DICHLOROMETHANE

Dichloromethane was reviewed previously by the Working Group in 1987 and 1998 (IARC, 1987, 1999). New data have since become available, and these have been incorporated, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

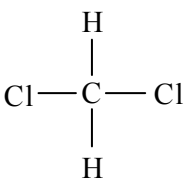
Chem. Abstr. Serv. Reg. No.: 75-09-2

Chem. Abstr. Serv. Name: Dichloromethane

IUPAC Systematic Name: Dichloromethane

Synonyms: Methane dichloride; methylene bichloride; methylene chloride; methylene dichloride

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: CH_2Cl_2

Relative molecular mass: 84.93

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless liquid with penetrating ether-like odour (O'Neil et al., 2006; Haynes, 2010)

Boiling point: 40 °C

Melting point: –97.1 °C

Density: d_4^{20} 1.327 g/mL

Solubility: Slightly soluble (1.38 g/100 mL) in water at 20 °C; soluble in carbon tetrachloride; miscible in ethanol, diethyl ether, and dimethylformamide

Volatility: Vapour pressure, 58.2 kPa at 25 °C; relative vapour density (air = 1), 2.93 (Verschueren, 1996)

Stability: Vapour is nonflammable and is not explosive when mixed with air, but may form explosive mixtures in atmospheres with higher oxygen content (Sax, 1984)

Reactivity: Reacts vigorously with active metals (lithium, sodium, potassium) and with strong bases (potassium *tert*-butoxide) (Sax, 1984)

Octanol/water partition coefficient (P): log P, 1.25 (Hansch et al., 1995)

Table 1.1 Methods for the analysis of dichloromethane

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	0.4 µg/sample	NIOSH (1998)
	Adsorb on charcoal; desorb with toluene	GC/ECD	0.002 µg/sample	
	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	94 µg/m^3	OSHA (1990)
	Adsorb on carbon-based molecular sieve; desorb with 99:1 mixture of carbon disulfide/dimethylformamide in anhydrous sodium sulfate	GC/FID	697 µg/m^3	
	Air collected in specially prepared canister; desorb on cold trap	GC/MS	0.84–1.38 ppm [2.97–4.87 µg/m^3]	EPA (1999a)
		GC/ECD	NR	
		GC/FID	NR	
		GC/PID	NR	
	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS	NR	EPA (1999b)
		GC/ECD	NR	
		GC/FID	NR	
		GC/PID	NR	
Water	Purge with inert gas and trap; desorb to GC	GC/PID	NR	EPA (1995a)
		GC/ECD	0.02 µg/L	EPA (2013)
		GC/MS	0.18 µg/L	EPA (2009)
		GC/MS	0.14 µg/L	
	Purge with inert gas and trap; desorb to GC	GC/MS	0.03 µg/L	EPA (1988)
	Add internal standard (isotope labelled dichloromethane); purge with inert gas and trap; desorb to GC	GC/ MS	10 µg/L	EPA (1996c)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	NR	EPA (1996b)
		GC/HECD	0.02 µg/L	
	Purge with inert gas and trap; and various other methods	GC/MS	5 µg/kg (soil/ sediment) 500 µg/kg (wastes) 5 µg/L (groundwater)	EPA (1996a)

ECD, electron capture detection; FID, flame ionization detection; GC, gas chromatography; HECD, Hall electrolytic conductivity detection; MS, mass spectrometry; NR, not reported; PID, photoionization detection

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 3.53 ppm; calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm.

1.1.4 Technical products and impurities

Dichloromethane is available in several grades according to intended end use: technical grade; aerosol; vapour degreasing; special; urethane; and Food Chemicals Codex/ National Formulary (food and pharmaceutical applications). Purity, when reported, ranges from 99% to 99.99%. Acidity (as hydrochloric acid) may be up to 5 mg/kg. The maximum concentration of water in these grades of dichloromethane is 100 mg/kg (Rossberg et al., 1986; Holbrook, 1993; Dow Chemical Co, 1995; Vulcan Chemicals, 1995, 1996a, b, c, d).

Small amounts of stabilizers are often added to dichloromethane at the time of manufacture to protect against degradation by air and moisture. The following substances in the listed concentration ranges are the preferred additives

(wt%): ethanol, 0.1–0.2; methanol, 0.1–0.2; cyclohexane, 0.01–0.03; and amylene (2-methyl-2-butene), 0.001–0.01. Other substances have also been described as being effective stabilizers, including phenols (phenol, hydroquinone, *para*-cresol, resorcinol, thymol, 1-naphthol), amines, nitroalkanes (nitromethane), aliphatic and cyclic ethers, epoxides, esters, and nitriles (Rossberg et al., 1986; Holbrook, 1993).

1.1.5 Analysis

Methods for the analysis of dichloromethane in air, solids, liquids, water, and food have been reviewed by ATSDR (2000) and HSDB (2012). Selected methods for the analysis of dichloromethane in various matrices are presented in Table 1.1. Exposures to dichloromethane can also be monitored in air using a direct-reading infrared analyser, with a minimum detectable concentration of 0.7 mg/m^3 (0.2 ppm) (Goelzer & O'Neill, 1985).

Exposure to dichloromethane can be monitored in samples of blood, breath, or urine (ATSDR, 2000; WHO, 2000; SCOEL, 2009). Urinary concentrations of dichloromethane in humans are reported to correlate well with exposure concentrations in air (Di Vincenzo et al., 1972; SCOEL, 2009). The concentration of dichloromethane or carboxyhaemoglobin (COHb) levels are measured in blood (SCOEL, 2009). Since the relationship between alveolar carbon monoxide (CO) and COHb has not been well established for workers exposed to dichloromethane, breath analysis for CO cannot be considered as providing definitive quantitative information regarding exposure to dichloromethane (WHO, 2000).

1.2 Production and use

1.2.1 Production

Dichloromethane was first prepared in 1840 by the chlorination of methyl chloride in sunlight. It became an industrial chemical of importance during the Second World War (Rossberg et al., 1986). Two commercial processes are currently used for the production of dichloromethane: hydrochlorination of methanol and direct chlorination of methane (Rossberg et al., 1986; Holbrook, 1993; ATSDR, 2000).

Global production of dichloromethane increased from 93 000 tonnes in 1960 to an estimated 570 000 tonnes in 1980 (IARC, 1986), and is estimated to range from 764 000 to 814 000 tonnes per year from 2005 to 2010 (OECD/SIDS, 2011). In 2009, dichloromethane was produced by 26 manufacturers worldwide and was available from 133 suppliers (NTP, 2011). Production and imports of dichloromethane in the USA totalled 45 000–227 000 tonnes between 1996 and 2006 (NTP, 2011). In the European Union, the total tonnage band for dichloromethane was reported to be 100 000 to 1 000 000 tonnes per year (ECHA, 2016). The production and import of dichloromethane reported in Japan was 58 000 tonnes in 2011 (METI, 2013).

1.2.2 Use

Most of the applications of dichloromethane are based on its solvent properties (IARC, 1999). The principal uses worldwide comprise paint stripper (23–50%), aerosol solvents and propellants (10–25%), process solvent in the chemical and pharmaceutical industry (10–20%), and metal degreasing (8–13%) (WHO, 1996; IARC, 1999). The distribution of uses varies considerably among countries (OECD, 1994). Dichloromethane has also been used in the production of cellulose fibre, in the manufacture of photographic film, in textile manufacturing, for extraction of food flavourings and decaffeination

of coffee, as a blowing agent for polymer foams, in production of hydrofluorocarbon refrigerants, and in pesticides (OECD, 1994; IARC, 1999; NTP, 2011; EPA, 2012). Use of dichloromethane in Europe and the USA has been declining since the 1970s (Holbrook, 1993; WHO, 1996; EPA, 2012).

(a) Paint stripper

For use in paint strippers, dichloromethane is typically blended with other chemical components (Holbrook, 1993; WHO, 1996). Dichloromethane has been the major component of nearly all solvent-based paint stripper formulations for industrial, professional, and consumer use; the aircraft industry and military are important users (OECD, 1994). Alternative paint strippers have come onto the market (Joe et al., 2013), and paint-strippers containing dichloromethane are no longer permitted for professional or consumer use in Europe, although they remain available elsewhere (European Commission, 2009; Joe et al., 2013).

(b) Aerosols

Dichloromethane is used as propellant and solvent in aerosol products including paints, automotive products, adhesives, and hair sprays (WHO, 1996; ATSDR, 2000; NTP, 2011). The use of dichloromethane in consumer aerosol products has declined in the USA (ATSDR, 2000), and dichloromethane is no longer permitted for use in cosmetic products in the USA since 1989 (FDA, 1989).

(c) Process solvent

In chemical processing, dichloromethane is used in the manufacture of polycarbonate plastic, the manufacture of photoresist coatings, and as a solvent carrier for the manufacture of insecticides and herbicides. It is used by the pharmaceutical industry as a process solvent in the manufacture of steroids, antibiotics, vitamins and, to a lesser extent, as a solvent in the coating of tablets. Other uses include oil de-waxing, in inks and adhesives, and in plastics manufacture (Rossberg et al., 1986; Holbrook, 1993; IARC, 1999).

(d) Metal cleaning

In the metalworking industries, dichloromethane is used as a vapour degreasing solvent, or blended with petroleum and other hydrocarbons as a dip-type cleaner (IARC, 1999). In the manufacture of metal products, cleaning is needed before painting, plating, plastic coating, etc. Degreasing in the engineering industry is normally carried out with special equipment in which dichloromethane is used either in the liquid or vapour phase. Dichloromethane is also used in the electronics industry in the production of circuit boards and as a stripper for photoresists (OECD, 1994). In Japan and elsewhere, dichloromethane has widely been used for metal cleaning as an alternative solvent to 1,1,1-trichloroethane after the implementation of the Montreal Protocol on Substances that Deplete the Ozone Layer (OECD, 1994).

(e) Printing industry

Dichloromethane is a major ingredient of cleaning solvent used to remove printer ink during the offset printing process. For efficient manual wiping with a cloth, dichloromethane is often blended with other halogenated hydrocarbons or kerosene to adjust its evaporation rate. Almost all the dichloromethane in the solvent evaporates into the working environment. It is to be noted that offset printing is usually carried out indoors, sometimes with limited ventilation to ensure that temperature and humidity are kept constant (Kumagai et al., 2013). Offset proof printing requires frequent cleaning interventions, and offset web printing sometimes includes manual wiping under the machine, both of which lead to high concentrations of vapour in the breathing zone.

Ink for a three-dimensional printing process has been developed using a fast-drying thermoplastic solution comprising polylactic acid dissolved in dichloromethane (Guo et al., 2013).

(f) Other uses

Dichloromethane is used as feedstock in the production of hydrofluorocarbon-32 (HFC-32) refrigerant (difluoromethane). The demand for HFC-32 as a replacement chemical for HFC-22 (chlorodifluoromethane) may increase the use of dichloromethane in the USA (EPA, 2012).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

(a) Natural occurrence

Dichloromethane is not known to occur naturally.

(b) Outdoor air

Background levels from remote monitors in the USA in operation since 2003 have shown that the concentration of dichloromethane in air in isolated locations is very low (mean, 0.1 μg/m³) (McCarthy et al., 2006).

Levels of dichloromethane are higher in urban areas than in rural areas. For example, at 13 urban monitoring centres in the USA in 1996, the geometric mean concentration of dichloromethane varied from 0.05 to 0.24 ppb by volume (0.28 to 0.85 μg/m³) (Mohamed et al., 2002). In the 1990s, the concentration of dichloromethane at 22 urban sites in Canada was reported as being between 0.5 μg/m³ and 10 μg/m³ (Government of Canada, 1993).

There is also seasonal variation. In China, dichloromethane was one of the five most abundant volatile organic compounds measured in air at 14 sites in 9 cities in the south-eastern coastal region. The average concentration of dichloromethane in air was 50.2 μg/m³ in winter (range, 12.4–113 μg/m³) and 10.1 μg/m³ in summer (range, 6.3–22.8 μg/m³) (Tong et al., 2013).

Generally, the concentrations of dichloromethane in industrial areas tend to be much higher than those in residential and administrative areas. In a study of six different areas within Haicang, China, the mean levels of dichloromethane in two industrial areas were 102.0 μg/m³ and 219.1 μg/m³, in the harbour was 69.80 μg/m³, in surrounding residential and administration areas were 119.60 μg/m³ and 112.00 μg/m³, while in the background site in forests at a distance of 20 km, the level was 8.2 μg/m³ (Niu et al., 2012). Similarly, mean concentrations of dichloromethane were 42.5 μg/m³ in a biopharmaceutical plant in China and 3.5 μg/m³ in a residential area nearby (Pan et al., 2011).

(c) Indoor air

Eight-hour average concentrations of dichloromethane were measured in a range of indoor environments in China as follows: home, 1.0–1.3 μg/m³; office, 0.03 μg/m³; school, 0.1 μg/m³; restaurant, 3.3 μg/m³; shopping mall, 0.7 μg/m³; city train, 0.8 μg/m³; and bus, 0.4 μg/m³ (Guo et al., 2004).

A report from Canada quoted a study from 1988 that found that the mean concentration of dichloromethane in 757 homes was 16.3 μg/m³ (Government of Canada, 1993).

(d) Water

Dichloromethane has been detected in surface water and groundwater samples taken at hazardous waste sites and in drinking-water in Europe, the USA, Canada, and Japan. Concentrations in many water samples are below the limit of detection (ATSDR, 2000). Dichloromethane was measured in more than 5000 wells in the USA between 1985 and 2002; in 97% of samples, concentrations of dichloromethane were below maximum contaminant levels (MCLs). Dichloromethane was detected in 3% of samples, with concentrations ranging from 0.02 to 100 μg/L. These positive samples were

mainly collected in agricultural areas, which may be a result of transformation of carbon tetrachloride used as a grain fumigant (Moran et al., 2007).

A report on dichloromethane in Canada summarized a range of measurements, and found that mean concentrations of dichloromethane in municipal drinking-water supplies in Canada during the 1980s ranged from 0.2 µg/L to 2.6 µg/L (Government of Canada, 1993). Measurements in groundwater near known spills were extremely high. For example, 25 years after the rupture of a storage tank near Toronto, the measured dichloromethane in groundwater was 25 × 106 µg/L. Mean concentrations in surface water were low (generally < 1 µg/L).

(e) Food

In the 1970s, dichloromethane was detected in decaffeinated coffee and tea, with levels ranging from < 0.05 to 4.04 mg/kg in coffee, and < 0.05 to 15.9 mg/kg in tea (Page & Charbonneau, 1984). Because of concern over residual solvent, most decaffeinators no longer use dichloromethane (ATSDR, 2000).

In an investigation of several halocarbons in table-ready foods, 8 of the 19 foods examined contained dichloromethane at concentrations above the quantification limit (0.008 ppb), with the following ranges reported: butter, 1.1–280 µg/kg; margarine, 1.2–81 µg/kg; ready-to-eat cereal, 1.6–300 µg/kg; cheese, 3.9–98 µg/kg; peanut butter, 26–49 µg/kg; and highly processed foods (frozen chicken dinner, fish sticks, pot pie), 5–310 µg/kg (Heikes, 1987).

1.3.2 Occupational exposure

The principal route of exposure in occupational settings is inhalation (ATSDR, 2000).

Occupational exposure to dichloromethane may occur in several industries. Workers may be exposed during the production and processing of dichloromethane, or during use of products containing dichloromethane, particularly when the end product is sprayed or otherwise aerosolized (ATSDR, 2000).

Monitoring data for dichloromethane up to 1999 have been reviewed previously (IARC, 1999). More than 1.4 million workers in the USA and approximately 250 000 workers in Europe were estimated to be potentially exposed to dichloromethane in the 1980s and 1990s (IARC, 1999; NIOSH, 2013). Exposure occurred across a range of industries, levels varying widely by operation and within operation. Concentrations of dichloromethane exceeding 1000 mg/m^3 were recorded in paint stripping, in the printing industry, and in the manufacture of plastics and synthetic fibres. Full-shift exposures to dichloromethane at concentrations exceeding 100 mg/m^3 were thought to have occurred in furniture-stripping shops, and in certain jobs in the aeronautical, pharmaceutical, plastic, and footwear industries (IARC, 1999).

In 2012, the United States Environmental Protection Agency (EPA) reviewed available historical studies that had monitored dichloromethane concentrations in workers stripping paint (EPA, 2012). Many of the studies included a very small numbers of exposed workers, and the results may not be generalizable. Exposure levels varied widely. For example, aircraft refinishing was reported to result in 8 hour time-weighted average (TWA) exposures of 86–3802 mg/m^3 (25–1096 ppm) in different studies between 1994 and 2002. Workers stripping paint from metal, wood, or aircraft and furniture refinishing were all potentially exposed to 8 hour TWA exposures exceeding 1000 mg/m^3.

Many of the industries in the EPA report do not now use dichloromethane (see Section 1.4). Data published since 2000 are summarized in Table 1.2. Levels now tend to be lower than earlier reports, with measured values in printing, polyurethane manufacture, and automotive and aircraft maintenance tending to be lower than 150 ppm. Studies in furniture-stripping

Table 1.2 Measured occupational exposures to dichloromethane

Industry (location)	Job classification	Concentration	Reference
Printing workers (USA)	Cleaning presses	7 ppm	Lee et al. (2009)
Furniture stripping (USA)	Stripping and rinsing using tank	39–332 ppm 6 ppm (with controls installed)	Estill et al. (2002)
	Spray stripping using compressed air	44–647 ppm TWA < 2 ppm (with controls installed)	Fairfax & Grevenkamp (2007)
Automotive industry, technicians (USA)	Chemical paint stripping	26–120 ppm TWA	Enander et al. (2004)
Polyurethane manufacture (USA)	Mix and heat ingredients in oven	8 ppm TWA	Fairfax & Porter (2006)
Aircraft maintenance (Taiwan, China)	Paint stripping	4 hours average varied from 14–84 ppm	Uang et al. (2006)
Laboratory workers (Japan)	No details given	Below LOD (about 1 ppm)	Nomura et al. (2006)

LOD, limit of detection; ppm, parts per million; TWA, time-weighted average

plants showed that the installation of exposure surveillance was effective in reducing exposures to below 10 ppm (Estill et al., 2002; Fairfax & Grevenkamp, 2007).

A new concern has been identified in connection with bathtub refinishing. In 2012, the United States Occupational Safety and Health Administration identified 13 fatalities associated with stripping agents containing dichloromethane that had been investigated in nine states during 2000–2011. These deaths occurred when products containing between 60% and 100% of dichloromethane were used to refinish bathtubs in bathrooms with inadequate ventilation and without use of respiratory protective equipment. Autopsy specimens showed blood concentrations of dichloromethane ranging from 18 to 223 mg/L in the six decedents for whom values were recorded; a concentration of < 2 mg/L is expected in a person working within the allowable air standard for the USA. Air concentrations of dichloromethane associated with such work were estimated to exceed 100 000 ppm (Chester et al., 2012).

Levels of exposure to dichloromethane were estimated in a printing company in Osaka, Japan, after the identification of a cluster of cancers of the biliary tract among workers at the plant (Kumagai et al., 2013). The circumstances of exposure were quite specific in that the workers removed ink from rollers using volatile solvents between 300 and 800 times per day, and the room was poorly ventilated. There was co-exposure for several years to both dichloromethane and 1,2-dichloropropane (see the *Monograph* on 1,2-Dichloropropane in the present volume). No monitoring was undertaken at the time, so the Japanese National Institute of Occupational Safety and Health undertook a reconstruction experiment to estimate exposure concentrations on the assumption that the exposure was proportional to the amount of chemical used. Estimated values of exposure to dichloromethane in the room where proofs were printed ranged from 80 to 210 ppm (278–728 mg/m^3], with a mean of 140 ppm (486 mg/m^3) in 1991–1992 and were higher in later years (mean, 360 ppm, equal to 1249 mg/m^3) (Table 1.3). The estimated exposures in the front room were estimated to be 50 ppm (173 mg/m^3) in 1991–1993, and 130 ppm (451 mg/m^3) in 1992–1998 (Kumagai et al., 2013).

In another case series of printing workers with cholangiocarcinoma in Japan, estimated concentrations of dichloromethane were modelled for the jobs in which the cases had worked (Yamada et al., 2014). The estimated shift TWA for two of

Table 1.3 Estimated exposure to dichloromethane and 1,2-dichloropropane among printers associated with clusters of cholangiocarcinoma in Japan[a]

Location	Job classification and years	Number of workers	Estimated shift-TWA of dichloromethane (ppm)	Estimated shift-TWA of 1,2-dichlorophenol (ppm)	Reference
Osaka	Proof printing (reconstruction)	50–100	130–360	60–210	JNIOSH (2012)
	1991–1993		80–210	120–430	Kumagai et al. (2013)
	1992–1998		190–540	100–360	
	1998–2006		NR	150–670	
Miyagi	Offset web printing 1992–2011	2	NR	80–170	Yamada et al. (2014) based on government survey data
Fukuoka	Offset web printing 1970–2008	3	0–150	62–200	Yamada et al. (2014) based on government survey data
				110–5200	Kumagai (2014)
Hokkaido	Proof printing 1985–1995	2	60–180	110–240	Yamada et al. (2014) based on government survey data
Aichi	Proof printing 1984–1995	1	240–6100	–	Kumagai (2014)

[a] The Working Group noted that the upper limits of these scenarios were estimated with the worst-case scenarios.
h, hour; NR, not reported; ppm, parts per million; TWA, time-weighted average

the six workers was below 1 ppm. The other four workers were exposed to estimated shift TWAs of between 28 ppm (97 mg/m^3) and 180 ppm (620 mg/m^3). The highest levels were estimated for years before 1995. Additional details of the Japanese case-series studies are given in Section 2 of the *Monograph* on 1,2-Dichloropropane in the present volume.

1.3.3 Exposure of the general population

There are few data on exposure levels to dichloromethane of the general population. People may be exposed to dichloromethane from air, water, food, or during the use of consumer products containing dichloromethane (ATSDR, 2000). Exposure of the general population to dichloromethane may be much higher from indoor air than from outdoor air, especially from spray painting or use of other aerosols or consumer products containing dichloromethane as a solvent (ATSDR, 2000).

In the United States National Health and Nutrition Examination Survey (NHANES) study in 2003–2004, only 7 of the 1165 blood samples (0.6%) collected showed detectable levels of dichloromethane (CDC, 2009).

1.4 Regulations and guidelines

Several jurisdictions have acted to reduce the use and release of various volatile organic compounds, including dichloromethane. The California Air Resources Board was one of the first jurisdictions to regulate dichloromethane; in 1995, it limited the levels of total volatile organic compounds (VOCs) contained in aerosol coating products. Subsequent regulations prevented manufacture, sale, supply, or application of any aerosol coating product containing dichloromethane (Air Resources Board, 2001). California has also prohibited the manufacture, sale, or use of automotive cleaning and degreasing products containing dichloromethane.

