series report of five male printers and one male coater diagnosed with CCA between 1993-2013 from seven print shops and two coating shops from four cities (Fukuoka, Aichi, Tokyo, Kyoto) Employment duration: 9–30 years	Exposure estimates were based on amounts of the chemicals reportedly used	6/6 cases exposed to 1,2-DCP, shift TWA in ppm in print shops: Shop 1: 74–170; Shop 3: 200; Shop 4: 230; Shop 5: 130–160; Shop 6: 13–65; Shop 7: 59; no exposure in Shop 2 Shift TWA in ppm in coating shops: Shop 8: 19; Shop 9: 5	5/6 cases exposed to DCM, shift TWA in ppm in print shops: Shop 1: 35–140; Shop 3: 300; Shop 4: 350; Shop 5: 240–470; Shop 6: 20–98; Shop 7: 170–370; no exposure in Shops 2, 8, 9 2/6 cases exposed to 1,1,1-TCE, used in print shops 6-7 (no exposure estimates)	No case overlap with Yamada et al. 2014 or 2015a. One printer worked in Shops 2–4, and the one coater worked in Shops 8-9.

Reference	Description	Exposure assessment	Exposure level of 1,2-DCP	Co-exposures and levels	Comments
				3/6 cases exposed to DCFE, used in print shops 1 and 6 (no exposure estimates), coating shops 8-9 (no exposure estimates)	
(Ogawa et al., 2020)	Case report of male 54 years old printer worked at a printing company in Nagoya and diagnosed with CCA. Employment duration: 11 years	Exposed to high concentrations of 1,2-DCP and DCM: exposures not assessed.			Diagnosed with CCA 22 years after last exposure.

Notes: CCA, cholangiocarcinoma; 1,2-DCP, 1,2-dichloropropane; DCM, dichloromethane; 1,1,1-TCE, 1,1,1-trichloroethane; TWA, Shift time-weighted average; DCFE, 1,1-dichloro-1-fluoroethane

Table 14: Summary of epidemiological studies on bile duct cancer (cholangiocarcinoma (CCA)) risk in Japanese printing industry workers exposed to 1,2-DCP. Risk estimates are expressed in decimal form where no increase of risk equals 1.0 (adapted in parts from (ATSDR, 2021))

Reference	Description and study design	Exposure assessment	Exposure level of 1,2-DCP	Co-exposures and levels	Cancer effect and risk estimate	Value of risk estimate and 95 % CI	Comments
Printing companies based in Osaka							
(Kumagai et al., 2013)	Retrospective cohort study of 51 male printers and 11 male workers from adjacent front room employed between 1991-2006. Employment duration: 7–17 years (mean 10 years). Comparison with the general population.	Generated based on amounts of the chemicals reportedly used between 1991-2006 using experimental data by JNIOSH.	11/11 cases exposed to 1,2-DCP. Print-shop: 190–310 ppm Front-room: 70–110 ppm	10/11 cases exposed to DCM (used from 1991 to 1997/1998). Print-shop: 140–360 ppm Front-room: 50–130 ppm	Mortality. 11/51 printers (22%) and 0/11 front-room workers. SMR.	2900 (1100–6400)	Female workers excluded from the analysis.
(Sobue et al., 2015)	Retrospective cohort study of 86 male and 20 female offset colour proof printing workers	Exposure to 1,2-DCP and DCM determined based on job history.	Years of cumulative exposure to 1,2-DCP	Years of cumulative exposure to DCM. Exposure to TCE	Incidence. 17/106 (16%). SIR.	All workers: 1320 (659–2362) Male workers: 1163 (678–1862)	SIR higher for cohorts started before 1993,

Reference	Description and study design	Exposure assessment	Exposure level of 1,2-DCP	Co-exposures and levels	Cancer effect and risk estimate	Value of risk estimate and 95 % CI	Comments
	employed between 1985-2012. 1452 PY. Employment duration: not reported. Comparison with the general population.			expected from 1985-1992.			SIR tended to increase in lagged analyses for 1,2-DCP but not for DCM with a 5-year lag time.
Printing companies in other Japanese cities							
(Okamoto et al., 2013)	Retrospective cohort study of 201937 workers employed in printing and related industries, identified in JHIA database (insurance claims) and followed up between 2009-2012. Employment duration: not reported. Comparison with workers in other industries.	Not assessed	Not available	Not available	Incidence. 76/201937 (0.04%). SPRR.	All workers: 1.3 (0.9–1.8) Males: 1.3 (0.9–1.9) Males aged 30–49: 1.8 (0.6–5)	SPRR for intrahepatic higher but insignificant
(Kumagai et al., 2016)	Retrospective cohort study of 78 male and 17 female printing workers employed in three print shops of Osaka and Tokyo between 1985-2006 (71 printers, 20 front room workers, 4 delivery workers). 1367 PY. Employment duration: not reported. Comparison with the general population/internal.	Generated based on amounts of the chemicals reportedly used between 1985-2006 using experimental data by JNIOSH.	62/95 workers exposed only to 1,2-DCP. 33/95 workers exposed to 1,2-DCP and DCM. Median (range) exposure to 1,2-DCP: 3 years (0.3–15 years) Printers: November 1987–February 1996 (Osaka Plants 1-2) 1,2-DCP: 130–210 ppm March 1996–October 2006	Printers: November 1987–February 1996 (Osaka Plants 1 and 2) DCM: 65–170 ppm Front room workers: April 1991–February 1996 (Osaka Plant 2) DCM: 45–100 ppm Exposure to kerosene: all workers, low concentrations. Exposure to 1,1,1-TCE in Osaka	Incidence. 17/95 (18%). SIR, RR.	SIR: All workers: 1171 (682–1875) Male workers: 1203 (701–1927) Workers exposed to 1,2-DCP only: 1019 (374–2218) Workers exposed to 1,2-DCP and DCM: 1275 (636–2280) RR per inter-tertile increase in cumulative exposure to 1,2-DCP (ppm-years; lag=5 years): Tertile 1 (1–1199): 1 (Referent) Tertile 2 (1200–2049): 11 (3–40) Tertile 3 (2050–3499): 32	Some workers were employed in multiple plants during working history. RR adjusted for sex, age, calendar year, and exposure to DCM.

