DICHLOROMETHANE

This substance was considered by previous Working Groups, in June 1978 (IARC, 1979), February 1982 (IARC, 1982), February 1986 (IARC, 1986), and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

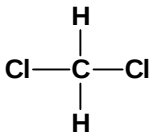
Chem. Abstr. Serv. Reg. No.: 75-09-2

Chem. Abstr. 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*



CH_2Cl_2 Relative molecular mass: 84.93

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with penetrating ether-like odour (Lewis, 1993; Budavari, 1996; Verschueren, 1996)

(*b*) *Boiling-point*: 40°C (Lide, 1995)

(*c*) *Melting-point*: –95.1°C (Lide, 1995)

(*d*) *Density*: d_4^{20} 1.3266 (Lide, 1995)

(*e*) *Spectroscopy data*: Ultraviolet (Grasselli & Ritchey, 1975), infrared (Sadtler Research Laboratories, 1995; prism [6620 (gas), 1011], grating [28523]), nuclear magnetic resonance (Sadtler Research Laboratories, 1995; proton [6401], ^{13}C [167]) and mass spectral data (Grasselli & Ritchey, 1975) have been reported.

(*f*) *Solubility*: Slightly soluble (1.38 g/100 mL) in water at 20°C; soluble in carbon tetrachloride; miscible in ethanol, diethyl ether and dimethylformamide (Lide, 1995; Budavari, 1996)

(*g*) *Volatility*: Vapour pressure, 58 kPa at 25°C (Lide, 1995); relative vapour density (air = 1), 2.93 (Verschueren, 1996)
(*h*) *Stability*: Vapour is nonflammable and is not explosive when mixed with air (Budavari, 1996) but may form explosive mixtures in atmospheres with higher oxygen content (Sax, 1984)
(*i*) *Reactivity*: Reacts vigorously with active metals (lithium, sodium, potassium) and with strong bases (potassium *tert*-butoxide) (Sax, 1984)
(*j*) *Octanol/water partition coefficient (P)*: log *P*, 1.25 (Hansch *et al*., 1995)
(*k*) *Conversion factor*: $mg/m^3 = 3.47 \times ppm$[1]

1.1.4 *Technical products and impurities*

Dichloromethane is available in several grades based on its intended end use: technical; aerosol; vapour degreasing; special; urethane; and decaffeination or Food Chemicals Codex/National Formula (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).

Trade names for dichloromethane include Aerothene MM, Narkotil, R30, Solaesthin, and Solmethine.

1.1.5 *Analysis*

Analytical methods are available for determination of dichloromethane in biological media and environmental samples. All methods involve gas chromatography in combination with a suitable detector. Very low detection limits have been achieved for most media (e.g., in food, 7 ng/sample; water, 0.01 μg/L; air, 1.76 $\mu g/m^3$; and blood, 0.022 mg/L) (WHO, 1996).

Selected methods for the analysis of dichloromethane in various matrices are identified in Table 1. The United States Environmental Protection Agency methods for analysing water (Methods 8010 and 8240) have also been applied to liquid and solid wastes (United States Environmental Protection Agency, 1982a,b). Volatile components of solid-

[1]Calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm, assuming a temperature of 25°C and a pressure of 101 kPa

Table 1. Methods for analysis of dichloromethane

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb on activated charcoal; desorb with carbon disulfide	GC/FID	10 µg/sample	Eller (1994) [Method 1005]
	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	94 µg/m^3	US Occupational Safety and Health Administration (1990) [Methods 59 & 80]
	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	
Water	Purge (inert gas); trap (OV-1 on Chromosorb-W/Tenax/silica gel); desorb as vapour (heat to 180°C, backflush with inert gas) onto GC column	GC/ECD	0.25 µg/L	US Environmental Protection Agency (1996a)
		GC/ MS	2.8 µg/L	US Environmental Protection Agency (1996b)
	Add internal standard (isotope-labelled dichloromethane); purge; trap and desorb as above	GC/ MS	10 µg/L	US Environmental Protection Agency (1996c)
	Purge (80°C, nitrogen); trap (Tenax-GC); desorb (flash-heat) and trap in 'mini-trap'(Tenax-GC, –30°C); desorb (flash-heat) onto GC column	GC/EC	0.1 µg/L (tap-water)	Piet *et al*. (1985a)
		GC/MS	< 0.05 µg/L (tap-water) 0.1 µg/L (surface water)	
	Equilibrate sealed water sample at 30°C; inject aliquot of head-space vapour	GC/EC	3–15 µg/L	Piet *et al*. (1985b)
	Inject aqueous sample directly onto calcium carbide precolumn (to remove water)	GC/EC	400 µg/L	Boos *et al*. (1985)
Food	Dissolve (toluene); distil under vacuum	GC/EC GC/ECD	0.35 mg/kg 0.5 mg/kg	US Food and Drug Administration (1983)

[a] Abbreviations: GC/EC, gas chromatography/electron capture detection; GC/ECD, gas chromatography/electrolytic conductivity detection; GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry

waste samples are first extracted with polyethylene glycol or methanol before purge/trap concentration and analysis (United States Environmental Protection Agency, 1982b).

Exposures to dichloromethane can also be monitored in air using a direct-reading infrared analyser, with minimum concentrations of 0.7 mg/m^3 (0.2 ppm) (Goelzer & O'Neill, 1985).

1.2 Production and use

1.2.1 *Production*

Dichloromethane was first prepared by Regnault in 1840 by the chlorination of methyl chloride in sunlight. It became an industrial chemical of importance during the Second World War. 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).

The predominant method of manufacturing dichloromethane uses as a first step the reaction of hydrogen chloride and methanol to give methyl chloride. Excess methyl chloride is then mixed with chlorine and reacts to give dichloromethane, with chloroform and carbon tetrachloride as co-products. This reaction is usually carried out in the gas phase thermally but can also be performed catalytically or photolytically. At low temperature and high pressure, the liquid-phase process is capable of giving high selectivity for dichloromethane (Rossberg *et al*., 1986; Holbrook, 1993).

The older and currently less used production method for dichloromethane involves direct reaction of excess methane with chlorine at high temperatures (400–500°C), or at somewhat lower temperatures either catalytically or photolytically. Methyl chloride, chloroform and carbon tetrachloride are also produced as co-products (Rossberg *et al*., 1986; Holbrook, 1993).

World production of dichloromethane increased from 93 thousand tonnes in 1960 to an estimated 570 thousand tonnes in 1980 (Edwards *et al*., 1982) and is believed to be still several hundred thousand tonnes. Production in the United States has shown a steady decline from 1981 to 1993, as shown by the following figures (thousand tonnes): 1981, 404; 1984, 275; 1987, 234; 1990, 209; 1993, 160 (Anon., 1994, 1997). The total amount produced in western Europe ranged from 331 500 tonnes in 1986 to 254 200 tonnes in 1991 (WHO, 1996).

1.2.2 *Use*

Most of the current applications of dichloromethane are based on its solvent properties. For use in paint strippers, one of its first applications, dichloromethane is blended with other chemical components to maximize its effectiveness against specific coatings. Typical additives include alcohols, acids, amines or ammonium hydroxide, detergents and paraffin wax (Rossberg *et al*., 1986; Holbrook, 1993; WHO, 1996).

Dichloromethane has been used as an extraction solvent for spices and beer hops and for decaffeination of coffee. It has also found use as a carrier solvent in the textile industry, in the manufacture of photographic film and as a blowing agent for polymer

foams. Dichloromethane is used as a solvent for vapour degreasing of metal parts and may also be blended with petroleum distillates and other chlorinated hydrocarbons for use as a dip-type cleaner in the metal-working industry, although consumption by this industry is declining because of recycling and recovery efforts on the part of end users. The reduction in use of 1,1,1-trichloroethane because of the Montreal Protocol and clean air legislation may increase the use of dichloromethane. It is also used as a component of low-pressure refrigerants, in air-conditioning installations, and as a low-temperature heat-transfer medium (Rossberg *et al*., 1986; Holbrook, 1993; WHO, 1996).

In chemical processing, dichloromethane is used in the manufacture of polycarbonate plastic from bisphenol and phosgene, the manufacture of photoresist coatings, and as a solvent carrier for the manufacture of insecticide and herbicide chemicals. 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 grain fumigation, oil dewaxing, in inks and adhesives and in plastics manufacture (Rossberg *et al*., 1986; Holbrook, 1993).

The use of dichloromethane in western Europe has shown a decrease from 200 thousand tonnes in 1975–85 to 175 thousand tonnes per year in 1989 and to 150 thousand tonnes per year in 1992 (WHO, 1996). Estimated use patterns for dichloromethane in the United States are presented in Table 2.

Table 2. Estimated use patterns (%) for dichloromethane in the United States[a]

Use	1986	1989	1992	1995
Paint removers/strippers	23	28	31	40
Aerosols	20	18	8	–
Chemical processing	20	11	16	10
Exports	10	15	–	–
Pharmaceuticals	–	–	11	6
Metal degreasing/cleaning	8	8	11	13
Electronics	7	7	4	3
Urethane blowing agent	5	9	14	6
Miscellaneous[b]	7	4	5	22

[a] From Anon. (1986, 1989, 1992, 1995)
[b] Includes pesticides, food processing, synthetic fibres, paints and coatings, aerosols (for 1995) and film processing

1.3 Occurrence

1.3.1 *Natural occurrence*

Dichloromethane is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The uses of dichloromethane reviewed in Section 1.2 can lead to human exposure.

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 250 000 workers in Europe and as many as 1.4 million workers in the United States were potentially exposed to dichloromethane (see General Remarks).

Information on numbers of workers potentially exposed in other countries was not available to the Working Group.

Concentrations of dichloromethane measured in 1968–73 in a plant producing plastic films in the United States were 458–2060 mg/m^3 in the casting area, 583–3350 mg/m^3 in the filtration area, 625–659 mg/m^3 in the winding area and 160–1130 mg/m^3 in offices (United States National Institute for Occupational Safety and Health, 1976).

Table 3 summarizes personal occupational exposures measured in various industries using dichloromethane. The levels vary widely by operation and within operations. Concentrations exceeding 1000 mg/m^3 have been measured, e.g., in paint stripping, in the printing industry and in the manufacture of plastics and synthetic fibres. Full-shift exposures to levels above 100 mg/m^3 of dichloromethane are possible, e.g., in furniture-stripping shops and in certain jobs in aeronautical, pharmaceutical, plastic and footwear industries.

Workers exposed to dichloromethane may be exposed also to various other agents, depending on their specific tasks and working environments.

1.3.3 *Air*

The principal route of exposure to dichloromethane for the general population is inhalation of ambient air. Average daily intake of dichloromethane from urban air has been estimated to range from about 33 to 309 μg. Exposure to dichloromethane in indoor air may be much higher, especially from spray painting or other aerosol uses and from paint removal and metal degreasing. Dichloromethane is degraded in the atmosphere by reaction with hydroxyl radicals, with an atmospheric lifetime of less than one year. The compound is highly mobile in soil and volatilizes rapidly from surface water to the atmosphere (Agency for Toxic Substances and Disease Registry, 1993).

Because dichloromethane is highly volatile, most environmental releases are into the atmosphere. It is released to the atmosphere during its production, storage and transport, but most (more than 99%) of the atmospheric releases result from industrial and consumer uses. Estimates of annual global emissions of 500 thousand tonnes have been reported for dichloromethane. It has been estimated that 85% of the total amount of dichloromethane produced in the United States is released to the environment, mostly to the atmosphere. Industrial dichloromethane emissions to the atmosphere in the United States fell from about 58 thousand tonnes in 1988 to approximately 25 thousand tonnes in 1995 (United States National Library of Medicine, 1997a). The total emission into the air in western Europe was estimated to be 173 thousand tonnes for 1989 and

Table 3. Personal occupational exposures to dichloromethane

Location	Job classification	Concentration (mg/m^3 air)	Reference
Plastics industry (six plants, 1971–81)	Mixing, moulding, preforming, pressing	18–35 17.5–130	Cohen & Vandervort (1972) Wagner (1974)
	Foam gun operator, waxer	690–1600	Burroughs & Moody (1982)
	Fabrication, assembly, finishing	< 0.4–40 < 0.3–35 59 16–325	Cohen & Vandervort (1972) Hollett (1977) Markel & Jannerfeldt (1981) Markel & Slovin (1981)
Polyester industry (one plant, 1991)	Preparation, five jobs laboratory	396–742[a] 161[a]	Post *et al*. (1991)
Flexible polyurethane manufacturing (one plant, 1991)	Pouring line	120–260	Boeninger (1991)
Synthetic fibres industry (two plants, 1977–79)	Pressman Extrusion area Bobbin stores Textile department	916–1300 239–1950 264–729 10.6–967	Cohen *et al*. (1980)
	Extrusion and preparation	486–1648	Ott *et al*. (1983a)
Footwear manufacture (four plants, 1975–82)	Four-part machine Crimping	118–597[a] 31[a]	Tharr *et al*. (1982)
	Assembly, moulding	< 5–104 2–319	Gunter (1975) Hervin & Watanabe (1981)
Pharmaceutical industry (one plant, 1993)	Washing of gelatine capsules	3–201[a]	Ghittori *et al*. (1993)
Photographic film (cellulose triacetate) industry (one plant, 1975)	Dichloromethane area	33[a]	Friedlander *et al*. (1978)

Table 3 (contd)

