

CHAPTER IV

METHYLENE CHLORIDE, CHLOROFORM AND CARBON TETRACHLORIDE

METHYLENE CHLORIDE

Methylene chloride (dichloromethane), CH_2Cl_2 , is a clear, colorless, non-flammable and highly volatile liquid. Methylene chloride is used industrially as a degreaser, solvent and extraction medium. Methylene chloride is also an important constituent of paint removers and may be used as an aerosol propellant (51, 299). Methylene chloride has also been used as a refrigerant and clinical anesthetic (51). The estimated world production capacity of methylene chloride in 1973 was about 400000 tons per year (267).

TOXICITY OF METHYLENE CHLORIDE

The early literature on methylene chloride has been summarized by Browning (51) and von Oettingen (299). The predominant effects of methylene chloride poisoning are due to its narcotic properties. Methylene chloride has a less pronounced narcotic action than chloroform and carbon tetrachloride (101, 301) and rather high atmospheric concentrations are necessary to produce anesthesia. The margin between anesthetic and lethal concentrations is small, which is the reason for the discontinued

use of methylene chloride as a clinical anesthetic (51, 286). The narcotic and lethal concentrations of methylene chloride have been determined for several animal species. Exposure to about 10000 ppm for 1 hour produces light to moderate narcosis in animals (178, 299). The LC_{50} is only slightly higher, amounting to about 16200 ppm for mice at 7 hours exposure (301) and 11600 ppm for guinea pigs at 6 hours exposure (30). Humans exposed to methylene chloride may detect its odor at 100-300 ppm (255). Concentrations over 2000 ppm cause nausea and lassitude after 30 minutes and concentrations between 7000 and 10000 ppm cause paresthesia of the extremities and eye irritation (255, 284). A concentration of 20000 ppm causes deep narcosis in man in 30 minutes (284). Several cases of acute or fatal occupational exposure to methylene chloride may be found in the early literature (51, 284, 299). Controlled human exposure to 100-500 ppm for 1-4 hours produced no untoward subjective responses (105, 426) but exposure to 1000 ppm gave a feeling of lightheadedness and CNS depression as evidenced by alterations in the visual evoked response (426). Similarly, Gamberale *et al.* (138) exposed human volunteers for four continuous 30 minute periods to increasing concentrations of methylene chloride (250-1000 ppm) and found signs of CNS depression, as evidenced by several psychological performance tests, only at the highest concentration. Rats exposed to 500 ppm methylene chloride 6 h/day for 4 days showed altered behavior (367). Although methylene chloride has definite narcotic properties and has been shown to affect both animal and human performances in behavioral or psychological tests, there are very few reports of injury to the nervous system at

chronic exposure. However, encephalopathy has been found in workers chronically exposed to high concentrations of methylene chloride (92, 168, 471).

High and acutely toxic or fatal concentrations of methylene chloride would only be expected to arise by accident in the occupational settings of today. Instead, the main toxicological hazard in the use of methylene chloride lies in the formation of CO during methylene chloride biotransformation. Elevations of carboxyhemoglobin (COHb) values in humans exposed to methylene chloride were first reported in 1972 (425, 426) and have later been demonstrated both at experimental (24, 314, 428) and occupational (333) exposure. Exposure to about 1000 ppm methylene chloride for 2-3 hours results in COHb values as high as 15% (425, 426, 428). The average COHb value of workers exposed to a mean concentration of 183 ppm methylene chloride for 8 hours rose from 4.5% to 9% (333). Additional toxic effects of methylene chloride may include sensitization of the myocardium to endogenous catecholamines with resulting cardiac arrhythmias (76, 180) and possibly liver and kidney injury. Exposure of human volunteers to 200-1000 ppm methylene chloride for 1-2 hours did not affect the liver as shown by several clinical tests (426). Acute fatal exposure did not produce any pronounced liver or kidney injury (284). Vauthey (464) states that daily exposure to low methylene chloride concentrations may cause digestive troubles secondary to hepato-renal injury with fatty degeneration of the liver and kidneys. Cases of hepatomegaly were found among chronically exposed workers but these were not definitely ascribed to methylene chloride exposure (168). The relative hepato-renal toxicity of methylene