Reference	Description and study design	Exposure assessment	Exposure level of 1,2-DCP	Co-exposures and levels	Cancer effect and risk estimate	Value of risk estimate and 95 % CI	Comments
			(Osaka Plants 2-3; Tokyo Plant) 1,2-DCP: 84–346 ppm. Front room workers: April 1991–February 1996 (Osaka Plant 2) 1,2-DCP: 51–76 ppm March 1996–October 2006 (Osaka Plant 2; Tokyo Plant) 1,2-DCP: 55–130 ppm	Plants 1-2 until 1992: exposure not reported		(6–164)	

Notes: CCA, cholangiocarcinoma; 1,2-DCP, 1,2-dichloropropane; DCM, dichloromethane; SMR, standardised mortality ratio; SIR, standardised incidence ratio; PY, person-years; JNIOSH, Japanese National Institute of Occupational Safety and Health; JHIA, Japan Health Insurance Association; RR, relative risk, 1,1,1-TCE, trichloroethane; SPRR, standardised prevalence ratio; CI, confidence interval

Several European countries investigated CCA risk within the Nordic Occupational Cancer Study (NOCCA) (Vlaanderen et al., 2013) and the Rare cancer dataset (Ahrens et al., 2014). In comparison with the general population, male "printers or related workers" in Finland, Iceland, Norway and Sweden showed a statistically significantly increased risk for intrahepatic CCA (SIR=2.34, 95% confidence interval (CI)=1.45–3.57). The risk in female printers was statistically non-significantly increased (SIR=1.95, 95% CI=0.84–3.85) (Vlaanderen et al., 2013). In the population-based case-control study in nine European countries the risk of extrahepatic CCA was significantly elevated among typesetters, compared with other occupations (OR=5.78, 95% CI=1.43–23.29), but not among printing workers (OR=2.42, 95% CI=0.81–7.24). Exposure to 1,2-DCP or other solvent was not assessed in either study. There is also a potential overlap between these two studies.

(Seeherunwong et al., 2022) performed a cross-sectional study of 200 CCA patients at a tertiary hospital in north-eastern Thailand. Among 11 cases of work-related CCA, six were exposed to asbestos as roof workers. Other five CCA patients were exposed to 1,2-DCP and dichloromethane and had various occupations (printing worker, mechanic, worker at film factory, vocational teacher and welder). Authors noted that mean duration of employment was 19 years and men latency period of 27 years.

Incidence rate of CCA varies according to genetic composition and geographical variation in risk factors. In Europe, the incidence rate varies from 0.45/100,000 in Switzerland to 3.36/100,000 in Italy (Bridgewater et al., 2014). Moreover, the high rate in some Asian countries seems to be related to high prevalence of liver fluke infections (Banales et al., 2016). Risk factors for cholangiocarcinoma include liver cholestatic disease, infections, inflammatory disorders, toxins, metabolic conditions, genetic disorders, and occupational exposure to chemicals (asbestos, vinyl chloride, etc.) (Farioli et al., 2018, Labib et al., 2019). The main molecular pathogenesis for chemical genotoxins is thought to be cholangiocyte DNA damage (Khan et al., 2008).

7.7.2 Animal data

The (NTP, 1986) study provided "*equivocal evidence for carcinogenicity*" in female F344/N rats, based on dose-related, marginally increased adenocarcinomas in mammary tissue (adjusted rates: 2.7%, 4.7% and 26.7% in the vehicle, 125 mg/kw bw/day and 250 mg/kg bw/day groups, respectively), concurrent with reduced survival and body weight gain. Mammary gland adenocarcinomas are relatively uncommon in female F344/N rats, however those observed at the toxic high dose level at a significant rate, were neither metastatic, nor anaplastic or highly invasive. Increased non-neoplastic liver lesions in dosed female rats (foci of clear cell change and necrosis) did not coincide with an increase in liver tumours. No significant or treatment-related increases in tumour incidences were observed in male rats treated with 1,2-DCP up to 125 mg/kg bw/day 1,2-DCP, thereby providing 'no evidence of carcinogenicity' in males.

In concurrent studies in B6C3F1 mice, *"some evidence of carcinogenicity"* was provided by dose-related increases in the incidence of hepatocellular neoplasms, primarily adenomas, in both males and females. Liver adenoma incidences were increased in treated males (adjusted rates: 29%-45%; significant increase in the high dose group) and in females (17-19%), relative to concurrent controls (20% in males and 3% in females). Non-significant numerical increases in the incidence of liver carcinomas were also observed in exposed animals of both sexes. The combined incidence of liver adenomas and/or carcinomas was significantly higher in high dose males and in all exposed females, compared to controls. Such hepatocellular tumours are common in control B6C3F_1 mice with a combined historical range of 14-46% for males and 2-14% for females ((adenomas and carcinomas; six NTP testing laboratories; (National Toxicology, 1986). Non-neoplastic liver lesions such as hepatocytomegaly and hepatic necrosis were also observed at an increased rate in treated male mice. Thyroid tumours (follicular cell adenomas or carcinomas, combined) were significantly higher in the high dose females compared to controls (adjusted rates 20.8% vs 2.9%), but no tumours were observed in the low dose

group or in any male mice at statistically significant incidences. As a result, NTP did not conclude whether these lesions were related to 1,2-DCP exposure. The major findings from the NTP studies and the principal target organs for 1,2-DCP carcinogenicity in both species are summarised in Table 15.

Table 15: Summary of animal carcinogenicity studies

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
F344/N rats (n=50/sex/dose	Oral administration of 1,2-DCP (>99% pure) in corn oil by gavage; 0, 125 or 250 mg/kg bw (F); 0, 62 and 125 mg/kg bw (M)[a]; 5 d/wk for 103 wk	Similar/comparable to OECD TG 451 Reduced survival relative to controls for high dose F (32% alive at end of study vs 74% and 86% in the low dose and vehicle control groups, respectively); 250 mg/kg bw/day in F was therefore deemed toxic); reduced mean body weight in high dose M (-14%)/F(-24%) vs controls **Mammary adenocarcinoma** (in the 0, 125 and 250 mg/kg bw/day dose groups): F: 1/50 (2.7%)[b], 2/50 (4.7%), 5/50 (26.7%)* dose-related, marginal increases in F, in the high dose group (>historical controls); concurrent with decreased survival and mean body; MTD was therefore exceeded; no other treatment-related effects in M; no increase in liver tumours in M/F NTP concluded: "*no evidence for carcinogenicity*" in M; NOAEL (carcinogenicity)=125 mg/kg bw/day "*equivocal evidence of carcinogenicity*" in F, based on mammary lesions in the high dose group	(NTP, 1986)
B6C3F$_1$ mice, M/F, (n=50/sex/dose)	Oral administration by gavage; 0, 125 or 250 mg/kg bw/day (M/F); 5 d/wk for 103 wk	Similar/comparable to OECD TG 451 Reduced survival relative to controls for the high dose F group (52% alive at termination of study vs 70% of vehicle controls), possibly related to reproductive tract infections; body weights unaffected in M/F **Liver adenoma** (in the 0, 125 and 500 mg/kg bw/day dose groups)**:** M: 7/50 (20%)[b], 10/50 (28.8%), 17/50 (45.5%)* F: 1/50 (2.9%), 5/50 (17.2%), 5/50 (19.2%) **Liver carcinoma** (in the 0, 125 and 500 mg/kg bw/day dose groups)**:** M: 11/50 (28.1%)[b], 17/50 (41.9%),	(NTP, 1986)