Location	Job classification	Concentration (mg/m^3 air)	Reference
General manufacturing (air-conditioning and refrigeration equipment, vending machines, pipes, welding wire, toys, fibreglass boats, sporting goods, paints and coatings, drugs, medical equipment) (14 plants, 1972–81)	Degreasing, stripping, flushing, cleaning	7–1930	Burton & Shmunes (1973)
		14–101	Markel & Shama (1974)
		< 3.5–403	Hervin *et al*. (1974)
		180–2190	Lee (1980)
		27	Ruhe (1981)
		52–141	Ruhe *et al*. (1981)
		1.3–467	Ruhe *et al*. (1982)
	Production, operations (fabrication, moulding, waxing, laminating)	4–38[a]	Rosensteel & Meyer (1977)
		22–85	Markel (1980)
	Solvent control	507	Ruhe *et al*. (1982)
Laboratories (three laboratories, 1978–89)	Laboratory technician	23–172	Ruhe (1978)
		236–455	Salisbury (1981)
	Preparation of samples	3–29[a]	McCammon (1990)
Aeronautical industry (one workshop, 1994)	Paint stripping of aircraft	86–1240[a]	Vincent *et al*. (1994)
Furniture stripping shops (five shops, 1991)	Strippers	663[a]	McCammon *et al*. (1991)
	Washers	503[a]	
	Refinishers	108[a]	
Maintenance/repair (automotive, aircraft, furniture, general contracting, miscellaneous) (10 plants, 1976–81)	Paint stripping, sanding	38–2820	Okawa & Keith (1977)
		94–4882	Chrostek (1980)
		45–698	Hartle (1980)
	Painting	25	Gunter (1976)
		30–503	Ruhe & Anderson (1977)
		22–233	Chrostek & Levine (1981)

Table 3 (contd)

Location	Job classification	Concentration (mg/m^3 air)	Reference
Maintenance/repair (contd)	Other	2	Gunter (1976)
		30–412	Ruhe & Anderson (1977)
		2.61	White & Wegman (1978)
		3.4 –31.1	Albrecht (1982)
Printing (five plants, 1975–81)	Printing operation	17	Ahrenholz (1980)
		24–410	Lewis & Thoburn (1981)
		5–560	Quinn (1981)
		8.2–37	Gorman (1982)
	Press checking and cleaning	360–1550	Quinn (1981)
	Tank cleaning	84–17 890	Rivera (1975)
	Darkroom, drafting, folding, collating, office work	< 6–248	Quinn (1981)
Coffee decaffeination (one plant, 1978)	Processing/extracting/evaporating	1.4 –115	Cohen *et al*. (1980)
	Coffee handling, drying	1–86	

[a] Eight-hour time-weighted average. For data not so indicated, the basis of measurement was not reported.

180 thousand tonnes in 1991 (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Dichloromethane has been detected in ambient air samples taken around the world, with background levels usually at about 0.17 μg/m^3. Concentrations in urban areas and in the vicinity of hazardous waste sites may be one to two orders of magnitude higher (up to 43 μg/m^3). Even higher levels (mean, 670 μg/m^3; peak level, 5000 μg/m^3) have been found in the indoor air of residences (WHO, 1996).

A large do-it-yourself consumer population uses paint strippers containing dichloromethane on furniture and woodwork. Formulations are available mainly in liquid form, but also, occasionally, as an aerosol. Exposures have been estimated on the basis of investigations of the use of household liquid products in the United States. The estimated levels ranged from less than 35 mg/m^3 to a few short-term exposures of 14 100–21 200 mg/m^3. The majority of the concentration estimates were below 1770 mg/m^3 (WHO, 1996).

Dichloromethane is also formed during water chlorination and is emitted into the air from wastewater in treatment plants (Agency for Toxic Substances and Disease Registry, 1993).

1.3.4 *Water*

About 2% of environmental releases of dichloromethane are to water. Industrial releases of dichloromethane to surface water and underground injection (potential groundwater release) reported to the United States Toxic Chemical Release Inventory in 1988 totalled 158 tonnes. Dichloromethane has been identified in industrial and municipal wastewaters from several sources at concentrations ranging from 0.08 μg/L to 3.4 g/L (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Dichloromethane has been detected in surface water, groundwater and finished drinking-water throughout the United States. It was detected in 30% of 8917 surface water samples recorded in the STORET database of the United States Environmental Protection Agency, at a median concentration of 0.1 μg/L. In a New Jersey survey, dichloromethane was found in 45% of 605 surface water samples, with a maximum concentration of 743 μg/L. Dichloromethane has also been identified in surface waters in Maryland, in Lakes Erie and Michigan, and at hazardous waste sites (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Dichloromethane is also present in small amounts in seawater. It has been found at up to 2.6 μg/L in coastal waters of the Baltic Sea. Levels up to 0.20 μg/L have been found in North Sea coastal waters. Dichloromethane is generally not detected in open ocean; a mean concentration of 2.2 ng/L has been reported in the southern Pacific Ocean (WHO, 1996).

Since volatilization is restricted in groundwater, concentrations of dichloromethane are often higher there than in surface water. Occurrence of dichloromethane in groundwater has been reported in several surveys across the United States, with concentrations ranging from 0 to 3600 μg/L (Agency for Toxic Substances and Disease Registry, 1993).

Dichloromethane has been detected in drinking-water supplies in numerous cities in the United States, with reported mean concentrations generally below 1 µg/L. It has also been identified in commercially bottled artesian water. Water chlorination in treatment plants appears to increase both the concentration and the frequency of occurrence of dichloromethane in drinking water supplies (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Samples from 128 drinking-water wells in the United States showed that 3.1% of them had dichloromethane levels of 1–5 µg/L. Dichloromethane was detected in 98.4% of drinking-water samples from Santiago de Compostela, Spain, in 1987; the average concentration was 14.1 µg/L, with a range of 1.2–93.2 µg/L. A sampling of 630 public community water supplies (serving 690 million people in New Jersey, United States) in 1984 and 1985 detected dichloromethane in 2.6–7.1% of the samples; the median concentration ranged from 1.1 to 2.0 µg/L and the range for the whole sampling period was 0.5–39.6 µg/L (WHO, 1996).

Dichloromethane has been detected in both surface water and groundwater samples taken at hazardous waste sites. Data from the Contract Laboratory Program Statistical Database of the United States Environmental Protection Agency indicate that dichloromethane was present at geometric mean concentrations of 68 and 98 µg/L in surface water and groundwater samples, respectively, at about 30% of the sites sampled (Agency for Toxic Substances and Disease Registry, 1993).

Wastewater from certain industries has been reported to contain dichloromethane at average concentrations in excess of 1000 µg/L. Such industries include coal mining, aluminium forming, photographic equipment and supplies, pharmaceutical manufacture, organic chemical/plastics manufacture, paint and ink formulation, rubber processing, foundries and laundries. The maximal concentration measured was 210 mg/L in wastewater from the paint and ink industry and the aluminium-forming industry. In leachate from industrial and municipal landfills, dichloromethane concentrations were reported to range up to 184 mg/L (WHO, 1996).

1.3.5 *Soil/sediment*

The principal sources of dichloromethane releases to land are disposal of dichloromethane products and containers to landfills. Industrial releases of dichloromethane to land and off-site transfers to landfills reported to the Toxic Chemical Release Inventory in 1988 totalled about 71 tonnes. It is estimated that about 12% of dichloromethane releases to the environment are to land (Agency for Toxic Substances and Disease Registry, 1993).

Dichloromethane has been detected in soil and sediment samples taken at 36% of the hazardous waste sites included in the Contract Laboratory Program Statistical Database at a geometric mean concentration of 104 µg/kg (Agency for Toxic Substances and Disease Registry, 1993).

The levels of dichloromethane found in samples of sediment from Lake Pontchartrain, Louisiana, United States, ranged from not detectable to 3.2 µg/kg wet weight. In

Germany, levels found in sediments from the River Rhine in 1987–88 varied from not detectable to 30–40 μg/kg. At one site, concentrations of 220–2200 μg/kg were measured (WHO, 1996).

1.3.6 *Aquatic organisms*

Concentrations of dichloromethane in freshwater organisms have been reported for oysters and clams from Lake Ponchartrain, Louisiana, United States; levels ranging from 4.5 to 27 μg/kg wet weight were detected. Levels up to 700 μg/kg wet weight were found in marine bottom fish taken from Commencement Bay, Washington, United States. Data on biota collected in the STORET database of the United States Environmental Protection Agency showed an average level of 660 μg/kg in the 28% of the samples in which dichloromethane was detected (WHO, 1996).

1.3.7 *Foodstuffs*

Although dichloromethane has been used as a grain fumigant and in processing certain raw food commodities, there is little information on residual levels in food. At a large decaffeinating plant in the United States in 1978, monthly average residues in dichloromethane-decaffeinated coffee beans ranged from 0.32 to 0.42 mg/kg dichloromethane (115–295 samples analysed per month) (Cohen *et al.*, 1980). In seven types of decaffeinated ground coffee, the dichloromethane content ranged from < 0.05 to 4.04 mg/kg; in eight instant coffee samples, it ranged from < 0.05 to 0.91 mg/kg; and in 10 decaffeinated tea samples, it ranged from < 0.05 to 15.9 mg/kg (Page & Charbonneau, 1984). Dichloromethane apparently is no longer used for decaffeination in the United States (Agency for Toxic Substances and Disease Registry, 1993).

In an investigation of several halocarbons in table-ready foods, eight of the 19 foods examined contained dichloromethane levels above the quantification limit (not given). The following ranges were reported (μg/kg): butter, 1.1–280; margarine, 1.2–81; ready-to-eat cereal, 1.6–300; cheese, 3.9–98; peanut butter, 26–49; and highly processed foods (frozen chicken dinner, fish sticks, pot pie), 5–310 (Heikes, 1987).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for dichloromethane in several countries are given in Table 4.

In the United States, dichloromethane may be present as an extractant or process solvent residue in spice oleoresins at a level not to exceed 30 mg/kg [ppm] (including all chlorinated solvents), in hops extract at less than or equal to 2.2% and in coffee at a level not to exceed 10 mg/kg [ppm] (United States Food and Drug Administration, 1996).

The Joint FAO/WHO Expert Committee on Food Additives (WHO, 1983) withdrew the previously allocated temporary allowable daily intake (ADI) of 0–0.5 mg/kg body weight and recommended that the use of dichloromethane as an extraction solvent be limited, in order to ensure that its residues in food are as low as practicable.

Table 4. Occupational exposure limits and guidelines for dichloromethane[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1991	350 (C3)	TWA
Austria	1993	360	TWA
Belgium	1991	174 (C2)	TWA
Czechoslovakia	1991	500	TWA
		2500	STEL
Denmark	1991	175 (C, sk)	TWA
Finland	1998	350	TWA
		870	STEL
France	1991	360	TWA
		1800	STEL
Germany	1998	350 (C3)	TWA
Hungary	1993	10 (Ca)	STEL
Italy	1991	10	STEL
Japan	1991	350	TWA
The Netherlands	1993	350	TWA
		1740	STEL
The Philippines	1993	1740	TWA
Poland	1991	50	TWA
Russia	1991	350	TWA
		50	STEL
Sweden	1991	120 (sk)	TWA
		250	STEL
		500	STEL
Switzerland	1991	360	TWA
		1800	STEL
Thailand	1993	500	TWA
		1000	STEL
Turkey	1993	1740	TWA
United Kingdom	1991	350	TWA
		870 (MEL)	STEL
United States			
ACGIH (TLV)[c]	1997	174 (A3)	TWA
NIOSH (REL)	1997	(Ca, lfc)	
OSHA (PEL)	1997	87 (Ca)	TWA
		435	STEL

[a] From International Labour Office (1991); American Conference of Governmental Industrial Hygienists (ACGIH) (1997a,b); United States National Library of Medicine (1997b); United States Occupational Safety and Health Administration (OSHA) (1997); Deutsche Forschungsgemeinschaft (1998); Ministry of Social Affairs and Health (1998)

[b] TWA, time-weighted average; STEL, short-term exposure limit; MEL, maximum exposure limit; TLV, threshold limit value; REL, recommended exposure limit; PEL, permissible exposure limit; A3, animal carcinogen; C, suspected of being a carcinogen; C2, probable human carcinogen; C3, suspected of having a carcinogenic potential; Ca, potential occupational carcinogen; lfc, lowest feasible concentration; sk, skin notation

[c] Countries that follow the ACGIH recommendations for threshold limit values include Bulgaria, Colombia, Jordan, Korea, New Zealand, Singapore and Viet Nam.

The use of dichloromethane in hair sprays was banned in the United States by the Food and Drug Administration in 1989 (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

2. Studies of Cancer in Humans

2.1 Industry-based studies (Table 5)

Hearne *et al*. (1990) reported on mortality among a cohort of 1013 photographic film (cellulose triacetate) workers in the United States who were chronically exposed to dichloromethane. This subsumed earlier analyses by Friedlander *et al*. (1978), Hearne and Friedlander (1981) and Hearne *et al*. (1987). The cohort consisted of male workers employed between 1964 and 1970 who had worked for at least one year. Exposure was classified on the basis of work history abstracted from company records and industrial hygiene measurements. The 1975 mean personal time-weighted average (TWA) exposure was 33 ppm [115 mg/m^3], while the range of exposures based on area samples collected during 1959 to 1975 was 0 to 350 ppm [0–1215 mg/m^3] (Friedlander *et al*., 1978). Four categories of cumulative exposure were used (< 400, 400–799, 800–1199, > 1200 ppm-years). Follow-up was from 1964 through 1988 and was reported to be more than 99% complete. There were 238 deaths in total [standardized mortality ratio (SMR), 0.7 using state rates; SMR, 0.8 using company rates] and 55 cancer deaths [SMR, 0.7 using both rates]. The numbers of observed cancers were less than or similar to the number expected for all sites except pancreas [SMR, 1.9, based on 8 deaths versus 4.2 expected from both state and company rates], but relative risk was not related to estimated cumulative exposure. No other significant association was reported.