In Japan, the environmental quality standards for dichloromethane state that outdoor air levels shall not exceed 0.15 mg/m³ (Ministry of the Environment Government of Japan, 2014).

A guideline value of 3 mg/m³ for 24-hour exposure is recommended by WHO. In addition, the weekly average concentration should not exceed one seventh (0.45 mg/m³) of this 24-hour guideline (WHO, 2000).

In the European Union, the VOC Solvent Emissions Directive (Directive 1999/13/EC) was implemented for new and existing installations on 31 October 2007 (European Commission, 1999). The Directive aims to reduce industrial emissions of VOCs from solvent-using activities, such as printing, surface cleaning, vehicle coating, dry cleaning, and manufacture of footwear and pharmaceutical products. Installations conducting such activities are required to comply either with emission limit values or with a reduction scheme. Reduction schemes allow the operator to reduce emissions by alternative means, such as by substituting products with a lower solvent content or changing to solvent-free production processes. The Solvents Directive was implemented in 2010 into the Industrial Emission Directive 2010/75/EU (IED).

The European Union has also restricted the use of paint strippers containing dichloromethane as of 2009 (Decision 455/2009/EC of the European Parliament amending Council Directive 76/769/EEC) as regards restrictions on the marketing and use of dichloromethane (European Commission, 2009). As noted above, dichloromethane-based paint strippers are banned for consumer and professional use. They may still be used in certain industrial applications with improved labelling and safety measures.

In the USA, the EPA National Emission Standards for Hazardous Air Pollutants (NESHAP) in 2008 adopted specific management practices to minimize emissions of dichloromethane in area sources that engage in paint stripping and spray application of coatings (EPA, 2008).

Occupational exposure limits for dichloromethane in air tend to be 50 ppm [176.5 mg/m³] over 8 hours, with United Kingdom permitting up to 100 ppm [353 mg/m³] (Table 1.4).

Table 1.4 International limit values for occupational exposure

Country	Limit value (8 hours)	
	ppm	mg/m³
Australia	50	174
Austria	50	175
Belgium	50	177
Canada, Québec	50	174
China	NR	200
Denmark	35	122
France	50	178
Germany, AGS	75	260
Hungary	NR	10
Ireland	50	174
Japan[a]	50	NR
Latvia	NR	150
New Zealand	50	174
Poland	NR	88
Singapore	50	174
Republic of Korea	50	175
Spain	50	177
Sweden	35	120
Switzerland	50	180
USA, OSHA	25	NR
United Kingdom	100	350

From GESTIS (2014)

[a] Notification on Standards for Work Environment Evaluation (No. 79 issued in 1988, amended in 2004) http://jaish.gr.jp/horei/hor1-18-2-1-2.html

AGS, Committee on Hazardous Substances (Ausschuss für Gefahrstoffe); NR, not reported; OSHA, Occupational Health and Safety Administration; ppm, parts per million

Biological monitoring regulations and recommendations

SCOEL (2009) recommended a biological monitoring limit value for dichloromethane in blood of 1 mg/L, and for dichloromethane in urine of 0.3 mg/L, both for samples collected at

the end of a working shift. These figures were considered comparable to an 8-hour limit value of 100 ppm (353 mg/m^3) for dichloromethane in air. The ACGIH recommended a Biological Exposure Index of 0.3 mg/L in urine at the end of a shift (ACGIH, 2012).

The Swiss authorities recommended a limit of 0.5 mg/L in blood (Suva, 2014). The Deutsche Forschungsgemeinschaft has provided the correspondence between concentrations in air and dichloromethane in blood (DFG, 2012).

2. Cancer in Humans

2.1 Introduction

Information about the risk of cancer associated with exposure to dichloromethane is available from cohort studies of occupational exposure among workers producing cellulose triacetate fibres and films, a cohort study of aircraft workers exposed to multiple solvents including dichloromethane, and case–control studies of several different cancers and occupational exposure to solvents. In addition, several studies have been conducted to investigate the occurrence of cancer of the liver among workers in the printing industry in Japan who were exposed to dichloromethane, 1,2-dichloropropane, and other solvents. Those studies are reviewed in the *Monograph* on 1,2-Dichloropropane in the present volume. While some other studies have been conducted in facilities where dichloromethane was mentioned as having been used (Ott et al., 1985; Shannon et al., 1988), only studies that reported estimates of association specifically for cancer and dichloromethane are reviewed here.

Only the cohort studies of cellulose-triacetate facilities provide quantitative measures of exposure to dichloromethane. While the availability of such information on exposure is the principal strength of these studies, the relatively small number of exposed workers is an important limitation. Among the case–control studies, most investigated cancers of the lymphohaematopoietic system, or cancers of the brain and central nervous system. The case–control studies typically assessed exposure to multiple solvents, including dichloromethane, in a semi-quantitative or qualitative manner, using expert judgment, job-exposure matrices or occupational titles. These studies consequently have limited ability to evaluate exposure–response patterns. However, those case–control studies that involved interviews with the subjects may provide improved ability to developed detailed work histories and account for non-occupational risk factors, to the extent those are relevant.

2.2 Occupational cohort studies of workers exposed to dichloromethane

Table 2.1 summarizes cohort studies of workers exposed to dichloromethane.

Lanes et al. (1993) conducted a cohort study of mortality among workers employed in the production of cellulose triacetate fibre in the USA who were potentially exposed to dichloromethane, extending earlier analyses by Ott et al. (1983a, b) and Lanes et al. (1990). The cohort consisted of 1271 workers employed between 1954 and 1976, and followed until 1990. Based on a combination of personal and area samples, median exposure levels (8-hour TWA) in 1977 were reported to be 140, 280, and 475 ppm [486, 971, 1650 mg/m^3] in three main work areas, but no dose–response analysis was performed. The workers had been also exposed to acetone and methanol. Standardized mortality ratios (SMRs) were elevated for cancer of the liver and biliary tract (SMR, 2.98; 95% CI, 0.81–7.63; 4 cases). Each of the deaths due to cancers of the liver and biliary tract occurred among employees with ≥ 10 years of employment and ≥ 20 years since

Table 2.1 Cohort studies on cancer and occupational exposure to dichloromethane

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Lanes et al. (1993) USA, 1954–1990	1271 (551 men and 720 women)	Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1954–76	Malignant neoplasms	Overall	39	0.82 (0.77–1.04)	Results based on mortality records; adjusted for age, sex, race and calendar period Co-exposures: acetone, methanol
			Biliary passages and liver	Overall	4	2.98 (0.81–7.63)	
				≥ 10 yrs of employment, ≥ 20 yrs since first exposure	4	5.83 (1.59–14.9)	
			Bronchus, trachea, and lung	Overall	13	0.80 (0.43–1.37)	
Gibbs et al. (1996) USA, 1970–1989	3211 white workers (2187 men and 1024 women)	Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1970–81	Malignant neoplasms	Men, high (350–700 ppm)	57	0.75 (0.57–0.98)	Results based on mortality records; adjusted for age, sex, race, and calendar period Co-exposures: acetone, methanol
				Women, high	5	1.09 (0.35–2.53)	
				Men, low (50–100 ppm)	64	0.91 (0.70–1.17)	
				Women, low	37	0.83 (0.58–1.14)	
				Men, none	23	0.82 (0.52–1.23)	
				Women, none	2	0.48 (0.06–1.74)	
			Biliary tract and liver (155–56)	Men, high (250–750 ppm)	1	0.81 (0.02–4.49)	
				Women, high	0	(0–374)	
				Men, low (50–100 ppm)	1	0.75 (0.02–4.20)	
				Men, none	0	(0.0–6.88)	
				Women, none	0	(0–35.50)	
			Bronchus, trachea, and lung	Men, high (250–750 ppm)	15	0.55 (0.31–0.91)	
				Women, high	2	2.29 (0.28–8.29)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Gibbs et al. (1996) USA, 1970–1989 (cont.)				Men, low (50–100 ppm)	20	0.78 (0.48–1.20)	
				Women, low	9	1.09 (0.50–2.07)	
				Men, none	6	0.59 (0.22–1.29)	
				Women, none	0	(0–4.92)	
			Cervix	Women, high	1	5.40 (0.14–30.10)	
				Women, low	5	3.00 (0.96–6.92)	
Hearne & Pifer (1999) USA, 1964–1994	1311 male white workers	Workers from a plant producing cellulose triacetate film, engaged for ≥ 1 yr in one of three areas in which dichloroethane was used (roll coating, doping, distilling) in 1946–70	All malignant neoplasms	Overall	93	0.88 (0.71–1.08)	Referent population (mortality) from New York, excluding New York City Co-exposures: acetone, methanol, 1,2-dichloropropane, 1,2-dichloroethane
				< 150 ppm	20	0.67 [0.41–1.03]	
				150–349 ppm	19	0.93 [0.56–1.45]	
				350–799 ppm	28	0.95 [0.63–1.37]	
				≥ 800 ppm	26	1.00 [0.65–1.47]	
			Liver and biliary ducts (155–156)	Overall	1	0.42 (0.01–2.36)	
			Lymphatic tissue, overall (200–203)	Overall	5	0.75 (0.24–1.76)	
			NHL (200 202)	Overall	2	0.49 (0.06–1.78)	
			Hodgkin disease (201)	Overall	2	1.82 (0.20–6.57)	
			Multiple myeloma (203)	Overall	1	0.68 (0.01–3.79)	
			Leukaemia (204–208)	Overall	8	2.04 (0.88–4.03)	
				< 150 ppm	2	1.61 [1.20–5.83]	
				150–349 ppm	0	0.00	
				350–799 ppm	1	0.98 [0.03–5.46]	
				≥ 800 ppm	5	5.89 [1.89–13.6]	
			Brain and other CNS	Overall	6	2.16 (0.79–4.69)	
				< 150 ppm		1.10 [0.03–6.12]	
				150–349 ppm		1.77 [0.05–9.95]	
				350–799 ppm		3.99 [0.83–11.7]	
				≥ 800 ppm		1.78 [0.05–9.95]	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Hearne & Pifer (1999) USA, 1964–1994 (cont.)			Trachea, bronchus, and lung	Overall	27	0.75 (0.49–1.09)	
				< 150 ppm	5	0.52 [(0.17–1.21)]	
				150–349 ppm	6	0.90 [(0.33–1.96)]	
				350–799 ppm	9	0.86 [(0.57–2.37)]	
				≥ 800 ppm	7	0.77 [(0.31–1.59)]	
Tomenson (2011) England, 1946–2006	1785 male employees	Workers producing cellulose triacetate film base in 1946–88, and exposed to dichloromethane; the reference group comprised 312 male workers unexposed to dichloromethane	All cancers	All exposed	120	0.70 (0.58–0.83)	Age, calendar period Co-exposures: acetone, methanol
				< 400 ppm-yr	54	0.61 [(0.53–1.58)]	
				400–700 ppm-yr	12	0.82 [(0.54–2.59)]	
				≥ 800 ppm-yr	11	0.87 [(0.55–2.80)]	
				1000 ppm-yr	77	1.23 (0.71–2.11)	
			Biliary passages and liver (155–156)	Overall	0		
			Lymphatic and haematopoietic (200–208)	All exposed	11	0.89 (0.44–1.59)	
			Leukaemia (204–208)	All exposed	5	1.11 (0.36–2.58)	
			Brain and CNS	All exposed	8	1.83 (0.79–3.60)	
				< 400 ppm-yr	4	1.56 [(0.43–3.99)]	
				400–700 ppm-yr	2	7.21 [(0.87–26.1)]	
				≥ 800 ppm-yr	0	NR	
				1000 ppm-yr	6	1.11 (0.12–10.4)	
			Bronchus, trachea, and lung	All exposed	27	0.48 (0.31–0.69)	
				< 400 ppm-yr	10	0.35 [(0.17–0.64)]	
				400–700 ppm-yr	4	0.80 [(0.22–2.05)]	
				≥ 800 ppm-yr	1	0.24 [(0.01–1.34)]	
				1000 ppm-yr	15	1.04 (0.28–3.78)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Radican et al. (2008) USA, 1952–2000	1222 workers	Employees from Hill Air Force Base; exposure to 21 solvents and chemicals assessed by job and organization combinations	NHL (200 202 & C82-C85)	Overall, men	8	2.02 (0.76–5.42)	Age, race Internal comparison of deaths Co-exposures: several organic solvents, in particular trichloroethylene, and other occupational exposures
				Overall, women	0		
			Multiple myeloma (203 & C90)	Overall, men	7	2.58 (0.86–7.72)	
				Overall, women	0		
			Breast	Overall, women	6	2.35 (0.98–5.65)	

CI, confidence interval; CNS, central nervous system; ICD, International Classification of Diseases; mo, month; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million; yr, year

first employment (SMR, 5.83; 95% CI, 1.59–14.92). Three out of these four deaths were attributed to cancer of the biliary tract, with durations of exposure to dichloromethane of < 1 to 28 years. These four cases were also observed in the initial analysis by Lanes et al. (1990) with an SMR of 5.75 (95% CI, 1.82–13.8) for cancers of the liver and biliary tract combined; the SMR estimated for cancer of the biliary tract alone was 20 (95% CI, 5.2–56) compared with a national referent population. [Although some of the subjects were also exposed to acetone and methanol, the Working Group considered these to be unlikely explanations for the observed risks because they were not known to be linked to cancer of the liver.] Results for other cancers were unremarkable; no results were reported for non-Hodgkin lymphoma (NHL).

Gibbs et al. (1996) conducted a cohort study of mortality among cellulose-triacetate fibre workers exposed to dichloromethane at a facility in the USA similar to that reported by Lanes et al. (1993). The cohort consisted of 3211 white workers who had been employed between 1970 and 1981 and followed until 1989. Comparisons were made with county mortality rates. The cohort was divided into three exposure groups; none; low (50–100 ppm [174–347 mg/m^3]) and high (350–700 ppm [1215–2430 mg/m^3]) based on the working area and exposure levels reported by Ott et al. (1983a, b). The workers had been also exposed to acetone and methanol. The risk of mortality from cancers of liver and biliary tract was not increased [SMR, 0.78; 95% CI, 0.09–2.81, for high and low exposure combined]. The two deaths in the group "liver and biliary tract cancer" were actually cancers of the biliary tract. Except for cancer of the prostate, for which there was a non-significant excess, SMRs for other cancers were < 1.0 for all exposure categories among men. The SMRs for women were based on very small numbers and were unstable. No data were reported for NHL. [The exposures observed in the studies by Lanes et al. (1993) and Gibbs et al. (1996) were higher than in other cohort studies. The proportion of cancers of the liver that occurred in the biliary tract in this study population was larger than would normally be expected (between 5% and 10% based on current data for the USA). While Gibbs et al. (1996) did not report an SMR for cancer of the biliary tract, if the value were to be computed, it might be higher than that reported for liver and biliary tract combined.]

Hearne & Pifer (1999) reported on mortality among a cohort of 1311 workers at a plant producing cellulose triacetate film base, in the USA. The cohort consisted of male workers who began working in the roll coating, or doping and distilling departments between 1946 and 1970, and were followed until 1994. Dichloromethane was introduced before the mid-1940s. Exposure to dichloromethane (8-hour TWA) was 0–520 ppm [0–1800 mg/m^3] in 1946–1965, 0–300 ppm [0–1040 mg/m^3] in 1966–1985, and 0–100 ppm [0–347 mg/m^3] in 1986–1994. Workers may have also been exposed to methanol, 1,2-dichloropropane, 1,2-dichloroethane, acetone, and benzene, but exposure levels were not reported for these agents. Malignant neoplasms with elevated SMRs were cancer of brain and central nervous system (SMR, 2.16; 95% CI, 0.79–4.69; 6 cases), leukaemia (SMR, 2.04; 95% CI, 0.88–4.03; 8 cases), and Hodgkin disease (SMR, 1.82; 95% CI, 0.20–6.57; 2 cases). Mortality from leukaemia increased with cumulative exposure among four exposure categories: for the group with the highest cumulative exposure, the SMR for leukaemia was 5.89 (95% CI, [1.89–13.6]; 5 cases) (Table 2.1). Three of the eight cases of leukaemia had also been exposed to benzene in the past. SMRs for cancer of the liver and NHL were less than unity, based on very small numbers (one and two cases, respectively). [The small numbers of exposed cases, which hampers analysis of exposure–response patterns, were an important limitation of this study.]

The above article (Hearne & Pifer, 1999) also reported on mortality among 1013 male workers who had been employed in the roll-coating department at any time between 1964 and 1970, and were followed until 1994. This superseded earlier analyses by Friedlander et al. (1978), Hearne & Friedlander (1981), Hearne et al. (1987), and Hearne et al. (1990). Because about 70% of the subjects in this cohort were also included in the larger cohort of cellulose triacetate workers, the description of this subcohort was omitted from this review.

Tomenson (2011) performed a cohort study of mortality among workers at a plant producing cellulose triacetate film base, in England. This extended earlier analyses by Tomenson et al. (1997). The cohort comprised 1785 male workers who had been employed at the site at any time between 1946 and 1988, and followed until 2006, of whom 1473 had been employed in jobs with potential exposure to dichloromethane. Exposure levels were estimated from area samples according to time period and work group. TWA exposures were estimated to range from 2 to 20 ppm [7–69 mg/m^3] before 1960, 6 to 127 ppm [21–441 mg/m^3] during the 1960s, 10 to 165 ppm [35–573 mg/m^3] during the 1970s, and 7 to 88 ppm [24–305 mg/m^3] during the 1980s Tomenson et al. (1997). The workers had been also exposed to acetone and methanol. Four exposure categories were established based on cumulative exposure, but 30% of the exposed could not be classified because employment histories were insufficiently precise. Only for cancer of the brain and central nervous system (SMR, 1.83; 95% CI, 0.79–3.60, among exposed workers) was the number of deaths more than 1.2 times that expected. No cancers of the liver were observed among exposed or unexposed workers (expected, 3.3 cases), and there was a significant deficit of cancer of the lung. Data for NHL were reported. Analysis of cumulative exposure for four cancer sites, including brain, did not show any significant trends with the level of exposure to dichloromethane. [The major weakness of this study was the small number of deaths, which limited the ability to conduct exposure–response analysis.]

Radican et al. (2008) performed a retrospective cohort study of mortality among workers at a military-aircraft maintenance facility in the USA, updating earlier studies (Spirtas et al., 1991; Blair et al., 1998). The cohort consisted of civilian employees employed between 1952 and 1956, and followed until 2000. Workers were exposed to numerous chemicals. Exposure was assessed quantitatively for trichloroethylene, and qualitatively (ever/never) to other agents including dichloromethane. The number of workers exposed to dichloromethane was 1222 (Stewart et al., 1991). Exposure to dichloromethane was associated with increased risks (hazard ratio, HR) of NHL (HR, 2.02; 95% CI, 0.76–5.42; 8 exposed cases) and multiple myeloma (HR, 2.58; 95% CI, 0.86–7.72; 7 exposed cases) for male workers, and cancer of the breast (HR, 2.35; 95% CI, 0.98–5.65; 6 exposed cases) for female workers. Results for other cancer sites in relation to dichloromethane exposure were not reported. [The strengths of this study included a large number of the subjects and a long follow-up period; however, because the primary analysis was for trichloroethylene, the exposure assessment and analysis for dichloromethane were limited.]

2.3 Case–control studies

Table 2.2 summarized case–control studies on the relationship between occupational exposure to dichloromethane and cancer.