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
		16/50 (37.3%) F: 1/50 (2.9%), 3/50 (9.7%), 4/50 (12.6%) **Combined adenoma or carcinoma** (in the 0, 125 and 500 mg/kg bw/day dose groups): M: 18/50 (46.7%), 26/50 (62.9%), 33/50 (74.7%)** F: 2/50 (5.7%), 8/50 (26.4%)*, 9/50 (30.8%)* NTP concluded: *"some evidence of carcinogenicity"* in M/F based on the incidence of hepatocellular neoplasms	
F344/DuCrj (SPF) M/F rats (n=50/sex/dose)	Inhalation, 0, 80, 200, or 500 ppm (v/v); 6 h/day, 5 days/wk for 104 wk (2 years) (500 ppm identified as the MTD); DCP of analytical grade (>99.5% pure) used for airflow by vaporisation	Studies conducted in accordance with OECD principles of GLP; no significant differences in survival rates in either M/F; significant decreases in body weights (-11% in M; -8% in F) in the high dose (MTD) groups, compared to controls **Nasal cavity lesions: neoplastic; papilloma** (in the 0, 80, 200 and 500 ppm groups): M: 0/50 (0%)[c], 0/50 (0%), 3/50 (6%), 15/50 (30%)** F: 0/50 (0%), 0/50 (0%), 0/50 (0%)54, 9/50 (18%)** 3 cases of esthesioneuroepitheliomas (2 in the 80 and one in the 200 ppm dose groups) in M only; deemed 'exposure-related' by the authors as no occurrences in historical controls. **Pre-neoplastic** (in the 0, 80, 200 and 500 ppm groups)**:** squamous cell hyperplasia M: 0/50, 2/50, 6/50*, 27/50** F: 0/50, 0/50, 3/50, 20/50** Hyperplasia of the transitional epithelium: M: 0/50, 31/50**, 39/50**, 48/50** F: 2/50, 21/50**, 39/50**, 48/50**	(Umeda et al., 2010)
$B6D2F_1$ mice, M/F, (n=50/sex/dose)	Inhalation, 0, 32, 80 or 200 ppm (v/v); 6h/day , 5 days/wk for 104 wk; DCP of analytical grade (>99.5% pure)	GLP study conducted according to OECD TG 451; no adverse effects in survival or body weights **Lung; bronchiolo-alveolar adenoma and/or carcinoma** (in the 0, 32, 80 and 200 ppm dose groups): M: 9/50, 18/50*, 14/50, 18/50* (>historical control data; 'not clear if	(Matsumoto et al., 2013)

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
	used for airflow by vaporisation	exposure-related'[d] F: 2/50, 4/50, 5/50, 8/50* (>historical control data; 'exposure-related'[d]) **Harderian gland adenoma** (in the 0, 32, 80 and 200ppm dose groups)**:** M: 1/50, 2/50, 3/50, 6/50 (>historical control data; significant positive trend; 'exposure-related'[d]) **Liver; Histiocytic sarcoma** (in the 0, 32, 80 and 200ppm dose groups)**:** M: 1/50, 4/50, 7/50*, 0/50 **Spleen; haemangioma and/or haemangiosarcoma** (in the 0, 32, 80 and 200ppm dose groups)**:** M: 0/50, 4/50, 3/50, 6/50* (within the maximum incidences of historical control data; 'not clear' if exposure related)	
Syrian hamster/n=24/group	gavage; In the 1st wk, hamsters in groups 1-3 (n=24/group) were injected s.c with BOP 4x (on d. 1, 3, 5, and 7) at a dose of 10 mg/kg bw to initiate hepatobiliary and pancreatic carcinogenesis; hamsters in groups 4 and 5 received vehicle (0.9% saline); 1 wk after the last BOP/vehicle treatment, BOP initiated hamsters in (groups 1-3) were administered 1,2-DCP by gavage at doses 0, 62.5, and 125 mg/kg b.w., 5 d/wk; 15 wk (9	17-and 19-wk experiment, final body weights were significantly decreased in the group treated with 125 mg/kg b.w. 1,2-DCP after BOP initiation (group 3) compared with the group given BOP alone; **Liver:** no significant differences in incidence or multiplicity of atypical biliary hyperplasia or hepatocellular adenomas between the groups treated with 1,2-DCP after BOP initiation and the group given BOP alone in wk17 and 19. 1,2-DCP treatment had no effect on the development of cholangioma; one cholangioma was observed in the group treated with 62.5 mg/kg b.w. 1,2-DCP after BOP initiation in the 19-week experiment but not in any other group. No atypical biliary hyperplasia, cholangioma or hepatocellular adenoma was observed in the group treated with 1,2-DCP alone in the 19-wk experiment **Pancreas:** 17-wk; increase in the incidence of pancreatic ductal adenocarcinoma in the group treated with 125 mg/kg b.w. 1,2- DCP after BOP initiation (3/9, 33.3%) compared with the group given BOP alone (0/9, 0%); however no significant difference in 19-wk between BOP alone and any combination groups	(Gi et al., 2015b)

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
	animals from each group) or 17 wk (15 animals from each group); non-initiation groups were administered 125 mg/kg b.w. 1,2-DCP (group 4; 9 animal) or corn oil vehicle (group 5; 6 animals) in the same manner for 17 weeks	**Lung:** no significant differences in the incidence or multiplicity of lung tumours between the combination groups and BOP-only **Kidney:** One renal cell tumour observed in the group treated with 62.5 mg/kg b.w. after BOP initiation No lung/kidney tumours in the group receiving 125 mg/kg b.w. 1,2-DCP alone	