Lanes *et al*. (1993) conducted a cohort mortality study of workers employed in the United States in the production of cellulose triacetate fibres, who were potentially exposed to dichloromethane. This extended earlier analyses by Ott *et al*. (1983a,b) and Lanes *et al*. (1990). The cohort consisted of 1271 workers who were employed for at least three months between 1954 and 1976. Based on a combination of personal and area samples, median TWA exposures in 1977 were reported to range from 140 to 745 ppm [486–2590 mg/m^3] in the exposed groups. Follow-up was from 1954 through 1990. Completeness of follow-up was not reported and those not known to have died were assumed to be alive at the end of follow-up. SMRs were calculated using county rates. In total, 172 deaths (SMR, 0.9) including 39 cancer deaths (SMR, 0.8; 95% confidence interval (CI), 0.6–1.5) were observed. The numbers of observed cancers were less than or similar to the number expected for all sites. The highest SMR was observed for cancer of the liver and biliary tract (SMR, 3.0; 95% CI, 0.8–7.6). Three deaths out of the four attributed to cancer of the liver and biliary tract were cancer of the biliary tract. Each of these deaths occurred among employees with longer than 10 years of employment and more than 20 years since first employment (SMR, 5.8; 95% CI, 1.6–14.9).

Table 5. Epidemiological results from industry-based studies relevant to the evaluation of dichloromethane carcinogenicity

Reference	Country	Cohort size/ no. of deaths	Cancer site[a]	Observed	RR	95% CI	Comment
Hearne *et al*. (1990)	United States	1013/238	All cancers	55	[0.7]	[0.6–1.0]	Expected numbers based on company-wide rates
			Lung	18	[0.8]	[0.5–1.2]	
			Liver	0	[0.0]	[0.0–6.2]	
			Pancreas	8	[1.9]	[0.8–3.8]	
			Brain	2	[1.0]	[0.1–3.6]	
Lanes *et al*. (1993)	United States	1271/172	All cancers	39	0.8	0.6–1.5	
			Lung	13	0.8	0.4–1.4	
			Liver/biliary	4	3.0	0.8–7.6	
			Pancreas	2	0.8	0.1–3.0	
			Breast	3	0.5	0.1–1.6	
Gibbs *et al*. (1996)	United States	Males: 1931/500[b]	All cancers	163	[0.8]	[0.7–1.0]	
			Lung	46	[0.7]	[0.5–1.0]	
			Liver/biliary	2	[0.5]	[0.1–2.0]	
			Pancreas	4	[0.6]	[0.2–1.5]	
			Prostate	22	[1.6]	[0.9–2.4]	
		Females: 978/124[b]	Cervix	6	[3.2]	[1.2–6.9]	
Tomenson *et al*. (1997)	United Kingdom	1473/287[b]	All cancers	68	0.7	0.5–0.8	
			Lung	19	0.5	0.3–0.8	
			Liver/biliary	0	0.0	[0.0–2.5]	
			Pancreas	3	0.7	0.1–2.0	
			Prostate	4	0.6	0.2–1.6	
			Brain	4	1.5	0.4–3.7	

Table 5 (contd)

Reference	Country	Cohort size/ no. of deaths	Cancer site[a]	Observed	RR	95% CI	Comment
Ott *et al*. (1985)	United States	226/42	All cancers	9	0.7	0.3–1.3	Expected from US rates
					[0.8]	[0.4–1.5]	Expected from company rates
			Respiratory	3	[0.7]	[0.1–2.0]	
			Digestive	6	[1.8]	[0.7–4.0]	
			Pancreas	3	[3.3]	[0.7–9.7]	
Blair *et al*. (1998)	United States	14 457/5 727	All cancers	1048	0.96	0.90–1.02	SMR, full cohort
			Non-Hodgkin lymphoma				
			men	6 exposed	3.0	0.9–10.0	Incident cancer,
			women	0 exposed	0.0	–	RR from Poisson regression
			Multiple myeloma				
			men	5 exposed	3.4	0.9–13.2	
			women	0 exposed	0.0	–	
			Breast, women	4 exposed	3.0	1.0–8.8	
Shannon *et al*. (1988)	Canada	Females: 203/19 incident cancers	All cancers	19	[1.6]	[0.9–2.4]	Incident cancers
			Breast	8	2.0	0.88–4.0	
			Cervix	1	1.1	0.03–5.9	

[a] Results are presented for all cancers, lung, liver/biliary, pancreas, prostate, breast, cervix and brain when reported.
[b] Results are presented only for the exposed portion of the cohort.

Gibbs *et al*. (1996) conducted a cohort mortality study of cellulose triacetate fibre workers exposed to dichloromethane at a facility in the United States similar to that reported by Lanes *et al*. (1993). The cohort consisted of 3211 workers who had been employed between 1970 and 1981 and had worked in the plant for three or more months. Follow-up was from 1970 through 1989 and comparisons were made with county rates. Completeness of follow-up was not reported and those not known to have died were assumed to be alive at the end of follow-up. In the areas of highest exposure, levels were reported to range from 300 to 1250 ppm [1040–4340 mg/m^3]. The cohort was divided into three exposure groups; none, low (50 to 100 ppm [174–347 mg/m^3]) and high (350 to 700 ppm [1215–2430 mg/m^3]) based on the area worked in and exposure levels reported by Ott *et al*. (1983). There were 302 workers classified as having no exposure. Among the 1931 dichloromethane-exposed male workers, there were 500 deaths [SMR, 0.8] and 163 cancer deaths [SMR, 0.8] observed. Results were reported for the following sites: trachea, bronchus and lung; liver and biliary tract; pancreas; prostate; and cervix. Only the SMR for prostate cancer [1.6; 95% CI, 0.9–2.4] was elevated and appeared to increase with level of exposure (SMR, 1.8; 95% CI, 1.0–3.0 for high exposure). There was also an excess of prostate cancer among workers who had been employed at the facility for 20 or more years (SMR, 2.9; $p < 0.05$). Among the 978 exposed women there was an excess of cervical cancer [SMR, 3.2; 95% CI, 1.2–6.9, based on 6 cases], which did not appear to be related to duration of employment or level of dichloromethane exposure. [The Working Group noted that the interpretation of the exposure measurements is not clear, since the units are in ppm and not ppm-years and it is not clear whether all jobs held at the facility were considered.]

Tomenson *et al*. (1997) performed a cohort mortality study of workers at a plant producing cellulose triacetate film base in the United Kingdom. The cohort comprised 1785 male workers who had been employed at the site at any time between 1946 and 1988, among whom 1473 had been employed in jobs with potential exposure to dichloromethane. Exposure assessment was based on time period and work group, and exposure levels were estimated from area samples. 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. Four exposure categories were established based on cumulative exposure to dichloromethane (never, < 400 ppm-years, 400–799 ppm-years, > 800 ppm-years). However, 30% of exposed workers could not be classified. Follow-up was from 1946 through 1994 and was reported to be > 99% complete. SMRs were calculated using rates for England and Wales. During the follow-up period there were 287 deaths (SMR, 0.7) and 68 cancer deaths (SMR, 0.7; 95% CI, 0.5–0.8) observed among the exposed workers. Only brain and central nervous system cancer (SMR, 1.5; 95% CI, 0.4–3.7; based on 4 cases) had appreciably more observed than expected cases and the excess was not limited to highly exposed workers. [The Working Group noted that 31 of the 68 cancer deaths among exposed workers were not assigned an exposure level, limiting the utility of the cumulative exposure analyses. The all-cancer SMR was unusually low.]

Ott *et al*. (1985) conducted a cohort mortality study of 1919 men employed for one year or longer between 1940 and 1969 at a chemical manufacturing facility in the United States. This cohort included 226 workers assigned to a unit which produced chlorinated methanes (methyl chloride (see this volume), dichloromethane, chloroform (IARC, 1987b) and carbon tetrachloride (see this volume)) and, recently, tetrachloroethylene (IARC, 1995a). Exposure levels were not reported. The follow-up period was from 1940 to 1979 and follow-up was 94% complete. Expected numbers were based on rates among white males in the United States for the full cohort and on the full cohort for sub-cohort analyses. Among the 226 workers producing chlorinated methanes, there were 42 deaths observed (SMR, 0.6, based on national rates) [SMR, 0.8, based on company rates], including nine from cancers [SMR, 0.8; 95% CI, 0.4–1.5, based on company rates] and three from pancreatic cancer [SMR, 3.3; 95% CI, 0.7–9.7, based on company rates]. Two of these three cases had been employed for less than five years, all three were first assigned to the chlorinated methane unit between 1942 and 1946, and the interval between first assignment to that unit and death was between 20 and 31 years. [The Working Group noted that the mix of exposures and the lack of information regarding exposure levels limits the ability to draw conclusions regarding the carcinogenicity of dichloromethane]

Blair *et al*. (1998) performed a retrospective cohort mortality study of 14 457 workers at a military aircraft maintenance facility in the United States, among whom 1222 were exposed to dichloromethane (Stewart *et al*., 1991). This was an update of an earlier study (Spirtas *et al*., 1991). The cohort consisted of civilian employees employed at a military air force base for at least one year between 1952 and 1956. Follow-up of the cohort was from 1952 through 1990 and comparisons were made with state rates and between exposed and unexposed cohort members. Mortality follow-up was 97% complete. In addition, incident cancers were identified using a statewide tumour registry with follow-up from 1973 to 1990. There were 5727 deaths (SMR, 0.97) and 1048 cancer deaths (SMR, 0.96; 95% CI, 0.90–1.02) identified in the full cohort. An extensive exposure assessment was performed to quantitatively classify exposure to trichloroethylene (IARC, 1995b) and to qualititatively classify exposure (ever/never) to other chemicals including various solvents (Stewart *et al*., 1991). Relative risks (RR) from chemicals other than trichloroethylene were examined by Poisson regression analyses of the cancer incidence data. Exposure to dichloromethane was associated with an increased risk of non-Hodgkin lymphoma (RR, 3.0; 95% CI, 0.9–10.0; 6 exposed cases) and multiple myeloma (RR, 3.4; 95% CI, 0.9–13.2; 5 exposed cases) among men, but not among women (0 exposed cases for both). Among women, exposure to dichloromethane was associated with the risk of breast cancer (RR, 3.0; 95% CI, 1.0–8.8; 4 exposed cases). Results for other cancer sites in relation to dichloromethane exposure were not reported. [The Working Group noted that overlapping exposures to solvents, some of which also showed associations with the cancers evaluated, and the lack of information about exposure levels limit the ability to draw conclusions regarding dichloromethane].

Shannon *et al*. (1988) reported the results of a study of cancer morbidity among Canadian lamp-manufacturing workers. The study had been initiated because of a reported cluster of five cancers among workers in the coiling and wire drawing (CWD) area. Although the study focused on exposure to dichloromethane, potential exposure to other solvents (e.g., trichloroethylene (IARC, 1995b)), strong acids (e.g., sulfuric or nitric acids (IARC, 1992)) and metals (e.g., arsenic (IARC, 1987c), chromium (IARC, 1990)) was also reported in the CWD area. The study population included 203 women and 46 men who had been employed for six or more months at some time between 1960 and 1975 in the CWD department. Incident cancers from 1964 to 1982 were ascertained using a population-based tumour registry and follow-up was stated to be over 90% complete. Among the 203 women who had been employed in the CWD department, the standardized incidence ratio (SIR) for all cancers was [1.6] [95% CI, 0.9–2.4 (n = 19)]. The SIR for breast cancer was 2.0 (95% CI, 0.9–4.0 (n = 8)) and was significantly elevated among those with ≥ 5 years employment and ≥ 15 years since first employment (SIR, 3.2; 95% CI, 1.1–7.5 (n = 5)). Only three cancers were observed among the 46 men who had been employed in the CWD department and their results were not reported. [The Working Group noted that one breast cancer was reported to be part of the initial cluster that led to the study and that the potential for exposure to multiple chemicals limits the ability to draw conclusions regarding dichloromethane.]

2.2 Community-based studies

Heineman *et al*. (1994) performed a case–control study to examine the relationship between occupational exposure to six chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Cases were 741 white men who died from central nervous system tumours in three areas of the United States (southern Louisiana, northern New Jersey, and Philadelphia) over a three-year period. Controls were 741 randomly selected men matched on age, year of death and study area, who died from other causes. The next-of-kin of 654 cases (88%) and 612 controls (83%) were located and 483 cases (74% of traced) and 386 of controls (63% of traced) were interviewed to ascertain detailed work history and other possible risk factors. The final data-set consisted of 300 cases and 320 controls, after exclusion of cases without a hospital diagnosis of astrocytic brain tumour and controls whose death might be linked to occupational exposure to chlorinated aliphatic hydrocarbons. Exposure was assessed using a semi-quantitative job–exposure matrix developed for the study (Gomez *et al*., 1994), and probability of exposure (unexposed, low, medium and high), duration of exposure (2–20 years and > 21 years), average intensity (low-medium, high) and cumulative exposure (low, medium and high) were examined. One hundred and nineteen cases and 108 controls were classified as having ever been exposed to dichloromethane. After adjustment for age and study area, there was a trend of increasing risk with increasing probability, duration and average intensity of exposure ($p < 0.05$), but not with cumulative exposure. The odds ratios for the highest categories were 2.4 (95% CI, 1.0–5.9, 19 exposed cases) for high probability, 1.7 (95% CI, 0.9–3.6, 24 exposed cases) for > 21 years, 2.2 (95% CI, 1.1–4.4, 28

exposed cases) for high average intensity, and 1.2 (95% CI, 0.6–2.5, 19 exposed cases) for high cumulative exposure. No association was found with any of the other five chlorinated hydrocarbons examined.

