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Joint Assessment of Commodity Chemicals

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METHYLENE CHLORIDE

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THE ECETOC SCHEME FOR THE "JOINT ASSESSMENT OF COMMODITY CHEMICALS " (JACC)

This report has been produced as part of a programme for making critical reviews of the toxicology, including ecotoxicology, of selected industrial chemicals.

A number of organisations, world-wide, have produced and are continuing to produce such reviews with the aim of ensuring that, based on an up-to-date knowledge of the toxicological, and other relevant, information regarding existing chemicals they can continue to be produced and used safely. ECETOC is contributing to this activity with its JACC reviews.

In general, commodity chemicals, ie. those produced in large tonnage by several companies and having widespread and multiple uses, are reviewed jointly by experts from a number of the companies concerned. Before it is decided to review a chemical, every effort is made to discover whether an adequate review exists already, in which case no work is necessary.

It should be noted that in a JACC review only the uses of the chemical as such are considered, ie. its occurrence as an impurity in other products is not normally taken into account.

In this document a critical assessment of the toxicology and ecotoxicology of methylene chloride is presented. Methylene chloride was chosen as one of four, varied chemicals on which trial exercises were carried out to aid in developing the JACC scheme.

CONTENTS

A. CHEMICAL IDENTITY AND PHYSICAL-CHEMICAL PROPERTIES

Methylene chloride (syn. dichloromethane, methylene dichloride, methylene bichloride) CH_2Cl_2.

1. Physical Properties

Methylene chloride is a colourless volatile liquid with a penetrating ether-like smell, which is detectable at about 200-300 ppm (DFG, 1981).

physical form		: volatile liquid
colour		: water clear
odour threshold, ppm		: 200-300
relative molecular mass		: 84.93
density (20°C/4°C)		: 1.3266
boiling point (1.013 bar)*, °C		: 40
freezing point, °C		: -97
viscosity, Pa.s* at 20°C		: 4.37×10^{-4}
viscosity, cP at 20°C		: 0.437
refractive index, n_D		: 1.4244
vapour pressure, in bar*, at 10°C		: 0.31
	at 20°C	: 0.47
	at 30°C	: 0.71
surface tension in air, in $N.m^{-1}$		: 28×10^{-3}
	in $dynes.cm^{-1}$	: 28
heat of vaporisation at 40°C and 1 bar,		
	in $kJ.kg^{-1}$	: 331
	in $kcal.kg^{-1}$	: 79
limit of explosion in air at 25°C		: none
limit of flammability in air at 25°C and 1 bar*		: not flammable
flash point (closed cup), °C		: will not flash
ignition temperature in air, °C		: 605
solubility of water in CH_2Cl_2 in $g.kg^{-1}$		: 1.5
solubility of CH_2Cl_2 in water in $g.kg^{-1}$		: 18.5
water azeotrope at 1.013 bar*		
composition, % H_2O		: 1.5
boiling point, °C		: 38
conversion factors (25°C, 1.013 bar)	1 ppm	: 3.474 $mg.m^{-3}$
	1 $mg.m^{-3}$	: 0.288 ppm

* 1 bar = 10^5 Pa = 10^5 Newton.m^{-2}; 1 Torr = 1.33×10^{-3} bar = 133 Pa.

2. Reactivity

Methylene chloride is a rather stable chemical. With water at ambient temperatures it very slowly hydrolyses, liberating hydrogen chloride. Elevated temperature, alkali, and metals accelerate this reaction. Although rather resistant, it can be oxidized in the vapour phase at elevated temperatures, or on exposure to UV light, to form hydrogen chloride, carbon monoxide and dioxide, and phosgene. When dry it does not attack mild or galvanized steel but becomes slightly aggressive in the presence of water. It may react with aluminium, magnesium and their alloys if not specially stabilized. It reacts with amines to form an ammonium salt, chloride and a free organic radical.