[a]Lower doses were chosen for the M rats compared to F because of the greater mortality observed for M during a preceding 13-week, dose-range finding study; [b]Incidence data are expressed as number of animals bearing lesions at a specific anatomic site/total number of animals in which that site was examined or animals necropsied; in brackets the incidence rate adjusted for survival unless otherwise stated; [c]overall incidence rate; [d] as per authors *statistically significant ($p \leq 0.05$) or **($p \leq 0.01$) by Fisher's Exact test or χ^2-test

The carcinogenic potential of 1,2-DCP was further probed in two 2-year inhalation studies in male and female F-344 rats and B6C3F1 mice (Umeda et al., 2010, Matsumoto et al., 2013)

Chronic exposure by the inhalation route of F344 rats to 500 ppm 1,2-DCP significantly increased the incidences of nasal papillomas in rats of both sexes, with no effect on survival (Umeda et al., 2010). Three cases of esthesioneuroepitheliomas were reported in the nasal cavity of exposed male rats only, with no occurrence in the high dose group, nor in any female rats at any exposure level. However, since the historical control data showed no cases of esthesioneuroepithelioma in 48 two-year carcinogenicity studies, it was concluded that the esthesioneuroepithelioma was induced by inhalation exposure to 1,2-DCP. Significant, dose-dependent increases in the incidences and severity of pre-neoplastic lesions comprising hyperplasia of the transitional epithelium (at all dose levels) and squamous cell hyperplasia were observed in both sexes. Squamous cell metaplasia, inflammation of the respiratory epithelium, and atrophy of the olfactory epithelium were also seen in all exposed animals and were evident at the lowest DCP concentration. No exposure-related lesions were observed in any other organs in the DCP-exposed rat groups of either sex. The (Umeda et al., 2010) inhalation study findings suggest that 1,2-DCP is a nasal carcinogen.

A subsequent 2-year inhalation carcinogenicity study in B6D2F1 male and female mice exposed to 32-200 ppm 1,2-DCP was conducted by (Matsumoto et al., 2013). The incidence of bronchiolo-alveolar carcinomas in the lungs of females increased dose-dependently, compared to controls, but since this increase was within the maximum incidences of the historical control data, it could not be unequivocally attributed to DCP exposure. In contrast, the combined incidences of bronchiolo-alveolar adenomas and carcinomas in females was both significantly higher in the 200 ppm group compared to controls and exceeded the maximum incidences of the historical control data. This finding was therefore deemed "*exposure-related*". The combined bronchiolo-alveolar lesions were also significantly increased in males exposed to 32 and 200 ppm, compared to control animals, exceeding the maximum incidences of historical controls. However, as these neoplasms did not display dose-dependency, this effect in male mice was deemed

equivocal. Male mice also presented a concentration-dependent increase in the incidence of Harderian gland adenomas, exceeding the historical control data and attributed therefore to 1,2-DCP exposure. In the liver, the incidence of histiocytic sarcoma was significantly increased only in male mice exposed to 80 ppm. There were no other increased incidences of substance-related neoplastic hepatic lesions, including hepatocellular adenomas and/or carcinomas, in any 1,2-DCP-exposed group of either sex. In the spleen, the incidence of haemangiosarcomas alone or in combination with haemangiomas was significantly increased only in males exposed to 200 ppm, but this observation was within the maximum incidences of historical control data and was therefore considered an equivocal finding. No other tumour induction was reported in any other organs, including mammary gland, of 1,2-DCP-exposed male or female mice. Different types of non-neoplastic lesions were observed in both sexes. In the nasal cavity, atrophy in the olfactory epithelium was increased in both males (significantly) and females exposed to ≥80 ppm, while respiratory metaplasia of the submucosal gland significantly increased in in both males and females exposed to 200 ppm. In the kidney, basophilic change in the proximal tubules and mineralisation of the cortex were significantly increased in all male-exposed groups. No other increases in pre-neoplastic lesions in the lungs (e.g. bronchiolo-alveolar hyperplasia) or in the liver (altered cell foci) were reported. The difference in the target organs for carcinogenicity in rodents between NTP and the two subsequent studies has been attributed by the authors of the latter to the different routes of exposure. The nasal and lung carcinogenicity of inhaled 1,2-DCP in rats and mice, respectively, is thought to result from direct exposure of the corresponding tissues to the inhaled substance entering through the lungs and the nasal cavity, while the induction of hepatocellular tumours in mice in the NTP study, is thought to result, at least in part, from the orally administered 1,2-DCP entering the liver after gastrointestinal absorption. The distinct sites of carcinogenicity between rats and mice, when both species were exposed via inhalation were in turn attributed to interspecies differences.

In an attempt to demonstrate cholangiocarcinogenesis in experimental animals, (Gi et al., 2015b) assessed the effects of 1,2-DCP on N-nitrosobis(2-oxopropyl)amine (BOP)-induced cholangiocarcinogenesis in male hamsters in a short-term study (up to 19 weeks). 1,2-DCP did not enhance the development of BOP-induced atypical biliary hyperplasia, associated with the development of bile duct cancer and did not induce any lesions in liver bile duct when administered alone. Additionally, 1,2-DCP had no effect on the proliferative activity of bile duct epithelial cells regardless of BOP-initiation, suggesting that 1,2-DCP lacks a promoting effect on BOP-induced cholangiocarcinogenesis and is not cholangiocarcinogenic to the hamster, in that model. Finally, 1,2-DCP lacked modifying effects on BOP-induced pancreatic or lung carcinogenesis in hamsters.

7.7.3 Summary

Small epidemiological studies, case reports and small case series in Japanese printing houses indicate that 1,2-DCP is associated with a rare type of bile duct /biliary tract cancer known as cholangiocarcinoma (CCA). However, the interpretation of these studies is challenging because the populations were small and workers were exposed not only to 1,2-DCP, but also to more than 20 other chemicals, including other chlorinated solvents (such as dichloromethane and 1,1,1-trichloroethane), gasoline, kerosene and printing inks. However, as summarised by IARC, 1,2-DCP was known to be common to all except one of the 24 cases of CCA. Exposure estimated in those studies were mainly based on indirect information, like amount of solvent use, instead of direct industrial hygiene measurements. Studies outside Japan have identified an increased risk of intra- an extrahepatic CCA in the printing industry but have not evaluated to risk by exposure to 1,2-DCP or any other specific exposure.