chloride in animals as compared to other halogenated hydrocarbons is low as shown by repeated investigations. Plaa and co-workers (316) could not produce liver injury in mice injected with high doses of methylene chloride as evidenced by bromo-sulphophthalein retention or elevation of serum transaminase activity. Prolongation of barbiturate hypnosis was observed in mice and dogs only with doses of about half the subcutaneous or intraperitoneal LD₅₀. Gehring (143) found elevations of serum glutamic pyruvic transaminase activity in mice only at exposure to lethal vapor concentrations. Compared to other chlorinated hydrocarbons, such as carbon tetrachloride or chloroform, methylene chloride was a very weak hepatotoxic agent. The biotransformation of hexobarbital or aminopyrine *in vitro* was not impaired in liver microsomes from rats poisoned with methylene chloride in contrast to the decreased biotransformation observed with liver microsomes from rats given equimolar doses of carbon tetrachloride or chloroform (99). Compared to several other halomethanes, methylene chloride was without adverse effects on the function or composition of rat endoplasmic reticulum *in vivo* ascribed to its low tendency towards homolytic cleavage, which results in the formation of free radicals (343). Oral administration of methylene chloride to rats produced no evidence of liver injury, such as depression of glucose-6-phosphatase activity, calcium influx, increased cell sap RNA content or necrosis, in contrast to the effects of equimolar doses of carbon tetrachloride or chloroform (345). Methylene chloride given at four dose levels from 100 to 1000 mg/kg intraperitoneally to guinea pigs failed to cause elevation of serum ornithine carbamoyl transferase activity or microscopic

liver injury (103). However, single 6 hour exposures of guinea pigs to 5000 or 11100 ppm methylene chloride produced hepatic triglyceride accumulation as well as moderate fatty changes in the liver at microscopic examination, whereas repeated exposure to 560 ppm (6 h/day, 5 days) was needed to produce the same effects (30). The lungs were congested and hemorrhagic after 6 hours exposure to 11100 ppm and pneumonitis was observed at repeated exposure to lower concentrations. All kidneys were normal upon microscopic examination. Intraperitoneal administration of near lethal doses of methylene chloride to dogs (233) and mice (222, 317) produced slight tubular changes in both species and proteinuria in mice. Mice continuously exposed to 5000 ppm methylene chloride for up to 7 days showed weight loss, an initially 12-fold increase of liver triglycerides followed by a decrease to a plateau at 2-3 times normal values on the 6th day of exposure with concomitant microscopic fatty changes of the liver and loss of liver glycogen as well as swelling and necrosis of scattered hepatocytes (470). The kidneys also showed mild fatty change appearing on the second day of exposure whereas sections of heart, lung, spleen, pancreas and intestine were normal. Heppel *et al.* (178) performed exposures of monkeys, dogs, rabbits, guinea pigs and rats 7 h/day, 5 days/week for up to 6 months to 5000 ppm and 4 h/day, 5 days/week for 8 weeks to 10000 ppm methylene chloride. Exposure to the lower concentration was well tolerated by all species but extensive pneumonitis and centrilobular fatty degeneration was noted in two guinea pigs which died during the exposures. At 10000 ppm, although the narcotic effect was obvious, only a few dogs and guinea pigs showed fatty changes

of the liver. Methylene chloride has thus rarely been associated with severe specific organ injury except maybe liver and kidney damage at exposure to large amounts. However, a recent study by Jongen *et al.* (212), where methylene chloride was shown to be mutagenic causing frame-shift as well as base substitution mutations in *Salmonella* strains both with and without metabolic activation, may be indicative of an over-looked aspect of methylene chloride toxicity.

UPTAKE, DISTRIBUTION, BIOTRANSFORMATION AND ELIMINATION OF METHYLENE CHLORIDE

Methylene chloride is absorbed by the lungs at inhalation and from the gastro-intestinal tract at oral administration (51, 299). Methylene chloride may also be absorbed through intact human skin although probably not in quantities sufficient to produce toxic effects (422). The average uptake of methylene chloride by human subjects was 33.6% at exposure to 2600 mg/m³ for 1 hour with an increasing amount taken up with increasing body fat content (118). In another study, the average uptake at rest was found to be 55% at exposure to 870 or 1740 mg/m³ for 30 minutes with increasing amounts taken up with increasing physical work load (24). Von Oettingen (299) cites the uptake to be higher, about 70-75%, and 90% was taken up in 30 minutes by rats exposed to 1000 ppm methylene chloride in a closed rebreathing system (350). Retained methylene chloride has been assumed to be exhaled unchanged to a large extent (51, 299). Rats given single intraperitoneal doses of 412 to 930 mg/kg methylene chloride exhaled 91.5% of the dose unchanged in 24 hours the major part of which was recovered in the first