Methylene chloride vapour exposed to intense heat (flame, sparks, red-hot surfaces) decomposes, forming toxic and corrosive substances. During welding in atmospheres contaminated with methylene chloride (used as a degreasing solvent) at concentrations largely in excess of occupational exposure limits, minor amounts of chlorine, hydrogen chloride and phosgene may be formed (Rinzema and Silverstein,1972).

3. Composition

Commercial products are of high purity, containing only minute amounts of impurities and stabilizers. They are normally inhibited with between 0.0005 and 0,2% of a stabilizer, typically methanol, ethanol, amylene, cyclohexane or tertiary butylamine. Applications in aggressive conditions such as metal cleaning require more sophisticated stabilizer mixtures. A less pure grade for general uses may contain up to 200 ppm of impurities, mostly C_1 and C_2 chlorinated hydrocarbons. For use in the food industry, the content of impurities is less than 100 ppm.

B. PRODUCTION AND USES

World wide production is estimated as 570,000 tonnes in 1980, and Western European production as 270,000 t in 1981 (CEFIC, 1982).

Two processes are used for producing methylene chloride. One starts from methane which is chlorinated to methyl chloride, methylene chloride, chloroform, and

carbon tetrachloride. In the other route methyl chloride, obtained by hydrochlorination of methanol, is converted into methylene chloride, chloroform and carbon tetrachloride by chlorination.

Most of the applications of methylene chloride are based on its high solvent power and low boiling point. These applications (CEFIC, 1982) include its use as :

- a solvent in aerosol formulations
- an extractant in food and pharmaceutical industries
- an extractant for fats and paraffins
- a process solvent in cellulose ester production and fibre and film forming
- a component of paints, varnish strippers and adhesive formulations
- a process solvent in polycarbonate production
- a blowing agent in flexible polyurethane foams.

It is also used in plastics processing, and in metal and textile treatment.

C. HUMAN EXPOSURE

The physical properties and pattern of use of methylene chloride are such that inhalation is the most likely route of human exposure.

1. Exposure Limits

The finding that methylene chloride metabolism raises the blood carboxy--haemoglobin level led to the setting of the following limit values for occupational exposure :

USA, Threshold Limit Value (TLV-TWA; 8 h time-weighted average), 100 ppm (ACGIH, 1982).
W. Germany, MAK value, 100 ppm (DFG, 1983).
Netherlands, proposed MAC value, 100 ppm.
Sweden, Limit Value, 100 ppm (National Swedish Board of Occupational Safety and Health, 1981).
USSR, Limit Value, 14 ppm (INRS, 1982). The basis for this limit is unclear.

A TLV-STEL (Short-term Exposure Limit) of 500 ppm for 15 minutes is recommended by the ACGIH (1982) and proposed in the Netherlands. The

short-term limit (15 min.) in Sweden is 150 ppm (National Swedish Board of Occupational Safety and Health, 1981). NIOSH (1979) recommended an environmental exposure limit of 75 ppm (time-weighted-average) and a 500 ppm ceiling (15 min). Each of these limit values should be related to its own system of definitions and implementation requirements.

Methylene chloride is allowed in cosmetic preparations and aerosols in the European Economic Community at concentrations not exceeding 35% (EEC, 1982).

The Council of Europe (1978) has recommended a maximum content in food of 5 $mg.kg^{-1}$. The US Food and Drug Administration (FDA, 1977) permits its presence in the following foods : i) spice oleoresins, as a residue after spice extraction, at a maximum level of 30 $mg.kg^{-1}$; ii) hops extracts, at a maximum level of 2.2%; and iii) coffee, as a residue from its use as a solvent in caffeine extraction, at a maximum level of 10 $mg.kg^{-1}$. The FDA also allows its use in adhesives and in the production of polycarbonate resins intended for use in producing, manufacturing, packaging, processing, preparing or holding food.

2. Exposure Data

There seem to be little available data on atmospheric concentrations at production plants. NIOSH (1980) reported that at one production facility the atmospheric concentrations were between 5 and 25 ppm.