2.3.1 Cancers of the lympho-haematopoietic system

Miligi et al. (2006) conducted a case–control study in Italy to evaluate the association between risk of lymphoma and exposure to dichloromethane and nine other organic solvents. The

Table 2.2 Case–control studies on lympho-haematopoietic cancer and exposure to dichloromethane

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Miligi et al. (2006) Italy, 1991–1993	NHL, 1428 cases Controls, 1530	Population	Person-to-person interview, structured questionnaire, and industrial hygiene experts who assessed exposure to eight specific organic solvents	NHL	Very low/low	23	0.9 (0.5–1.6)	Sex, age, education and area Co-exposures: benzene, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane OR not reported for follicular NHL, diffuse NHL, and other NHL
					Medium/high	13	1.7 (0.7–4.3) *P* for trend, 0.46	
					≤ 15 yr	8	1.4 (0.5–4.4)	
					> 15 yr	4	NR	
				Small lymphocytic NHL	Medium/high, excluding proxy respondents	8	3.2 (1.0–10.1)	
Seidler et al. (2007) Germany, 1999–2003	Malignant lymphoma, 710 cases Controls, 710	Population	Interview; exposure to eight organic solvents assessed by one industrial physician		*Exposure (ppm-yrs)*			Smoking and alcohol Co-exposure: trichloroethene, tetrachloroethylene, carbon tetrachlorine, benzene, toluene, xylene and styrene
				Lymphoma	0	681	1	
					> 0–≤ 26.3	8	0.4 (0.2–1.0)	
					> 26.3– ≤ 175	9	0.8 (0.3–1.9)	
					> 175	5	2.2 (0.4–11.6)	
							P for trend, 0.40	
				Hodgkin lymphoma	> 0–≤ 26.3	2	0.7 (0.2–3.6)	
					> 26.3–≤ 175		NR	
					> 175		NR	
				B-cell NHL	> 0–≤ 26.3	6	0.4 (0.2–1.1)	
					> 26.3–≤ 175	8	0.9 (0.3–2.3)	
					> 175	5	2.7 (0.5–14.5)	
				T-cell NHL	> 0–≤ 26.3		NR	
					> 26.3–≤ 175	1	1.2 (0.1–10.9)	
					> 175		NR	

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Costantini et al. (2008) Italy, 1991–1993	Leukaemia, 586 cases Controls, 1278	Population	Person-to-person interview, structured questionnaire, and industrial hygiene experts who assessed exposure to eight specific organic solvents	Leukaemia (204–208)	Very low/low	7	0.7 (0.3–1.7)	Sex, age, education and area Co-exposures: benzene, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane
					Medium/high	2	0.5 (0.1–2.3)	
				Acute myeloid leukaemia (205.0)	Very low/low	3	NR	
					Medium/high	0	NR	
				Chronic lymphocytic leukaemia (204.1)	Very low/low	2	0.4 (0.1–2.0)	
					Medium/high	2	1.6 (0.3–8.6)	
	Multiple myeloma, 263 cases Controls, 1100			Multiple myeloma (203)	Very low/low	4	NR	
					Medium/high	0	NR	
Gold et al. (2011) USA, 2000–2002	Multiple myeloma, 180 cases	Population	Interview and JEM	Multiple myeloma (ICD-O-2/3: 9731:9732)	Primary analysis *Ever exposed*	47	1.5 (0.9–2.3)	Age, race, study site, and years of education
					1–4 yr	9	1.2 (0.5–2.9)	
					5–11 yr	11	1.8 (0.8–4.1)	
					12–29 yr	17	1.8 (0.9–3.5)	
					30–51 yr	10	1.1 (0.5–2.6)	
					P for trend	0.35		
					Cumulative exposure score			
					1–318	7	1.2 (0.5–2.9)	
					319–2218	17	2.2 (1.1–4.6)	
					2219–7793	7	0.8 (0.3–1.9)	
					7794–57 000	14	1.6 (0.8–3.4)	
					P for trend	0.27		
					Cumulative exposure score, 10-yr lag			
					1–311	8	1.4 (0.6–3.3)	
					312–2089	12	1.6 (0.7–3.6)	
					2090–7285	10	1.2 (0.5–2.8)	
					7286–50 000	12	1.5 (0.7–3.2)	
					P for trend	0.39		

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Gold et al. (2011) USA, 2000–2002 (cont.)					Secondary analysis *Ever exposed*	37	2.0 (1.2–3.2)	In secondary analyses, jobs assessed with low confidence are considered unexposed
					1–4 yr	8	2.0 (0.8–5.1)	
					5–7 yr	6	1.1 (0.4–3.1)	
					8–24 yr	13	2.7 (1.1–6.5)	
					25–47 yr	10	2.1 (0.9–5.2)	
					P for trend	0.01		
					Cumulative exposure score			
					1–102	5	1.6 (0.5–4.7)	
					103–1122	13	2.8 (1.2–6.6)	
					1123–5493	8	1.6 (0.6–3.8)	
					5494–57 000	10	2.4 (1.0–5.9)	
					P for trend	0.08		
					Cumulative exposure score, 10-yr lag			
					1–71	4	1.3 (0.4–4.4)	
					72–437	10	2.9 (1.1–7.5)	
					438–3903	9	1.9 (0.7–5.0)	
					3904–49 500	10	2.4 (1.0–6.1)	
					P for trend	0.06		

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Wang et al. (2009) USA, 1996–2000	601 female cases 717 female controls	Population	Person-to person interview, structured questionnaire, and JEM	NHL	Ever	52	1.5 (1.0–2.3)	Adjusted for age, family history of haematopoietic cancer, alcohol consumption, and race Co-exposures: benzene, formaldehyde, chloroform, carbon tetrachloride, dichloroethane, trichloroethylene
					Low intensity	37	1.5 (0.9–2.4)	
					Medium high intensity	15	1.6 (0.7–3.3)	
					P for trend	0.11		
					Low probability	48	1.6 (1.0–2.4)	
					Medium high probability	4	1.2 (0.3–4.4)	
					P for trend	0.34		
					Low intensity and medium and high probability	1		
					Medium and high intensity and medium and high probability	3	0.9 (0.2–3.8)	
Barry et al. (2011) USA, 1996–2000	NHL, 518 cases Diffuse large B-cell lymphoma, 161 cases Follicular lymphoma, 119 cases Controls, 597	Population	Person-to person interview, structured questionnaire, and JEM	NHL	Ever	47	1.7 (1.1–2.7)	Women from the study by Wang et al. (2009) who provided a blood or buccal cell sample for genotyping; adjusted for age and race Co-exposures: benzene, formaldehyde, chloroform, carbon tetrachloride, dichloroethane, trichloroethylene
				Diffuse large B-cell lymphoma	Ever	33	2.10 (1.15–3.85)	
				Follicular lymphoma	Ever	19	1.27 (0.58–2.76)	
				NHL	Ever	30	4.42 (2.03–9.62)	
				Diffuse large B-cell lymphoma	Ever (with *CYP2E1* rs2070673 TT)	11	4.71 (1.85–12.0)	
				Follicular lymphoma	Ever (with *CYP2E1* rs2070673 TT)	5	2.67 (0.86–8.30)	
				NHL	Ever (with *CYP2E1* rs2070673 *TT + AA)*	13	0.80 (0.36–1.75)	
				Diffuse large B-cell lymphoma	Ever (with *CYP2E1* rs2070673 *TT + AA)*	6	1.12 (0.40–3.19)	
				Follicular lymphoma	Ever (with *CYP2E1* rs2070673 *TT + AA)*	4	0.96 (0.29–3.20)	

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Christensen et al. (2013) Canada, 1979–85	215 cases 2341 controls			NHL (200 202)	Never exposed to chlorinated solvents	155	1 (reference)	Adjustment by age, census tract median income, educational attainment (yrs), ethnicity (French Canadian vs others), questionnaire respondent (self vs proxy), smoking (cigarettes-yrs) using only population controls. For bladder additionally: coffee intake, exposure to aromatic amines
					Any	3	0.6 (0.2–2.2)	
					Substantial	0	NR	

CI, confidence interval; ICD-O, International Classification of Diseases for Oncology; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; ppm, parts per million; vs, versus; yr, year

study included 1428 cases of NHL (including 285 with small lymphocytic lymphoma, 308 with diffuse lymphoma, 100 with follicular lymphoma, and 315 with other lymphomas), and 1530 controls. Information about occupational history and other potential risk factors was obtained by in-person interview, and probability and intensity of occupational exposure to individual chemicals and chemical classes were assigned by expert assessment. Odds ratios were adjusted by area, sex, age, and education, excluding subjects with low probability of exposure. The odds ratio (OR) for NHL in the category for combined medium- and high-intensity exposure to dichloromethane was 1.7 (95% CI, 0.7–4.3; 13 cases; *P* for trend, 0.46). Among the NHL subtypes, an odds ratio for dichloromethane was reported only for small lymphocytic NHL: for medium or high exposure, the odds ratio was 3.2 (95% CI, 1.0–10.1). The study also included cases of Hodgkin lymphoma, but odds ratios for exposure to dichloromethane were not reported.

Seidler et al. (2007) conducted a case–control study to examine the relationship between malignant lymphoma and exposure to eight organic solvents including dichloromethane. The study included 710 cases (including 554 cases with B-cell NHL, 35 cases with T-cell NHL, and 1 case with combined B-cell and T-cell NHL), and 710 general-population controls matched for area, sex, and age collected from six areas in Germany. In-person interview obtained occupational history, medical history, and lifestyle. Exposure was assessed for several chlorinated solvents, with metrics of intensity, frequency, and confidence assigned by an industrial hygienist, and cumulative exposure was calculated. Odds ratios were adjusted for smoking and alcohol consumption. The odds ratio for high cumulative exposure to dichloromethane was 2.2 (95% CI, 0.4–11.6; *P* for trend, 0.40) for all lymphomas, and 2.7 (95% CI, 0.5–14.5; *P* for trend, 0.29) for B-cell NHL.

Costantini et al. (2008) conducted a case–control study of 586 cases of leukaemia and 1278 controls from seven areas in Italy, to evaluate the risks associated with exposure to ten organic solvents including dichloromethane. In-person interviews obtained occupational history, other exposures to chemicals, and other potential risk factors. Exposure was assessed by expert rating to assign metrics of probability and intensity of exposure to several solvents. Subjects with a low probability of exposure were excluded from the analysis and odds ratios were adjusted by area, sex, age, and education. No associations between acute leukaemia or myeloma and dichloromethane were seen. Four cases of chronic lymphocytic leukaemia (now classified as a type of NHL) were observed, with a non-significant odds ratio of < 1 for very low/low exposure, and an odds ratio of 1.6 (95% CI, 0.3–8.6) for medium/high exposure.

Gold et al. (2011) conducted a case–control study to evaluate the associations between risk of multiple myeloma and exposure to dichloromethane and other chlorinated solvents. During 2000–2002, 180 cases were collected from Seattle–Puget Sound region of Washington and Detroit metropolitan area of Michigan in the USA and 481 controls were collected from the general population in the same areas. In-person interviews obtained occupational history and additional job-specific modules were applied when solvent exposure was likely. Exposure metrics of probability, frequency, intensity, confidence, and cumulative exposure were assigned using a job-exposure matrix. Odds ratios were adjusted by area, race, sex, age, and education. Ever-exposure to dichloromethane entailed elevated risk of multiple myeloma (OR, 1.5; 95% CI, 0.9–2.3). Significant trends with exposure duration were observed when occupations that had low confidence scores were included in the unexposed category: the odds ratio for ever exposure was 2.0 (95% CI, 1.2–3.2) and odds ratios of 2.7 (95% CI, 1.1–6.5), and 2.1 (95% CI, 0.9–5.2), were observed for workers employed for 12–29 years

and 30–51 years, respectively (*P* for trend, 0.01). No such trend was seen for cumulative exposure.

Wang et al. (2009) conducted a case–control study to examine the association between NHL and exposure to nine organic solvents including dichloromethane. The study included 601 female cases, and 717 controls, matched for age, collected from the general population in Connecticut, USA. Information about occupational history and other potential risk factors was obtained by in-person interview and probability and intensity of exposure to solvents were assigned using a previously developed job-exposure matrix. Odds ratios were adjusted by race, age, family history of haematopoietic cancer, and alcohol consumption. Subjects ever-exposed to dichloromethane had an increased risk of NHL (OR, 1.5; 95% CI, 1.0–2.3). Analyses by intensity and probability of exposure indicated elevated ORs, but trends were not statistically significant.

Barry et al. (2011) conducted a further study in a subset of the population studied by Wang et al. (2009) to evaluate whether genetic variation in four genes involved in metabolism (*CYP2E1*, *EPHX1*, *NQO1*, *MPO*) modifies associations between exposure to organic solvents and risk of NHL or five major histological subtypes of NHLL (diffuse large B-cell lymphoma, follicular lymphoma, chronic lymphocytic leukaemia/small lymphocytic lymphoma, marginal zone lymphoma, and T-cell lymphoma). Ever-exposure to dichloromethane entailed elevated risk of NHL (OR, 1.69; 95% CI, 1.06–2.69). The risk associated with ever-exposure to dichloromethane was higher (OR, 4.42; 95% CI, 2.03–9.62) among women with the TT genotype for *CYP2E1* rs2070673. In contrast, no effects with dichloromethane was observed among women with the TA or AA genotype (OR, 0.80; 95% CI, 0.36–1.75). Similar patterns were observed for diffuse large B-cell lymphoma and follicular lymphoma. No interactions with other single-nucleotide polymorphisms (SNPs) in the studied genes, including *CYP2E1*, *EPHX1*, *NQO1*, or *MPO*, were statistically significant. [The Working Group noted that the functional role of the CYP2E1 polymorphism is unclear.]

2.3.2 Cancers of brain and central nervous system

See Table 2.3

Heineman et al. (1994) examined associations between astrocytic cancer of the brain and exposure to six chlorinated solvents including dichloromethane in a study of 300 men who died from astrocytic cancer of the brain in Louisiana and Pennsylvania, USA, and 320 men who died from other causes not associated with occupational exposure to chlorinated hydrocarbons. Information including occupational history and risk factors for cancer of the brain was obtained by interview of next-of-kin and exposure estimates were assigned using a job-exposure matrix. After adjusting for age at death and study area, significant trends in risk were observed with increasing probability and intensity of exposure, as well as with increasing exposure duration and cumulative exposure when the probability of exposure was high. [The reliability of the exposure assessment was judged to be relatively low because occupational information was obtained from the next of kin.]

Cocco et al. (1999) conducted a case–control study to examine associations between mortality from the cancer of the brain and other parts of central nervous system and exposure to 11 factors including dichloromethane. Cases were 12 980 women who died due to cancer of central nervous system in 24 states of the USA. Controls were 51 920 randomly selected women who died from non-malignant diseases, excluding neurological disorders. Probability and intensity of exposure were assigned using occupation and industry titles from subjects' death certificates and a job-exposure matrix. After adjusting for age at death, marital status, and socioeconomic status, the odds ratio for the association of exposure to

Table 2.3 Case–control studies of cancer of the brain and central nervous system and exposure to dichloromethane

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Heineman et al. (1994) Louisiana, New Jersey, and Philadelphia, USA, 1979–81	Cases, 300 white men from death certificates Controls, 320 white men	Death certificates from men who died from causes other than brain cancer	Next-of-kin interview and JEM	Brain or other CNS (ICD-9 191, 192, 225, 239.7)	All, ever	108	1.3 (0.9–1.8)	Age, study area Covariates: organic solvents, carbon tetrachloride, methyl chloroform, tetrachloroethylene, trichloroethylene
					Low probability, ever	71	1.0 (0.7–1.6)	
					Medium probability, ever	21	1.6 (0.8–3.0)	
					High probability, ever	10	2.4 (1.0–5.9)	
					P trend < 0.05			
					All, 2–20 yrs	80	1.2 (0.8–1.7)	
					Low probability, 2–20 yr	49	1.0 (0.6–1.6)	
					Medium probability, 2–20 yr	22	1.5 (0.7–3.2)	
					High probability, 2–20 yr	9	1.8 (0.6–6.0)	
					All 21+ yr	24	1.7 (0.9–3.6)	
					Low probability 21+ yr	12	1.2 (0.5–3.0)	
					Medium probability 21+ yr	4	1.5 (0.3–9.0)	
					High probability 21+ yr	8	6.1 (1.1–43.8)	
					P trend < 0.01 for duration (high probability)			
					All, low cumulative exposure	37	0.9 (0.5–1.5)	
					Low probability, low cumulative exposure	24	0.7 (0.4–1.3)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Heineman et al. (1994) Louisiana, New Jersey, and Philadelphia, USA, 1979–81 (cont.)					Medium probability, low cumulative exposure	9	1.3 (0.4–3.8)	
					High probability, low cumulative exposure	4	2.0 (0.3–16.7)	
					All, medium cumulative exposure	48	1.9 (1.1–3.2)	
					Low probability, medium cumulative exposure	29	1.6 (0.8–3.0)	
					Medium probability, medium cumulative exposure	13	2.3 (0.8–7.0)	
					High probability, medium cumulative exposure	6	4.2 (0.7–31.4)	
					All, high cumulative exposure	19	1.2 (0.6–2.5)	
					Low probability, high cumulative exposure	8	0.9 (0.3–2.5)	
					Medium probability, high cumulative exposure	4	0.9 (0.2–4.0)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Heineman et al. (1994) Louisiana, New Jersey, and Philadelphia, USA, 1979–81 (cont.)					High probability, high cumulative exposure	7	2.5 (0.6–11.0)	
					P trend < 0.05 for cumulative exposure (high probability)			
					Low-medium average intensity, total	76	1.1 (0.7–1.6)	
					High intensity, total	28	2.2 (1.1–4.4)	
Cocco et al. (1999) 24 states in USA, 1984–92	Cases, 12 980 women Controls, 51 920 women who died from non-malignant diseases	Death certificates	Usual occupation or industry from death certificate and JEM	Brain and other CNS (191, 192)	Any	867	1.2 (1.2–1.3)	State, race Co-exposures: electromagnetic fields, solvents, chlorinated aliphatic hydrocarbons, benzene, lead, nitrosamines, polyaromatic hydrocarbons, insecticides and fungicides, herbicides, contact with the public
					Low probability	756	1.2 (1.1–1.3)	
					Medium probability	83	1.2 (1.0–1.6)	
					High probability	28	1.3 (0.9–1.3)	
					Low intensity	370	1.3 (1.1–1.5)	
					Medium intensity	345	1.2 (1.1–1.4)	
					High intensity	152	1.0 (0.8–1.2)	
				Meningioma	Any	13	1.2 (0.7–2.2)	
De Roos et al. (2001) USA and Canada, 1 May 1992, and 30 April 1994	Cases, 538 from hospitals in the USA and Canada	Population controls from random-digit dialling	Self-reported exposure by parents and review by industrial hygienists	Neuroblastoma	Self-reported by parent (paternal exposure)	10	0.7 (0.3–1.6)	Adjusted for child's age, maternal race, maternal age, and maternal education
					Industrial hygienists reviewed exposure	4	0.7 (0.2–0.8)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Neta et al. (2012) Arizona, Massachusetts and Pennsylvania, USA, 1994–98	Cases, 489 glioma, 197 meningioma, Controls, 799	Hospital	Personal interviews and expert assessment	Glioma or other neuroepitheliomatous neoplasm (ICD-O-2 9380–9473 and 9490–9506)	Possible, all	126	0.8 (0.6–1.1)	Age group, race sex, hospital site and proximity of residence to hospital
					Probable, all	21	0.5 (0.3–0.9)	
					Possible, men	90	0.7 (0.5–1.0)	
					Probable, men	16	0.4 (0.2–0.8)	
					Possible, women	36	1.1 (0.7–1.1)	
					Probable, women	5	1.0 (0.3–2.9)	
					Unexposed, all	534	1	
					Years exposed, low	9	0.4 (0.2–0.8)	
					Years exposed, high	12	0.7 (0.3–1.4)	
					Cumulative low	11	0.5 (0.2–1.0)	
					Cumulative high	10	0.5 (0.2–1.1)	
					Average weekly exposure low	15	0.7 (0.3–1.3)	
					Average weekly exposure high	6	0.3 (0.1–0.8)	
					Highest exposure low	12	0.5 (0.3–1.1)	
					Highest exposure high	9	0.5 (0.2–1.0)	
				Meningioma (ICD-O-2 9530–9538) or acoustic neuroma (ICD-O-2 9560)	Possible	42	1.6 (0.7–3.5)	Age, race, sex, hospital and proximity to hospital and all other solvents
					Probable	8	0.8 (0.2–3.0)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Ruder et al. (2013) Iowa, Michigan, Minnesota, Wisconsin, USA, 1995–97	798 cases 1175 controls	Population	Personal interview and industrial hygienist evaluation	Glioma (9380–948)	All	304	0.80 (0.66–0.97)	Age, education, sex
					Men	222	0.88 (0.69–1.13)	
					Women	82	0.69 (0.50–0.95)	
					Cumulative exposure (ppm-yrs), including unexposed participants, including proxy-only interviews	798	0.98 (0.97–0.99)	
					Cumulative exposure (ppm-yrs), excluding unexposed participants, including proxy-only interviews	304	0.96 (0.89–1.03)	

CI, confidence interval; CNS, central nervous system; ICD-O, International Classification of Diseases for Oncology; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; ppm, parts per million; yr, year

dichloromethane and all cancer of the central nervous system was 1.2 (95% CI, 1.1–1.3). Odds ratios were generally similar for all categories of probability and intensity of exposure. [Because this study, like others using similar methods, assessed exposure from occupational information from death certificates, the specificity for dichloromethane was poor.]

De Roos et al. (2001) analysed occupations of 405 case fathers and 302 control fathers to identify paternal occupational exposures associated with an increased risk of cancer of the brain in children. When considering paternal exposure to dichloromethane as assessed by an industrial hygienist, the odds ratio for neuroblastoma was 0.70 (95% CI, 0.2–2.8; 4 exposed cases; adjusted by age, maternal race, maternal age, and maternal education).

Neta et al. (2012) conducted a hospital-based case–control study to examine associations between glioma and meningioma and exposure to six chlorinated solvents including dichloromethane. Cases were 484 patients with glioma and 197 patients with meningioma diagnosed in Massachusetts, Pennsylvania, and Arizona, USA. Controls were 797 patients admitted to the same hospitals for non-malignant conditions and were frequency-matched to cases by sex, age, race, hospital, and proximity to the hospital. Exposure to solvents was assessed by an industrial hygienist based on detailed occupational histories collected by interview. Odds ratios adjusted for the matching factors did not show any association between glioma or meningioma and overall exposure to dichloromethane or other metrics, including duration, intensity, and cumulative exposure.

Ruder et al. (2013) conducted a population-based case–control study to examine associations between glioma and exposure to six chlorinated solvents including dichloromethane. Cases were 798 patients with intracranial glioma in Iowa, Michigan, Minnesota, and Wisconsin, USA, and controls were 1175 residents selected from the same area. Lifetime occupational histories were obtained by interview and several exposure metrics were assigned by an industrial hygienist. Odds ratios adjusted for the frequency-matching variables (age group and sex), and for age and education. There were no associations between glioma and overall exposure to dichloromethane, or exposure probability and cumulative exposure.

In a multicentre case–control study of meningioma conducted in seven countries (INTEROCC) and including 1906 cases and 5565 controls, there were no subjects classified as exposed to dichloromethane after assessment of lifetime occupational histories using a modified version of the Finnish national job-exposure matrix (INTEROCC-JEM) (McLean et al., 2014).

2.3.3 Other cancer sites

The Working Group also reviewed case–control studies on dichloromethane and several other cancer sites. These included a case–control study on cancer at many sites (Christensen et al., 2013), and studies on cancer of the breast (Cantor et al., 1995), pancreas (Kernan et al., 1999), kidney (Dosemeci et al., 1999), and lung (Vizcaya et al., 2013). However, no remarkable excess of cancer was reported in these studies and the evidence for these cancer sites was regarded as inadequate.

2.3.4 Case report from Japan

In a case report, Kumagai (2014) described two cases of cholangiocarcinoma in workers employed in two different printing plants in Japan. One of the two cases had been exposed to dichloromethane and 1,1,1-trichloroethane, and the other had been exposed to 1,2-dichloropropane.

2.4 Meta-analysis

Liu et al. (2013) conducted a meta-analysis to examine the relationship between occupational exposure to dichloromethane and risk of cancer, with a focus on NHL and multiple myeloma. However, the population for one of the included studies on NHL was a subset of another (Wang et al., 2009; Barry et al., 2011) and one potentially informative study on multiple myeloma (Costantini et al., 2008) was not reviewed. The meta-analysis was consequently not considered further.

3. Cancer in Experimental Animals

The carcinogenicity of dichloromethane in experimental animals was reviewed previously by the Working Group (IARC, 1999).

3.1 Mouse

There were six studies of carcinogenicity with dichloromethane in mice (dichloromethane was administered orally in two studies, by inhalation in three studies, and by intraperitoneal injection in one study).

See Table 3.1

3.1.1 Oral administration

Groups of 50–200 male and female B6C3F$_1$ mice (age, 7 weeks) were given drinking-water containing dichloromethane (purity, 99%) at a dose of 0 (first control group), 0 (second control group), 50, 125, 185, or 250 mg/kg body weight (bw) per day for 104 weeks (Serota et al., 1986a). Two vehicle-control groups were run simultaneously. No significant exposure-related trend in survival was found in males; in females, a significant trend towards longer survival in exposed groups was reported. In male mice, there was an increased incidence of hepatocellular carcinoma at the highest dose compared with the first control group. A dose-related increase in the incidence of hepatocellular adenoma or carcinoma (combined) was also observed.