1,2-DCP is carcinogenic in experimental animals following both chronic inhalation and oral exposure. There is evidence for respiratory tract carcinogenesis following inhalation exposure (nasal tumours in rats, lung tumours in mice) and some evidence for neoplastic lesions in the Harderian gland and spleen in male mice. Following oral administration,

there is equivocal evidence of mammary tumours in female rats and some evidence on hepatocellular neoplasms in male and female mice. 1,2-DCP induced cholangiocarcinogenicity, observed in humans, was not demonstrated experimentally in a model system in the hamster, while no occurrences were observed in rats and mice.

7.8 Reproductive toxicity

7.8.1 Human data

There are no human data regarding effects of 1,2-DCP on fertility or developmental toxicity.

7.8.2 Animal data

Male Sprague-Dawley rats were exposed by gavage to 1,2-DCP in corn oil at 0, 100, 250, 500, or 750 mg/kg bw/d for 10 consecutive days or 13 weeks (5 d/week). See section 7.3.2.2 for general toxicity. Histopathology of the animals in two highest doses revealed effects in the testis (degeneration, reduction in sperm production), and in the epididymis (increased number of degenerate spermatogonia) within 10 days and 13 weeks for the high and mid dose, respectively (Bruckner et al., 1989, as reported in the abstract and in (WHO, 2003, ATSDR, 2021, Fan and Alexeeff, 1999)).

No effects in the testes were reported in other repeated dose or in carcinogen studies in rats or mice, see section 7.3.2.2. In female rats, significantly increased incidences of mammary gland hyperplasia and mammary tumours was reported in animals exposed from 250 mg/kg bw/d in a 2 year cancer study (5 d/week), no effects on reproductive organs were reported in repeated dose studies at doses up to 1000 mg/kg bw/d for 13 weeks (WHO, 2003).

F344 rats were exposed from 3 weeks (7 d/week, 8 h/d) to 1,2-DCP at concentrations of 0, 50, 100 or 200 ppm (8, 6, 6, 9 females, respectively). Prior to exposure, three consecutive oestrous cycles were monitored using a vaginal smear test, only rats exhibiting regular cycles were used in the experiment. The number of cycle per rat decreased (non-significant) in a dose dependent manner (0, -7, -11, -16%, respectively), and the total cycles lasting ≥6 days (all rats combined/group) was significantly increased at ≥100 ppm (8.3, 12, 54, 47%, respectively). The number of ovulated ova per rat decreased in a dose dependent manner and significantly at the top dose (0, -21, -28, -35%, respectively), in addition rats with no ovulation were reported. The author concluded that exposure to 1,2-DCP was associated with disruption of the oestrous cycle through the inhibition of normal ovulation which resulted from the injury of preovulatory antral follicles (Sekiguchi et al., 2002).

Groups of Sprague-Dawley rats (30, sex, dose) received 1,2-DCP in drinking water at concentrations of 0, 0.24, 1, or 2.4 g/L (w/v), over two generations (equivalent to 0, 33.6, 140, or 336 mg/kg bw/d). The highest concentration of 2.4 g/L is the maximum attainable concentration due to water solubility. Decreased water consumption, due to low palatability, was reported at all dose levels in both generations, which also caused a dose-related decreases in F0 body weight at the two highest doses levels. The decrease in body weight was significant in both F0 and F1 high doses. These differences in water intake and body weights were also evident among females during gestation and/or lactation. In the low dose, a minor effect on water consumption and body weights was observed in the absence of other adverse effects. There were no treatment-related gross pathological changes reported in any dose groups, and the histological changes were limited to increased hepatocellular granularity in both sexes in both generations at all dose levels. In both generations and sexes, no effects were observed on the reproductive function and morphology. The study authors attributed the significantly lower neonatal body weight and slightly increased neonatal mortality observed in the high dose to the decreased maternal water intake, rather than a direct effect of 1,2-DCP exposure. There were no external

malformations at in the mid and low dose groups, however offspring were not assessed for skeletal or visceral malformations or variations. In addition, no evidence of dominant lethal toxicity was observed in all dosed males (Kirk et al., 1990; as summarized by (Fan and Alexeeff, 1999, WHO, 2003, OECD SIDS, 2005)).

In a range finding teratogenic study, Sprague-Dawley rats (10, dose) received 0, 50, 125, 250 or 500 mg/kg bw/d of 1,2-DCP by gavage on GD6-15. Dose-related toxic effects were noted at all treatment levels on day 1 in some of the battery of observational tests including decreased respiration, movement, muscle tone and extensor thrust reflex and increased salivation, lacrimation and perineal urine staining. However, there seemed to be accommodation to the effects so that only the highest two doses elicited any of these effects subsequently. The three highest dose groups showed decreased maternal weight gain and feed consumption on GD6-9 of gestation while the highest dose group also had decreased haematological values and continued to have a decreased body weight through day 16. No effects on reproduction (numbers of implants, resorptions, litter size, foetal population composition) were reported (Dow Chemical, 1989 as reported in (EPA, 2016)).

In a developmental study, SD female rats (30, group) received 0, 10, 30, or 125 mg/kg bw/d of 1,2-DCP by gavage in corn oil from GD6 to 15. In the high dose, clinical signs of toxicity some related to transient CNS depression (decreased movement, muscle tone, and extensor thrust reflex and increased salivation and lacrimation) were observed the first day of exposure in 6 to 23 dams (0 to 1 in control). These signs were less frequent (1 to 3 dams) after the second dose, and not observed in the other treated groups. Food and water consumption was significantly decrease (~25% both) in the high dose dams between GD6-9 or GD6-15 for food and water, respectively. In this dose, decreased body weight and body weight gain was reported, however no information is available on corrected body weight. There were no significant differences in organ weight between treated animals and controls. Significant increase in the incidence of delayed ossification of skull bones was observed on the pups from high dosed dams which the study authors attributed to maternal toxicity. No effects were reported at the low and mid doses in dams or pups ((Hanley et al., 1990), (Kirk et al., 1995) as reported in (EPA, 2016, Fan and Alexeeff, 1999)).

In a developmental study, New Zealand white rabbits (18, group) received 1,2-DCP by gavage (0, 15, 50, or 150 mg/kg bw/d) on GD7-19. At the high dose, decreased food consumption (with intermittent episodes of anorexia), significantly lower weight gain, and anaemia were reported. Significant increase in the incidence of delayed ossification of skull bones observed in pups from the high dose dams, which was considered by the study authors to be secondary to maternal toxicity. No effects were reported at the low and mid doses in dams or pups ((Hanley et al., 1990), (Kirk et al., 1995) as reported in (EPA, 2016, Fan and Alexeeff, 1999)).