In user industries the following values were reported : in a paint-stripping operation, 200-300 ppm (NIOSH, 1978); servicing diesel engines, 11 ppm; spray-painting booths, 1-74 ppm; plastic tank construction, a few ppm; ski manufacture, 0-36 ppm; cleaning foam heads, 3-29 ppm; cleaning nozzles in plastics manufacture, 5-37 ppm (NIOSH, 1976); chemical user-plant, 140 ppm to 475 ppm TWA (Ott et al., 1983). Beauticians using hair sprays were exposed to a daily mean background concentration of less than 1-2 ppm methylene chloride (Hoffman, 1973), but this information is somewhat outdated. Such background concentrations will depend upon the methylene chloride concentration in the hairspray used.

Methylene chloride-extracted spice oleoresins were found to contain concentrations in the range 0.3-3 ppm (Page and Kennedy,1975). Methylene

chloride has been detected in drinking water at concentrations of up to 1.4 ppm in the past (IARC, 1979), but present levels are very low (ppb).

D. TOXICOLOGICAL DATA

1. Human

1.1. Inhalation of vapour.

The odour of methylene chloride is detectable at concentrations around 200-300 ppm (DFG, 1982), i.e. well above the present TLV-TWA values.

Methylene chloride depresses the central nervous system but its potency is significantly lower than that of chloroform. Its poor muscle-relaxant potential and pronounced excitation phase limited its early use in anaesthesia. Dizziness may occur at concentrations between 900 and 1000 ppm, and nausea, headache and vomiting at concentrations exceeding 2000 ppm. At above 7000 ppm, numbness and tingling in the limbs, and tachycardia, occur. Loss of consciousness has been reported, with complete recovery if exposure is terminated before anaesthetic death occurs. Very few fatalities have been reported from accidental gross overexposure (Moskowitz and Shapiro, 1952).

1.1.1. Studies in volunteers. Volunteers exposed to 300 ppm for 2.5 h developed a reduction in vigilance as measured by flicker fusion frequency (Schlipkoeter et al.,1970). Subjects exposed to 1000 ppm for 1 to 2 hours presented symptoms of central nervous system depression. These were not observed on exposure to 500 ppm for 1 hour (Stewart et al.,1972).

An extensive laboratory study of healthy adult volunteers "repeatedly" exposed for 7.5 h/d to 250 ppm showed no untoward subjective or objective health effects (Stewart et al.,1974).

Airborne methylene chloride resulting from typical home-use application of commercial spray paint did not cause clinically significant elevation of blood carboxyhaemoglobin in male and female volunteers (Stevenson et al., 1978) (See specific section on metabolism).

1.1.2. Case reports. After exposure to methylene chloride vapour over a period of five years, at concentrations frequently exceeding 500 ppm (up to 3600 ppm) and with dermal contact as well, a chemist developed toxic encephalopathy with acoustical and optical delusions and hallucinations (Weiss, 1967).

Two days after an accidental inhalation of methylene chloride resulting in narcosis, creatine phosphokinase values in the blood increased. No signs of heart failure or heart-rhythm changes were observed (Ossenberg, 1971).

A single case of recurrent acute myocardial infarction followed ultimately by death has been described in a 66 year-old man who had been stripping paint under conditions requiring considerable physical exertion during his leisure time (Stewart and Hake, 1976).

A man of 60 developed bilateral temporal lobe degeneration, tentatively attributed to three years of work in a poorly-ventilated laboratory in which methylene chloride was routinely used. Exposure was estimated to have been in the range 300-1000 ppm (Barrowcliff and Knell,1979).

1.1.3. Morbidity studies in an occupational setting. A cross-sectional morbidity study was carried out at two fibre production plants in the US. Time-weighted-average methylene chloride exposures were up to 500 ppm. The employee health study consisted of a comprehensive questionnaire, physical examination, clinical evaluation of blood chemistry and urinalysis. Twenty-four hour electrocardiograms were taken on 50 men selected from a group on the basis of personal or family health history (cardiovascular disease) and other heart risk criteria. Statistical and clinical assessment of the data revealed no effects from methylene chloride exposure (Ott et al.,1983).