Cantor *et al*. (1995) performed a case–control study to examine the relationship between occupational exposures and female breast cancer mortality in the United States. Cases and controls were identified from a 24-state death certificate surveillance system. The case group comprised all deaths from female breast cancer between 1984 and 1989 (n = 59 515). For each case, four controls were selected from non-cancer deaths. Usual occupation and industry were coded from death certificates for cases and controls. After excluding those coded as homemakers, there were 29 397 white female cases and 4112 black female cases matched to 102 955 white and 14 839 black controls. Probability and level of workplace exposure to 31 chemical and physical agents were estimated using a job–exposure matrix and results for white women and black women were reported separately. No association was observed with probability of exposure to dichloromethane. However, a small elevated risk was observed for the highest exposure level among both white women (odds ratio, 1.2; 95% CI, 1.1–1.3) and black women (odds ratio, 1.5; 95% CI, 1.2–1.7) after adjustment for age and socioeconomic status. [The Working Group noted that usual occupation as recorded on death certificates may be a poor indicator of exposure to dichloromethane].

A population-based case–control study of cancer among male residents of Montreal, Canada, aged 35–70 years, included histologically confirmed cases of several types of cancer, newly diagnosed between 1979 and 1985 in 20 major hospitals (Siemiatycki, 1991). Interviews were carried out with 3730 cancer patients (response rate, 82%) and 533 age-stratified controls from the general population (response rate, 72%). The main cancer sites included were: oesophagus (99), stomach (251), colon (497), rectum (257), pancreas (116), lung (857), prostate (449), bladder (484), kidney (177), skin melanoma (103) and non-Hodgkin lymphoma (215). For each site of cancer analysed, two control groups were available: population controls and a cancer control group selected from among cases of cancer at the other sites studied. The interview was designed to obtain detailed lifetime job histories and information on potential confounders. Each job was reviewed by a team of chemists and industrial hygienists who translated jobs into occupational exposures using a checklist of 293 occupational substances. Analyses were carried out to estimate the odds ratio between cancer at each site and exposure to each substance. For each association, eight separate estimates were made: two control groups (population and cancer), two target populations (the entire population of Montreal and the 65% subset of the population who were French Canadian and who had a much more homogeneous genetic, social and environmental exposure profile than the population as a whole) and two exposure levels (any exposure and ‘substantial exposure’, defined on the basis of duration, intensity and frequency of purported exposure). The publication did not present all the odds ratios computed; while a set of results in the entire population and using cancer controls was presented for a subset of possible associations, for others, only the most significant results have been published. Dichloromethane was one of the

substances. About 2% of the study subjects had ever been exposed to dichloromethane. Among the main occupations to which dichloromethane exposure was attributed in this study were construction painters, paint mixers and cabinet makers. For most types of cancer examined (oesophagus, stomach, colon, pancreas, prostate, bladder, kidney, skin melanoma, lymphoma), there was no indication of an excess risk due to dichloromethane. However, for rectal cancer, based on five cases exposed at the 'substantial level', the odds ratio was 4.8 (90% CI, 1.7–13.8). For lung cancer, based on seven cases exposed at the 'substantial level', the odds ratio was 3.8 (90% CI, 1.2–12.0). [The interpretation of null results has to take into account the small numbers and low power. Workers typically had multiple exposures. This is particularly true among workers exposed to dichloromethane.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of male and female B6C3F$_1$ mice, seven weeks of age, were administered dichloromethane (containing < 300 mg/kg cyclohexane, < 20 mg/kg *trans*-1,2-dichloroethylene, < 10 mg/kg chloroform, < 2 mg/kg vinyl chloride and < 1 mg/kg each methyl chloride, ethyl chloride, vinylidene chloride, carbon tetrachloride and trichloroethylene) in the drinking-water for 104 weeks according to the study design shown in Table 6. 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, the incidences of hepatocellular adenoma were: 6/60 (10%), 4/65 (8%), 20/200 (10%), 14/100 (14%), 14/99 (14%) and 15/125 (12%); and the incidences of hepatocellular carcinomas were: 5/60 (8%), 9/65 (14%), 33/200 (17%), 18/100 (18%), 17/99 (17%) and 23/125 (18%) in control 1, control 2, low-dose, mid-dose 1, mid-dose 2 and high-dose groups, respectively. A slight but significant [$p = 0.035$] dose-related increase in the incidence of hepatocellular adenomas and/or carcinomas (combined) was observed in male mice: 11/60 (18%), 13/65 (20%), 51/200 (25%), 30/100 (30%), 31/99 (31%) and 35/125 (28%). However, the authors noted that tumour incidences in exposed groups were similar to those reported in historical controls (mean, 32.1%; range, 7–58%) (Serota *et al.*, 1986a).

Groups of 50 male and 50 female Swiss mice, nine weeks of age, were administered 100 (low-dose) or 500 (high-dose) mg/kg bw dichloromethane (purity, > 99.9%) in olive oil by gavage once per day on four to five days per week for 64 weeks. Groups of 60 mice of each sex were given olive oil (vehicle-control). Animals were then kept under observation for their lifespan. Excess mortality was observed in male and female mice exposed to the high dose ($p < 0.01$). An increase in mortality appeared after 36 weeks of treatment and led to withdrawal of the treatment at 64 weeks. In mice that died by 78 weeks, the incidence of lung tumours in males was 1/14 control, 4/21 low-dose and 7/24

Table 6. Design of studies of dichloromethane in drinking-water

Group	No. of animals		Dose (mg/kg bw/day)
	Males	Females	
Mice[a]			
Control 1	60	50	0
Control 2	65	50	0
Low-dose	200	100	60
Mid-dose 1	100	50	125
Mid-dose 2	100	50	185
High-dose	125	50	250
Rats[b]			
Control 1	85	85	0
Control 2	50	50	0
Low-dose	85	85	5
Mid-dose 1	85	85	50
Mid-dose 2	85	85	125
High-dose	85	85	250
High-dose (78 weeks)	25	25	250

[a] From Serota *et al*. (1986a)
[b] From Serota *et al*. (1986b)

high-dose ($p < 0.05$) mice, respectively. At the end of the experiment, the cumulative incidence of lung tumours in males was 5/47, 5/28 and 9/36. No treatment-related increase in the incidence of any tumour in females or other type of tumour in males was reported (Maltoni *et al*., 1988). [The Working Group noted the short period of exposure and the high numbers of animals lost for examination.]

3.1.2 *Rat*

Groups of male and female Fischer 344 rats, seven weeks of age, were administered dichloromethane (containing < 300 mg/kg cyclohexane, < 20 mg/kg *trans*-1,2-dichloroethylene, 26 mg/kg chloroform and < 1 mg/kg each methyl chloride, vinyl chloride, ethyl chloride, vinylidene chloride and trichloroethylene) in the drinking-water for 104 weeks according to the study design shown in Table 6. Interim terminations were carried out at 26, 52 and 78 weeks in control group 1 and in the low-, mid-1 and -2 and high-dose groups, such that 50 males and 50 females per group received the treatment for 104 weeks. There was no significant difference in survival between the exposed and control groups. In females, the incidences of hepatocellular carcinomas after 104 weeks were: 0/85, 0/50, 0/85, 2/83, 0/85 and 2/85; those of neoplastic nodules [now classified as hepatocellular adenomas] were: 0/85, 0/50, 1/85, 2/83, 1/85 and 4/85; and those of

neoplastic nodules and/or hepatocellular carcinomas (combined) were: 0/85, 0/50, 1/85, 4/83, 1/85 and 6/85 in the six groups, respectively. This increasing trend was significant [$p < 0.01$]; however, tumour incidences in exposed groups were similar to those reported in historical controls in this laboratory (mean, 8%; range, 0–16%). In male rats, no increased incidence of liver tumours was observed at 104 weeks (neoplastic nodules: 4/85, 5/50, 2/85, 3/84, 3/85 and 1/85; carcinomas: 2/85, 2/50, 0/85, 0/84, 0/85 and 1/85; neoplastic nodules and/or carcinomas combined: 6/85, 7/50, 2/85, 3/84, 3/85 and 2/85). No other significant increase in tumour incidence was found (Serota *et al*., 1986b).

Groups of 50 male and 50 female Sprague-Dawley rats, 12 weeks of age, were administered 100 (low-dose) or 500 (high-dose) mg/kg bw dichloromethane (purity, > 99.9%) in olive oil by gavage once per day on four or five days per week for 64 weeks. A group of 50 rats of each sex was given olive oil (vehicle controls) and an additional group of 20 males and 26 females was kept untreated (untreated controls). Animals were then kept under observation for their lifespan. Excess mortality was observed in male and female rats administered dichloromethane at the high 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 (Maltoni *et al*., 1988). [The Working Group noted the short period of treatment and the inadequate reporting of the data.]

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, eight to nine weeks of age, were exposed to 0, 2000 or 4000 ppm [0, 6940 or 13 900 mg/m^3] dichloromethane (> 99% pure) by whole-body inhalation for 6 h per day on five days per week for 102 weeks and were killed after 104 weeks on study. The mean body weight of the high-dose male mice was generally comparable to that of controls until week 90, and that of high-dose females was somewhat lower from weeks 51 to 95. Survival to the end of the study period in males was: control, 39/50; low-dose, 24/50; and high-dose, 11/50; and that in females was: 25/50, 25/49 and 8/49. Significant dose-related increases in the incidence of lung and liver tumours were observed in exposed mice. The incidences of alveolar/bronchiolar adenomas were: males—3/50, 19/50 and 24/50 ($p < 0.001$); and females—2/50, 23/48 and 28/48 ($p < 0.001$). Those of alveolar/bronchiolar carcinomas were: males—2/50, 10/50 and 28/50 ($p < 0.001$); and females—1/50, 13/48 and 29/48 ($p < 0.001$). The incidences of hepatocellular adenomas were: males—10/50, 14/49 and 14/49 ($p = 0.075$); and females — 2/50, 6/48 and 22/48 ($p < 0.001$). The incidences of hepatocellular carcinomas were: males—13/50, 15/49 and 26/49 ($p = 0.016$); and females—1/50, 11/48 and 32/48 ($p < 0.001$) (United States National Toxicology Program, 1986).

Groups of 68 female B6C3F_1 mice, eight to nine weeks of age, were administered dichloromethane (> 99% pure) by whole-body inhalation at concentrations of 0 ppm (control) or 2000 ppm [6940 mg/m^3] for various lengths of time over a 104-week period (Table 7). Lung and liver were evaluated histopathologically. Survival was reduced

Table 7. Effect of various dichloromethane inhalation exposure regimens on survival and pulmonary and liver tumours in female B6C3F$_1$ mice

	Dichloromethane treatment							
	0 ppm 104 weeks	2000 ppm 26 weeks/ 0 ppm 78 weeks	0 ppm 78 weeks/ 2000 ppm 26 weeks	2000 ppm 52 weeks/ 0 ppm 52 weeks	0 ppm 52 weeks/ 2000 ppm 52 weeks	2000 ppm 78 weeks/ 0 ppm 26 weeks	0 ppm 26 weeks/ 2000 ppm 78 weeks	2000 ppm 104 weeks
Survival (%) (Kaplan–Meier)	58.8	47.1	54.1	34.4**	58.8	35.3**	47.1	40.4
Lung								
Adenomas								
Incidence	1/67	8/68	0/67	12/63	5/67	19/68	7/67	18/67
Carcinomas								
Incidence	4/67	17/68	3/67	36/63	6/67	25/68	7/67	31/67
Combined								
No. animals with adenomas or carcinomas/no. animals at risk	5/67	21/26**	3/67	40/63**	10/67	38/68**	13/67*	42/67**
Liver								
Adenomas								
Incidence	8/67	16/67	16/67	14/64	9/67	28/68	17/67	24/68
Carcinomas								
Incidence	11/67	14/67	13/67	18/64	12/67	25/68	20/67	35/68
Combined								
No. animals with adenomas or carcinomas/no. animals at risk	18/67	27/67	23/67	28/64*	21/67	42/68**	32/67*	47/68**

Incidence = No. of animals with tumour/No. of animals at risk
*p < 0.05; **p < 0.01 against control group
From Kari *et al.* (1993)

compared with controls in groups exposed to dichloromethane for the first 52, 78 or the complete 104 weeks of the study. The incidences of mice with lung adenomas, carcinomas or adenomas and carcinomas combined and the incidences of mice with hepatocellular adenomas, carcinomas or adenomas and carcinomas combined were increased in all groups in which exposure was begun during the first 26 weeks of the study [statistical analyses were reported only for the combined tumour incidences] (Table 7) (Kari *et al.*, 1993).