7.8.3 Summary

In a repeated dose study, rats exposed to 1,2-DCP at and above 500 mg/kg bw/d showed effects on the testis (degeneration, reduction in sperm production), and in the epididymis (increased number of degenerate spermatogonia). These findings were not observed in other repeated dose or in carcinogenicity studies. In another repeated dose study via inhalation, exposure to 1,2-DCP was associated with disruption of the oestrous cycle. No effects on sexual function and fertility were observed in a 2-generation study in rats exposed via drinking water up to 336 mg/kg bw/d; the effects on pups observed at the highest dose were attributed to lower water intake in dams. No significant reprotoxic effects were reported in rats or rabbits in developmental studies. In these studies, the maternal toxicity (decrease body weight or body weight gain, transient CNS depression) was considered the cause for the decreased skull ossification reported in the pups of high dosed dams. Overall, there is some evidence that 1,2-DCP interferes with the animal fertility by altering sperm production or quality and by prolonging oestrus cycles.

8. Other considerations

8.1 Mode of action (MoA) considerations

Studies on the genotoxicity of 1,2-DCP, presented in sections 7.6.2 and 7.6.3 yielded conflicting results in *in vitro* and *in vivo* assessments. 1,2-DCP was not positive in the dominant-lethal assay in Sprague-Dawley rats exposed through drinking water, yet *in vitro*, it induced sister chromatid exchange and chromosomal aberration in CHO and V79 cells, both with and without exogenous metabolic activation. To date, there is no direct evidence for the formation of DNA adducts by 1,2-DCP or its metabolites, and the mechanisms by which 1,2-DCP induces DNA damage observed in cultured cells, in animals but also in specimens from occupationally exposed workers who developed cholangiocarcinoma, remain elusive. Furthermore, oral exposure to 1,2-DCP by gavage was not found be cholangiocarcinogenic in hamsters (Gi et al., 2015b).

Genomic DNA damage, considered to be an initiating factor to carcinogenicity has been assessed mainly by the comet assay and the phosphorylation of H2AX. γ-H2AX is considered a sensitive marker of mainly DNA double-strand breaks, with 1,2-DCP-related increases in expression demonstrated *in vitro*, *in vivo* and in cholangiocytes from specimens from workers exposed to 1,2-DCP in the offset printing factory (Sato et al., 2014). As 1,2-DCP belongs to the dihalogenated hydrocarbons, it could be assumed that the above changes are due to the formation of episulfonium ion after conjugation with glutathione, which is highly reactive to DNA (Guengerich et al., 1987, Kumagai et al., 2013, Zhang et al., 2015). However, a study using deuterium DCP did not support the formation of episulfonium ion from 1,2-DCP in rats (Bartels and Timchalk, 1990).

The relevance and potential contribution of human 1,2-DCP exposure to mutagenesis and cholangiosarcoma carcinogenesis was proposed by (Mimaki et al., 2016), following genomic analysis of tumour tissues from four cases of occupationally exposed printing workers. Whole-exome sequencing revealed a characteristic mutational profile, shared by, and unique to the four occupational cholangiosarcoma samples investigated, comprising high somatic mutation burden, substantial strand bias in C:G to T:A mutations and unique trinucleotide mutational changes. Mutations with transcriptional strand bias are known to occur in cancer genomes as a result of the formation of bulky DNA adducts, such as in smoking-related lung cancer and ultraviolet-associated melanoma (Mimaki et al., 2016); references therein). The authors speculated that DNA adducts on G residues, preferentially processed by transcription-coupled DNA repair mechanisms could induce strand-biased mutations. One of the predominant mutational signatures detected in this study, NpCpY (the complement to RpGpN) is a target site for electrophillic agents such as alkylating agents and platinum-derived drugs, which at highly reactive nucleophilic sites such as the N^7 and O^6 positions of guanine, can form alkyl-DNA adducts or intra- and inter-strand DNA cross-links, and exert cytotoxicity and mutagenicity. A similar mutational signature to that of 1-2 DCP was reported by (Olivier et al., 2014). This signature was encountered in acquired mutations caused by overexpression of activation-induced cytidine deaminase (AID) in immortalized human TP53 knock-in mouse fibroblasts. Aberrant AID expression, previously implicated in somatic hypermutation and lymphoid and non-lymphoid malignancies has been associated with chronic tissue injury in parenchymal cells such as hepatocytes and gastric epithelial cells. 1,2-DCP induced inflammation could therefore be a contributing factor to the mutational profile observed in the printing workers' cholangiocarcinomas, by modulating AID expression (Mimaki et al., 2016); references therein).

The genotoxic potential of 1,2-DCP as a result of DNA damage was investigated in studies, examining specifically the induction of phosphorylated histone H2AX (Toyooka et al., 2017). Previously, immunohistological analysis of specimens obtained from 1,2-DCP cholangiocarcinoma cases had showed an increase in γ-H2AX foci of BilIN, IPNB, invasive carcinoma, and non-neoplastic biliary epithelial cells, compared to specimen from control of common cholangiocarcinoma (Sato et al., 2014). Toyooka et al. (2017) reported 1,2-

DCP-related increase in γ-H2AX foci both *in vitro* in treated human hepatocytes and cholangiocytes and *in vivo*, in the liver of mice exposed by inhalation. The induction of γ-H2AX and a related increase in intracellular reactive oxygen species (ROS) were significantly attenuated *in vitro* by CYP2E1 inhibition. It was suggested therefore that ROS produced via a cytochrome P450 2E1-mediated metabolic process is a major causal factor for the 1,2-DCP-induced DNA damage Subsequent studies however did not corroborate the ability of 1,2-DCP to directly produce γ-H2AX in cholangiocytes at (lower) concentrations believed to better correspond to the occupational exposure concentration levels.