Clinical examination, ECG and motor conduction velocity measurements, and psychological tests, revealed no evidence of long-term damage attributable to methylene chloride in a group of workers exposed for up to 10 years or more at concentrations

below 100 ppm in a factory making acetate film (Cherry et al.,1981). Workers exposed for up to 2 years at concentrations of up to 5000 ppm complained of headache, fatigue, irritation of the eyes and respiratory passages, neurasthenic disorders and digestive disturbances (Kuzelova and Vlasak,1966).

1.2. Contact of liquid methylene chloride with skin and eye.

Liquid methylene chloride is painful and irritating only if confined on the skin by gloves, clothing or paint remover formulations. Repeated contact does not sensitize the skin. Transient corneal burns may result from splashes in the eye (ACGIH, 1981). Methylene chloride may be absorbed through the intact skin (Stewart and Dodd, 1964) but because of its irritating action, significant exposure by this route is unlikely (DFG, 1982).

2. Experimental : Acute, Sub-acute and Chronic.

2.1. Inhalation of vapour.

The numerous acute inhalation toxicity studies, on various species, have been reviewed by DFG (1982) and Norris (1981).

LC 50 values for a single 6-7 h exposure were about 15,000 ppm in rats (Laham et al.,1978; Bonnet et al.,1980) and mice (Svirbely et al.,1947; Gradiski et al.,1978). Methylene chloride depresses the central nervous system. Slight narcosis occurred at 4,000 to 6,000 ppm after 2.5-6 h exposure in several species (Weinstein et al.,1972). Rats exposed at 1,000 or 3,000 ppm for 24 h showed a reduction of paradoxical sleep (REM-sleep) during exposure. This was not observed at 500 ppm (Schlipkoeter et al.,1970). At concentrations of several thousand ppm, methylene chloride depressed myocardial contractility (Aviado, 1975; Taylor et al.,1976; Aviado et al.,1977). It did not cause respiratory depression in monkeys (Aviado, 1975).

Inhalation of 5,200 ppm for 6 h induced fatty infiltration in the liver of guinea pigs. Slight hepatotoxicity and fatty changes in the liver have been observed in several species (Morris et al., 1979). Mice exposed continuously to 5,000 ppm methylene chloride for up to 7 days

showed transient fatty changes in the liver and swelling of the hepatocytic rough endoplasmic reticulum. As observed from serial killings, these lesions were "partially reversible" (Weinstein et al., 1972).

Rats exposed to 3,700 ppm of methylene chloride for 5 h/d, 5 d/wk for 4 wk showed a significant increase of protein secretion in the cell-free lavage effluent from their lungs, interpreted as increased damage to pulmonary cells (Sahu et al., 1980).

Chronic toxicity studies have given the following results. Exposure at 5,000 ppm, (7h/d, 5d/wk, 6 m) had no discernible effect on rats, dogs or rabbits, but led to a reduction in growth rate and to a centrolobular degeneration of the liver in guinea pigs . At 10,000 ppm, 4 h/d, 5d/wk for 7.5 wk, dogs and guinea pigs, but not monkeys, rabbits or rats, developed slight fatty degeneration of the liver (Heppel et al.,1944). Male and female Sprague-Dawley rats were exposed to 0, 50, 200 or 500 ppm of methylene chloride for 6 h/d, 5 d/wk for 2 years. Metabolism to CO resulted in some elevation of carboxyhaemoglobin in all treated groups. The male rats showed no methylene chloride-related toxicity at any level. In the liver of female rats inhaling 500 ppm there was an increased incidence of multi-nucleated hepatocytes. The number of rats bearing mammary tumours did not increase, but the ratio of the number of benign mammary tumours to the number of rats bearing these tumours increased. These findings were not observed in female rats at lower exposure levels (Burek et al., 1980).