Groups of 50 male and 50 female Swiss mice (age, 9 weeks) were given dichloromethane (purity, > 99.9%) at a dose of 100 or 500 mg/kg bw in olive oil by gavage once per day, for 4 or 5 days per week, for 64 weeks (Maltoni et al., 1988). Groups of 60 male and 60 female mice were given olive oil only (vehicle controls). The mice were then kept under observation for their lifespan. Excess mortality was observed in male and female mice exposed to dichloromethane at the lowest and the highest dose. An increase in mortality appeared after week 36 of treatment and led to withdrawal of the treatment at week 64. In mice that died by week 78, the incidence of pulmonary adenoma or adenocarcinoma (combined) was significantly increased in the group at the highest dose. At the end of the experiment, the cumulative incidences of pulmonary adenoma or carcinoma (combined) in males were 5/50, 5/50, and 9/50. No treatment-related increase in the incidence of any tumour type in females, or of any type of tumour other than pulmonary in males was reported. [The Working Group noted the short period of exposure and the high numbers of animals lost due to mortality and thus not available for examination at the end of the experiment.]

3.1.2 Inhalation

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 8–9 weeks) were exposed to dichloromethane (purity, > 99%) at concentrations of 0, 2000, or 4000 ppm (0, 6940 or 13 900 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for up to 102 weeks and were killed after 104 weeks (NTP, 1986). The final body weights of male mice at the highest dose and of female mice at the lowest and highest dose were 10–17% lower than those of the respective

Table 3.1 Studies of carcinogenicity with dichloromethane in mice

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Serota et al. (1986a) B6C3F$_1$ (M) 24 mo	Drinking-water 0, 0, 60, 125, 185, 250 mg/kg bw per day in deionized drinking-water continuously for 104 wk. Controls received deionized water 60–200 mice/group	Hepatocellular adenoma: 6/60 (10%), 4/65 (6%), 20/200 (10%), 14/100 (14%), 14/99 (14%), 15/125 (12%) Hepatocellular carcinoma: 5/60 (8%), 9/65 (14%), 33/200 (17%), 18/100 (18%), 17/99 (17%), 23/125 (18%)* Hepatocellular adenoma or carcinoma (combined): 11/60 (18%), 13/65 (20%), 51/200 (26%), 30/100 (30%), 31/99 (31%), 35/125 (28%)	NS[a] *P = 0.0114 (250 mg/kg vs control 1) NS	Purity, 99% Two vehicle -control groups were run concurrently No significant exposure-related trend in survival. Historical controls for hepatocellular adenoma or carcinoma (combined): mean, 32.1%; range, 7–58%
Serota et al. (1986a) B6C3F$_1$ (F) 24 mo	Drinking-water 0, 0, 60, 125, 185, 250 mg/kg bw per day in deionized drinking-water continuously for 104 wk. Controls received deionized water 50–100 mice/group	NR	NS	Purity, 99% Two vehicle-control groups were run concurrently Significant trend towards longer survival
Maltoni et al. (1988) Swiss (M) Lifetime	0, 100, 500 mg/kg bw by gavage in olive oil, once per day, 4–5 days/wk, for 64 wk Kept under observation for lifespan 60 or 50 mice/group	Pulmonary adenomas or adenocarcinomas (combined) in mice that died at 78 weeks: 1/14 (7%), 4/21 (19%), 7/24 (29%)* Pulmonary adenomas or adenocarcinomas (combined) at end of experiment: 5/50 (10%), 5/50 (10%), 9/50 (18%)	*P < 0.05	Purity, 99.9% Excess mortality (P < 0.01) was observed in male mice exposed to the lowest and highest dose Histopathology of tumours not further specified
Maltoni et al. (1988) Swiss (F) Lifetime	0, 100, 500 mg/kg bw by gavage in olive oil, once per day, 4–5 days/wk, for 64 wk 60 or 50 mice/group	NR	NS	Purity, 99.9% Excess mortality was observed in female mice at the lowest and highest dose
NTP (1986) B6C3F$_1$ (M) 24 mo	0, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk for 102 wk 50 mice/group	Bronchiolo-alveolar adenoma: 3/50 (6%)*, 19/50 (38%)**, 24/50 (48%)** Bronchiolo-alveolar carcinoma: 2/50 (4%)*, 10/50 (20%)***, 28/50 (56%)** Hepatocellular adenoma: 10/50 (20%), 14/49 (29%), 14/49 (29%) Hepatocellular carcinoma: 13/50 (26%), 15/49 31%), 26/49 (53%)*** Hepatocellular adenoma or carcinoma (combined): 22/50 (44%)*, 24/49 (49%), 33/49 (67%)***	*P < 0.001 (trend)[a] **P < 0.001 ***P < 0.05	Purity, 99%, Survival: 78%, 48%, 22%, 40%

Table 3.1 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
NTP (1986) B6C3F$_1$ (F) 24 mo	0, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk for 102 wk 50 mice/group	Bronchiolo alveolar adenoma: 2/50 (4%)*, 23/48 (48%)**, 28/48 (58%)** Bronchiolo alveolar carcinoma: 1/50 (2%)*, 13/48 (26%)**, 29/48 (58%)** Hepatocellular adenoma: 2/50 (4%)*, 6/48 (13%), 22/48 (46%)** Hepatocellular carcinoma: 1/50 (2%)*, 11/48 (23%)***, 32/48 (67%)** Hepatocellular adenoma or carcinoma (combined): 3/50 (6%)*, 16/48 (33%)***, 40/48 (83%)**	*$P < 0.001$ (trend)[a] **$P < 0.001$ ***$P < 0.004$	Purity, 99%, Survival: 50%, 50%, 16%, 40%
Kari et al. (1993) B6C3F$_1$ (F) 24 mo	Inhalation, 6 h/days, 5 days/wk: 0 ppm, for 104 wk 2000 ppm, 26 wk/0 ppm, 78 wk 0 ppm, 78 wk/2000 ppm, 26 wk 2000 ppm, 52 wk/0 ppm, 52 wk 0 ppm, 52 wk/2000 ppm, 52 wk 2000 ppm, 78 wk/0 ppm, 26 wk 0 ppm, 26 wk/2000 ppm, 78 wk 2000 ppm, 104 wk 68 mice/group	Bronchiolo alveolar adenoma: 1/67 (1%), 8/68 (12%), 0/67, 12/63 (19%), 5/67 (7%), 19/68 (28%), 7/67 (10%), 18/67 (27%) Bronchiolo alveolar carcinoma: 4/67 (6%), 17/68 (25%), 3/67 (4%), 36/63 (57%), 6/67 (9%), 25/68 (37%), 7/67 (10%), 31/67 (46%) Bronchiolo alveolar adenoma or carcinoma (combined): 5/67 (7%), 21/68 (31%)*, 3/67 (4%), 40/63 (63%)*, 10/67 (15%), 38/68 (56%)*, 13/67 (19%)**, 42/67 (63%)* Hepatocellular adenoma: 8/67 (12%), 16/68 (24%), 16/67 (24%), 14/64 (22%), 9/67 (13%), 28/68 (41%), 17/67 (25%), 24/68 (35%) Hepatocellular carcinoma: 11/67 (16%), 14/67 (21%), 13/67 (19%), 18/64 (28%), 12/67 (18%), 25/68 (37%), 20/67 (30%), 35/68 (51%) Hepatocellular adenoma or carcinoma (combined): 18/67 (27%), 27/67 (40%), 23/67 (34%), 28/64 (44%)**, 21/67 (31%), 42/68 (62%)*, 32/67 (48%)**, 47/68 (69%)*	*$P < 0.01$[b] **$P < 0.05$	Purity, > 99% Survival: 59%, 47%, 54%, 34%, 59%, 35%, 47%, 40% Histopathological examination of the lung and liver only Statistical analysis applied to combined incidence only

Table 3.1 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
JBRC (2000a), Aiso et al. (2014) Crj:BDF$_1$ (M) 24 mo	0, 1000, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk, for 104 wk 50 mice/group	Bronchiolo alveolar adenoma: 7/50 (14%)*, 3/50 (6%), 4/50 (8%), 14/50 (28%) Bronchiolo-alveolar carcinoma: 1/50 (2%)*, 14/50 (28%)**, 22/50 (44%)**, 39/50 (78%)** Bronchiolo-alveolar adenoma or carcinoma (combined): 8/50 (16%)*, 17/50 (34%)***, 26/50 (52%)**, 42/50 (84%)** Hepatocellular adenoma: 10/50 (20%)*, 13/50 (26%), 14/50 (28%), 15/50 (30%) Hepatocellular carcinoma: 10/50 (20%)*, 9/50 (18%), 14/50 (28%), 20/50 (40%)*** Hepatocellular adenoma or carcinoma or hepatoblastoma (combined): 15/50 (30%)*, 20/50 (40%), 25/50 (50%)***, 29/50 (58%)*** Liver haemangioma: 0/50, 4/50 (8%), 3/50 (6%), 5/50 (10%)*** Adrenal gland pheochromocytoma: 1/50 (2%)****, 0/50, 1/50 ((2%), 3/50 (6%) Haemangioma (all organs): 1/50 (2%)****, 5/50 (10%), 6/50 (12%), 7/50 (14%)***	*$P < 0.001$ (trend)[c] **$P < 0.001$ ***$P < 0.05$ ****$P < 0.05$ (trend)	Purity, 99.9% Survival: 76%, 70%, 52%, 40% (statistical analysis, NR) The incidence of haemangioma (all organs) in males at the highest dose did not exceed the upper limit of the historical controls of the laboratory
JBRC (2000a), Aiso et al. (2014) Crj:BDF$_1$ (F) 24 mo	0, 1000, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk 50 mice/group	Bronchiolo-alveolar adenoma: 2/50 (4%), 4/50 (8%), 5/49 (10%), 12/50 (24%)** Bronchiolo-alveolar carcinoma: 3/50 (6%)*, 1/50 (2%), 8/49 (16%), 20/50 (40%)** Bronchiolo-alveolar adenoma or carcinoma (combined): 5/50 (10%)*, 5/50 (12%), 12/49 (24%)***, 30/50 (60%)**	*$P < 0.001$ (trend)[c] **$P < 0.001$ ***$P < 0.05$ ****$P < 0.01$ (trend)	Purity, 99.9% Survival: 52%, 52%, 34%, 42% (statistical analysis, NR)

Table 3.1 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
JBRC (2000a), Aiso et al. (2014) Crj:BDF$_1$ (F) 24 mo (cont.)		Hepatocellular adenoma: 1/50 (2%)*, 7/49 (9%)***, 4/49 (8%), 16/50 (32%)** Hepatocellular carcinoma: 1/50 (2%)*, 1/49 (2%), 5/49 (10%), 19/50 (38%)** Hepatocellular adenoma or carcinoma (combined): 2/50 (4%)*, 8/49 (16%)***, 9/49 (18%)***, 30/50 (60%)** Liver haemangioma or haemangiosarcoma (combined): 3/50 (6%)****, 2/49 (4%), 0/49, 7/50 (14%)		
Theiss et al. (1977) A/St (M) 24 wk	0, 160, 400, 800 mg/kg bw by intraperitoneal injection, 3 × per wk; 24, 17, 17 or 16 times 50 or 20 mice/group	Multiplicity of bronchiolo-alveolar tumours: 0.27, 0.94, 0.80, 0.50	NS	Purity, > 95% No tumour incidence provided Histopathological examination of the lung only. Full histopathology not performed Survival: 47/50, 18/20, 5/20, 12/20

[a] Incidental tumour test
[b] Likelihood ratio score test
[c] Peto test, Fisher exact test
bw, body weight; F, female; h, hour; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; wk, week

controls. The survival of exposed male rats was comparable to that of the controls. The survival of exposed male mice and of female mice at the highest dose was reduced relative to that of the controls. The incidences of bronchiolo-alveolar adenoma and of bronchiolo-alveolar carcinoma were significantly increased in exposed males and females. The incidence of hepatocellular adenoma was significantly increased in females at the highest dose, and the incidence of hepatocellular carcinoma was significantly increased in males and females at the highest dose.

Groups of 68 female B6C3F$_1$ mice (age, 8–9 weeks) were given dichloromethane (purity, > 99%) at a concentration of 0 ppm (control) or 2000 ppm [6940 mg/m^3] by whole-body inhalation for 6 hours per day on 5 days per week for various lengths of time over a 104-week period (Kari et al., 1993). Only the lung and liver were evaluated histopathologically. Survival was reduced compared with controls in groups exposed to dichloromethane for 52, 78, or 104 weeks. The incidences of bronchiolo-alveolar adenoma, bronchiolo-alveolar carcinoma, and adenoma or carcinoma (combined), and the incidences of hepatocellular adenoma, hepatocellular carcinoma, and adenoma or carcinoma (combined) were significantly increased in all groups in which exposure was begun during the first 26 weeks of the study. [The Working Group noted that statistical analyses were reported only for the combined tumour incidences.]

Groups of 50 male and 49 or 50 female Crj:BDF$_1$ mice (age, 6 weeks) were exposed to dichloromethane (purity, > 99.9%) at a concentration of 0, 1000, 2000, or 4000 ppm [0, 3470, 6940, or 13 900 mg/m^3] by whole-body inhalation for 6 hours per day on 5 days per week for up to 104 weeks (JBRC, 2000a; Aiso et al., 2014). Survival rates and body weights of both males and females exposed to 2000 and 4000 ppm were decreased [no statistical analysis reported]. The incidences of bronchiolo-alveolar carcinoma were significantly increased in exposed males and females. The incidences of bronchiolo-alveolar adenoma or carcinoma (combined) were significantly increased in exposed males and females. The incidences of hepatocellular carcinoma were significantly increased in males and females at the highest dose. The incidence of hepatocellular carcinoma, hepatoblastoma, or hepatocellular adenoma (combined) was significantly increased in exposed males, and the incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in females at the highest dose. The incidence of liver haemangioma was increased in males at the highest dose. The incidence of liver haemangioma or haemangiosarcoma (combined) was significantly increased in females at the highest dose. In males, the incidence of pheochromocytoma of the adrenal gland was increased with a positive trend. Hyperplasia in the terminal bronchiole of the lung [this lesion may be classified as a preneoplastic lesion capable of developing into bronchiolo-alveolar adenoma and carcinoma] and peripheral vacuolar change in the liver were increased in males and females at 4000 ppm.

3.1.3 Intraperitoneal injection

In a screening assay based on the production of bronchiolo-alveolar adenoma in strain A mice, groups of 20 male mice (age, 6–8 weeks), were given reagent-grade dichloromethane (purity, > 95%; impurities unspecified) at a dose of 0, 160, 400, or 800 mg/kg bw in tricaprylin by intraperitoneal injection three times per week for a total of 16–17 injections (total doses, 2720, 6800, and 12 800 mg/kg bw in the treated groups, respectively) (Theiss et al., 1977). After 24 weeks, 18, 5, and 12 mice were still alive in the three treated groups, respectively; these and 15 out of 20 surviving mice in the vehicle-control group were killed. Lungs were examined for macroscopic nodules. No significant increase was found in the multiplicity of bronchiolo-alveolar adenoma in exposed mice. [The Working Group

noted that histopathology was not performed on all of those nodules, and multiplicity was the only type of data reported in this study.]

3.2 Rat

There were seven studies of carcinogenicity with dichloromethane in rats (dichloromethane was administered orally in two studies, and by inhalation in five studies).

See Table 3.2

3.2.1 Oral administration

Groups of 25–85 male and female Fischer 344 rats, (age, 7 weeks) were given drinking-water containing dichloromethane (purity, 99%) at a dose of 0 (control group 1), 0 (control group 2), 5, 50, 125, 250 (highest dose), or 250 (recovery group) mg/kg bw per day for 104 weeks (Serota et al., 1986b). Interim terminations were carried out at 26, 52, and 78 weeks in control group 1 and in the groups at the lowest, intermediate, and highest dose, such that 50 males and 50 females per group received treatment for 104 weeks. There was no significant difference in survival between the exposed and control groups. In females, the incidence of hepatocellular carcinoma after 104 weeks was: 0/85, 0/50, 0/85, 2/83, 0/85, 2/85, and 0/25; the incidence of neoplastic nodules [hepatocellular adenomas] was: 0/85, 0/50, 1/85, 2/83, 1/85, 4/85, and 2/25; and the incidence of neoplastic nodules [hepatocellular adenomas] or hepatocellular carcinoma (combined) was: 0/85, 0/50, 1/85, 4/83, 1/85, 6/85, and 2/25 in the seven groups, respectively. This increasing trend was statistically significant (the recovery group was excluded). In male rats, no increased incidence of hepatocellular tumours was observed at 104 weeks. No other significant increase in tumour incidence was found.

Groups of 50 male and 50 female Sprague-Dawley rats (age, 12 weeks), were given dichloromethane (purity, > 99.9%) at a dose of 100 or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 64 weeks (Maltoni et al., 1988). A group of 50 males and 50 females was given olive oil only (vehicle controls) and additional groups of 20 males and 26 females were kept untreated (controls). The rats were then kept under observation for their lifespan. Excess mortality was observed in male and female rats given dichloromethane at the highest dose. An increase in mortality started to appear after 36 weeks of treatment and led to cessation of exposure after 64 weeks [details on mortality not reported]. There was no significant increase in tumour incidence associated with exposure. [The Working Group noted the short period of treatment and the inadequate reporting of the data.]

3.2.2 Inhalation

Groups of approximately 95 male and 95 female Sprague-Dawley rats (age, 8 weeks) were given dichloromethane (purity, > 99%) at a concentration of 0, 500, 1500, or 3500 ppm [0, 1740, 5200, or 12 100 mg/m^3] by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (Burek et al., 1984; EPA, 1985). The numbers of rats per group still alive at the end of the study were 14, 14, 6, 7 for males, and 21, 24, 13, 4 for females, respectively. From the 18th month onwards, the mortality among females at the highest dose was significantly increased. There was no significant increase in the incidence of benign or malignant tumours of the mammary gland; however, the total number of benign tumours of the mammary gland [type not specified] showed a small dose-related increase in males, and a dose-related increase in females [statistics not reported]. The incidence of sarcoma located around the salivary glands was increased in males at the highest dose (1/92, 0/95, 5/95, and 11/97). [The Working Group noted the reported occurrence of sialodacryoadenitis of the salivary

Table 3.2 Studies of carcinogenicity with dichloromethane in rats

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Serota et al. (1986b) F344 (M) 24 mo	0 (control), 0 (control), 5, 50, 125, 250 (highest dose), 250 (recovery group) mg/kg bw per day in drinking-water for 104 wk 50–85 rats/group	Liver neoplastic nodules [hepatocellular adenoma]: 4/85 (5%), 5/50 (10%), 2/85 (2%), 3/84 (3%), 3/85 (3%), 1/85 (1%), 4/25 (16%) Hepatocellular carcinoma: 2/85 (2%), 2/50 (4%), 0/85, 0/84, 1/85 (1%), 0/25 Liver neoplastic nodules [hepatocellular adenoma or hepatocellular carcinoma (combined)]: 6/85 (7%), 7/50 (14%), 2/85 (2%), 3/84 (3%), 3/85 (3%), 2/85 (2%), 4/25 (16%)	NS[a]	Purity, 99% Two vehicle-control groups were run concurrently. No significant exposure-related trend in survival was found in males. The recovery group was exposed for 78 wk
Serota et al. (1986b) F344 (F) 24 mo	0 (control), 0 (control), 5, 50, 125, 250 (highest dose), 250 (recovery group) mg/kg bw per day in drinking-water for 104 wk 25–85 rats/group	Liver, neoplastic nodules [hepatocellular adenoma]: 0/85, 0/50, 1/85 (1%), 2/83 (2%), 1/85 (1%), 4/85 (4%), 2/25 (8%) Hepatocellular carcinoma: 0/85, 0/50, 0/85, 2/83 (2%), 0/85, 2/85 (2%), 0/25 Liver, neoplastic nodules [hepatocellular adenoma] or hepatocellular carcinoma (combined): 0/85*, 0/50, 1/85 (1%), 4/83 (5%)**, 1/85 (1%), 6/85 (7%)**, 2/25 (8%)**	NS[a] NS *$P = 0.0041$ (trend) **$P \leq 0.05$	Purity, 99% Two vehicle-control groups were run concurrently. No significant exposure-related trend in survival was found in females. The recovery group was exposed for 78 wk Incidences within the range of historical controls
Maltoni et al. (1988) Sprague-Dawley (M) Lifetime	0 (untreated control), 0 (olive oil), 100, 500 mg/kg bw by gavage in olive oil, 4–5 days/wk, for 64 wk 20 or 50 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% Excess mortality was observed in male rats at the highest dose ($P < 0.01$) [The period of treatment was short and reporting of data was inadequate]
Maltoni et al. (1988) Sprague-Dawley (F) Lifetime	0 (untreated control), 0 (olive oil), 100, 500 mg/kg bw, by gavage in olive oil, 4–5 days/wk for 64 wk 26 or 50 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% Excess mortality was observed in female rats at the highest dose [The period of treatment was short and reporting of data was inadequate]
Burek et al. (1984), EPA (1985) Sprague-Dawley (M) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, for 6 h/day, 5 days/wk, for 104 wk 92–97 rats/group	Salivary gland sarcoma: 1/92 (1%), 0/95, 5/95 (5%), 11/97 (11%)* Total number of benign mammary gland tumours: 8, 6, 11, 17	*$P = 0.002$[b] NR	Purity, > 99% No exposure-related effect on mortality

Table 3.2 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Burek et al. (1984), EPA (1985) Sprague-Dawley (F) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 95–97 rats/group	Total number of benign mammary gland tumours: 165, 218, 245, 287	NR	Purity, > 99% Mortality among females at the highest dose was significantly increased
NTP (1986) F344 (M) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/day, 5 days/wk, for 102 wk 50 rats/group	Mammary gland adenoma or fibroadenoma (combined): 0/50*, 0/50, 2/50 (4%), 5/50 (10%)** Subcutis, fibroma or sarcoma (combined): 1/50 (2%)***, 1/50 (2%), 2/50 (4%), 5/50 (10%)	*$P < 0.001$ (trend)[c] **$P = 0.023$ ***$P = 0.026$ (trend)	Purity, 99% Survival: 32%, 32%, 34%, 18%
NTP (1986) F344 (F) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/day, 5 days/wk, for 102 wk 50 rats/group	Mammary gland adenoma or fibroadenoma (combined): 5/50 (10%), 11/50 (22%), 13/50 (26%), 23/50 (26%)	$P < 0.001$ (trend)[c] $P < 0.001$ (high dose) $P < 0.05$ (mid-dose) $P < 0.05$ (low dose)	Purity, 99% Survival: 60%, 44%, 44%, 30%
Maltoni et al. (1988) Sprague-Dawley (F) Lifetime	0, 100 ppm, by inhalation, 4 h/day, 5 days/wk, for 7 wk, then 7 h/day, 5 days/wk, for 97 wk Start at age 13 wk (breeders) 60, 54 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% No excess in mortality was found in the exposed group [Low exposure concentration and inadequate reporting of data]
Maltoni et al. (1988) Sprague-Dawley (M) Lifetime	0, 60 ppm, by inhalation, 4 h/day, 5 days/wk, for 7 wk, then 7 h/day, 5 days/wk, for 97 wk; or 7 h/day, 5 days/wk, for 8 wk Start at day 12 of gestation 158 or 60 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity. 99.9% No excess in mortality was found in the exposed groups [Low exposure concentration and inadequate reporting of data]
Maltoni et al. (1988) Sprague-Dawley (F) Lifetime	0, 60 ppm, by inhalation, 4 h/day, 5 days/wk for 7 wk, then 7 h/day, 5 days/wk for 97 wk or 7 h/day, 5 days/wk for 8 wk Start at day 12 of gestation 149, 69 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% No excess in mortality was found in the exposed groups [Low exposure concentration and inadequate reporting of data]

Table 3.2 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nitschke et al. (1988) Sprague-Dawley (M) 20 mo	0, 50, 200, 500 ppm by inhalation, 6 h/day, 5 days/wk 90 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, > 99.5% No exposure-related adverse effect on body weight or mortality was observed
Nitschke et al. (1988) Sprague-Dawley (F) 24 mo	0, 50, 200, 500 ppm, by inhalation, 6 h/day, 5 days/wk 108 rats/group Fifth group: 500 ppm for 12 mo, then to 0 ppm for 12 mo, 30 rats/group Sixth group: 0 ppm for 12 mo, then to 500 ppm for 12 mo, 30 rats/group	Mammary gland adenoma or fibroadenoma: 52/70 (74%), 58/70 (82%), 61/70 (71%)*, 55/70 (78%), 23/30 (77%), 23/30 (77%)	*$P < 0.05$[b]	Purity, > 99.5% No exposure-related adverse effect on body weight or mortality was observed
JBRC (2000b), Aiso et al. (2014) F344/DuCrj (M) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 50 rats/group	Subcutis fibroma: 1/50 (2%), 4/50 (8%), 7/50 (14%), 12/50 (24%) Mammary gland fibroadenoma: 1/50 (2%), 2/50 (4%), 3/50 (6%), 8/50 (16%) Peritoneal mesothelioma: 3/50 (6%), 1/50 (2%), 0/50, 7/50 (14%)	$P < 0.001$ (trend), $P < 0.001$ (high dose), $P < 0.05$ (mid-dose)[d] $P < 0.001$ (trend), $P < 0.05$ (high dose)[b] $P < 0.05$ (trend)[d]	Purity, 99.9% Survival: 64%, 86%, 76%, 56%
JBRC (2000b), Aiso et al. (2014) F344/DuCrj (F) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/days, 5 days/wk, for 104 wk 50 rats/group	Mammary gland fibroadenoma: 7/50 (14%), 7/50 (14%), 9/50 (18%), 14/50 (28%)	$P < 0.01$ (trend)[d]	Purity, 99.9% Survival: 90%, 80%, 86%, 60%

[a] Cochran-Armitage, χ^2 test
[b] Fisher exact test
[c] Incidental tumour test
[d] Peto test, Fisher exact test
bw, body weight; F, female; h, hour; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; wk, week

gland early in the study. The effect of this viral infection on tumour formation is unknown.]