2 hours (102). The very rapid expiration of unchanged methylene chloride after cessation of inhalational exposure of human subjects has been noted in several studies (24, 104, 105, 118, 314, 347, 426) although traces of methylene chloride may be detected in the exhaled air as long as 17-18 hours after exposure (24, 347, 426). The breath concentration of methylene chloride decreases in a multi-exponential way. Riley *et al.* (426) noted four different slopes in the breath decay curve 60 minutes after cessation of exposure supposed to reflect the elimination of methylene chloride from various tissue compartments. DiVincenzo *et al.* (105) and Engström and Bjurström (118) observed two-compartment pharmacokinetics of methylene chloride during 3 hours after exposure to 100 ppm for 2-4 hours and during 4 hours after exposure to 750 ppm for 1 hour, respectively. Unchanged methylene chloride has also been found to be eliminated in the urine although in a negligible amount compared to that exhaled (104, 105, 178).

The rapid and extensive expiration of unchanged methylene chloride has been taken to indicate that the risk of accumulation of methylene chloride in the body would be small during repeated exposure (105, 314). However, it has also been suggested that methylene chloride could be stored in adipose tissues on the basis of both animal (367) and human (118) experimentation. In contrast, several recent distribution studies of methylene chloride have failed to reveal any specific retention of methylene chloride in body fat or other tissues. Fat and livers of rats exposed to about 600 ppm ¹⁴C-methylene chloride for 7-15 hours in a closed rebreathing system contained no radioactivity (349). In another study, the

highest radioactivity level was found in white adipose tissue of rats immediately after exposure to 1935 mg/m³ (about 550 ppm) ¹⁴C-methylene chloride for 1 hour (63). The fat radioactivity level had decreased by more than 90% 2 hours after exposure. The lowest amount of radioactivity was found in the brain immediately after exposure whereas the liver, kidneys and adrenals contained slightly higher amounts. All tissue levels declined rapidly. Six hours after exposure the highest radioactivity level was registered in the liver followed in decreasing order by the kidney, adrenal, white adipose tissue and brain levels. Two hours after intraperitoneal administration of 412 to 930 mg/kg ¹⁴C-methylene chloride to rats radioactivity was registered in a large number of tissues although no specific accumulation in body fat was noted (102). The liver and kidney levels increased considerably from 2 hours to 8 hours after injection. Fractionation of liver and kidney homogenates revealed that the largest proportion of the tissue radioactivities was present in the acid-soluble fraction but relatively high amounts of radioactivity were also found in proteins and lipids. Very little radioactivity was recovered in the nucleic acid fractions. The total carcass radioactivity was very low amounting to about 3% of the dose at 2 hours and less than 2% of the dose at 24 hours.

The formation of CO shown to occur at human methylene chloride exposure has also been shown to take place *in vivo* in several animal species. Rats given methylene chloride intraperitoneally or by inhalation show increased COHb-levels (63, 183, 235). Similarly, increased COHb-levels have been observed after inhalational exposure of rabbits (352) and guinea pigs

(30) to methylene chloride. Evidence that CO is a biotransformation product of methylene chloride has been obtained by the use of isotope-tagged methylene chloride. Expiration of ^{14}CO (102, 349) and the presence of $^{14}\text{COHb}$ or $^{13}\text{COHb}$ (63, 235) has been established in rats administered ^{14}C - or ^{13}C -methylene chloride by inhalation. The amount of CO formed from a dose of methylene chloride as recovered in the expired air varies considerably with the experimental conditions. Rats given a single intraperitoneal injection of ^{14}C -methylene chloride (412 to 930 mg/kg) exhaled about 2% of the dose as ^{14}CO in 24 hours (102) whereas rats exposed to ^{14}C -methylene chloride in a closed rebreathing system for up to 15 hours (calculated dose 0.2 mmol/kg) transformed 47% of the dose to ^{14}CO (349). In these two studies $^{14}\text{CO}_2$ was also identified in the exhaled air in amounts corresponding to 3% and 29% of the dose respectively. In addition, 24 hours after intraperitoneal injection of ^{14}C -methylene chloride about 1.5% of the dose was recovered in the exhaled air and about 1% of the dose in the urine as uncharacterized metabolites (102). A number of *in vitro* studies have also been performed to elucidate the sites and pathways of methylene chloride biotransformation but no complete information is as yet available on the fate of methylene chloride. Heppel and Porterfield (179) reported that methylene chloride was dechlorinated by glutathione-dependent enzymes in tissue slices and homogenates, especially from liver, to formaldehyde, hydrogen and chloride ions. The glutathione-dependent enzymatic breakdown of methylene chloride to formaldehyde and chloride was later shown to take place in the hepatic cytosol fraction (6, 234). Formaldehyde has not been