A series of subsequent studies employed a model of co-cultures of cholangiocytes and differentiated macrophages. (Zong et al., 2018) highlighted the potential role of AID in 1,2-DCP-induced cholangiocarcinoma, as previously proposed by (Mimaki et al., 2016). 1,2-DCP exhibited pro-inflammatory properties, increasing cytokine TNF-α expression in THP-1 macrophages, while TNF-α treatment upregulated the expression of AID, through the NF-kB pathway. The 1,2-DCP-induced ectopic over-expression of AID resulted in an increase in DNA damage in the co-cultures. These findings pointed to a critical role of macrophages and a cross-talk between inflammatory responses mediated through cytokine release and an aberrant expression of AID and the resulting genotoxicity. Histopathological examination of surgically-obtained tissue specimens from eight patients who developed cholangiocarcinoma after exposure to 1,2-DCP, had previously revealed proliferation and high infiltration of inflammatory cells in various sites of the bile ducts in the noncancerous hepatic tissues (Kubo et al., 2014b).

Furthermore, oral treatment of experimental animals with 1,2-DCP resulted in inflammatory cell infiltration in the liver parenchyma (Bruckner et al., 1989). AID on the other hand, as a DNA editing enzyme, deaminates cytidine residues into uracil (C to U), causing U:G mismatches, which can potentially lead to a C to T mutation. Such alterations could evade repair systems such as mismatch repair and excision repair or result in error-prone repair leading to mutations in critical, cancer-related genes. AID has indeed been reported to produce mutations and translocations through induction of DSBs in such genes including the tumour suppressor gene p53, protooncogene c-Myc, and BCL6 gene (B-cell lymphoma 6) (Chesi et al., 2008, Robbiani et al., 2008, Takai et al., 2009, Shen et al., 1998). In addition, under inflammatory conditions, macrophage-generated cytokines can also indirectly contribute to DNA damage increasing the mutational burden and ultimately contributing to tumour development. On the other hand, exposure to 1,2 DCP may enhance the proliferation of cholangiocytes, which has been demonstrated to be mediated by CYP450 in mice and in cultured cholangiocytes (Zhang et al., 2018, Zong et al., 2019, Ekuban et al., 2021).

The induction of DNA damage only in the presence of macrophages in exposed co-cultures, was further confirmed by (Takizawa et al., 2021). In this studyIL-1b was also identified as one of the cytokines produced by macrophages which would lead to 1,2-DCP-induced DNA double strand breaks, detected as γ-H2AX. (Ekuban et al., 2021) employing the same co-culture model showed that total DNA strand breaks and double strand breaks were increased in the presence of macrophages, compared to monocultures. Additionally, (Ekuban et al., 2021) demonstrated a significantly enhanced production of ROS in exposed co-cultures. The authors proposed that the increased ROS could be attributed to inflammation, as both TNF-α and IL-1b are known to stimulate the production of ROS in cells. Alternatively, ROS could result as a by-product of a CYP2E1 metabolic process. The elevated ROS levels could also partly account for the increased DNA damage observed in 1,2-DCP-exposed co-cultured cells via oxidative stress Oxidative base lesions such as the highly mutagenic guanine derivative 7,8-dihydro-8-oxoguanine (8-oxoG) and the corresponding ring fragmented purine formamidopyrimidine derivative (FapyG) or abasic sites are predominantly repaired by base excision repair (BER) and to a lesser extent by nucleotide excision repair.

Oxidative DNA lesions can lead to DNA double-strand break (DSB) formation originating from single strand breaks (SSB) during repair, excision of base, topoisomerase cleavage, DNA replication or transcription ((Ekuban et al., 2022); references therein). Over time, this could result in genomic instability, diseases, and cancer.

In a recent follow-up study, Ekuban et al. (2022) performed transcriptomic analysis of supernatant from treated co-cultures. 1,2-DCP upregulated the expression of BER genes in cholangiocytes in the co-cultures, whereas it upregulated the expression of cell cycle-related genes in THP-1 macrophages. These findings suggest that the 1,2-DCP-induced DNA damage is substrate for BER, which has been previously shown to be required for the processing of AID-induced lesions into DNA double strand breaks (Stratigopoulou et al., 2020). The involvement of other mechanisms such as DNA replication, cell death or other types of DNA repair was not excluded, considering the multifaceted roles of repair enzymes. LIG1 and POLD1 for example which were differentially upregulated, are also related to replication and other DNA repair pathways and PARP4 is involved to apoptosis or transportation as a vault protein. The dose-dependent upregulation of DNA repair genes suggests an increase in DNA damage which could enhance mutations in cells. Indeed, the DNA damage in cholangiocytes co-cultured with THP-1 macrophages has been shown to be 1,2-DCP dose-dependent. Additionally, 1,2-DCP might directly induce the proliferation of the THP-1. It should be noted that this study did not show a significant change in the mRNA expression levels, as previously reported, of some key cytokines, such as TNF-α, IL-1β, and IL-6 in the co-cultures. Similarly, no AICDA upregulation was detected under the experimental conditions of this study.

8.1.1 Summary

Even though the exact mechanism of action is not fully understood at present and data on genotoxicity are not uniform across test systems and species, 1,2-DCP has to be regarded as a genotoxic, non-threshold carcinogen. This is based especially on human data, where defined signature mutations have been identified in exposed workers, even though specific underlying DNA lesions have not been identified. Additionally, there is evidence for additional DNA damage amplifying effects. Thus, recent studies employing a co-culture model suggest a cross-talk between cholangiocytes and macrophages upon exposure to 1,2-DCP. Inflammatory responses through TNF-α, IL-1β and IL-6 signalling, overexpression of AID and ROS production can lead to the potentiation of DNA damage; proliferation of cholangiocytes but also macrophages. The accumulation of the latter at the site of injury following exposure to 1,2-DCP may modulate inflammatory responses, which can potentially create an immune milieu favourable for cholangiocarcinogenesis.

8.2 Lack of specific scientific information

Not identified.

8.3 Groups at Extra Risk

No groups at extra risk were identified.

9. Evaluation and recommendations

9.1 Cancer risk assessment

9.1.1 Published approaches for cancer risk assessment

No published cancer risk assessments with quantitative dose-responses and cancer risk estimate calculations were found.

(Hartwig and MAK Commission, 2021) concluded that the critical effect of 1,2-DCP is the occurrence of bile duct tumours in humans. There are irritations after inhalation as well as liver and kidney dysfunctions after oral and dermal absorption in humans and rodents.