Groups of 50 male and 50 female Fischer 344/N rats (age, 7–8 weeks) were exposed to dichloromethane (purity, 99%) at a concentration of 0, 1000, 2000, or 4000 ppm (0, 3470, 6940, or 13 900 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for 102 weeks and were killed after 104 weeks (NTP, 1986). Mean body weights of control and dosed males and females were similar throughout the study. Survival of treated males was similar to that of controls. Survival at termination of the study was reduced in females at the highest dose compared with controls. Significantly increased incidences of benign tumours of the mammary gland (all fibroadenoma, except for one adenoma in the group at the highest dose) were observed in treated females (5/50, 11/50, 13/50, 23/50). In males, there was a positive trend in the incidences of adenoma or fibroadenoma (combined) of the mammary gland, and of fibroma or sarcoma (combined) of the subcutis. There was no difference in the distribution of other types of tumours in the control and treated groups.

Groups of 54–70 male and female Sprague-Dawley rats (age, 13 weeks), were given dichloromethane (purity, > 99.9%) at a concentration of 100 ppm [347 mg/m^3] or 60 ppm [208 mg/m^3] by whole-body inhalation for 7 hours per day, 5 days per week (Maltoni et al., 1988). The exposure was started in female breeders, and male and female offspring (12-day embryos). The breeders and a first group of offspring were exposed for 104 weeks, and a second group of offspring was exposed for 15 weeks only. Control groups were composed of 60 female rats (untreated breeders controls), and 158 males and 149 females (untreated offspring controls). The rats were observed for their lifespan. No excess in mortality was found in the exposed groups. No significant increase in the incidence of any tumour type was noted. [The Working Group noted the low concentration of exposure.]

Groups of 90 male and 108 female Sprague-Dawley rats [age unspecified] were given dichloromethane (technical-grade; purity, > 99.5%) at a concentration of 0, 50, 200, or 500 ppm [0, 174, 694, or 1740 mg/m^3] by whole-body inhalation for 6 hours per day, 5 days per week, for 20 (males) or 24 (females) months (Nitschke et al., 1988). An additional group of 30 female rats was exposed to dichloromethane at 500 ppm for the first 12 months and to room air for the last 12 months of the study. An additional group of 30 female rats was exposed to room air for the first 12 months, followed by dichloromethane at 500 ppm for the last 12 months of the study. Subgroups of five rats per sex per exposure level were scheduled for interim termination after 6, 12, 15, or 18 months of exposure to dichloromethane. No exposure-related adverse effect on body weight or mortality was observed. In females, the incidences of benign tumours of the mammary gland (adenomas and fibroadenomas, combined) were 52/70, 58/70, 61/70 [significant increase], and 55/70 in the control group, and the groups at the lowest, intermediate, and highest dose, respectively. No significant increase in the incidence of any other tumour type was seen in the exposed groups. There was no significant increase in the incidence of any tumour type in males.

Groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were exposed to dichloromethane (purity, 99.9%) at concentrations of 0, 1000, 2000, or 4000 ppm (0, 3470, 6940, or 13 900 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2000b; Aiso et al., 2014). Survival rates of females exposed to dichloromethane at 4000 ppm were decreased compared with the controls [no statistical analysis reported]. The incidence of fibroma of the subcutis was significantly increased in exposed males. The incidence of fibroadenoma of the mammary gland was significantly increased in males at the highest dose and with a positive trend in females. The incidence

Table 3.3 Studies of carcinogenicity with dichloromethane in hamsters

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Burek et al. (1984), EPA (1985) Syrian golden (Ela:Eng) (M) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 95 hamsters/group	No significant differences in tumour incidence between control and treated hamsters	NS	Purity, > 99%
Burek et al. (1984), EPA (1985) Syrian golden (Ela:Eng) (F) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 95 hamsters/group	Lymphosarcoma [malignant lymphoma]: 1/91 (1%), 6/92 (6%), 3/91 (3%), 7/91 (8%)*	$P < 0.05$[a]	Purity, > 99% Survival at the end of experiment: 0, 4, 10, 9

[a] Fischer exact test
F, female; h, hour; M, male; mo, month; NS, not significant; ppm, parts per million; wk, week

of peritoneal mesothelioma was significantly increased with a positive trend in males.

3.3 Hamster

There was one study of carcinogenicity in hamsters treated with dichloromethane by inhalation.

See Table 3.3

Groups of 95 male and 95 female Syrian golden hamsters (*Mesocricetus auratus*) (age, 8 weeks), were given dichloromethane (purity, > 99%) at a concentration of 0, 500, 1500, or 3500 ppm (0, 1740, 5200, or 12 100 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (Burek et al., 1984; EPA, 1985). The numbers of hamsters surviving to the end of the study were 16, 20, 11, and 14 in males, and 0, 4, 10, and 9 in females. The incidence of lymphosarcoma [malignant lymphoma] was significantly higher in females at the highest dose than in controls (1/91, 6/92, 3/91, and 7/91). [The Working Group noted that the higher survival in treated hamsters may have contributed to this non-dose-dependent result for which historical control data were not available.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Dichloromethane is a lipophilic solvent of low relative molecular mass, which can readily cross biological membranes. Pulmonary uptake is rapid, approaching steady state within a few hours after the start of exposure (Riley et al., 1966; DiVincenzo et al., 1971, 1972; Astrand et al., 1975; DiVincenzo & Kaplan, 1981). Measured values of pulmonary uptake are about 55–75% at rest and 30–40% during physical exercise (Astrand et al., 1975; DiVincenzo & Kaplan, 1981). The blood:air partition coefficient for dichloromethane describes the ratio of the concentrations in the two media at steady state, and is a factor in determining pulmonary uptake. The partition coefficient has been measured in vitro using vial equilibrium methods. Mean reported values range from around 8 to 10 for humans (Sato & Nakajima, 1979; Gargas et al., 1989; Meulenberg & Vijverberg, 2000). However, these data might have been influenced

by the presence of glutathione *S*-transferase T1 (GSTT1) in human erythrocytes (Schröder et al., 1996).

Data on oral absorption in humans are limited to case reports of accidental ingestion, and suggest that dichloromethane is also readily absorbed by this route of exposure (Hughes & Tracey, 1993; Vetro et al., 2012). Quantitative estimates of oral bioavailability in humans are not available because the ingested amounts are not known precisely.

Ursin et al. (1995) report that the permeability of human skin to dichloromethane is 24 g/m^2 per hour. No other information on human dermal absorption of dichloromethane was available to the Working Group.

(b) Experimental systems

Inhalation studies in experimental animals provide clear evidence that dichloromethane is readily absorbed via the lungs into the systemic circulation (Carlsson & Hultengren, 1975; Anders & Sunram, 1982; McKenna et al., 1982; Andersen et al., 1991). The blood:air partition coefficient for dichloromethane, measured in vitro using vial equilibrium methods, has been reported to range from 19 to 23 for rodents (Gargas et al., 1989; Marino et al., 2006).

Absorption from the gut after oral doses is rapid and nearly complete, according to reports of several studies with radiolabel in mice and rats (McKenna & Zempel, 1981; Angelo et al., 1986a, b). For instance, Angelo et al. (1986b) reported that on average 97% of the radiolabel was recovered in expired air as dichloromethane, CO, and carbon dioxide (CO_2) in the 24 hours after each repeated oral dose of 50 or 200 mg/kg per day in rats. Angelo et al. (1986a) reported absorption in mice to be more rapid (but equally extensive) with an aqueous vehicle than with an oil-based vehicle, consistent with studies on other chlorinated solvents.

No studies of dermal uptake of dichloromethane in experimental animals were available to the Working Group.

4.1.2 Distribution and body burden

(a) Humans

Once absorbed, dichloromethane enters blood circulation and undergoes rapid systemic distribution to tissues. The highest concentrations are expected in adipose tissue and other fatty tissues, due to the lipophilicity of the compound. Engström & Bjurström (1977) detected dichloromethane in fat biopsy specimens obtained from men exposed to dichloromethane for 1 hour during light exercise. Other data in humans on tissue distribution in vivo are limited to tissues taken from autopsies after accidental fatalities, which showed wide systemic distribution in blood and across all tested tissues, including the fat, lung, liver, heart, kidney, spleen, and brain (Moskowitz & Shapiro, 1952; Winek et al., 1981; Shinomiya & Shinomiya, 1985; Manno et al., 1989; Leikin et al., 1990; Kim et al., 1996; Goullé et al., 1999). Goullé et al. (1999) and Leikin et al. (1990) measured the largest number of tissues, and found the highest concentrations in brain, spleen, and fat.

Engström & Bjurström (1977) also measured an in-vitro partition coefficient of 51 between adipose tissue and air using a vial equilibrium method. This value is about five times the blood:air partition coefficient, consistent with the lipophilicity of dichloromethane. Partition-coefficient measurements for other human tissues were not available to the Working Group.

(b) Experimental systems

Studies in experimental animals provide clear evidence that dichloromethane distributes widely to all tissues of the body. After in-vivo oral and/or intravenous exposures in mice and/or rats, dichloromethane has been measured in the liver, kidney, lung, and whole carcass, with

the highest concentrations in the liver (Angelo et al., 1986a, b). Several inhalation experiments with radiolabeled dichloromethane detected the presence of radiolabel in all tissues, including the liver, kidney, adrenals, brain, fat, lung, muscle, and testes (Carlsson & Hultengren, 1975; McKenna et al., 1982). While part of the radiolabel is likely to be metabolites, it is likely that a substantial portion also represents dichloromethane. Experiments in animals show that dichloromethane readily crosses the blood–brain barrier and the placenta (Savolainen et al., 1981; Anders & Sunram, 1982).

Tissue:air partition coefficients have also been measured in vitro for several tissues in rats and mice, including fat, liver, muscle, skin, kidney, and brain (Andersen et al., 1987; Gargas et al., 1989; Clewell et al., 1993). The highest reported values are for fat (60–120), with values for the remaining tissues ranging from 8 to 40, as compared with blood:air partition coefficients of around 20.

4.1.3 Metabolism

(a) Overview

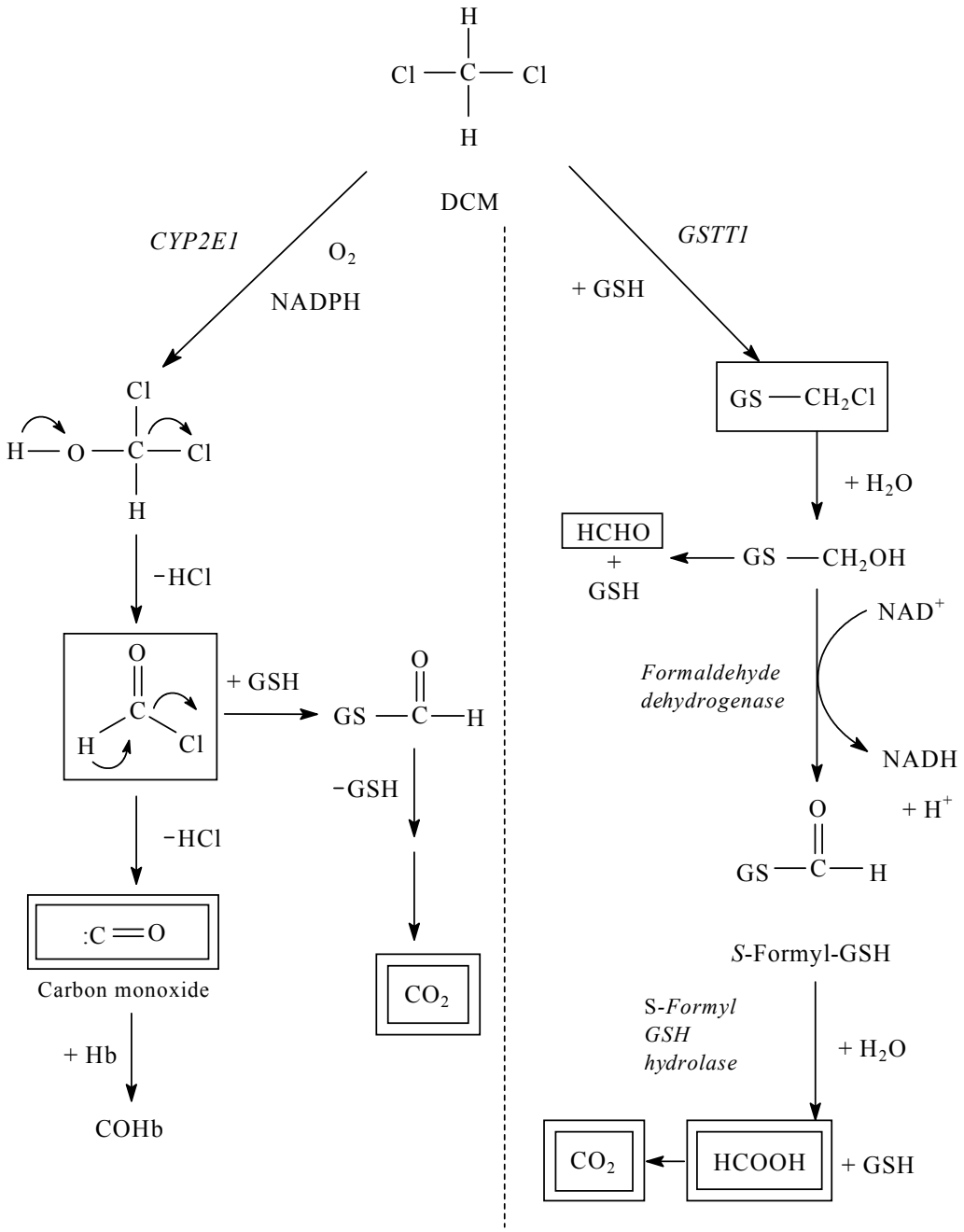
The pathways for metabolism of dichloromethane were initially characterized nearly 40 years ago in the mid-1970s and are widely considered to be well established (Kubic & Anders, 1975, 1978; Ahmed & Anders, 1976, 1978). Dichloromethane is metabolized by either of two pathways, as summarized in Fig. 4.1.

One pathway, a reductive dehalogenation that is a mixed-function oxygenation, was subsequently shown to be catalysed by cytochrome P450 2E1 (CYP2E1) (Guengerich et al., 1991), and ultimately generates CO and CO_2 as stable end products. The initial product of the reaction, chloromethanol, spontaneously rearranges to form formyl chloride, which is reactive and can spontaneously generate CO or react with nucleophiles such as glutathione (GSH) to generate formylglutathione; the latter rearranges to release CO_2. CO avidly reacts with haemoglobin, displacing oxygen and forming COHb.

The other pathway for dichloromethane metabolism involves conjugation with GSH, forming *S*-chloromethyl GSH. The conjugation is catalysed by GSTs, with the GSTT1 isoform being the most active (Mainwaring et al., 1996; Sherratt et al., 1997). *S*-Chloromethyl GSH is reactive and is believed to be one of the dichloromethane metabolites responsible for DNA binding and mutagenicity (Graves & Green, 1996). Alternatively, *S*-chloromethyl GSH can be hydrolysed to form hydroxymethyl GSH, which can either decompose to release formaldehyde or be oxidized by formaldehyde dehydrogenase to form *S*-formyl GSH. The latter is subsequently hydrolysed to release formic acid and GSH. Formic acid further decomposes to release CO_2. Thus, while both the CYP and GST pathways can generate CO_2, only the CYP pathway produces CO from dichloromethane. Although both pathways can generate reactive and unstable metabolites that are mechanistically linked to dichloromethane-induced genotoxicity and carcinogenesis, it is thought that these come primarily from the GST pathway (Andersen et al., 1987).

Despite the wealth of data over more than three decades from in-vivo and in-vitro studies in humans and experimental animals, which supports the function of both CYP2E1 and GSTT in dichloromethane metabolism, Evans & Caldwell (2010a) proposed a different explanation for dichloromethane metabolism that involves only CYP2E1. As precedent for this alternative metabolic pathway, the authors cited studies by Harrelson and colleagues (Harrelson et al., 2007, 2008) and Tracy (2006) and two studies by Guengerich and colleagues (Watanabe & Guengerich, 2006; Watanabe et al., 2007). Their conclusion was that the available data support a limited role for GST-dependent metabolism. Anders et al. (2010) criticized this proposal by noting that the authors misinterpreted the data from Watanabe & Guengerich (2006) and

Fig. 4.1 Pathways for the metabolism of dichloromethane



Dichloromethane is metabolized by both cytochrome P450 (CYP) and glutathione (GSH) conjugation. Reaction products surrounded by single rectangles are chemically reactive and are believed to be mechanistically linked to dichloromethane-induced mutagenesis and carcinogenesis. Reaction products surrounded by double rectangles are stable end products

CoHb, carboxyhaemoglobin; CDNB, 1-chloro-2,4-dinitrobenzene; CYP, cytochrome P450; CYP2E1, cytochrome P450 2E1; DCM, dichloromethane; GSH, glutathione; GSTT1, glutathione S-transferase theta-1; Hb, haemoglobin; MOA, mode of action; PBPK, physiologically based pharmacokinetic

Based on Ahmed & Anders (1978) and Kubic et al. (1974)

Adapted from Andersen et al. (1987) with permission from Toxicology and applied pharmacology, Vol 87, Andersen ME, Clewell HJ 3rd, Gargas ML, Smith FA, Reitz RH, Physiologically based pharmacokinetics and the risk assessment process for methylene chloride, Page Nos 185–205, Copyright Elsevier (1987)

Watanabe et al. (2007), for the limited in-vitro data supporting the alternative mechanism, for dismissing the wealth of data on the role of GSTT in dichloromethane metabolism, mutagenicity, and carcinogenicity, and for rejecting without any sound basis the several well-established and validated physiologically based pharmacokinetic models of dichloromethane metabolism in humans and rodents. Although Evans & Caldwell (2010b) maintained the validity of their interpretations, no data supporting metabolism of dichloromethane that exclude GST, particularly at higher dichloromethane concentrations, were identified by the Working Group.

Specific studies on dichloromethane metabolism and mechanisms in humans and human-derived tissues and in experimental systems are summarized below.

(b) Humans or human-derived tissues

Oxidative metabolism of dichloromethane to CO was first demonstrated in occupationally exposed humans (Stewart et al., 1972a, b; Ratney et al., 1974; Astrand et al., 1975). DiVincenzo & Kaplan (1981) measured dichloromethane metabolism using COHb in nonsmoking volunteers exposed to dichloromethane vapour at concentrations of up to 200 ppm for 7.5 hours (once, or daily for 5 days). Dose-dependent COHb formation was readily demonstrated, with the single-day exposures resulting in peak CoHb saturations of 1.9%, 3.4%, 5.3%, and 6.8%, respectively, at 0, 50, 100, and 200 ppm. A comparative study of the effects in humans exposed to either CO or dichloromethane up to concentrations that produce 5% COHb saturation was performed; both substances impaired performance (Putz et al., 1979), this was consistent with evidence that about 70% of dichloromethane at relatively low doses is metabolized to CO (Andersen et al., 1991).

Metabolic parameter estimates made by Clewell (1995) show that the oxidative pathway in human liver has a capacity of 100- to 200-fold that of the GST pathway, although in-vitro studies by Reitz et al. (1989) generally showed a much more modest difference in capacity of the two pathways, with the CYP pathway having a two- to fourfold higher capacity than the GST pathway in most of the human liver samples studied.

Bogaards et al. (1993) measured GST activity with dichloromethane and 1-chloro-2,4-dinitrobenzene (CDNB) in nine human liver cytosol samples, finding three distinct activity groups. Specifically, with dichloromethane, two exhibited no detectable activity, four exhibited relatively low activity (0.2–0.4 nmol/min per mg protein), and three exhibited relatively high activity (0.9–1.1 nmol/min per mg protein). Interestingly, although metabolic activity with CDNB as substrate also exhibited an approximately five-fold variation among the nine samples, there were no apparent null variants and the pattern of metabolism with CDNB and dichloromethane did not coincide. While CDNB is a substrate for multiple GST isoforms (Habig et al., 1974), it is now widely accepted that dichloromethane is selectively metabolized by GSTT (see below).