3.2.2 *Rat*

Groups of approximately 95 male and 95 female Sprague-Dawley rats, eight weeks of age, were administered dichloromethane by whole-body inhalation at concentrations of 0, 500, 1500 or 3500 ppm [1740, 5200 or 12 100 mg/m^3] for 6 h per day on five days per week for two years. The dichloromethane was > 99% pure, with ≤ 706 mg/kg (ppm) *trans*-1,2-dichloroethylene, ≤ 467 mg/kg cyclohexane, ≤ 576 mg/kg chloroform, ≤ 90 mg/kg vinylidene chloride, ≤ 20 mg/kg carbon tetrachloride, ≤ 23 mg/kg methyl bromide, ≤ 11 mg/kg ethyl chloride, ≤ 4.5 mg/kg methyl chloride and ≤ 1 mg/kg vinyl chloride. The numbers of animals still alive at the end of the study were 14, 14, 6 and 7 control, low-, mid- and high-dose males and 21, 24, 13 and 4 females, respectively. Mortality among high-dose females was significantly increased from the 18th month onwards. Non-neoplastic pathological changes in the liver and kidney were more frequently observed in treated animals. There was no significant increase in the incidence of benign or malignant mammary tumours; however, the total number of benign mammary tumours [type not specified] showed a slight dose-related increase in males (control, 8/95; low-dose, 6/95; mid-dose, 11/95; and high-dose, 17/97; $p = 0.046$), and a dose-related increase [$p < 0.001$] in the total number of benign mammary tumours [type not specified] was observed in females (165/96, 218/95, 245/95 and 287/97). The incidence of sarcomas located around the salivary glands was increased in mid- and high-dose males (1/93, 0/94, 5/91 and 11/88; $p = 0.002$ [$p < 0.001$, trend test]) (Burek *et al*., 1984; United States Environmental Protection Agency, 1985). [The Working Group noted the reported occurrence of salivary gland sialodacryoadenitis early in the study.]

Groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks of age, were administered dichloromethane (> 99% pure) by whole-body inhalation at concentrations of 0, 1000, 2000 or 4000 ppm [0, 3470, 6940 or 13 900 mg/m^3] for 6 h per day on five days per week for 102 weeks and were killed after 104 weeks on study. Mean body weights of control and dosed rats of both sexes were comparable throughout the study. Survival of treated males was similar to that of controls. Survival at termination of the study was reduced in high-dose females compared with controls: control, 30/50; low-dose, 22/50; mid-dose, 22/50; and high-dose, 15/50. Increased incidences of benign mammary gland tumours (all fibroadenomas, except for one adenoma in the high-dose group) were observed in treated females (5/50, 11/50, 13/50 and 23/50; $p < 0.001$). There was a positive trend in the incidence of mammary gland adenoma or fibroadenoma

combined in males (0/50, 0/50, 2/50 and 5/50; $p < 0.01$). There was no difference in the distribution of other types of tumour between the control and treated groups (United States National Toxicology Program, 1986).

Groups of 54–70 male and female Sprague-Dawley rats, 13 weeks old, were administered 100 ppm [347 mg/m^3] dichloromethane (purity, > 99.9%) by whole-body inhalation for 7 h per day on five days per week. The exposure was started on breeders, and male and female offspring (12-day embryos). The breeders and a group of offspring were exposed for 104 weeks, another group of offspring was exposed for 15 weeks only. Control groups were composed of 60 female rats (breeder controls) and 158 males and 149 females (untreated controls). Animals 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 (Maltoni *et al*., 1988). [The Working Group noted the low exposure concentration.]

Groups of 90 male and 108 female Sprague-Dawley rats [age unspecified] were administered 0, 50, 200 or 500 ppm [0, 174, 694 or 1740 mg/m^3] dichloromethane (technical-grade; purity, > 99.5%) by whole-body inhalation for 6 h per day on five days per week for 20 (males) or 24 (females) months. A further group of 30 female rats was exposed to 500 ppm dichloromethane for the first 12 months and to room air for the last 12 months of the study (denoted 500/air). An additional group of 30 female rats was exposed to room air for the first 12 months followed by 500 ppm dichloromethane for the last 12 months of the study (denoted air/500). Subgroups of five rats per sex per exposure level were scheduled for interim terminations after 6, 12, 15 and 18 months of exposure to dichloromethane. No exposure-related adverse effect on body weight or mortality was observed. In females, the incidence of benign mammary tumours (adenomas and fibroadenomas combined) was 52/70, 58/70, 61/70 ($p < 0.05$, Fisher's exact test) and 55/70 in control, low-, mid- and high-dose groups, respectively. The multiplicity of benign mammary tumours was 1.8, 2.1, 2.0 and 2.2 ($p < 0.05$) in the control, low-, mid- and high-dose groups, respectively, and 2.3 ($p < 0.05$) and 2.7 ($p < 0.05$) in the air/500 and 500/air groups. No significant increase in the incidence of any other tumour type was seen in the exposed groups (Nitschke *et al*., 1988).

3.2.3 *Hamster*

Groups of 95 male and 95 female Syrian golden hamsters, eight weeks of age, were administered dichloromethane by whole-body inhalation at concentrations of 0, 500, 1500 or 3500 ppm [0, 1740, 5200 or 12 100 mg/m^3] for 6 h per day on five days per week for two years. The dichloromethane was > 99% pure, with ≤ 706 mg/kg *trans*-1,2-dichloroethylene, ≤ 467 mg/kg cyclohexane, ≤ 576 mg/kg chloroform, ≤ 90 mg/kg vinylidene chloride, ≤ 20 mg/kg carbon tetrachloride, ≤ 23 mg/kg methyl bromide, ≤ 11 mg/kg ethyl chloride, ≤ 4.5 mg/kg methyl chloride and ≤ 1 mg/kg vinyl chloride. The numbers of animals 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 lymphosarcomas was slightly higher in exposed females than in controls: control, 1/91; low-dose, 6/92; mid-dose, 3/91; and high-dose, 7/91

($p = 0.032$) (Burek *et al.*, 1984; United States Environmental Protection Agency, 1985). [The Working Group noted that the higher survival in treated animals may have contributed to this non-dose-dependent result for which historical control data were not available.]

3.4 Intraperitoneal administration

Mouse: In a screening assay based on the production of lung adenomas in strain A mice, groups of 20 male mice, six to eight weeks of age, were administered thrice-weekly intraperitoneal injections of 0, 160, 400 or 800 mg/kg bw reagent-grade dichloromethane (purity, > 95%; impurities unspecified) in tricaprylin for a total of 16–17 injections (total doses: 2720, 6800 and 12 800 mg/kg bw in the treated groups, respectively). After 24 weeks, 18, 5 and 12 animals were still alive in the three treated groups, respectively; these and 15/20 surviving vehicle controls were killed and their lungs examined for tumours. No significant increase was found in the multiplicity of lung adenomas: vehicle-control, 0.27; low-dose, 0.94; mid-dose, 0.80; and high-dose, 0.50 (Theiss *et al.*, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The metabolism of dichloromethane has been extensively reviewed (WHO, 1984; United States Environmental Protection Agency, 1985).

4.1.1 *Humans*

Liquid dichloromethane is absorbed through human skin, with maximum concentrations in expired air being reached 30 min after exposure (Stewart & Dodd, 1964). After 0.5–8 h inhalation exposure, concentrations of dichloromethane in the blood and expired air were directly proportional to dose, over the concentration range 173–1740 mg/m^3 (DiVincenzo *et al.*, 1972). It is distributed principally to adipose tissue. In male volunteers exposed to 2600 mg/m^3 for 1 h at a work intensity of 50 W, mean adipose tissue concentrations after 1, 4 and 22 h were 10.2, 8.4 and 1.7 mg/kg, respectively (Engström & Bjurström, 1977). Elevated carboxyhaemoglobin saturation and increased urinary formic acid concentrations have been found in exposed workers (Ku elová & Vlasák, 1966; DiVincenzo & Kaplan, 1981), but inhaled dichloromethane is excreted principally unchanged in expired air (Riley *et al.*, 1966).

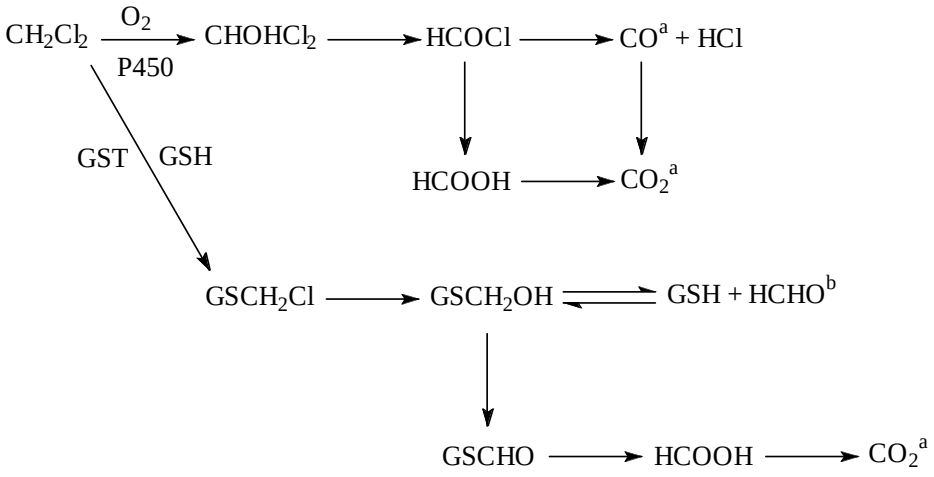
Dichloromethane can be conjugated with glutathione by human θ-class glutathione *S*-transferase (GST) T1-1, which is expressed in many human organs. However, the tissue-specific expression pattern differs from that of the α-, μ- and π-forms of GST, the θ-class being expressed only at very low levels in a small number of Clara cells and ciliated cells at the alveolar/bronchiolar junctions in human lung (Mainwaring *et al.*, 1996a; Sherratt *et al.*, 1997).

4.1.2 *Experimental systems*

Dichloromethane is rapidly absorbed through the lungs and distributed throughout the body reaching all organs, including the brain. Dichloromethane has a particular affinity for fat; concentrations in adipose tissues may be seven- to eight-fold higher than in other tissues (Savolainen *et al*., 1977). After inhalation or oral exposure of rats, the majority of the dose is exhaled unchanged (McKenna & Zempel, 1981; McKenna *et al*., 1982). Dichloromethane metabolites are also largely excreted via the lungs as carbon monoxide and carbon dioxide (DiVincenzo & Hamilton, 1975).

Two pathways for the metabolism of dichloromethane are known, one catalysed by a cytochrome P450 and the other by a GST (Kubic & Anders, 1975; Ahmed & Anders, 1976, 1978; Kubic & Anders, 1978; Gargas *et al.*, 1986). The P450-mediated oxidative pathway produces carbon monoxide and inorganic chloride, presumably via the highly unstable intermediate, formyl chloride. The glutathione pathway produces carbon dioxide after the formation of a postulated glutathione conjugate and formaldehyde (Figure 1).

Figure 1. Metabolic pathways for dichloromethane



[a] Metabolites identified *in vivo*
[b] Metabolite identified *in vitro*
All other metabolites are postulated intermediates
GSH, glutathione; GST, glutathione *S*-transferase

More recently the specific isoenzymes involved in the metabolism of dichloromethane have been identified as the cytochrome CYP2E1 (Kim & Kim, 1996) and the θ-class GST, GSTT1-1 (Meyer *et al*., 1991; Mainwaring *et al*., 1996b).

Early studies suggested that the relative activities of each pathway could be determined by measuring exhaled carbon monoxide (or carboxyhaemoglobin in blood) and carbon dioxide, resulting from the P450 and glutathione pathways respectively. How-

ever, it is now known that this is an unreliable method, since significant quantities of carbon dioxide are also derived from the oxidative pathway (Gargas *et al*., 1986).

The metabolism of dichloromethane is a saturable process. For example, 48 h after inhalation exposure of rats to 50 ppm, 500 ppm or 1500 ppm [74, 1740 or 5200 mg/m^3] dichloromethane, 5, 30 and 55% respectively of the chemical was expired unchanged (McKenna *et al*., 1982). Detailed investigations, in both rats and mice, have indicated that the cytochrome P450-mediated metabolism is a saturable high-affinity/low-capacity pathway. Saturation occurs at relatively low dose levels (< 500 ppm) in both rats and mice and results in similar levels of carboxyhaemoglobin in the blood (12–15%). Conversely, the GST-mediated pathway is low-affinity/high-capacity and exhibits dose-dependent linear kinetics (Gargas *et al*., 1986; ECETOC, 1987). This pathway is particularly active in mice; indeed it is the major pathway at the dose levels (2000 and 4000 ppm [6940 and 13 900 mg/m^3]) used in the carcinogenicity bioassays. *In vitro*, the relative rates for this pathway (as indicated by the yield of RNA–formaldehyde adducts) are 1, 2, 4 and 14 in Syrian hamster hepatocytes, in human hepatocytes with functional *GSTT1* genes and in rat and mouse hepatocytes, respectively (Casanova *et al*., 1997). Marked human inter-individual differences have been described for dichloromethane metabolism via the GST pathway. In 39 human samples, this activity varied between 0 and 3 nmol/min/mg cytosolic protein (Green, 1989; Reitz *et al*., 1989; Bogaards *et al*., 1993; Graves *et al*., 1995). Indeed a genetic polymorphism has been described for a θ-class GST found in erythrocytes (Bogaards *et al*., 1993; Hallier *et al*., 1994; Schroder *et al*., 1996). Some 10–40% of human samples studied appear to be deficient in this transferase activity.

The distribution of GSTT1-1 has been examined in mouse, rat and human liver and lung (Pemble *et al*., 1994; Mainwaring *et al*., 1996a; Sherratt *et al*., 1997; Mainwaring *et al*., 1998). GSTT1-1 mRNA and protein were visualized by in-situ hybridization and immunocytochemistry, respectively. High levels of both GSTT1-1 protein and mRNA were observed in mouse hepatocytes and mouse Clara cells, while much less was seen in rat or human liver and lung (Mainwaring *et al*., 1996a, 1998). Additionally, GSTT1-1 mRNA and protein were concentrated preferentially in certain cell types (mouse lung Clara cells, limiting plate hepatocytes) and, particularly in the nuclei of these cells in mice, whereas in rat and man, the distribution was more generalized.