2.2. Contact of liquid methylene chloride with skin and eye.

Methylene chloride was severely irritating when applied to rabbit skin under an occlusive dressing (Duprat et al.,1976). It is absorbed through the skin (Tsuruta, 1975 and 1977). No adverse local or systemic effect resulted from the application of 0.5 g/kgbw/d, 5 d/wk to intact and abraded rabbit skin over a period of 90 days (Industrial Bio-Test Laboratories Inc., 1957). Methylene chloride does not sensitize the skin (Industrial Bio-Test Laboratories Inc., 1972).

In rabbits, the liquid is a severe eye irritant, and transient corneal thickening may develop (Ballantyne et al.,1976; Duprat et al.,1976).

2.3. Ingestion

The acute oral LD 50 for rats and mice is about 2 g/kgbw (AIHA, 1965; Aviado et al., 1977). A higher value (3.05/kgbw) was found in adult mice than in young adults (2.12 g/kgbw) (Kimura et al., 1971). CNS depression was the main sign of intoxication.

Rats fed up to 2.2 g/kgbw showed only minimal centrolobular suppression of Glucose-6-phosphatase activity in the liver after 24 h (Reynolds and Yee, 1968) with no significant subcellular alterations involving the endoplasmic reticulum (Reynolds, 1972). Subchronic studies showed no adverse effects in rats receiving drinking water containing 0.112 g/l of methylene chloride for 91 days (Bornmann and Loeser, 1967).

2.4. Intraperitoneal route

The intraperitoneal LD 50 in the rat is between 1,500 and 1,900 mg/kgbw, and in the mouse is about 1,900 mg/kgbw (Klaassen and Plaa, 1966; Gehring, 1968; Gradiski et al., 1974 and 1978). For mice, a much lower value of 448 mg/kgbw was found by Aviado et al. (1977). The i.p. LD 50 in dogs is 0.95 ml/kgbw (1.25 g/kgbw) with evidence of slight hepatotoxicity, but no kidney dysfunction (Klaassen and Plaa,1967).

Concentrations of up to 1,000 mg/kgbw injected intraperitoneally into guinea-pigs caused no increase in serum ornithine carbamyl transferase 2 h after dosing. Slight hepatocellular damage was observed at the highest concentration. Phenobarbital pretreatment of rats given 0.2, 0.5 and 1.0 ml/kgbw (0.26, 0.65 and 1.3 g/kgbw) did not enhance hepatotoxicity as indicated by serum SGOT levels and liver histopathology (Cornish et al., 1973). Mice injected with 1 ml/kgbw (1.3 g/kgbw) showed necrosis and swelling of proximal convoluted tubules in the kidney (Plaa and Larson,1965).

There is a correlation between the i.p. administration of methylene chloride to rats in the range 1-6 mmol/kgbw (0.09 - 0.55 g/kgbw) and a reduction in sciatic motor conduction velocity,"presumably because of endogenous carbon monoxide production" (Pankow et al.,1979).

3. Carcinogenicity

3.1. Observations in animals

Rats and hamsters were exposed to methylene chloride (containing between 250 and 450 ppm of cyclohexane as stabiliser) at concentrations of 500, 1500 and 3500 ppm, 6h/d, 5d/wk for 2 years. No evidence of carcinogenic potential or other toxic effects was found in the hamsters. Despite the occurrence of slight liver toxicity in the rat, there was no increase in malignant liver cell tumours. Rats, but not hamsters, showed an increase in the incidence of benign mammary tumours, especially at high levels. The significance of this response is unknown. The Sprague-Dawley rats used in this study reportedly have a high spontaneous incidence of mammary tumours (Burek et al., 1980).

In the same study, male rats exposed to 1,500 ppm or 3,500 ppm of methylene chloride appeared to have an increased number of sarcomas in and around the salivary glands. The relevance of this finding is uncertain. A relationship between methylene chloride exposure and effects on the salivary gland is unusual in the light of the presently-available toxicity data. The rats had experienced a common viral infection (sialodacryoadenitis) early in the treatment period. This infection primarily affects the salivary glands and was present in both control and exposed rats.