Mainwaring et al. (1996) determined mRNA and protein expression of GSTT1 in cells from human liver and lung, both of which are target organs for dichloromethane in the mouse. While expression of GSTT1 was readily detected in the liver, very low levels were detected in the lungs. Furthermore, GSTT1 activity with dichloromethane was measured in three samples of lung at 0.06, 0.21, and 0.23 nmol/min per mg protein, which was about one order of magnitude less than that in human liver.

Casanova et al. (1997) detected RNA–formaldehyde adducts in human hepatocytes with functional GST genes and incubated with dichloromethane, which is evidence that formaldehyde is formed in human cells as a metabolite of dichloromethane.

GST activity in human liver was further related to carcinogenic risk with dichloro-

methane in studies of GSTT1 polymorphism (El-Masri et al., 1999; Sherratt et al., 2002; Olvera-Bello et al., 2010). Although the importance of genetic polymorphisms in determining carcinogenic risk is discussed elsewhere (see Section 4.5.1), it is mentioned here as providing further evidence of the presence and importance of GST activity in dichloromethane metabolism.

In addition to absolute levels of GSTT protein expression in target organs, another important issue is the subcellular localization of the expressed enzyme. While GSTT11 in mouse liver is readily found in cytoplasm and nuclei of hepatocytes, it is found at lower levels in nuclei of bile-duct epithelial cells, and in cytoplasm and nuclei of some human hepatocytes (Sherratt et al., 2002). This less intense nuclear localization is thought to be of significance for carcinogenic risk because less *S*-chloromethyl GSH and formaldehyde will be generated near DNA.

GST is also present in human erythrocytes and is thought to play a role in toxicity of dichloromethane in lymphocytes (Hallier et al., 1993, 1994). Erythrocyte GSTT is polymorphic, as further discussed in Section 4.5.

(c) Experimental systems

(i) Rat

The metabolism of dichloromethane has been extensively studied in several experimental systems, predominantly those derived from rodents. This is particularly important in that mouse liver and lung have been identified as prominent target organs for dichloromethane, and toxicity has been clearly linked to metabolism. Some of the earliest studies that established the basic outlines of dichloromethane metabolism were conducted in rat liver microsomes (Kubic & Anders, 1975, 1978), rat liver cytosol (Ahmed & Anders, 1976, 1978), and rat lung microsomes (Kubic & Anders, 1975).

As noted above, the CYP-dependent oxidative pathway is considered to be a high-affinity, low-capacity pathway for dichloromethane metabolism, while the GST pathway is a low-affinity, high-capacity pathway. An in-vivo study of metabolism after oral administration of ^{14}C-labelled dichloromethane in rats showed dose-dependent metabolism primarily to CO and CO_2, with clear evidence of saturation (McKenna & Zempel, 1981). While rats given a dose of dichloromethane at 1 mg/kg metabolized approximately 88% of the administered dose over 48 hours, those given dichloromethane at 50 mg/kg only metabolized about 28% of the administered dose over the same period. Saturation of dichloromethane metabolism after inhalation in rats was also demonstrated by Kurppa & Vainio (1981), who showed that blood COHb levels stabilized at dichloromethane exposures of 500 ppm.

Gargas et al. (1986) measured COHb levels in rats given dichloromethane or other dihalomethanes by inhalation in a closed-atmosphere exposure system. The bromine-containing dihalomethanes exhibited the highest activities, while fluorine-containing dihalomethanes exhibited no detectable activity. Maximal rates of COHb formation from dibromomethane, chlorobromomethane, and dichloromethane were 72, 54, and 47 μmol/kg per hour, respectively. Pretreatment with pyrazole, which inhibits microsomal oxidation, abolished production of CO. Depletion of GSH with 2,3-epoxypropanol increased the steady-state levels of COHb generated from dichloromethane.

Takano & Miyazaki (1988) applied dichloromethane to perfused livers of male Wistar rats previously given phenobarbital to induce CYP, and examined spectral changes by scanning reflectance spectrophotometry. Both with and without addition of exogenous CO, a type-I spectral change with a peak at 450 nm was observed, demonstrating CYP-dependent metabolism of dichloromethane to CO in the intact rat liver.

Kim & Kim (1996) further explored the role of CYP2E1 in dichloromethane metabolism by

Table 4.1 Reaction rates for dichloromethane metabolism by CYP and GST in liver and lung tissue from different species

Enzyme activity	Organ	Concentration of dichloromethane (mM)	Reaction rate (nmol product formed/min per mg protein)			
			Mouse	Rat	Hamster	Human[a]
CYP	Liver	1	5.87	2.40	7.18	1.57
		5	11.4	4.10	14.5	3.90
		10	14.4	4.91	18.2	4.69
	Lung	5	4.62	0.16	0.99	< 0.1
GST	Liver	10	7.24	1.11	0.31	–
		25	18.5	3.19	0.76	2.41
		50	33.2	6.17	1.24	3.73
		100	48.6	12.1	2.64	4.34
	Lung	40	7.3	1.0	0.0	0.37

[a] Average value from two samples of human tissue for liver, and value from a single sample of lung

CYP, cytochrome P450; GST, glutathione *S*-transferase

Adapted from Toxicology Letters, Volume 43, issue 1–3, Reitz et al. (1988). Incorporation of in vitro enzyme data into the physiologically-based pharmacokinetic (PB-PK) model for methylene chloride: implications for risk assessment, pp. 97–116, Copyright (1988), with permission from Elsevier

examining the effect of prior administration of organic solvents that induce CYP2E1 on COHb levels in adult female rats after intraperitoneal administration of dichloromethane (3.0 mmol/kg). Peak COHb levels in blood reached 21%, 16%, and 23% in rats pretreated with benzene, toluene, or m-xylene, respectively, compared with only about 10% in rats given dichloromethane alone. The selective CYP2E1 inhibitor disulfiram (3.4 mmol/kg) blocked the elevations in COHb. No effects on hepatic GSH levels were observed with the single administration of the solvents, indicating no involvement with changes in the GST pathway in the observed responses.

(ii) Mouse

Reitz and colleagues analysed dichloromethane metabolism by the CYP and GST pathways in the liver and lung of male B6C3F$_1$ mice, F344 rats, Syrian golden hamsters, and humans (Table 4.1; Reitz et al., 1988). Several striking species-dependent differences are clearly evident from the data. First, mice exhibit similar rates of CYP-dependent metabolism in liver as hamsters and nearly threefold higher rates than rats or humans. Second, in lung tissue CYP-dependent metabolism of dichloromethane in mice was ~30-fold higher than in rats and ~5-fold higher than in hamsters. No CYP-dependent metabolism was detected in the human lung sample. Third, even greater species-dependent differences in addition to interindividual differences were observed in the liver and lung for GST-dependent dichloromethane metabolism. In this case, rates of GSH conjugation in mouse liver were ~4-fold faster than in rats, ~20-fold faster than in hamsters, and ~10-fold faster than in humans. Finally, perhaps the greatest species-dependent metabolic difference was observed for GST metabolism in the lung. Here, rates in mice were ~7-fold faster than in rats and ~20-fold faster than in humans. These metabolic differences have been interpreted to explain species-dependent differences as well as interindividual differences in target-organ specificity and sensitivity to dichloromethane-induced mutagenesis and carcinogenicity (Green, 1990; Starr et al., 2006). Furthermore, data on tumour incidence across species show a correlation with

the amount of dichloromethane metabolized by GST but not by CYP (Andersen et al., 1987).

Ottenwälder et al. (1989) gave two specific CYP inhibitors (i.e. pyrazole, 320 mg/kg, and diethyldithiocarbamate, 300 mg/kg) to male B6C3F$_1$ mice also exposed to dichloromethane at 1000 or 3000 ppm, or a mixture of dichloromethane at 1000 ppm and methyl chloride at 1000 ppm. For those mice given only dichloromethane, uptake by inhalation was markedly decreased by the CYP inhibitors. In contrast, CYP inhibitors had no effect on the uptake of methyl chloride by inhalation. Because methyl chloride is metabolized solely by GSTs, these results showed that even at relatively high exposures, dichloromethane is predominantly metabolized by CYP. These results contrasted with those of Andersen et al. (1987) described above, who concluded that GST-dependent, rather than CYP-dependent, metabolism was critical for dichloromethane-induced liver tumorigenesis.

The in-vivo metabolism of dichloromethane by CYP was further demonstrated by Casanova et al. (1992), who pre-exposed male B6C3F$_1$ mice to dichloromethane at 4000 ppm for 6 hours per day for 2 days, and then on day 3 to ^{14}C-labelled dichloromethane at a declining concentration (4500–2500 ppm). DNA–protein cross-links and incorporation of ^{14}C derived from dichloromethane into DNA was observed in the liver of these mice.

Foster et al. (1994) also showed that modulation of pulmonary CYP activity can also alter responses of the lung to dichloromethane.

4.1.4 Excretion

(a) Humans

In humans, the main route of excretion of dichloromethane is by exhalation of the parent compound and its primary metabolites CO_2 and CO, with lesser amounts as dichloromethane excreted in the urine (DiVincenzo et al., 1971, 1972; DiVincenzo & Kaplan, 1981). DiVincenzo & Kaplan (1981) estimated that only 5% of absorbed dichloromethane is exhaled unchanged, 25–34% excreted converted as CO, and the balance excreted as CO_2. After cessation of exposure, the half-life of dichloromethane in the blood has been estimated to be about 40 minutes, with concentrations of parent and metabolites returning the preexposure levels within a few days (DiVincenzo et al., 1972; DiVincenzo & Kaplan, 1981). Urinary excretion occurs mostly during and/or within the first hour after cessation of exposure, and in total accounts for less than 0.1% of uptake (DiVincenzo et al., 1971, 1972).

(b) Experimental systems

As in humans, the main route of excretion of dichloromethane in experimental animals is by exhalation of the parent compound and its primary metabolites CO_2 and CO, with lesser amounts excreted in the urine. As exposure levels increase, the percentage excreted as unchanged parent compound increases, reflecting saturation of metabolism. For instance, McKenna et al. (1982) reported that in rats exposed to dichloromethane at 50 ppm via inhalation, elimination in expired air consists of about 5% parent compound, and 26% and 27% CO_2 and CO, respectively. At 500 and 1500 ppm, elimination of parent compound increased to 30% and 55%, respectively, with declines in the amount of CO_2 and CO expired. Similarly, for oral doses of 1 mg/kg, McKenna & Zempel (1981) reported that rats exhaled 12% of the administered dose as parent compound, and 35% and 31% as CO_2 and CO, respectively. At higher oral doses (50 mg/kg or greater), rats and mice exhale greater amounts as parent compound (60–80%), and lesser amounts as CO_2 and CO (McKenna & Zempel, 1981; Angelo et al., 1986a, b).

Overall, experimental studies in rodents have found that > 90% of absorbed dichloromethane is eliminated within 24 or 48 hours of exposure, regardless of dose. McKenna et al. (1982) reported

Table 4.2 Studies of genotoxicity with dichloromethane in human cell lines in vitro

Test system	Results[a]		Concentration (LEC or HIC)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Single-strand breaks, human primary hepatocytes	–	NT	5100	Graves et al. (1995)
DNA–protein cross-links, human hepatocytes (expressing GSTT1)	–	NT	425	Casanova et al.(1997)
Unscheduled DNA synthesis, human AH fibroblasts	–	NT	65 000	Jongen et al. (1981)
Micronucleus test, human MCL-5 and h2E1 lymphoblastoid cells	+[c]	NT	200	Doherty et al. (1996)
Micronucleus test, human AHH-1 lymphoblastoid cells	–	NT	850	Doherty et al. (1996)
Sister-chromatid exchanges, human lymphocytes	+[d]	NT	290	Hallier et al. (1993)
Sister-chromatid exchange, human peripheral blood mononuclear cells	+	NT	60 ppm	Olvera-Bello et al. (2010)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LEC, lowest effective concentration; HIC, highest ineffective concentration; in-vitro tests, µg/mL (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg /mL in atmosphere)
[c] Induction of kinetochore-positive and -negative micronuclei
[d] Positive results were reported in lymphocytes from donors lacking GST activity

that after inhalation exposure in rats, a low percentage of the initial body burden of dichloromethane remained at 48 hours. After a single intravenous dose in mice, Angelo et al. (1986a) reported 92–94% recovery within 4 hours after dosing. After repeated oral exposures in mice, Angelo et al. (1986a) reported 90–96% recovery of within 24 hours after each dose.

4.2 Genetic and related effects

Dichloromethane has been studied for genotoxic potential in a variety of assays. The genotoxicity of dichloromethane has been reviewed previously by the Working Group (IARC, 1999).

4.2.1 Humans

(a) In vivo

No data were available to the Working Group.

(b) In vitro

See Table 4.2

Dichloromethane did not induce DNA single-strand breaks in human primary hepatocytes (Graves et al., 1995). There was no induction of DNA–protein cross-links in vitro in human hepatocytes with functional *GSTT1* genes (Casanova et al., 1997) or unscheduled DNA synthesis in AH fibroblasts (Jongen et al., 1981) after treatment with dichloromethane in vitro.

In a study by Doherty et al. (1996), dichloromethane induced the formation of kinetochore-staining micronuclei (which are indicative of aneuploidy) and kinetochore-negative micronuclei in human MCL-5 cells that stably express cDNA encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1, and epoxide hydrolase and in h2E1 cells, which contains a cDNA for CYP2E1. The increased frequency of micronucleus formation is combined with the fact that MCL-5 and h2El cell lines showed the capacity to produce metabolites in the presence of dichloromethane. AHH-1 cells, constitutively expressing CYP1A1, showed no increase in the total frequency of

micronucleus formation or in the frequency of kinetochore-staining micronuclei.

Hallier et al. (1993) showed that sister-chromatid exchanges were induced in human peripheral blood lymphocyte cultures from non-conjugator donors lacking GST activity, but not in those from conjugators. This study did not provide details on the type of GST activity that was monitored. Sister-chromatid exchanges were also induced by dichloromethane in vitro in human peripheral blood mononuclear cells (Olvera-Bello et al., 2010). This study also demonstrated that the group with high GSTT1 activity showed a larger increase in the frequency of sister-chromatid exchanges induced by dichloromethane than did the groups with low and medium GSTT1 activity.

4.2.2 Experimental systems

(a) Mammalian systems

See Tables 4.3 and 4.4

(i) DNA damage

Exposure of B6C3F_1 mice to dichloromethane by inhalation induced DNA single-strand breaks in the lung and liver (Graves et al., 1995). Prior treatment of the mice with buthionine sulfoximine (a depletor of GSH) immediately before exposure to dichloromethane reduced the amount of DNA damage to control levels.

Dichloromethane induced DNA single-strand breaks in vivo in AP rat primary hepatocytes and B6C3F_1 mouse hepatocytes (Graves et al., 1994b), and in Clara cells (Graves et al., 1995). DNA damage was reduced in Clara cells co-treated with buthionine sulfoximine. DNA single-strand breaks were not observed in the liver or lung of AP rats treated by inhalation (Graves et al., 1994b, 1995), but were induced in the liver of CD rats treated by gavage (Kitchin & Brown, 1994), and in the liver of B6C3F_1 mice treated by inhalation (Graves et al., 1994b). Dichloromethane did not cause DNA damage as measured by the comet assay in male B6C3F_1 mice exposed by inhalation for 6 weeks (6 hours per day, 5 days per week) at 400, 800, or 1600 ppm (Suzuki et al., 2014).

The frequency of DNA single-strand breaks was increased in vitro in Chinese hamster ovary cells cultured with dichloromethane in the presence, but not in the absence, of an exogenous metabolic activation system (Graves et al., 1994b). DNA single-strand breaks were also induced in Chinese hamster ovary cells exposed to dichloromethane with or without exogenous metabolic activation, the effect being stronger with metabolic activation (Graves & Green, 1996). Conversely, DNA single-strand breaks were not induced in Syrian hamster hepatocytes (Graves et al., 1995).

Hu et al. (2006) performed the standard and proteinase K-modified comet assay to measure DNA damage and DNA–protein crosslinks in untreated V79 cells and in V79 cells transfected with the murine *GSTT1* gene (V79 mGSTT1). Dichloromethane induced DNA damage in both cell types. However, the study showed the presence of dichloromethane-induced DNA–protein crosslinks in the V79 mGSTT1 cell line and not in standard V79 cell line, which indicates that *GSTT1* was instrumental for the induction of DNA–protein crosslinks. Moreover, dichloromethane formed significantly higher amounts of cytosolic formaldehyde in V79 in GSTT1 cells.

No DNA binding was observed in vivo in the liver or kidney of male rats or male and female mice after intraperitoneal administration of dichloromethane (Watanabe et al., 2007). Covalent binding of dichloromethane to DNA was not observed in the liver, kidney, or lung of rats or mice exposed by inhalation, although metabolic incorporation of ^{14}C was found in normal deoxyribonucleosides in both species (Ottenwälder & Peter, 1989).

DNA–protein cross-links were induced in vivo in the liver, but not the lung of B6C3F_1/CrlBR mice exposed to dichloromethane (Casanova

Table 4.3 Studies of genotoxicity with dichloromethane in mammalian systems in vivo

Test system	Results[a]	Dose (LED or HID)	Reference
DNA single-strand breaks, B6C3F$_1$ mouse liver	+	4831 ppm, inh., 6 h	Graves et al. (1994b)
DNA single-strand breaks, AP rat liver	–	4527 ppm, inh., 6 h	Graves et al. (1994b)
DNA single-strand breaks, CD rat liver	+	1275 µg/mL, po × 1	Kitchin & Brown (1994)
DNA single-strand breaks, B6C3F$_1$ mouse liver	+[c]	4000 ppm, inh., 6 h	Graves et al. (1995)
DNA single-strand breaks, B6C3F$_1$ mouse lung	+[c]	2000 ppm, inh., 3 h	Graves et al. (1995)
DNA single-strand breaks, AP rat lung	–	4000 ppm, inh., 3 h	Graves et al. (1995)
DNA damage, male B6C3F$_1$ mouse liver, comet assay	–	1600 ppm, inh., 6 h/day, 5 days/wk, 6 wk	Suzuki et al. (2014)
DNA binding, rats (male) or mice (male and female), liver or kidney	–	5 mg/kg bw per day, ip	Watanabe et al. (2007)
DNA binding, rat or mouse liver, lung, or kidney	–	NR, inh.	Ottenwälder & Peter (1989)
DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver	+[b]	4000 ppm, inh., 6 h/day, 2 days	Casanova et al. (1992)
DNA–protein cross-links, Syrian hamster liver and lung	–	4000 ppm, inh., 6 h/day, 2 days	Casanova et al.(1992)
DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver	+	498 ppm, inh., 6 h/d, 2 days	Casanova et al. (1996)
DNA–protein cross-links, Syrian golden hamster liver	–	3923 ppm, inh., 6 h/d, 2 days	Casanova et al.(1996)
Sister-chromatid exchange, B6C3F$_1$ mouse lung cells	+[d]	2000 ppm, inh., 6 h/day, 5 days/wk 12wk	Allen et al. (1990)
Sister-chromatid exchange, B6C3F$_1$ mouse bone marrow	–	5000 µg/mL, sc × 1	Allen et al. (1990)
Sister-chromatid exchange, C57BL/6J mouse bone marrow	–	1500 µg/mL, ip × 1	Westbrook-Collins et al. (1990)
Unscheduled DNA synthesis, F344 rat hepatocytes	–	1000 µg/mL, po × 1	Trueman & Ashby (1987)
Unscheduled DNA synthesis, F344 rat hepatocytes	–	4000 ppm, inh., 6 h	Trueman & Ashby (1987)
Unscheduled DNA synthesis, B6C3F$_1$ mouse liver	–	4000 ppm, inh., 6 h	Trueman & Ashby (1987)
Gene mutation, *Pig-a* assay, male B6C3F$_1$ mouse, erythrocytes	–	1600 ppm, inh., 6 h/day, 5 days/wk, 6 wk	Suzuki et al. (2014)
Gene mutation, transgenic rodent, male *Gpt* Delta C57BL/6J mouse liver	–	800 ppm, inh., 6 h/day, 5 days/wk, 4 wk	Suzuki et al. (2014)
Chromosomal aberrations, B6C3F$_1$ mouse bone marrow	–	5000 µg/mL sc × 1	Allen et al. (1990)
Chromosomal aberrations, C57BL/6J mouse bone marrow	–	1500 mg/kg ip × 1	Westbrook-Collins et al. (1990)
Chromosomal aberrations, B6C3F$_1$ mouse bone marrow	(+)	8000 ppm, inh., 6 h/day, 5 days/wk, 2 wk	Allen et al. (1990)
Chromosomal aberrations, Sprague-Dawley rat bone marrow	–	3500 ppm, inh., 6 h/day, 5 days/wk, 2 yr	Burek et al. (1984)
Chromosomal aberrations, B6C3F$_1$ mouse lung cells	(+)	8000 ppm, inh., 6 h/day, 5 days/wk, 2 wk	Allen et al. (1990)
Micronucleus test, NMRI mouse bone marrow	–	1700 mg/kg, ip × 2	Gocke et al. (1981)
Micronucleus test, C57BL/6J/Alpk mouse bone marrow	–	4000 mg/kg, po × 1	Sheldon et al. (1987)

Table 4.3 (continued)

Test system	Results[a]	Dose (LED or HID)	Reference
Micronucleus test, CD-1 mouse bone marrow	–	1720 mg/kg, ip × 1	Morita et al. (1997)
Micronucleus test, $B6C3F_1$ mouse erythrocytes	(+)[e]	2000 ppm, inh., 6 h/day, 5 days/wk, 12 wk	Allen et al. (1990)
Micronucleus test, male $B6C3F_1$ mouse reticulocytes and normochromatic erythrocytes	–	1600 ppm, inh., 6 h/days, 5 days/wk, 6 wk	Suzuki et al. (2014)

[a] +, positive; (+), weakly positive; –, negative
[b] Negative in mouse lung
[c] Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage
[d] The highest dose tested (8000 ppm, 6 hours per day, 5 days per week, for 2 weeks) gave positive results in erythrocytes and lung cells, but negative results in bone marrow
[e] Negative in lung cells at this dose; positive in erythrocytes after exposure to 8000 ppm for 6 hours per day [10 000 mg/kg bw], 5 days per week, for 2 weeks
h, hour; HID, highest ineffective dose; inh., inhalation; ip, intraperitoneal; LED, lowest effective dose; NR, not reported; NT, not tested; po, oral; ppm, parts per million; sc, subcutaneous; wk, week; yr, year

et al., 1992). No DNA–protein cross-links were detected in Syrian hamster liver or lung after inhalation of dichloromethane (Casanova et al., 1992). DNA–protein cross-links were not induced in the liver of Syrian golden hamsters, but were observed in the liver of $B6C3F_1$/CrlBR mice treated with dichloromethane by inhalation (Casanova et al., 1996).