A number of physiological toxicokinetic models have been developed to describe the metabolism of dichloromethane in mouse, rat, Syrian hamster and man following exposure either by inhalation or in drinking-water (Andersen *et al*., 1987; ECETOC, 1988; Reitz *et al*., 1989; Andersen *et al*., 1991).

4.2 Toxic effects

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

4.2.1 *Humans*

The odour threshold of dichloromethane is about 200 ppm [694 mg/m^3] (Stahl, 1973). One of the products of dichloromethane metabolism is carbon monoxide, and the acute effects of dichloromethane poisoning are mainly due to carbon monoxide, which binds to haemoglobin and thus decreases the oxygen-transporting capacity of blood, and simultaneously increases the affinity of haemoglobin toward oxygen, thereby decreasing the liberation of oxygen to tissues (Shusterman *et al*., 1990; Dhillon & Von Burg, 1995).

Fatalities have been associated with acute or prolonged exposure to dichloromethane (Moskowitz & Shapiro, 1952; Ku elová *et al*., 1975; Stewart & Hake, 1976; Bonventre *et al*., 1977; Bakinson & Jones, 1985; Manno *et al*., 1989). Temporary neurobehavioural effects have been reported after exposure to doses as low as 200 ppm [694 mg/m^3] by some (Winnekke, 1974; Putz *et al*., 1976) but not by others (Gamberale *et al*., 1975).

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*., 1983a). A cross-sectional study of 24 employees at a fibre production plant showed no excess of electrocardiographic abnormalities among those exposed to 60–475 ppm [208–1650 mg/m^3] (time-weighted average) dichloromethane and monitored for 24 h (Ott *et al*., 1983b).

The following mortality studies are described in more detail in Section 2.1. No significant increase in overall mortality or deaths due to ischaemic heart disease was found among 1271 male and female employees exposed to 140–475 ppm [486–1650 mg/m^3] dichloromethane compared to the mortality of the general United States population (Ott *et al*., 1983c). An increased risk of ischaemic heart disease was found in comparison with an internal reference group. In another study in the United States of America on cellulose triacetate fibre workers (Gibbs *et al*., 1996), mortality from cardiovascular disease as a whole was not elevated, and in addition showed an inverse relationship with duration of exposure. In a further study on cellulose triacetate fibre workers in the United Kingdom (Tomenson *et al*., 1997), mortality from ischaemic heart disease was similarly less than expected from national rates. However, it was higher among the exposed than in the non-exposed cohort, and showed an association with the estimated lifetime exposure to dichloromethane. There was a statistically significant deficit in the mortality from non-malignant lung disease and cerebrovascular disease. In a third cohort study on dichloromethane-exposed workers (Hearne *et al*., 1990), which had a 90% probability of detecting an 1.3-fold risk of ischaemic heart disease, no elevated risk was observed. The risk of non-malignant pulmonary disease was not elevated either, and there was a (non-significant) deficit of cerebrovascular disease.

4.2.2 *Experimental systems*

Intraperitoneal LD_{50} values for dichloromethane are approximately 1.5 mL/kg bw (2000 mg/kg bw) in mice (Klaassen & Plaa, 1966) and 0.95 mL/kg bw (1300 mg/kg bw) in dogs (Klaassen & Plaa, 1967); the oral LD_{50} in rats ranges from 1.6 to 2.3 mL/kg bw (2100–3000 mg/kg bw) (Kimura *et al*., 1971); and the subcutaneous LD_{50} in mice is

approximately 76 mmol/kg bw (6400 mg/kg bw) (Kutob & Plaa, 1962). The LC_{50} values in mice, rats and guinea-pigs are 16 000 ppm [55 500 mg/m^3] (7-h exposure plus 1-h observation), 5.7% [200 000 mg/m^3] (15-min exposure) and 11 600 ppm [40 300 mg/m^3] (6-h exposure plus 18-h observation), respectively (Sviberly *et al*., 1947; Balmer *et al*., 1976; Clark & Tinston, 1982).

The acute toxicity of dichloromethane is expressed mainly as disturbances of the central nervous system, involving sleep disturbance and reductions in spontaneous activity (Heppel & Neal, 1944; Schumacher & Grandjean, 1960; Fodor & Winneke, 1971; United States National Institute for Occupational Safety and Health, 1976).

Hepatotoxic effects are seen after exposure to near-lethal concentrations of dichloromethane (Gehring, 1968). Inhalation exposure of guinea-pigs to 5200 ppm [18 000 mg/m^3] dichloromethane for 6 h increased hepatic triglyceride concentrations (Morris *et al*., 1979). Exposure of guinea-pigs to approximately 11 000 ppm [38 200 mg/m^3] dichloromethane for 6 h also increased hepatic triglyceride concentrations, but concomitant exposure to 21 400–24 100 ppm [40 200–45 300 mg/m^3] ethanol blocked this effect (Balmer *et al*., 1976). Continuous exposure of mice by inhalation to 5000 ppm [17 400 mg/m^3] dichloromethane 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 following administration of dichloromethane by gavage (133–665 mg/kg bw) to mice (Condie *et al*., 1983). In Sprague-Dawley rats, two doses of 1250 mg/kg dichloromethane by gavage 21 and 4 h before killing the animals did not affect serum alanine aminotransferase, or hepatic glutathione or cytochrome P450 content, but increased the hepatic ornithine decarboxylase activity in 3/15 animals (Kitchin & Brown, 1989). In a long-term bioassay of dichloromethane, increased incidences of haemosiderosis, cytomegaly, cytoplasmic vacuolation, necrosis, granulomatous inflammation and bile-duct fibrosis were observed in the livers of treated male and female Fischer 344/N rats (United States National Toxicology Program, 1986). Increased liver weight associated with glycogen accumulation in the hepatocytes, but no hepatotoxicity, was observed in another carcinogenicity study in mice, in which an elevated frequency of hepatic tumours was observed (Kari *et al*., 1993). 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 of the altered foci from non-treated animals (Foley *et al*., 1993). Administration of dichloromethane to B6C3F$_1$ mice by gavage (1000 mg/kg, once) or inhalation (4000 ppm [13 900 mg/m^3] dichloromethane for 2 h) did not induce DNA synthesis, as measured by the number of cells in S-phase ([^{3}H]thymidine incorporation) (Lefevre & Ashby, 1989) and, when female B6C3F$_1$ mice were exposed to 1000, 2000, 4000 or 8000 ppm [3470, 6940, 13 900 or 27 800 mg/m^3] dichloromethane for 6 h per day on five days per week for up to four 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 4000- and 8000-ppm groups at two weeks and in the 1000-ppm group at one week. The labelling index of bronchiolar epithelium (in two branches proximal to the terminal

bronchiole and in the terminal bronchioles themselves) of female B6C3F$_1$ mice exposed to 2000 ppm dichloromethane for 2–26 weeks decreased to 40–60% of the value found in unexposed control mice. Exposure to 8000 ppm dichloromethane led to a smaller decrease in labelling index. No pathological change was found in the exposed lungs (Kanno *et al.*, 1993).

Inhalation exposure of male B6C3F$_1$ mice to dichloromethane (6 h, once) led to vacuolation of bronchiolar cells 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 cytochrome P450 inhibitor piperonyl butoxide (300 mg/kg intraperitoneally) 1 h before the exposure practically abolished the toxic effect upon bronchiolar cells, while buthionine sulfoximine (1 g/kg intraperitoneally), which decreased the pulmonary glutathione content by 50%, had no such protective effect. In Clara cells isolated after exposure to dichloromethane exposure (≥ 1000 ppm), the proportion of cells in the S-phase was increased.

Following intraperitoneal administration of dichloromethane at near-lethal doses, hydropic degeneration was observed in the kidneys of mice (Klaassen & Plaa, 1966), while no kidney damage was observed following administration of dichloromethane by gavage at dose levels of 133–665 mg/kg bw (Condie *et al.*, 1983). Slight calcification of the renal tubules in dogs was seen after intraperitoneal administration of dichloromethane at near-lethal doses (Klaassen & Plaa, 1967). In rats, intraperitoneal administration of 1330 mg/kg bw dichloromethane produced renal proximal tubular swelling (Kluwe *et al.*, 1982). After a similar dose by gavage, a transient elevation of blood urea nitrogen 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 gerbils exposed continuously by inhalation to 350 ppm [1210 mg/m^3], but not in those exposed to 210 ppm [730 mg/m^3], dichloromethane for up to three months, increased brain concentrations of two astroglial proteins (S-100 and GFA) and decreased cerebellar DNA concentrations were observed. Decreased hippocampal DNA concentrations were observed at both exposure levels (Rosengren *et al.*, 1986).

Dichloromethane (≥ 6.3 mmol/kg bw) administered to rats by gavage induced increased urinary excretion of catecholamines in the urine in rats; cytomorphological changes and a decrease in chromaffin reaction were observed in the adrenal medulla at a dose level of 15.6 mmol/kg bw (1330 mg/kg) (Marzotko & Pankow, 1987).

In a two-year carcinogenicity test of inhaled dichloromethane, an increased incidence of testicular atrophy was observed in B6C3F$_1$ mice exposed to 4000 ppm [13 900 mg/m^3] for 6 h per day on five days a week (United States National Toxicology Program, 1986).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A case–control study on 44 women who had had a spontaneous abortion was performed within a cohort of female workers employed in Finnish pharmaceutical

factories during 1973 or 1975–80. Three controls matched for age at conception within 2.5 years were chosen for each case (except two). Information about pregnancy outcome was collected from hospital data, and data on exposures from health personnel at the factories. The odds ratio for dichloromethane exposure, based on 11 exposed cases, was 2.3 (95% CI, 1.0–5.7); the odds ratio was also increased for exposure to many other solvents. The odds ratio for those exposed once a week or more during the first trimester of pregnancy was 2.8, and that for those exposed less often was 2.0 (Taskinen *et al*., 1986).

4.3.2 *Experimental systems*

In a teratology study, groups of Swiss Webster mice and Sprague-Dawley rats were exposed by inhalation to 0 or 1225 ppm [0 or 4250 mg/m^3] dichloromethane (purity, 97.9%) for 7 h per day on gestation days 6–15. Exposure of female mice resulted in a significant increase in body weight during and after exposure, while absolute but not relative liver weights were increased in both species. There was no significant increase in visceral anomalies in the fetuses of either species, but skeletal anomalies included decreased incidence of lumbar spurs and delayed ossification of the sternebrae in rats and increased incidence of a single extra sternal ossification centre in mice (Schwetz *et al*., 1975).

Female Long-Evans rats were exposed to 0 or 4500 ppm [0 or 15 600 mg/m^3] dichloromethane (> 97% pure) during either a three-week pregestational period or during the first 17 days of gestation or both. Ten females per group were allowed to give birth, and the offspring were examined for abnormal growth and behaviour. Dams exposed to dichloromethane during gestation had increased absolute and relative liver weights. There was no effect on litter size or viability, but fetal weight was reduced in both groups exposed during gestation. No treatment-related visceral or skeletal abnormality was detected in the fetuses of any exposure group, but a greater proportion of litters exposed during both the pregestational and gestational periods had fetuses with rudimentary lumbar ribs (Hardin & Manson, 1980). No difference in pup birth weight, viability or growth rate was observed, but alterations in spontaneous locomotor activities were seen in all exposure groups. No change was observed in running-wheel activity or acquisition of an avoidance response (Bornschein *et al*., 1980).

In a two-generation reproduction study (Nitschke *et al*., 1988), male and female Fischer 344 rats were exposed to 0, 100, 500 or 1500 ppm [0, 347, 1740 or 5200 mg/m^3] dichloromethane for 6 h per day on five days per week for 14 weeks and then mated to produce F_1 litters. After weaning, 30 randomly selected pups of each sex and dosage were exposed to dichloromethane for 17 weeks and subsequently mated to produce F_2 litters. Reproductive parameters examined included fertility, litter size, neonatal growth and survival. All adults and selected (10 per group) weanlings were examined for grossly visible lesions. Tissues from the selected weanlings were examined histopathologically. No adverse effects were observed on reproductive parameters, neonatal survival or neonatal growth; there were no gross or histopathological lesions.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 8 for references)

Gene mutations were induced in *Salmonella typhimurium* strains TA100, TA1535 and TA98 exposed to dichloromethane vapour in a closed chamber with or without the addition of exogenous metabolic activation. Glutathione-deficient strains of TA100 (NG 11 and NG 54) were less responsive to the effects of dichloromethane than were the parent strains. Studies using the liquid plate incorporation assay were negative, with the exception of one study which reported positive results in strain TA1535 transfected with rat θ-class GST 5-5+. Dichloromethane also induced mutation in *Escherichia coli* and gene conversion and mutation in *Saccharomyces cerevisiae*. In *Drosophila melanogaster* it did not induce sex-linked recessive lethal mutations.

Dichloromethane induced DNA–protein cross-links *in vitro* in hepatocytes of male $B6C3F_1$ mice but not in hepatocytes of Fischer 344 rats, Syrian hamsters or in human hepatocytes with functional *GSTT1* genes. DNA–protein cross-links were also induced in Chinese hamster ovary CHO cells exposed to dichloromethane with or without exogenous metabolic activation. DNA damage was greater, however, in the presence of metabolic activation.

Dichloromethane induced DNA single-strand breaks in AP rat primary hepatocytes and $B6C3F_1$ mouse hepatocytes and Clara cells, but not in Syrian hamster hepatocytes *in vitro*. DNA damage was reduced in Clara cells co-treated with buthionine sulfoximine, a glutathione-depleting agent. In one study, DNA single-strand breaks were increased in CHO cells cultured with dichloromethane in the presence, but not in the absence, of an exogenous metabolic activation system.