Haemolytic effects are observed in mice after inhalation. Furthermore, very high concentrations can lead to central nervous system depression in humans. A unique mutational signature found in the cholangiocarcinoma tissue of occupationally exposed printing workers indicates a genotoxic mode of action and/or the involvement of a perturbed immune response for the aetiology of these tumours. In inhalation studies, 1,2-DCP caused an increased incidence of lung adenomas in mice at 32 ml/m^3 and tumours in the nasal cavity in rats at 500 ml/m^3 with a statistically significant trend. *In vivo* studies in animals did not find any significant clastogenic or mutagenic effects after prolonged inhalation or oral administration. Therefore, the substance was not considered to be a germ cell mutagen. 1,2-DCP is absorbed via the skin in toxicologically relevant amounts and remains designated with a skin notation by DFG. A sensitizing potential is not expected according to DFG.

(EPA, 2016) concluded that the 1,2-DCP is 'Likely to Be Carcinogenic to Humans' and summarised the human data as "Recent human epidemiological studies and case-series reports in Japanese workers indicate a potential correlation between occupational exposure to 1,2-DCP (and other solvents) and cholangiocarcinoma". However, EPA considered that the data are insufficient to support their highest weight-of-evidence descriptor ('Carcinogenic to Humans').

As noted in Section 7.7.1, (ATSDR, 2021) performed a systematic review of health hazards and noted that cancer is among the most sensitive health effects of 1,2-DCP. The available evidence suggests that 1,2-DCP is not a potent mutagen, but it can cause DNA and chromosomal damage under certain conditions.

9.1.2 Cancer risk assessment

There is clear evidence of carcinogenic effects of 1,2-DCP in humans. In particular, increased incidences of bile duct tumours have been reported among exposed workers. The available data indicates a genotoxic, non-threshold mode of action, and a unique mutational signature has been found in the cholangiocarcinoma tissue of occupationally exposed workers. However, no robust human exposure data is available, and therefore it is not possible to any derive exposure-risk relationship (ERR) on the basis of human data.

Instead, ERR was derived from animal data. The 2-year mouse inhalation study by (Matsumoto et al., 2013) was identified as the key study, showing carcinogenic effects (bronchoalveolar adenomas/carcinomas) at low dose levels. The dose-response correlations reported were however not very clear and were not suitable for benchmark dose modelling. Therefore, T25 was used to identify the point-of-departure for bronchoalveolar adenoma/carcinoma findings (LOAEC 32 ppm). Calculations included the following steps:

1) T25 was calculated as:

T25 = C*(reference incidence (0.25) / (incidence at C – control incidence) * (1-control incidence) / 1

T25 = 32 ppm * (0.25/(18/50 – 9/50)) * (1 – 9/50)/1

= 36.5 ppm (171.5 mg/m^3) 1,2-DCP

2) The T25 value was adjusted to correspond to worker exposure conditions (40 years, 48 weeks/year, 8 h/day, and correction for the inhalation volume for workers at light physical activity. No allometric scaling is needed for inhalation exposure

T25(worker)= 171.5 mg/m^3 * (75/40 years) * (52/48 weeks) * (6/8 h) * (6.7/10 m^3) = 175 mg/m^3

3) Additional lifetime cancer risks were calculated as follows according to a linearised approach (high to low dose extrapolation)[14]

Exposure concentration representing a $1*10^{-5}$ risk = 175 mg/m^3 / 25.000=

0.007 mg/m^3

Assuming linearity, excess life-time cancer risks were calculated and are presented in Table 16.

Table 16: Cancer exposure-risk relationship (bronchoalveolar adenomas/ carcinomas) after working life exposure to a given 8-hour air concentration for five working days a week over a 40-year working life period

1,2-DCP (mg/m^3)	Excess life-time cancer risk (Cases per 100 000 exposed)
0.007	1
0.028	4
0.07	10
0.28	40
0.7	100
2.8	400

9.2 Derived Occupational Exposure Limit (OEL) Values

9.2.1 Published approaches to establishing OELs

No published approaches to establishing OELs for 1,2,3-TCP were found.

9.2.2 Occupational Exposure Limits (OELs) - 8h TWA

9.2.2.1 Derivation of OEL (8 h TWA)

Sufficient information is available to conclude on a non-threshold MoA for carcinogenic action, which is considered the critical effect for exposure to 1,2-DCP. For that reason, it is not possible to derive a health-based OEL, and exposure-risk relationships (ERR) were calculated from animal data (see section 9.1.2). A quantitative cancer risk assessment based on human data was not considered feasible because human cancer studies lack exposure data.

If an OEL was derived from data on threshold effects, the nasal hyperplasia findings observed at 15 ppm (70.5 mg/m^3; LOAEC) in the 13-week rat (inhalation exposure 6 h /day, 5 days/week) study (Dow Chemical, 1988a as reported in (ATSDR, 2021, EPA, 2016) could be used as the starting point. Other studies had higher NOAEC/LOAEC values.

Correction of the starting point to correspond to worker exposure conditions: 70.5 mg/m^3 * 6h/8h * 6.7/10 mg/m^3= 35 mg/m^3. Assessment factors proposed to be applied include: a factor of 3 for LOAEC to NOAEC extrapolation, a factor of 2 for subchronic to chronic, 2.5 to cover interspecies differences, and 5 for worker intraspecies differences. Application of these factors would lead to:

OEL (8h TWA): 35 mg/m^3 / 3*2*2.5*5 ≈ 0.5 mg/m^3.

14 https://echa.europa.eu/documents/10162/17224/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258?t=1353935239897

9.2.2.2 Uncertainties

Reports on exposed workers clearly indicate that exposure to 1,2-DCP is associated with a rare type of bile duct /biliary tract cancer (CCA). Unfortunately, there is no information on exposure levels and therefore this data cannot be used to set an OEL or derive ERR. Bile duct findings were not reported in chronic animal inhalation or oral studies. Therefore, there is some level of uncertainty on the human relevance of the ERR that was derived on the basis of lung adenoma/carcinoma findings.

9.2.3 Short Term Exposure Limits (STELs)

The available data does not indicate a need to propose a STEL.

9.2.4 Biological Limit Value (BLV)

There are some methods available to measure urinary levels of 1,2-DCP and its (unspecific) metabolite, but biomonitoring has not been commonly used at workplaces. No BLV is proposed.

9.2.5 Biological Guidance Value (BGV)

No robust information on background levels of 1,2-DCP or specific metabolites in the general population was found. No BGV is proposed.

9.3 Notations

Accidental dermal exposure to a paint containing 1,2-DCP caused significant renal and hepatic function effects in the exposed person. This indicates marked systemic uptake via the skin, and therefore a ‘skin’ notation is proposed.

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