Dichloromethane induced DNA–protein cross-links in vitro in hepatocytes of male $B6C3F_1$ mice, but not in hepatocytes of Fischer 344 rats or Syrian hamsters (Casanova et al., 1997). DNA–protein cross-links were also induced in Chinese hamster ovary cells exposed to dichloromethane with or without exogenous metabolic activation, with DNA damage being greater in the presence of metabolic activation (Graves & Green, 1996). Using the proteinase K-modified comet assay, it was demonstrated that dichloromethane induced DNA–protein cross-links in V79 cells transfected with the murine *GSTT1* gene, but not in standard V79 cells (Hu et al., 2006). [The Working Group noted that this suggests a key role for GST in genotoxicity induced by dichloromethane.]

In a study in vivo, mice treated with dichloromethane at 2000 ppm [6940 mg/m^3] for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of sister-chromatid exchange in lung cells (Allen et al., 1990). Exposure to higher concentrations (8000 ppm [27 800 mg/m^3] for 2 weeks) also induced an increase in the frequency of sister-chromatid exchange in peripheral blood erythrocytes. Dichloromethane did not induce sister-chromatid exchange in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins et al., 1990; Allen et al., 1990). Dichloromethane did not increase the frequency of sister-chromatid exchange in Chinese hamster ovary cells in the presence or absence of an exogenous metabolic system (Thilagar & Kumaroo, 1983; Anderson et al., 1990). When tested in Chinese hamster lung V79 cells in the absence of exogenous metabolic activation, dichloromethane induced a slight increase in the frequency of sister-chromatid exchange (Jongen et al., 1981).

Dichloromethane did not induce unscheduled DNA synthesis in vivo in Fischer 344 rats treated by gavage or inhalation, or in $B6C3F_1$ mouse hepatocytes treated by inhalation (Trueman & Ashby, 1987).

Table 4.4 Studies of genotoxicity with dichloromethane in mammalian systems in vitro

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA–protein cross-links, B6C3F$_1$ mouse hepatocytes	+	NT	43	Casanova et al. (1997)
DNA–protein cross-links, F344 rat hepatocytes	–	NT	425	Casanova et al. (1997)
DNA–protein cross-links, Syrian hamster hepatocytes	–	NT	425	Casanova et al. (1997)
DNA–protein crosslinks, V79 cells	–	NT	850	Hu et al. (2006)
DNA–protein cross-link, murine GSTT1 transfected V79 cells	+[c]	NT	212	Hu et al. (2006)
DNA–protein cross-links, Chinese hamster ovary cells	(+)	+	3975	Graves & Green (1996)
DNA single-strand breaks, B6C3F$_1$ mouse hepatocytes	+	NT	34	Graves et al. (1994b)
DNA single-strand breaks, AP rat hepatocytes	+	NT	2550	Graves et al. (1994b)
DNA single-strand breaks, Chinese hamster ovary cells	–	+	5100	Graves et al. (1994b)
DNA single-strand breaks, Syrian hamster hepatocytes	–	NT	5100	Graves et al. (1995)
DNA single-strand breaks, B6C3F$_1$ mouse lung Clara cells	+[d]	NT	425	Graves et al. (1995)
DNA single-strand breaks, Chinese hamster ovary cells	(+)	+	3975	Graves & Green (1996)
DNA damage, V79 cells, comet assay	+[e]	NT	425	Hu et al. (2006)
DNA damage, murine *GSTT1* transfected V79 cells, comet assay	+[f]	NT	212	Hu et al. (2006)
Unscheduled DNA synthesis, Chinese hamster lung V79 cells	–	NT	65 000	Jongen et al. (1981)
Sister-chromatid exchange, Chinese hamster V79 cells	(+)	NT	13 000	Jongen et al. (1981)
Sister-chromatid exchange, Chinese hamster ovary cells	–	–	13 000	Thilagar & Kumaroo (1983)
Sister-chromatid exchange, Chinese hamster ovary cells	–	–	5000	Anderson et al. (1990)
Gene mutation, Chinese hamster ovary cells, *Hprt* locus	–	NT	65 000	Jongen et al. (1981)
Gene mutation, Chinese hamster ovary cells, *Hprt* locus	–	+	3975	Graves & Green (1996)
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus	–	NT	52 000	Jongen et al. (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	?	?	3300	Myhr et al. (1990)
Chromosomal aberrations, Chinese hamster ovary CHO cells	+	+	6500	Thilagar & Kumaroo (1983)
Chromosomal aberrations, Chinese hamster ovary CHO cells	–	–	5000	Anderson et al. (1990)
Cell transformation, RLV/Fischer rat	+	NT	14	Price et al. (1978)
Cell transformation, SA7/Syrian hamster embryo cells	+	NT	73	Hatch et al. (1982)

[a] +, positive; (+), weakly positive; –, negative; ?, inconclusive; NT, not tested
[b] LEC, lowest effective concentration; HIC, highest ineffective concentration; in-vitro tests, µg/mL
[c] DNA–protein crosslinks were demonstrated by increase in DNA migration following post-treatment with proteinase K
[d] Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage
[e] Concentration-dependent increase in DNA migration
[f] Concentration-dependent decrease in DNA migration; post-incubation with proteinase K increased DNA migration

(ii) Chromosomal aberration

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

Dichloromethane induced chromosomal aberration in vitro in Chinese hamster ovary cells in the presence and absence of an exogenous metabolic system in one of two studies (Thilagar & Kumaroo, 1983; Anderson et al., 1990).

(iii) Micronucleus formation

Dichloromethane did not induce micronucleus formation in vivo in bone marrow of mice treated by gavage or intraperitoneal injection (Gocke et al., 1981; Sheldon et al., 1987; Morita et al., 1997). Mice treated with dichloromethane at 2000 ppm [6940 mg/m^3] for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of micronuclei in peripheral blood erythrocytes (Allen et al., 1990). The highest dose tested (8000 ppm, 6 hours per day, 5 days per week, for 2 weeks) gave positive results in erythrocytes and lung cells, but negative results in bone marrow. On the other hand, dichloromethane did not cause micronucleus formation in male B6C3F$_1$ mice exposed at 400, 800 and 1600 ppm by inhalation for 6 weeks (6 hours per day, 5 days per week) (Suzuki et al., 2014).

(iv) Mutagenicity

Dichloromethane did not cause gene mutation in two inhalation experiments in vivo: a Pig-a assay in male B6C3F$_1$ mice exposed to dichloromethane at 400, 800, or 1600 ppm for 6 weeks (6 hours per day, 5 days per week); and a transgenic rodent gene mutation assay on *Gpt* Delta C57BL/6J mouse liver treated for 4 weeks (6 hours per day, 5 days per week) with dichloromethane at 800 ppm (Suzuki et al., 2014).

In vitro, dichloromethane was mutagenic in Chinese hamster ovary cells at the *Hprt* locus in one study, in the presence of exogenous metabolic activation (Graves & Green, 1996), and gave equivocal results in the mouse lymphoma Tk$^{+/-}$ assay in another study (Myhr et al., 1990). DNA sequence analysis of the *Hprt* mutants of Chinese hamster ovary cells treated with dichloromethane indicated that most mutations were GC→AT transitions (4 out of 8), with two GC→CG transversions and two AT→TA transversions. This pattern was more similar to that of 1,2-dibromoethane (ethylene dibromide) (IARC, 1999) (7 out of 9 being GC→AT transitions) than that of formaldehyde, a metabolite of dichloromethane that has been identified in vitro (see Section 4.1), for which all mutations were single-base transversions and 5 out of 6 arose from AT base pairs (Graves et al., 1996). When tested in Chinese hamster lung fibroblast V79 cells in the absence of exogenous metabolic activation, dichloromethane did not induce gene mutations at the *Hprt* locus (Jongen et al., 1981).

(v) Cell transformation

Virus-infected Fischer rat and Syrian hamster embryo cells were transformed after treatment with dichloromethane in vitro (Price et al., 1978; Hatch et al., 1982).

(b) Bacterial and other systems

See Table 4.5

Mutagenicity

Gene mutations were induced in *Salmonella typhimurium* strains TA100, TA1535, and TA98 exposed to dichloromethane vapour in a closed chamber with or without exogenous metabolic activation (JETOC, 1997).

The relationship between the metabolism of dichloromethane and mutagenicity has been examined in several studies with various assays for bacterial mutation. For example, Jongen et al. (1982) showed that while dichloromethane was directly mutagenic in *S. typhimurium* TA100, mutagenic activity was enhanced by addition of rat liver microsomes or cytosolic fraction (this implicated enhanced metabolism of dichloromethane by CYP and GST, respectively). In contrast, Green (1983) tested the mutagenicity of dichloromethane in the same *S. typhimurium* strain and observed an increase in mutagenic activity only when rat liver post-mitochondrial S9 fraction was added and not rat liver microsomes.

To further illustrate the complexities of how the two metabolic pathways interact to promote mutagenesis, Dillon and colleagues examined the involvement of endogenous and exogenous GSH using wild-type *S. typhimurium* TA100 and a GSH-deficient strain (NG54) that contains approximately 25% of the GSH content as the wild-type strain (Dillon et al., 1992). The influence of addition of rat liver S9 fraction, microsomes, or cytosol fractions was also studied. The NG54 strain was slightly less responsive to dichloromethane exposure, addition of rat liver cytosol marginally increased the mutagenic response to dichloromethane, but addition of GSH had little effect (Dillon et al., 1992).

DeMarini and colleagues assessed dichloromethane mutagenicity by using a *Salmonella* TA1535 strain that had been modified by the cloning of the rat gene for GSTT11 into its genome (DeMarini et al., 1997). This modified strain, called RSJ100, showed a positive mutagenic response to dichloromethane that was predominantly (96–100%) due to mutations that were GC→AT transitions. Interestingly, only 15% of the mutations were GC→AT transitions in the TA100 strain, a homologue strain that lacks the rat GSTT11 gene. These results suggested that different reactive metabolites are formed in the two strains, which leads to different mutations.

Studies using the liquid plate incorporation assay gave negative results (e.g. Zeiger & Dellarco, 1990), with the exception of one study reporting positive results in strain TA1535 transfected with rat Gstt1 (Thier et al., 1993). Dichloromethane also induced mutation in *Escherichia coli* (Dillon et al., 1992; Zielenska et al., 1993; Graves et al., 1994a; JETOC, 1997) and gene conversion and mutation in *Saccharomyces cerevisiae* (Callen et al., 1980). In *Drosophila melanogaster* dichloromethane did not induce sex-linked recessive lethal mutations (Gocke et al., 1981; Kramers et al., 1991).

4.3 Other mechanistic data relevant to carcinogenicity

Few experimental studies have examined the potential for non-genotoxic mechanistic events to play a role in carcinogenesis caused by dichloromethane in tissues that are targets for carcinogenesis in studies in experimental animals. In long-term studies of dichloromethane exposure in mice, elevations in liver-cell proliferation were not observed (Foley et al., 1993; Casanova et al., 1996). In the mouse lung, exposure to dichloromethane results in toxicity to Clara cells, which are secretory cells in the primary bronchioles. Acute exposure to dichloromethane produces vacuolization of Clara cells, which is not sustained with long-term exposure (Foster et al., 1992).

One recent genomics study in vitro compared the effects of dichloromethane and other volatile organic solvents (benzene, toluene, o-xylene, ethylbenzene, and trichloroethylene) on gene expression in human promyelocytoc leukaemia HL-60 cells (Sarma et al., 2010). Equi-toxic concentrations of all solvents were used in studies of gene expression (80% and 50% cell viability). Based on the overall changes in gene expression, dichloromethane exhibited a response that was distinct from other solvents; however, common signatures were identified. These included

Table 4.5 Studies of genotoxicity with dichloromethane in non-mammalian systems in vitro

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prokaryotes (Bacteria)				
Salmonella typhimurium BA/3, forward mutation, Ara resistance	+	(+)	325	Roldán-Arjona & Pueyo (1993)
Salmonella typhimurium TA100, reverse mutation	+	NT	14	Simmon et al. (1977)
Salmonella typhimurium TA100, reverse mutation	+	+	19	Jongen et al. (1978)
Salmonella typhimurium TA100, reverse mutation	+	+	18	Gocke et al. (1981)
Salmonella typhimurium TA100, reverse mutation	+	+	23	Jongen et al. (1982)
Salmonella typhimurium TA100, reverse mutation	+	+	95	Green (1983)
Salmonella typhimurium TA100, reverse mutation	(+)	NT	6 800	Osterman-Golkar et al. (1983)
Salmonella typhimurium TA100, reverse mutation	(+)[c]	NT	3 700	Hughes et al. (1987)
Salmonella typhimurium TA100, reverse mutation	+[c]	+	150	Zeiger & Dellarco (1990)
Salmonella typhimurium TA100, reverse mutation	+	+	8.5	Dillon et al. (1992)
Salmonella typhimurium TA100, reverse mutation	+	NT	17 667	Graves et al. (1994a)
Salmonella typhimurium TA100, reverse mutation	+	+	34	JETOC (1997)
Salmonella typhimurium TA1535, reverse mutation	+	NT	300	McGregor (1979)
Salmonella typhimurium TA1535, reverse mutation	– [d]	NT	170	Thier et al. (1993)
Salmonella typhimurium TA1535 transfected with rat GST 5-5, reverse mutation	+ [d]	NT	42	Thier et al. (1993)
Salmonella typhimurium TA1535, reverse mutation	+	+	170	JETOC (1997)
Salmonella typhimurium TA1537, reverse mutation	–	–	340	JETOC (1997)
Salmonella typhimurium TA98, reverse mutation	+	+	19	Jongen et al. (1978)
Salmonella typhimurium TA98, reverse mutation	+	+	72	Gocke et al. (1981)
Salmonella typhimurium TA98, reverse mutation	?[c]	?	1500	Zeiger & Dellarco (1990)
Salmonella typhimurium TA98, reverse mutation	+	+	34	JETOC (1997)
Escherichia coli NR3835, forward mutation	+	NT	26 500	Zielenska et al. (1993)

Table 4.5 (continued)

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli K12, forward mutation, Rif resistance	–	(+)[e]	5100	Graves et al. (1994a)
Escherichia coli WP2 uvrA, reverse mutation	+	+	170	IETOC (1997)
Escherichia coli WP2 uvrA/pKM101, reverse mutation	+	+	21	Dillon et al. (1992)
Escherichia coli WP2 uvrA/pKM101, reverse mutation	+	+	170	IETOC (1997)
Saccharomyces cerevisiae, gene conversion	+	NT	13 300	Callen et al. (1980)
Saccharomyces cerevisiae, homozygosis	+	NT	13 300	Callen et al. (1980)
Saccharomyces cerevisiae, reverse mutation	+	NT	13 300	Callen et al. (1980)
Insects				
Drosophila melanogaster, sex-linked mutation	–	NT	52 600	Gocke et al. (1981)
Drosophila melanogaster, sex-linked mutation	–	NT	19.2	Kramers et al. (1991)
Plants				
Tradescantia species, gene mutation	+	NT	100	Schairer &Sautkulis (1982)

[a] +, positive; (+), weakly positive; -, negative; ?, inconclusive; NT, not tested
[b] LEC, lowest effective dose; HIC, highest ineffective dose; in-vitro tests, µg/mL (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg /mL in atmosphere).
[c] Negative in liquid plate incorporation assay
[d] Liquid plate incorporation assay
[e] Positive with mouse liver S9, negative with rat liver S9

induction of the immune response, apoptosis, cell cycle regulation, and transport pathways. Select transcripts from these pathways were tested by real-time polymerase chain reaction (PCR) in two other cell lines, human erythromyeloblastoid leukaemia K562 and human leukaemic monocyte lymphoma U937. [The Working Group noted that these data were difficult to interpret as the study appeared not to use proper multiple-testing correction to determine significance of both individual genes and pathways.]

4.4 Organ toxicity

The toxicity of dichloromethane has been reviewed previously (Dhillon & Von Burg, 1995; WHO, 1996; Green, 1997).

4.4.1 Neurotoxicity

(a) Humans

Temporary neurobehavioural effects have been reported (Putz et al., 1979; Winneke, 1981), or not (Gamberale et al., 1975) after exposure to dichloromethane at doses as low as 200 ppm [694 mg/m^3]. Cerebral damage after exposure to dichloromethane has been reported (Barrowcliff & Knell, 1979).

(b) Experimental systems

Increase in concentrations of astroglial proteins S-100 and glial fibrillary acidic protein was found in the frontal and sensory motor cerebral cortex of gerbils exposed to dichloromethane at 210 or 350 ppm for 3 months (Rosengren et al., 1986). DNA concentration was also measured as

a possible index of astroglial proliferation. DNA concentration was not increased in the frontal and sensory motor cerebral cortex, but was decreased in the hippocampus at 210 and 350 ppm, and in the cerebellar hemispheres (Rosengren et al., 1986).

4.4.2 Liver

(a) Humans

An exposure-related increase in serum bilirubin was observed in workers exposed to dichloromethane, but no other sign of liver injury or haemolysis was reported (Ott et al., 1983)

(b) Experimental systems

A 2-year study of exposure to dichloromethane by inhalation in F344 rats reported that the incidence of some non-neoplastic liver lesions was significantly elevated in response to treatment when compared with concurrent controls (NTP, 1986). These liver lesions were haemosiderosis, focal necrosis, cytoplasmic vacuolization, and bile duct fibrosis in males, and focal granulomatous inflammation, haemosiderosis and cytoplasmic vacuolization in females. In the same study, liver cytological degeneration was observed in female B6C3F$_1$ mice.

A 2-year study of exposure to dichloromethane by inhalation in F344 rats reported that the incidence some non-neoplastic liver lesions (acidophilic, basophilic and vacuolated cell foci in males) was significantly elevated in response to treatment when compared with controls (JISHA, 2000a). In the same study, liver granulation and peripheral vacuolation were observed in male and female BDF1 mice.

Increased liver weight associated with glycogen accumulation in the hepatocytes, but no hepatotoxicity, was observed in another study of carcinogenicity in mice, in which an elevated incidence of hepatic tumours was observed (Kari et al., 1993). An experiment in female B6C3F$_1$ mice showed that the proportion of S-phase cells was frequently higher in altered foci than in cells from the areas of the liver with normal architecture, but similar to that in the altered foci from non-treated mice (Foley et al., 1993). Administration of dichloromethane to B6C3F$_1$ mice by gavage (1000 mg/kg, single dose) or inhalation (4000 ppm [13 900 mg/m^3] dichloromethane for 2 hours) did not induce DNA synthesis, as measured by the number of cells in S-phase ([^{3}H]thymidine incorporation) (Lefevre & Ashby, 1989). When female B6C3F$_1$ mice were exposed to dichloromethane at 1000, 2000, 4000, or 8000 ppm [3470, 6940, 13 900 or 27 800 mg/m^3] for 6 hours per day, 5 days per week, for up to 4 weeks, followed by a recovery period of 1–2 weeks (Foley et al., 1993), the hepatocyte labelling index was mostly decreased. There were, however, transient increases in the labelling index in the groups at 4000 and 8000 ppm at 2 weeks and in the group at 1000 ppm at 1 week.

In Sprague-Dawley rats, two doses of dichloromethane at 1250 mg/kg given by gavage for 4 and 21 hours, there was no effect on serum alanine aminotransferase levels, or hepatic GSH or CYP content, but hepatic ornithine decarboxylase activity increased in 3 out of 15 rats (Kitchin & Brown, 1989).

Hepatotoxic effects were seen after exposure to near-lethal concentrations of dichloromethane in mice (Gehring, 1968). Continuous exposure of mice to dichloromethane at 5000 ppm [17 400 mg/m^3] by inhalation caused swelling of the rough endoplasmic reticulum, fatty changes in the liver, and necrosis of individual hepatocytes (Weinstein et al., 1972). Slight liver damage was also observed after administration of dichloromethane (133–665 mg/kg bw) by gavage in mice (Condie et al., 1983).

Exposure of guinea-pigs to dichloromethane at 5200 ppm [18 000 mg/m^3] by inhalation for 6 hours increased hepatic concentrations of triglyceride (Morris et al., 1979). Exposure of guinea-pigs to dichloromethane at approximately 11 000 ppm [38 200 mg/m^3] for 6 hours

also increased hepatic concentrations of triglyceride, but concomitant exposure to ethanol at 21 400–24 100 ppm [40 200–45 300 mg/m^3] blocked this effect (Balmer et al., 1976).

4.4.3 Cardiovascular system

(a) Humans

Of four epidemiological studies on mortality from cardiovascular disease, two studies showed increased mortality from ischaemic heart disease in workers exposed to dichloromethane, compared with an internal reference group or a non-exposed cohort, although mortality did not increase compared with the general population (Tomenson et al., 1997; Tomenson, 2011).

(b) Experimental systems

No data were available to the Working Group.

4.4.4 Respiratory system

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Nasal cavity lesions of olfactory epithelium and hyperplasia of the terminal bronchiole have been reported in male and female BDF1 mice in a 2-year study of exposure to dichloromethane by inhalation (JISHA, 2000b). The incidence of eosinophilic changes in the respiratory epithelium was also elevated in female mice in this study.

F344/N rats were exposed to dichoromethane at a concentration of 0, 1000, 2000, or 4000 ppm by inhalation for 6 hours per day, 5 days per week, for 102 weeks. Squamous metaplasia of the nasal cavity was observed as a treatment-related non-neoplastic change in rats (Mennear et al., 1988).

The labelling index in bronchiolar epithelium (in two branches proximal to the terminal bronchiole and in the terminal bronchioles themselves) in female B6C3F$_1$ mice exposed to dichloromethane at 2000 ppm for 2–26 weeks decreased to 40–60% of the value for control mice. Exposure to dichloromethane at 8000 ppm led to a smaller decrease in labelling index. No pathological changes were found in the exposed lungs (Kanno et al., 1993). In male B6C3F$_1$ mice exposed to dichloromethane by inhalation (6 hours, single dose), vacuolation of bronchiolar cells was observed at exposure levels ≥ 2000 ppm [6940 mg/m^3], while no effect was observed at levels ≤ 1000 ppm [3470 mg/m^3] (Foster et al., 1994). Pretreatment with the CYP inhibitor piperonyl butoxide (300 mg/kg, administered intraperitoneally) 1 hour before exposure abolished the toxic effect in bronchiolar cells, while buthionine sulfoximine (1 g/kg, administered intraperitoneally), which decreased the pulmonary GSH content by 50%, had no protective effect. In Clara cells isolated after exposure to dichloromethane (≥ 1000 ppm), the proportion of cells in S-phase was increased.