When tested in Chinese hamster lung V79 cells in the absence of exogenous metabolic activation, dichloromethane did not induce unscheduled DNA synthesis or *hprt* locus gene mutations but did induce a slight increase in sister chromatid exchange frequencies. It was mutagenic in CHO cells at the *hprt* locus in one study, in the presence of exogenous metabolic activation, and gave equivocal results in the mouse lymphoma $tk^{+/-}$ assay in another study. DNA sequence analysis of the *hprt* mutants of CHO cells treated with dichloromethane indicated that most were GC→AT transitions (4/8), with two GC→CG transversions and two AT→TA transversions. This pattern was more similar to that of 1,2-dibromoethane (ethylene dibromide) (see this volume) (7/9 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 5/6 arose from AT base pairs (Graves *et al.*, 1996). Dichloromethane induced chromosomal aberrations in CHO cells in the presence and absence of an exogenous metabolic system in one of two studies, but did not increase sister chromatid exchange frequencies. Virus-infected Fischer rat and Syrian hamster embryo cells were transformed after treatment with dichloromethane *in vitro*. Neither DNA single-strand

Table 8. Genetic and related effects of dichloromethane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAF, *Salmonella typhimurium* BA/3, forward mutation, Ara resistance	+	(+)	325	Roldan-Arjona & Pueyo (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	14	Simmon *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	19	Jongen *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	18	Gocke *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	23	Jongen *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	95	Green (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	6800	Osterman-Golkar *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)[c]	NT	3700	Hughes *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+[c]	+	150	Zeiger (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	8.5	Dillon *et al*. (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	17667	Graves *et al*. (1994)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	34	JETOC (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	300	McGregor (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–[d]	NT	170	Thier *et al*. (1993)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	170	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	340	JETOC (1997)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	19	Jongen *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	72	Gocke *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	?[c]	?	1500	Zeiger (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	34	JETOC (1997)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
ECF, *Escherichia coli* NR3835, forward mutation	+	NT	26500	Zielenska *et al*. (1993)
ECK, *Escherichia coli* K12, forward mutation, Rif resistance	–	(+)[e]	5100	Graves *et al*. (1994a)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	170	JETOC (1997)
ECR, *Escherichia coli* WP2 *uvrA*/pKM101, reverse mutation	+	+	21	Dillon *et al*. (1992)
ECR, *Escherichia coli* WP2 *uvrA*/pKM101, reverse mutation	+	+	170	JETOC (1997)
SCG, *Saccharomyces cerevisiae*, gene conversion	+	NT	13300	Callen *et al*. (1980)
SCH, *Saccharomyces cerevisiae*, homozygosis	+	NT	13300	Callen *et al*. (1980)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	NT	13300	Callen *et al*. (1980)
TSM, *Tradescantia* species, gene mutation	+	NT	100	Schairer & Sautkulis (1982)
DMX, *Drosophila melanogaster*, sex-linked mutation	–		52600	Gocke *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked mutation	–		19.2	Kramers *et al*. (1991)
DIA, DNA–protein cross-links, B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	43	Casanova *et al*. (1997)
DIA, DNA–protein cross-links, Fischer 344 rat hepatocytes *in vitro*	–	NT	425	Casanova *et al*. (1997)
DIA, DNA–protein cross-links, Syrian hamster hepatocytes *in vitro*	–	NT	425	Casanova *et al*. (1997)
DIA, DNA–protein cross-links, human hepatocytes (expressing *GSTT1-1) in vitro*	–	NT	425	Casanova *et al*. (1997)
DIA, DNA single-strand breaks, B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	34	Graves *et al*. (1994b)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DIA, DNA single-strand breaks, AP rat hepatocytes *in vitro*	+	NT	2550	Graves *et al*. (1994b)
DIA, DNA single-strand breaks, Chinese hamster ovary cells *in vitro*	–	+	5100	Graves *et al*. (1994b)
DIA, DNA single-strand breaks, Syrian hamster hepatocytes *in vitro*	–	NT	5100	Graves *et al*. (1995)
DIA, DNA single-strand breaks, $B6C3F_1$ mouse lung Clara cells *in vitro*	+[f]	NT	425	Graves *et al*. (1995)
DIA, DNA single-strand breaks and DNA-protein cross-links, Chinese hamster ovary CHO cells *in vitro*	(+)	+	3975	Graves & Green (1996)
UIA, Unscheduled DNA synthesis, Chinese hamster lung V79 cells *in vitro*	–	NT	65000	Jongen *et al*. (1981)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	–	NT	65000	Jongen *et al*. (1981)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	–	+	3975	Graves & Green (1996)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	52000	Jongen *et al*. (1981)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	?	?	3300	Myhr *et al*. (1990)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	(+)	NT	13000	Jongen *et al*. (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	13000	Thilagar & Kumaroo (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Anderson *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	6500	Thilagar & Kumaroo (1983)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Anderson *et al.* (1990)
TRR, Cell transformation, RLV/Fischer rat	+	NT	14	Price *et al.* (1978)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	NT	73	Hatch *et al.* (1982)
DIH, Single-strand breaks, human primary hepatocytes *in vitro*	–	NT	5100	Graves *et al.* (1995)
UHF, Unscheduled DNA synthesis, human AH fibroblasts *in vitro*	–	NT	65000	Jongen *et al.* (1981)
SHL, Sister chromatid exchanges, human lymphocytes *in vitro*	+[g]	NT	290	Hallier *et al.* (1993)
MIH, Micronucleus test, human MCL-5 and h2E1 lymphoblastoid cells *in vitro*	+[h]	NT	200	Doherty *et al.* (1996)
MIH, Micronucleus test, human AHH-1 lymphoblastoid cells *in vitro*	–	NT	850	Doherty *et al.* (1996)
DVA, DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver *in vivo*	+[i]		4000 ppm inh 6 h/d, 3 d	Casanova *et al.* (1992)
DVA, DNA–protein cross-links, Syrian hamster liver and lung *in vivo*	–		4000 ppm inh 6 h/d 3 d	Casanova *et al.* (1992)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		4831 ppm inh 6 h	Graves *et al.* (1994b)
DVA, DNA single-strand breaks, AP rat liver *in vivo*	–		4727 ppm inh 6h	Graves *et al.* (1994b)
DVA, DNA single-strand breaks, CD rat liver *in vivo*	+		1275 po × 1	Kitchin & Brown (1994)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+[f]		4000 ppm inh 6 h	Graves *et al.* (1995)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse lung *in vivo*	+[f]		2000 ppm inh 3 h	Graves *et al.* (1995)
DVA, DNA single-strand breaks, AP rat lung *in vivo*	–		4000 ppm inh 3 h	Graves *et al.* (1995)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DVA, DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver *in vivo*	+		498 ppm inh 6 h/d, 2 d	Casanova *et al*. (1996)
DVA, DNA–protein cross-links, Syrian golden hamster liver *in vivo*	–		3923 ppm inh 6 h/d, 2 d	Casanova *et al.* (1996)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		1000 po × 1	Trueman & Ashby (1987)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		4000 ppm inh 6 h	Trueman & Ashby (1987)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse liver *in vivo*	–		4000 ppm inh 6 h	Trueman & Ashby (1987)
SVA, Sister chromatid exchange, B6C3F$_1$ mouse lung cells *in vivo*	+[j]		2000 ppm inh 6 h/d, 5 d/wk 12wk	Allen *et al*. (1990)
SVA, Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	–		5000 sc × 1	Allen *et al*. (1990)
SVA, Sister chromatid exchange, C57BL/6J mouse bone marrow *in vivo*	–		1500 ip × 1	Westbrook-Collins *et al*. (1990)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	–		1700 ip × 2	Gocke *et al*. (1981)
MVM, Micronucleus test, C57BL/6J/Alpk mouse bone marrow *in vivo*	–		4000 po × 1	Sheldon *et al*. (1987)
MVM, Micronucleus test, B6C3F$_1$ mouse erythrocytes *in vivo*	(+)[j]		2000 ppm inh 6h/d, 5 d/wk 12 wk	Allen *et al*. (1990)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	–		1720 ip × 1	Morita *et al*. (1997)
CBA, Chromosomal aberrations, Sprague-Dawley rat bone marrow *in vivo*	–		3500 ppm inh 6 h/d, 5 d/wk, 2 y	Burek *et al*. (1984)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CBA, Chromosomal aberrations, B6C3F$_1$ mouse bone marrow *in vivo*	(+)		8000 ppm inh 6 h/d, 5 d/wk, 2 wk	Allen *et al*. (1990)
CBA, Chromosomal aberrations, B6C3F$_1$ mouse bone marrow *in vivo*	–		5000 sc × 1	Allen *et al*. (1990)
CBA, Chromosomal aberrations, C57BL/6J mouse bone marrow *in vivo*	–		1500 ip × 1	Westbrook-Collins *et al*. (1990)
CVA, Chromosomal aberrations, B6C3F$_1$ mouse lung cells *in vivo*	(+)		8000 ppm inh 6 h/d, 5 d/wk, 2 wk	Allen *et al*. (1990)
BVD, DNA binding, rat or mouse liver, lung, or kidney *in vivo*	–		NG	Ottenwalder & Peter (1989)

[a] +, positive; (+), weakly positive; -, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg/mL in atmosphere); in-vivo tests, mg/kg bw /day; inh, inhalation; po, oral; sc, subcutaneous; ip, intraperitoneal; NG, not given

[c] Negative in liquid plate incorporation assay

[d] Liquid plate incorporation assay; cells transfected with rat GST 5-5+ were positive at 42 µg/mL

[e] Positive with mouse liver S9, negative with rat liver S9

[f] Pre- or co-treatment with buthionine sulfoximine, a GSH depletor, caused a decrease in DNA damage

[g] Positive results were reported in lymphocytes from donors lacking GST activity

[h] Induction of kinetochore-positive and -negative micronuclei

[i] Negative in mouse lung

[j] The highest dose tested (8000 ppm 6 h/d, 5 d/w × 2 wk) was positive in erythrocytes and lung cells but negative in bone marrow

[k] Negative in lung cells at this dose; positive in erythrocytes after exposure to 8000 ppm 6 h/d [10 000 mg/kg bw], 5d/wk × 2 wk

breaks nor unscheduled DNA synthesis were induced in human primary hepatocytes or AH fibroblasts, respectively, following dichloromethane treatment. Sister chromatid exchanges were induced in human peripheral blood lymphocyte cultures but only in those from donors lacking GST activity towards methyl bromide. In a single study, dichloromethane induced 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. AHH-1 cells constitutively expressing CYP1A1 showed neither an increase in total micronucleus frequencies nor kinetochore-staining micronuclei.

DNA–protein cross-links were induced in the liver but not the lung of $B6C3F_1$/CrlBR mice exposed to dichloromethane. No DNA–protein cross-links were detected in Syrian hamster liver or lung after inhalation. Inhalation exposure of $B6C3F_1$ mice to dichloromethane induced DNA single-strand breaks in both lung and liver. Prior treatment of the mice with buthionine sulfoximine immediately before dichloromethane exposure reduced the amount of DNA damage to control levels. DNA single-strand breaks were not induced in liver or lung of AP rats but were seen in liver of CD rats treated by gavage. Dichloromethane did not induce unscheduled DNA synthesis in Fischer 344 rat or $B6C3F_1$ mouse hepatocytes *in vivo*. In a single study, mice treated with 2000 ppm [6940 mg/m^3] dichloromethane for 6 h per day on five days per week for 12 weeks showed an increased sister chromatid exchange frequency in lung cells and an increased frequency of micronuclei in peripheral blood erythrocytes. Exposure to higher concentrations (8000 ppm [27 800 mg/m^3] for two weeks) also induced an increase in sister chromatid exchange frequency in peripheral blood erythrocytes. Dichloromethane did not induce sister chromatid exchanges, micronuclei or chromosomal aberrations in bone marrow of mice treated by gavage or intraperitoneal or subcutaneous injection. A small increase in chromosomal aberrations in mouse bone marrow and lung cells was reported in one study following inhalation exposure to 8000 ppm dichloromethane for 6 h per day on five days per week for two weeks. Dichloromethane gave negative results in the rat bone-marrow chromosomal aberration assay. Covalent binding of dichloromethane to DNA was not observed in 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.

4.5 Mechanistic considerations

The currently available data lead to the suggestion that it is metabolism via the glutathione pathway, and not the cytochrome P450 pathway, that is related to the liver and lung carcinogenicity of dichloromethane in mice. This hypothesis is supported by the observations that the major species differences correspond to those of GST distribution and activity, and that the dose-dependent behaviour of the two pathways is consistent with the results of the carcinogenicity bioassays. For example, at the two high dose levels (2000 and 4000 ppm [6940 and 13 900 mg/m^3]) used in the National Toxicology Program

(NTP) mouse bioassay, the GST pathway would predominate and liver and lung tumour incidence was increased. Conversely, in the mouse drinking-water study where, presumably, lower blood levels of dichloromethane would be reached than in the inhalation bioassay, the dichloromethane would have been metabolized primarily via cytochrome P450, while GST-mediated metabolism would have been minimal (predicted by pharmacokinetic modelling to be two orders of magnitude lower than at the high dose used in the NTP study; Andersen *et al*., 1987), and no increased incidence of tumours was observed.