identified as a metabolite of methylene chloride *in vivo* (102) probably because it is rapidly oxidized to formate which joins the one-carbon pool or to CO₂ (150, 306). However, Kuzelová and Vlasák (236) found formic acid in the urine of methylene chloride exposed workers. *In vitro* studies have also shown that the conversion of methylene chloride to CO is mediated by an enzyme system in liver microsomes but also in kidney and lung microsomal fractions requiring O₂ and NADPH (183, 234).

CHLOROFORM

Chloroform, CHCl₃, is a clear, colorless, highly volatile non-flammable liquid with a characteristic sweetish odor. As compared to methylene chloride, the addition of chlorine makes chloroform more fat soluble but less volatile (Appendices 1 and 2). Chloroform has never been an important industrial solvent but may be found in industrial solvent mixtures and as a laboratory solvent. Chloroform has been used as a preservative and sweetener of pharmaceutical preparations and as a clinical anesthetic agent (299). Although the use of chloroform may have become restricted, due to its hepatotoxic properties, its estimated world production capacity, 245000 tons per year, points out chloroform as a still important chemical (267).

TOXICITY OF CHLOROFORM

The literature on chloroform toxicity up to 1964 has been summarized by von Oettingen (299). Chloroform is a powerful CNS depressant and its acutely anesthetic, narcotic and lethal

concentrations have been repeatedly determined in a variety of experimental animals. The mouse seems to be the most sensitive species with slight narcosis appearing at exposure to about 3000 ppm for 1 hour, deep narcosis at exposure to about 4000 ppm for 30 minutes (419) and death by depression of the respiratory center at exposure to 6000-8000 ppm for 3-7 hours (299, 301). Guinea pigs, rabbits and dogs are more resistant to the narcotic action of chloroform with 12000-40000 ppm required for 40 minutes-2 hours to produce fatal narcosis (299). Humans may detect chloroform by odor at 200-300 ppm and the first signs of CNS depression such as vertigo, intracranial pressure and nausea appear after exposure to 1000-2000 ppm for about 10 minutes. The narcotic concentration approaches 15000 ppm for 30 minutes-1 hour and higher concentrations, about 25000 ppm, may be fatal if inhaled for 5-10 minutes (299, 419). At acute exposure to high chloroform concentrations death may occur not only in respiratory paralysis but also as a result of cardiac arrest, a feared complication of chloroform narcosis in humans. Like many other chlorinated hydrocarbons, chloroform sensitizes the myocardium to the effects of endogenous catecholamines (180, 299).

Very little is known about the nervous system effects of chronic chloroform poisoning. Continued occupational exposure to up to about 240 ppm chloroform has been reported to produce dizziness, lassitude, irritability, headache and inability to sleep, concentrate or stand erect and intentional chloroform sniffing for long periods of time has produced neuralgic pain, irritability, ataxia, tremors, hallucinations and delirium (70, 176, 299). Persons addicted to a pharmaceutical morphine

preparation containing 12.5% chloroform but also ethanol (12.5%) and ether (3%) showed severe mental changes but also signs of peripheral neuropathy (84).