4.4.5 Kidney

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a 2-year study in female F344 rats exposed to dichloromethane by inhalation, kidney tubular degeneration was reported to be significantly elevated in response to treatment when compared with controls (NTP, 1986). In the same study, kidney tubule casts were observed in male and female B6C3F$_1$ mice.

In a 2-year study in female F344 rats exposed to dichloromethane by inhalation, the incidence of chronic nephropathy was significantly elevated in response to treatment when compared with controls (JISHA, 2000a). In a study in similarly exposed BDF1 mice, basophilic change, lymphocytic infiltration and proximal tubule vacuolation were observed (JISHA, 2000b).

After intraperitoneal administration of dichloromethane at near-lethal doses, hydropic degeneration was observed in the mouse kidney (Klaassen & Plaa, 1966), no kidney damage was observed after administration of dichloromethane at doses of 133–665 mg/kg bw by gavage (Condie et al., 1983). Slight calcification of the renal tubules in mongrel dogs was seen after intraperitoneal administration of dichloromethane at near-lethal doses (Klaassen & Plaa, 1967).

In rats, intraperitoneal administration of dichloromethane at 1330 mg/kg bw produced renal proximal tubular swelling (Kluwe et al., 1982). After a similar dose administered by gavage, a transient elevation in blood urea nitrogen levels and decreased urine output, coinciding with cloudy swelling of tubular cells, were observed (Marzotko & Pankow, 1988). Urinary flow was already decreased at the lowest dose tested (3.1 mmol/kg bw; 263 mg/kg bw). In F344/N rats exposed to dichloromethane at 0, 1000, 2000, or 4000 ppm by inhalation, for 6 hours per day, 5 days per week, for 102 weeks, treatment-related degeneration of kidney tubules was reported (Mennear et al., 1988).

4.4.6 Spleen

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In F344/N rats were exposed by inhalation to dichloromethane at 0, 1000, 2000, or 4000 ppm, for 6 hours per day, 5 days per week, for 102 weeks, fibrosis of the spleen was observed as a treatment-related non-neoplastic change (Mennear et al., 1988).

4.5 Susceptible populations

4.5.1 Polymorphisms

(a) CYP2E1

The association between exposure to organic solvents including dichloromethane and NHL was investigated in relation to different genetic variations in four metabolic genes – CYP2E1, microsomal epoxide hydrolase (EPHX1), myeloperoxidase (MPO), and quinone oxidoreductase (NQO1) – using unconditional logistic regression models based on data collected from women in Connecticut, USA, in 1996–2000 (Barry et al., 2011). Overall associations between total NHL and dichloromethane (OR, 1.69; 95% CI, 1.06–2.69), carbon tetrachloride (OR, 2.33; 95% CI, 1.23–4.40), and methyl chloride (OR, 1.44; 95% CI, 0.94–2.20) were increased among women of genotype TT for rs2070673 in the CYP2E1 gene (dichloromethane: OR, 4.42; 95% CI, 2.03–9.62; *P* interaction < 0.01; carbon tetrachloride: OR, 5.08; 95% CI, 1.82–14.15; *P* interaction = 0.04; and methyl chloride: OR, 2.37; 95% CI, 1.24–4.51; P interaction = 0.03). In contrast, no effects of these solvents were observed among women of genotype TA/AA. Similar patterns were observed for dichloromethane and diffuse large B-cell lymphoma, follicular lymphoma, and marginal zone lymphoma (Barry et al., 2011). [The Working Group noted that the functional significance of this polymorphism was unknown.]

(b) GSTT1

GSTT1 polymorphisms may result in inter-individual variation in the ability to metabolize dichloromethane by GSH conjugation; some individuals (non-conjugators) completely lack GSH conjugation activity. Because GSH conjugation of dichloromethane leads to formation of reactive and genotoxic metabolites, it is plausible that diminished or lack of GSH conjugation activity will lead to reduced risk of carcinogenesis. For

instance, in the absence of GSTT1, exposure to dichloromethane did not lead to formaldehyde production in human erythrocytes (Hallier et al., 1994), and DNA–protein-cross-links were not detected in human liver cells (Casanova et al., 1997). This could be relevant to multiple target tissues that express GSTs, including the liver, kidney, brain, and lung (Sherratt et al., 1997, 2002).

Interindividual variation in the conjugation of dichloromethane with GSH by cytosolic GST in vitro was investigated in 22 samples of human liver (Bogaards et al., 1993). In nine of the liver samples, the α-, mu-, and pi-class GST subunits were quantified. In two of these samples, no activity was observed towards dichloromethane, while α-, mu-, and pi-class subunits were expressed in these human liver cytosolic samples, suggesting no relationship between enzymatic activities and dichloromethane with these classes of GST.

Hallier et al. (1993) found that dichloromethane induced sister-chromatid exchange in the human lymphocytes of non-conjugators donors lacking GST activity, but not in those of conjugators. However, Olvera-Bello et al. (2010) demonstrated that the group with high GSTT1 activity showed a larger increase in the frequency of sister-chromatid exchange induced by dichloromethane than did the groups with low and medium GSTT1 activity.

Garte et al. (2001) showed major and significant differences in the allele and genotypes frequencies between ethnic groups, especially between Asians and Caucasians (Table 4.6).

Table 4.6 Frequencies of *GSTT1*0* gene polymorphism in Caucasians and Asians

Ethnicity	No.	Homozygous	Range
Caucasians	5577	0.197	0.13–0.26
Asians	575	0.470	0.35–0.52

From Garte et al. (2001)

4.5.2 Life stage

Few studies have examined the influence of life stage on dichloromethane-induced toxicity or carcinogenesis. Most of the available studies related to potential differences in toxicokinetics across life stages, with no chemical-specific data on toxicodynamic differences. With respect to absorption and distribution, no age-dependent differences in the partition coefficient for mixtures of volatile organic solvents have been observed in rats (Mahle et al., 2007). No data on life-stage–dependent differences in elimination or excretion were available.

Although no direct data on life-stage–dependent differences in dichloromethane metabolism were available, based on information on the ontogeny of CYP2E1 and GSTT1, such differences are plausible. In humans, CYP2E1 activity is low during gestation and the early neonatal period (Choudhary et al., 2005), but no data were available on the ontogeny of GSTT1. Data in experimental animals suggested that both CYP2E1 (Choudhary et al., 2005) and GST (Cui et al., 2010) expression are low during gestation, and peak between 0 and 12 days after birth. Czekaj et al. (2010) found that CYP2E1 expression increases further in older adult rats. Although the qualitative patterns were similar, the available data were insufficient to estimate the magnitude of any differences in the proportion of oxidative metabolism versus conjugation during early life stages as compared with during adulthood. Therefore, there was inadequate evidence to conclude whether there are differences in susceptibility as a function of life stage as a result of changes in metabolism.

4.6 Mechanistic considerations

See Table 4.7

Two important metabolic pathways for the metabolism of dichloromethane have been

characterized in humans and experimental animals. One pathway is CYP2E1-mediated reductive dehalogenation, which ultimately generates CO and CO_2 as stable end products. One of the intermediates, formyl chloride, can react with nucleophiles. GSH conjugation, catalysed primarily by GSTT1, is the other important metabolic pathway of dichloromethane, resulting in the formation of reactive metabolites, including formaldehyde and *S*-chloromethyl GSH.

Supporting evidence for the GST pathway include in-vitro studies from human-derived tissue or cells, in-vivo studies in rodents, in-vitro studies in rodent-derived tissue or cells, in-vitro mutagenicity studies in microorganisms, and biochemical studies with purified enzymes. Humans are polymorphic for GSTT1, with a proportion of the population showing no activity towards dichloromethane. CYP2E1 catalytic activity predominates at relatively low concentrations of substrate, but there is ample evidence that GST-mediated metabolism eventually predominates at higher concentrations (Gargas et al., 1986; Clewell, 1995; Bos et al., 2006). Such higher concentrations of dichloromethane are readily observed in occupational settings and in some environmental exposures. Moreover, with continued exposure to dichloromethane, even at relatively low concentrations, CYP2E1 readily becomes saturated. Overall, evidence strongly supports qualitative similarities in both oxidative and GST-mediated metabolism of dichloromethane between humans and rodents.

Differences in activity levels and tissue and cellular distributions of GSTT1 exist across species. For instance, in the liver and lung, two sites where tumours are observed in mice in long-term bioassays (NTP, 1986), GSTT1 activity was greater in mice than in rats or humans (Reitz et al., 1989; Thier et al., 1998). Humans, however, have GSTT1 activity in erythrocytes that is comparable to that in the mouse liver, while neither rats nor mice exhibit GSTT1 activity in erythrocytes (Thier et al., 1998). Additionally, in the mouse liver, nuclear localization of GSTT1 was observed in hepatocytes, while in the human liver, nuclear localization of GSTT1 was observed in bile-duct epithelial cells (Quondamatteo et al., 1998; Sherratt et al., 2002). Thus, while the metabolic pathways are similar across species, the target tissues and cell types of GSTT1 metabolism differ across species.

Dichloromethane has been evaluated for genotoxicity in several test systems, both in the presence or absence of metabolic activation. In human cell lines or isolated cells, dichloromethane has been reported to induce micronucleus formation and sister-chromatid exchange (Hallier et al., 1993; Doherty et al., 1996; Olvera-Bello et al., 2010); but studies of DNA–protein cross-links, DNA single-strand binding proteins (SSBs), and unscheduled DNA synthesis have largely given negative results (Jongen et al., 1981; Graves et al., 1995; Casanova et al., 1997). In one study, the extent of sister-chromatid exchange was greater in cells from individuals without GST activity (Hallier et al., 1993). In another study, by contrast, the extent of sister-chromatid exchange was greater in cells from individuals with high GSTT1 activity (Olvera-Bello et al., 2010). In experimental animals, dichloromethane-induced genotoxicity also tended to correlate with GST activity, with positive results in cells derived from mouse liver and lung, which also exhibited the greatest GST activity (Graves et al., 1994b, 1995; Casanova et al., 1997). Similarly, after exposure to dichloromethane in vivo, although many studies gave negative results for genotoxicity, positive results in multiple measures of genotoxicity were reported in tissues with GST-mediated metabolism, such as the mouse liver and lung (Allen et al., 1990; Casanova et al., 1992, 1996; Graves et al., 1995; Sasaki et al., 1998). Finally, several studies in non-mammalian in-vitro systems showed evidence for mutagenicity, particularly in systems in which GST activity is present or exogenously enhanced (Jongen et al., 1978, 1982; Gocke et al., 1981;

Table 4.7 Relationship between the glutathione/glutathione *S*-transferase pathway and dichloromethane-induced genotoxicity

System	DNA damage without exogenous metabolic activation	Comments	Reference
Salmonella typhimurium TA1535 transfected with GSTT1 (GST5-5)	+	Increased number of revertants in transfected *GSTT1* strain compared with non-transfected strain	Thier et al. (1993)
Salmonella typhimurium TA100, reverse mutation	+	In strain NG54, GSH-deficient TA100, twofold reduction in the number of revertants was observed	Dillon et al. (1992)
Salmonella typhimurium TA100, reverse mutation	+	In strain NG11, GSH-deficient TA100, twofold reduction in the number of revertants was observed	Graves et al. (1994a)
Salmonella typhimurium TA1535 transfected with GSTT1	+	96–100% of mutations were GC → AT in TA1535 transfected with GSTT1 compared with 15% in *S. typhimurium* TA100 (homologue of TA1535 containing plasmid pKM101) without *GSTT1*gene	DeMarini et al. (1997)
Single-strand breaks, $B6C3F_1$ mouse and rat hepatocytes, in vitro	+	Pre-treatment of hepatocytes with BS decreased DNA damage	Graves et al. (1994b)
Single-strand breaks, $B6C3F_1$ Clara cells, in vitro	+	Cotreatment with BS, decreased DNA damage	Graves et al. (1995)
Single-strand breaks, $B6C3F_1$ mouse lung and liver, in vivo	+	Pre-treatment of mice with BS decreased DNA damage	Graves et al. (1995)
Sister-chromatid exchange, human peripheral blood mononuclear cells	+	Group with high GSTT1 activity group showed larger DCM-induced increase in frequency than groups with low or medium GSTT1 activity	Olvera-Bello et al. (2010)
Sister-chromatid exchange, human lymphocytes, in vitro	+	Positive results in lymphocytes from donors "non conjugators" lacking GST activity (not in lymphocytes from "conjugators") (type of GST, NR)	Hallier et al. (1993)
DNA–protein cross-links, $B6C3F_1$/CrlBR mouse liver, in vivo, inhalation	+	Mice (type, NR) formed DNA–protein cross-links in the liver	Casanova et al. (1992, 1996)
DNA–protein cross-links, human hepatocytes (expressing GSTT1), in vitro	–	RNA–formaldehyde adducts were detected in human hepatocytes expressing *GSTT1,* but not in those lacking *GSTT1*	Casanova et al. (1997)
DNA–protein crosslinks, murine GSTT1-transfected V79 cells, comet assay, in vitro	+	DNA–protein crosslinks not observed in parent V79 cell line	Hu et al. (2006)

+, positive;–, negative; BS, buthionine sulfoximine, a glutathione-depleting agent; DCM, dichloromethane; GST, glutathione *S*-transferase; GSTT1, glutathione S-transferase theta 1; NR, not reported

Green, 1983; Thier et al., 1993; DeMarini et al., 1997; Pegram et al., 1997). Overall, genotoxicity attributable to dichloromethane appears to be strongly associated with GST-mediated metabolism, consistent with the formation of reactive metabolites through this pathway. However, in two available studies in human cells, enhanced genotoxicity was observed without GSTT1 activity in one, and with high GSTT1 activity in another.

Increased liver weights and glycogen deposition were observed after long-term exposure to dichloromethane, but their relationship to carcinogenesis was not clear (NTP 1986; Kari et al., 1993). Several studies in mice have shown that liver cell proliferation does not increase with

exposure to dichloromethane, suggesting that proliferation does not play a role in hepatocarcinogenesis in the mouse (Lefevre & Ashby, 1989; Foley et al., 1993; Casanova et al., 1996). In the mouse lung, acute exposure to dichloromethane leads to vacuolization of Clara cells, but this effect appears to be transient (Foster et al., 1992), so is unlikely to be involved in carcinogenesis in the mouse lung. Mice exposed to dichloromethane for up to 26 weeks had no pathological changes in the lung, but exhibited a decrease in cell proliferation in this tissue. Neurological, renal, spleen, reproductive, and developmental toxicity have also been reported in humans or experimental animals, confirming the widespread distribution of dichloromethane or its metabolites.

Together, the relationship between GSTT1-mediated metabolism, formation of reactive metabolites, the association between GST activity and genotoxicity, and the presence of GSTT1 polymorphisms in the human population suggest that GSTT1 polymorphism may lead to differential susceptibility to dichloromethane-related carcinogenesis. However, no studies have directly investigated whether an association exists between GSTT1 polymorphism and the incidence of cancer. One study has reported an association between a CYP2E1 polymorphism and NHL in dichloromethane-exposed individuals (Barry et al., 2011). Whether this is due to differences in formation of CYP2E1-mediated metabolites, which may also be reactive, or to a shift in the proportion of GST-mediated reactive metabolites is unknown.

5. Summary of Data Reported

5.1 Exposure data

Dichloromethane is a chlorinated solvent that was first synthesized in the 1840s, and is produced by hydrochlorination of methanol or by direct chlorination of methane. Dichloromethane has been used in paint stripping, aerosol spray products, in the manufacture of polycarbonate plastic and hydrofluorocarbons, in the production of synthetic fibres, in metal cleaning, in printing-press cleaning, as an extraction solvent for certain foods, and in the production of refrigerants. Annual world production in 2005 to 2010 was estimated at between 764 000 and 814 000 tonnes.

The principal occupational exposures to dichloromethane have been from its use in paint stripping, spray painting, and metal and printing-press cleaning. Occupational exposures of more than 1000 mg/m^3 were measured in the paint, printing, and chemical manufacturing industries before 2000. More recently reported levels have been lower, except for some printing plants in Japan where values were estimated at being up to about 900 mg/m^3. The main current source of exposure to the general population is through the use of consumer products containing dichloromethane. Recent reports of ambient air concentrations around industrial areas in some countries are as high as 200 µg/m^3, and groundwater concentrations can remain high for many decades after spills. Several jurisdictions (including the USA, the European Union, and Japan) have moved to reduce the use and release of various volatile organic compounds, including dichloromethane. These measures have included reducing or banning dichloromethane use in paint strippers and cosmetics.

5.2 Human carcinogenicity data

Two cohort studies of workers exposed to dichloromethane (as well as acetone and methanol, but not 1,2-dichloropropane) in the USA reported findings for cancers of the liver and biliary tract, based on small numbers. One of the studies reported a positive association for cancer of the liver and biliary tract, while the other did not. Only one study reported a standardized mortality ratio separately for cancer of

the biliary tract (SMR, 20). Cancer of the biliary tract constituted three of the four liver cancers in the study with a positive association, and both of the liver cancers in the other. Given that cancer of the biliary tract normally represents a small proportion of cancers of liver and biliary tract combined, these proportions are very high. In a case series of cancer of the biliary tract (histologically identified as cholangiocarcinoma) among printing workers in Japan, most of the cases were exposed to dichloromethane, and all except one of these were also exposed to 1,2 dichloropropane. The high risk of this rare cancer in one cohort study of workers without exposures to other likely risk factors and among exposed printing workers in Japan is consistent with a causal association, but the number of exposed cases was small and the printing workers had other potentially confounding exposures, notably to 1,2 dichloropropane.

Two cohort studies and three case–control studies in several countries evaluated non-Hodgkin lymphoma (NHL), and all except one cohort study reported increased risks among workers exposed to dichloromethane. While positive associations for NHL were consistent among studies using different designs and in several countries, most subjects were exposed to several solvents (some of which have been previously associated with NHL) and the risk estimates were based on small numbers.

There were several studies that assessed other cancer sites, but these data were regarded as inadequate.

5.3 Animal carcinogenicity data

There were six studies of carcinogenicity with dichloromethane in mice: two studies of oral administration (one with drinking-water in males and females, and one by gavage in males and females), three studies of inhalation (two in males and females, one in females), and one study in which dichloromethane was injected intraperitoneally in males. Dichloromethane increased the incidence of hepatocellular carcinoma in three studies in male mice (two by inhalation, one in drinking-water), and in three studies of inhalation in female mice. Dichloromethane increased the incidence of hepatocellular adenoma or carcinoma (combined) in two studies of inhalation in male mice and three studies by inhalation in female mice. Dichloromethane increased the incidence of bronchiolo-alveolar carcinoma in two inhalation studies in male mice and three inhalation studies in female mice, and bronchiolo-alveolar adenoma or carcinoma (combined) in three inhalation studies in male mice and three inhalation studies in female mice. Dichloromethane increased the incidences of haemangioma of the liver and of all organs (including the liver) in one inhalation study in male mice, and may have increased the incidence of haemangioma or haemangiosarcoma (combined) in the liver in one inhalation study in female mice.

There were seven studies of carcinogenicity with dichloromethane in rats: two oral administration studies (one drinking-water study in males and females and one gavage study in males and females), five inhalation studies (four in males and females, one in pregnant females and their male and female offspring). Dichloromethane increased the incidence of fibroma of the subcutis in two inhalation studies in male rats and fibroma or fibrosarcoma of the subcutis in one inhalation study in male rats. Dichloromethane caused salivary gland sarcomas in one inhalation study in male rats (the sialodacryoadenitis virus was detected in these rats; the effect of this virus on carcinogenesis is unknown). Dichloromethane increased the incidence of mammary gland adenoma or fibroadenoma (combined) in two inhalation studies in female rats and one inhalation study in male rats. The incidence of mammary gland adenoma was also increased in another inhalation study in males and another one in females. Dichloromethane

caused a minimal increase (positive trend test) in hepatocellular adenomas and carcinomas (combined) in female rats in one oral administration (drinking-water) study.

There was one inhalation study on dichloromethane in male and female Syrian hamsters in which there was an increase in the incidence of malignant lymphoma in females.

5.4 Mechanistic and other relevant data

Dichloromethane is a volatile lipophilic compound that is readily absorbed after oral, inhalation, or dermal exposure, and distributed systemically. Two important metabolic pathways for the metabolism of dichloromethane have been characterized in humans and experimental animals. One pathway is CYP2E1-mediated, which ultimately generates carbon monoxide (CO) and carbon dioxide (CO_2) as stable end products. One of the intermediates, formyl chloride, is reactive with nucleophiles. glutathione conjugation, catalysed primarily by glutathione *S*-transferase theta-1 (GSTT1), is the other important metabolic pathway, and results in the formation of reactive metabolites, including formaldehyde and *S*-chloromethyl glutathione. CYP2E1-mediated metabolism is predominant at lower concentrations, but can be easily saturated, with glutathione *S*-transferase-mediated metabolism eventually predominating at higher concentrations.

Oxidative and glutathione *S*-transferase (GST)-mediated metabolism of dichloromethane are qualitatively similar between humans and rodents, but quantitative differences exist across species, tissues, and cell types, and among individuals. Differences in GSTT1 expression and localization may be important determinants of site-specific carcinogenicity caused by dichloromethane.

In human cells, dichloromethane induces micronucleus formation and sister-chromatid exchange, but not DNA–protein cross-links and DNA damage. In experimental animals, dichloromethane-induced genotoxicity is associated with the GST pathway. Studies in non-mammalian systems in vitro showed evidence of mutagenicity, particularly in systems with GST activity. Evidence for the role of GSTT1 in genotoxicity in humans is mixed. Overall, the genotoxicity of dichloromethane appears to be strongly associated with GST-mediated metabolism, consistent with the formation of reactive metabolites through this pathway.

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

There is little evidence for non-genotoxic mechanisms of carcinogenesis with dichloromethane.

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

Overall, given the extensive evidence for genotoxicity, particularly in association with a metabolic pathway that is operative in humans, the Working Group concluded that the mechanistic evidence for dichloromethane carcinogenesis is *strong*.

6. Evaluation

6.1 Cancer in Humans

There is *limited evidence* in humans for the carcinogenicity of dichloromethane. Positive associations have been observed between exposure to dichloromethane and cancer of the biliary tract and non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

There is *sufficient* evidence for the carcinogenicity of dichloromethane in experimental animals.

6.3 Overall evaluation

Dichloromethane is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

The overall evaluation of Group 2A was based on *sufficient evidence* in experimental animals and *limited evidence* in humans. In addition, a Group 2A evaluation was also supported by *sufficient evidence* in experimental animals, and the *strong* evidence that the metabolism of dichloromethane via GSTT1 leads to the formation of reactive metabolites, that GSTT1 activity is strongly associated with genotoxicity in vitro and in vivo, and that GSTT1-mediated metabolism of dichloromethane occurs in humans.

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