A variety of in-vivo (at dose levels used in the NTP studies) and in-vitro experiments using mouse hepatocytes and Clara cells have revealed DNA damage when the animals or cells were exposed to dichloromethane (Graves *et al*., 1994b, 1995). Cells depleted of glutathione, either *in vitro* or *in vivo*, had decreased DNA damage, thus strengthening the link with the GST pathway. DNA damage was not observed in hamster or human hepatocytes exposed to dichloromethane. This is consistent with the observed species differences in the degree of expression and the pattern of distribution of GSTT1-1, the enzyme responsible for the metabolic activation of dichloromethane.

A link has also been established between metabolism of dichloromethane by GST and mutagenicity in bacteria and Chinese hamster ovary (CHO) cells. Depletion of glutathione (*Salmonella typhimurium* and CHO cells) or expression of a rat GST (*S. typhimurium*) decreased or increased their mutagenicity respectively (Thier *et al*., 1993; Graves *et al*., 1994b). When liver subcellular fractions were used in these assays, only cytosol (GST), and not microsomes, supported the bioactivation of dichloromethane. Generally, throughout the mutagenicity assays, a good correlation is evident between glutathione and/or GST activity and genotoxicity (Table 9). Studies with CHO cells, measuring strand breaks and mutations at the *hprt* gene, suggest that the DNA damage was caused by *S*-chloromethylglutathione (Graves & Green, 1996; Graves *et al*., 1996).

DNA–protein cross-links caused by formaldehyde, a metabolite from the GST pathway, have been demonstrated in mice but not hamsters exposed to dichloromethane (Casanova *et al*., 1992). Similarly, in-vitro studies have not demonstrated DNA–protein cross-links in rat, hamster or human hepatocytes exposed to concentrations of dichloromethane of up to 5 mM. This is equivalent to the time-weighted average concentration predicted to occur in mouse liver during a 6-h inhalation exposure to a dichloromethane concentration of > 10 000 ppm [34 700 mg/m^3] (Casanova *et al*., 1997).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Dichloromethane is used principally as a solvent, in paint removers, degreasers and aerosol products, and in the manufacture of foam polymers. Widespread exposure occurs during the production and industrial use of dichloromethane and during the use of a variety of consumer products containing dichloromethane. Substantial losses to the environment lead to ubiquitous low-level exposures from ambient air and water.

Table 9. Relationship of glutathione *S*-transferase (GST) status and dichloromethane-mediated DNA damage

System	GST-mediated metabolism of dichloro-methane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Salmonella typhimurium BA13	ND	+	?		Roldan-Arjona & Pueyo (1993)
Salmonella typhimurium TA100	+	+	Yes	TA100 metabolizes dichloromethane	Simmon *et al.* (1977)
Salmonella typhimurium TA100	+	+	Yes		Jongen *et al*. (1978)
Salmonella typhimurium TA100	+	+	Yes		Gocke *et al*. (1981)
Salmonella typhimurium TA100	+	+	Yes		Jongen *et al*. (1982)
Salmonella typhimurium TA100	+	+	Yes		Green (1983)
Salmonella typhimurium TA100	+	(+)	(Yes)		Osterman-Golkar *et al*. (1983)
Salmonella typhimurium TA100	+	(+)	(Yes)		Hughes *et al*. (1987)
Salmonella typhimurium TA100	+	+	Yes		Zeiger (1990)
Salmonella typhimurium TA100	+	+	Yes		Dillon *et al.* (1992)
Salmonella typhimurium TA100	+	+	Yes		Graves *et al.* (1994a)
Salmonella typhimurium TA100	+	+	Yes		JETOC (1997)
Salmonella typhimurium TA1535	ND	+	?		McGregor (1979)
Salmonella typhimurium TA1535	ND	–	?		Thier *et al*. (1993)
Salmonella typhimurium TA1535+ transfected GST5-5	+	+	Yes	Transfected GST5-5 increased response	Thier *et al*. (1993)
Salmonella typhimurium TA1535	ND	+	?		JETOC (1997)
Salmonella typhimurium TA1537	ND	–	?		JETOC (1997)
Salmonella typhimurium TA98	ND	+	?		Jongen *et al*. (1978)
Salmonella typhimurium TA98	ND	+	?		Gocke *et al*. (1981)

Table 9 (contd)

System	GST-mediated metabolism of dichloro-methane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Salmonella typhimurium TA98	ND	?	?		Zeiger (1990)
Salmonella typhimurium TA98	ND	+	?		JETOC (1997)
Escherichia coli NR3835	ND	+	?		Zielenska *et al*. (1994)
Escherichia coli K12	ND	+	?		Graves *et al*. (1994a)
Escherichia coli WP2/pKM101	ND	+	?		Dillon *et al*. (1992)
Escherichia coli WP2	ND	+	?		JETOC (1997)
Escherichia coli WP2/pKM101	ND	+	?		JETOC (1997)
Saccharomyces cerevisiae D7	ND	+	?		Callen *et al*. (1980)
Tradescantia	ND	+	?		Schairer & Sauttkulis (1982)
Drosophila melanogaster	ND	–	?		Gocke *et al*. (1981)
Drosophila melanogaster	ND	–	?		Kramers *et al*. (1991)
Single strand breaks, B6C3F$_1$ mouse hepatocytes *in vitro*	+	+	Yes	Deplete GSH and DNA damage decreases	Graves *et al*. (1994b)
Single strand breaks, AP rat hepatocytes *in vitro*	–	+	No	Very high dose (> 30 mM)	Graves *et al*. (1994b)
Single strand breaks, Chinese hamster ovary CHO cells				Not tested	Graves *et al*. (1994b)
Single strand breaks, B6C3F$_1$ Clara cells *in vitro*	+	+	Yes	Buthionine sulfo-ximine decreased DNA damage	Graves *et al*. (1995)
Single strand breaks and DNA-protein cross-links, CHO cells	+	(+)	(Yes)		Graves & Green (1996)

Table 9 (contd)

System	GST-mediated metabolism of dichloromethane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Unscheduled DNA synthesis, Chinese hamster V79 cells *in vitro*	ND	–	?		Jongen *et al*. (1981)
Gene mutation, CHO cells *hprt* locus	–	–	Yes		Jongen *et al*. (1981)
Gene mutation, CHO cells *hprt* locus	–	–	Yes		Graves & Green (1996)
Gene mutation, Chinese hamster V79 cells *hprt* locus	ND	–	?		Jongen *et al*. (1981)
Gene mutation, mouse lymphoma L5178Y cells *tk* locus	ND	?	?		Myrh *et al*. (1990)
Sister chromatid exchange, Chinese hamster V79 cells	ND	–	?		Jongen *et al*. (1981)
Sister chromatid exchange, CHO cells	–	–	Yes		Thilagar & Kumaroo (1983)
Sister chromatid exchange, CHO cells	–	–	Yes		Anderson *et al*. (1990)
Chromosomal aberrations, CHO cells	–	+	No		Thilagar & Kumaroo (1983)
Chromosomal aberrations, CHO cells	–	–	Yes		Anderson *et al*. (1990)
Cell transformation, RLV/F344 cells	ND	+	?		Price *et al*. (1978)
Cell transformation, SA7/Syrian hamster cells	ND	+	?		Hatch *et al*. (1982)
Single strand breaks, human hepatocytes *in vitro*	–	–	Yes		Graves *et al*. (1995)
Unscheduled DNA synthesis, human AH fibroblasts	ND	–	?		Jongen *et al*. (1981)

Table 9 (contd)

System	GST-mediated metabolism of dichloromethane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Sister chromatid exchange, human lymphocytes *in vitro*	–	+	No		Hallier *et al*. (1993)
Micronucleus test, human MCL5 cells	ND	+	?		Doherty *et al*. (1996)
Micronucleus test, human AHH-1 cells	ND	–	?		Doherty *et al*. (1996)
DNA–protein cross-links, B6C3F$_1$ mouse liver *in vivo*	+	+	Yes		Casanova *et al*. (1992)
Single strand breaks, B6C3F$_1$ mouse liver *in vivo*	+	+	Yes		Graves *et al*. (1994b)
Single strand breaks, AP rat liver *in vivo*	–	–	Yes		Graves *et al*. (1994b)
Single strand breaks, AP rat liver *in vivo*	–	+	No	Large po dose, small response	Kitchin & Brown (1994)
Single strand breaks, B6C3F$_1$ mouse lung and liver *in vivo*	+	+	Yes	Buthionine sulfoximine decreased DNA damage	Graves *et al*. (1995)
Single strand breaks, AP rat lung *in vivo*	–	–	Yes		Graves *et al*. (1995)
Unscheduled DNA synthesis, F344 rat hepatocytes *in vivo*	ND	–			Trueman & Ashby (1987)
Unscheduled DNA synthesis, B6C3F$_1$ mouse liver *in vivo*	ND	–			Trueman & Ashby (1987)
Sister chromatid exchange, B6C3F$_1$ mouse lung *in vivo*	+	+	Yes		Allen *et al*. (1990)
Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	ND	–	?		Allen *et al*. (1990)
Sister chromatid exchange, C57BL/6J mouse bone marrow	ND	–	?		Westbrook-Collins *et al*. (1990)

Table 9 (contd)

System	GST-mediated metabolism of dichloromethane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Micronucleus test, NMRI mouse bone marrow	ND	–	?		Gocke *et al.* (1981)
Micronucleus test, C57BL/6J mouse bone marrow	ND	–	?		Sheldon *et al.* (1987)
Micronucleus test, B6C3F$_1$ mouse erythrocytes *in vivo*	ND	(+)	?		Allen *et al.* (1990)
Chromosomal aberrations, CD rat bone marrow	ND	–	?		Burek *et al.* (1984)
Chromosomal aberrations, B6C3F$_1$ mouse bone marrow	ND	(+)	?		Allen *et al.* (1990)
Chromosomal aberrations, B6C3F$_1$ mouse bone marrow	ND	–	?		Allen *et al.* (1990)
Chromosomal aberrations, C57BL/6J mouse bone marrow	ND	–	?		Westbrook Collins *et al.* (1990)
Chromosomal aberrations, B6C3F$_1$ mouse lung	+	(+)	(Yes)		Allen *et al.* (1990)

Additionally *Salmonella typhimurium* TA100 strains (NG11 and NG54) are deficient in glutathione and were less responsive than their parent strains to mutagenesis induced by dichloromethane.
Parentheses mean weak response/poor correlation.
ND, no data available; po, oral
–/+, in GST status column; –, absence/or presence of GST; ( ), –/+, in DNA damage column, as in Table 8

5.2 Human carcinogenicity data

Seven cohort studies have examined the risk of cancer among populations exposed to dichloromethane. Two studies observed an excess of pancreatic cancer, but the three others which reported on this tumour did not. One study observed an excess of liver and biliary tract cancers among longer-term employees. One study observed an excess of prostate cancer that appeared to increase with level of exposure. One study observed an excess of breast cancer and gynaecological cancers among women with the highest likelihood of exposure and another study observed an excess of cervical cancer. With the exception of the prostate cancer excess observed in one study, all the excesses were based on small numbers. No estimates of exposure levels were available for two of the six studies.

Three case–control studies have examined the risk of cancer associated with dichloromethane exposure and provided data adequate for evaluation. One observed an association between estimated intensity, probability and duration of exposure and the risk of astrocytic brain tumours. A second, which focused on female breast cancer, observed an elevated risk in the highest exposure category but no association with probability of exposure. The third indicated an increased risk of rectal cancer and possibly lung cancer.

For no type of cancer was there a sufficiently consistent elevation of risk across studies to make a causal interpretation credible.

5.3 Animal carcinogenicity data

Dichloromethane was tested by oral administration in the drinking-water in one study in mice and one study in rats, by inhalation exposure in two studies in mice, three studies in rats and one study in hamsters and by intraperitoneal injection in a lung adenoma assay in mice. In the study in mice by oral administration, no increase in tumour incidence was observed. The study in rats by oral administration gave inconclusive results. In the two inhalation studies in mice, increased incidences of benign and malignant lung and liver tumours were observed in both sexes. In the three inhalation studies in rats, the incidence of benign mammary tumours was increased in one study in females of a strain in which the incidence of spontaneous mammary tumours is low, and the multiplicity was increased in two studies in females of a high-incidence strain. In one study, in males, the incidence of mammary gland adenomas and fibroadenomas was increased. Negative results were obtained in the lung adenoma test in mice and in the inhalation study in hamsters.

5.4 Other relevant data

Two dose-dependent alternative pathways involving cytochrome P450 and glutathione *S*-transferases are responsible for the metabolism of dichloromethane in human and rodent cells.

Dichloromethane is consistently mutagenic in microorganisms. Weaker and less consistent responses are seen in mammalian systems, predominantly in mice, both *in vitro* and *in vivo*.

It induced sister chromatid exchanges, chromosome breakage and chromosome loss *in vitro* in human cells. In-vitro results in rodent cells were inconclusive or negative.

Dichloromethane induced DNA single-strand breaks in mammalian cell cultures, but inconclusive or negative effects were reported for induction of gene mutations. It did not induce unscheduled DNA synthesis either *in vivo* in rodents or in human fibroblast cultures. It was genotoxic in fungi but not in *Drosophila* in the sex-linked recessive lethal assay.

Mechanistic studies have established a link between glutathione *S*-transferase-mediated metabolism of dichloromethane and its genotoxicity and carcinogenicity in mice. The glutathione *S*-transferase responsible for the metabolism of dichloromethane is expressed to significantly greater extents in mouse tissues than in rat, hamster or human tissues.

The available data suggest a plausible mechanism for the development of liver and lung tumours which occur in mice but not in rats exposed to dichloromethane.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of dichloromethane.

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

Overall evaluation

Dichloromethane is possibly carcinogenic to humans (Group 2B).

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