Numerous acute animal studies have also shown that chloroform is a hepatotoxic agent. In contrast to other chlorinated hydrocarbon solvents, such as methylene chloride and trichloroethylene, where exposure to concentrations as high as those producing anesthesia or even death are necessary to produce liver injury, chloroform is often placed in the same group as carbon tetrachloride in investigations of the relative hepatotoxic potency of chlorinated hydrocarbon solvents since both produce liver injury at concentrations or doses considerably lower than those required to produce anesthesia. However, carbon tetrachloride exerts its hepatotoxic action with doses which are about 10-13 times lower than those of chloroform (316). The histopathological liver picture of chloroform poisoning is characterized by fatty infiltration and necrosis reaching a maximum at 24-48 hours after administration of chloroform. As demonstrated for mice by Jones *et al.* (211) using oral administration or by Kylin *et al.* (238) using inhalational exposure the severity of the liver damage increases with increasing dose or atmospheric concentration. The minimally hepatotoxic oral dose of chloroform in mice, i.e. the dose producing midzonal fatty change and midzonal and centrilobular fatty infiltration, was 0.035 g/kg whereas the moderately hepatotoxic dose producing massive fatty infiltration was 0.14 g/kg. Severe changes with massive fatty infiltration with superimposed centrilobular necrosis was observed with the narcotic dose 0.35 g/kg. The LD₅₀ was 1.1 g/kg. Similarly,

a single 4 hour exposure of mice to 100 ppm produced moderate fatty infiltration whereas moderate to massive fatty infiltration together with an increasing necrosis of liver cells appeared at exposure to 200, 400 and 800 ppm chloroform for 4 hours. This may be compared to the anesthetic concentration, 3000 ppm for 1 hour, as previously reported for mice (299). In addition to microscopic examination the acute hepatotoxicity of chloroform has also been evaluated by several biochemical tests of liver function and composition including determinations of serum enzyme activities, decreased activities of enzymes responsible for the biotransformation of drugs, clearance of dyes, amount of extractable liver fat, incorporation of radiolabeled amino acids into protein, cell sap RNA content or lipid diene conjugation (indicative of lipid peroxidation). Serum ornithine carbamoyl transferase activity was significantly increased in mice 24 hours after exposure to 200, 400 or 800 ppm chloroform for 4 hours but 3 days after exposure a significant increase was noted only in mice exposed to 800 ppm (238). The serum activity of this enzyme was also elevated in guinea pigs after intraperitoneal injection of 150 or 300 mg/kg chloroform with lipid deposition in hepatocytes observable at both dose levels but hepatocellular damage only at 300 mg/kg (103). Elevation of serum glutamic-pyruvic transaminase activity has been observed in mice and dogs after intraperitoneal injections of chloroform at dose levels corresponding to about 15-20% of the LD₅₀ (316). This enzyme was also used by Gehring (143) as an indicator of hepatic injury in mice inhaling chloroform. At 4500 ppm chloroform an increase of serum glutamic-pyruvic transaminase activity within

24 hours in 50% of the animals required exposure for 13.5 minutes whereas anesthesia of 50% of the animals required exposure for 35 minutes. Dingell and Heimberg (99) observed a reduction of aminopyrine biotransformation to 61% of control values in liver microsomes obtained from rats 24 hours after oral administration of 1.05 ml/kg chloroform. Hexobarbital biotransformation was not affected. An equimolar dose of carbon tetrachloride reduced aminopyrine and hexobarbital biotransformation to 14% and 29%, respectively, of control values. However, prolongation of barbiturate hypnosis has been observed by Plaa and co-workers (316) in mice given about 0.25 ml/kg chloroform subcutaneously. They also used bromosulfophthalein retention as an index of hepatic dysfunction in mice but found this to be less sensitive than prolongation of barbiturate sleeping time or elevation of serum glutamic-pyruvic transaminase activity. Chloroform increased the amount of total extractable liver fat in mice after 4 hours exposure to 400 or 800 ppm (238) and the amount of liver triglycerides in rats given high doses ($0.75 \times LD_{50}$) intraperitoneally (224). Reynolds (343), using several tests of function and composition of liver endoplasmic reticulum from rats fed single high doses (3.1 g/kg) chloroform, found a significantly reduced incorporation of ^{14}C -glycine into proteins and an increased cell sap RNA content indicating a disturbed protein synthesis. No lipid peroxidation as measured by lipid diene conjugate content was noted. Similarly, Klaassen and Plaa (224) did not observe lipid peroxidation either in rats given high doses of chloroform intraperitoneally or *in vitro* in rat liver homogenates incubated with chloroform.