As a follow-up to this chronic inhalation study, male and female Sprague-Dawley rats were exposed to 0, 50, 200 or 500 ppm of methylene chloride for 6 h/d, 5 d/wk for 2 years. With the exception of elevated carboxy-haemoglobin, male rats showed no methylene chloride-related effects. Female rats at 500 ppm had an increased incidence of multinucleated hepatocytes and an increased ratio of the number of benign mammary tumours to the number of rats bearing these tumours although the number of rats bearing mammary tumours did not increase. No detectable alterations in hepatocytic DNA synthesis or cell turnover were produced in females at any concentration (Nitschke et al., 1983).

Further long-term carcinogenicity studies have been completed in the USA under the National Toxicology Programme (NTP, 1982), but the results

have been determined by NTP to be scientifically unacceptable (Food Chemical News, 1983).

In a recently completed study, methylene chloride administered orally to Fischer 344 rats in their drinking water, at levels of 0, 50, 125 and 250 mg/kgbw/d for two years, did not induce a carcinogenic response (Hazleton Laboratories America, 1982).

3.2. Observations on humans

A proportionate mortality study covering 334 deaths among active, disabled or retired employees exposed for over 30 years to methylene chloride (30 - 125 ppm) was undertaken in an American plant for the period 1956-1976. Breathing-zone concentrations were measured in 1959 and thereafter, the highest (350 ppm) being found in 1959. No significant or suggestive differences were noted in the age-standardised proportionate mortality ratios in comparison with the New York State general population (Friedlander et al., 1978).

All of the 751 males who had worked in the above area between 1964 and 1976 were included in a retrospective cohort study. Of this group, 252 had a minimum of 20 years work exposure in this area at July 1, 1964. This long-exposure group was segregated for a separate analysis and followed up through July 1976. No increased incidence of malignant neoplasms, circulatory disease, or total mortality was identified when compared with industrial controls within the Company, and with New York State mortality figures (Friedländer et al., 1978). This cohort study was updated through 1980 (Hearne and Friedlander, 1981), and the earlier conclusions were confirmed. It should be noted that this study provided over 95% statistical power to detect relative risks as low as 1.5 for deaths from ischaemic heart disease and 2.0 for total malignant neoplasms. No such increases were observed.

Another retrospective mortality study involving 2,227 employees was conducted at two other plants in the U.S.A. (Ott et al., 1983). Methylene chloride had been used in fibre production at one plant since 1954, but not at a similar comparison plant.Time-weighted-average methylene chloride exposures were up to 500 ppm. No increase in

malignant neoplasms, circulatory disease or mortality was observed compared to U.S. mortality rates.

4. Kinetics and Metabolism

4.1. Kinetics

Absorption. The absorption of methylene chloride through the lungs leads to a rapid rise in human blood serum level during the first minutes of exposure, and a slower rise in the subsequent 40 to 50 min. Male volunteers at rest took up about 55% of the amount supplied. Under work loads of 50, 100 and 150 Watts, the uptake was 40, 30 and 35% respectively (Astrand et al., 1975). The concentration in venous blood rises more slowly and tends not to increase further after about 2 h exposure (Riley et al., 1966; DiVincenzo et al.,1971, 1972). Methylene chloride is absorbed through the skin of man (DFG, 1982) and animals (Tsuruta, 1975, 1977). After a single oral administration in the rat, peak blood levels occurred after 1 h., fell rapidly during the second hour, and thereafter fell more slowly (Laham and Potvin, 1976).

Distribution. Savolainen et al.(1977) found that in inhalation experiments (rats, 200 ppm, 6h/d, 5d) methylene chloride accumulated in the fat and brain. After i.p. administration of radio-labelled material (412 to 930 mg/kgbw) to the rat, widespread distribution in the tissues occurred, although the overall residue was low (Di Vincenzo and Hamilton, 1975). When radio-labelled methylene chloride was inhaled by rats, the largest concentration per gram of tissue was found in white adipose tissue, but accumulation was not significant. Two hours after a 1h exposure (500ppm) by inhalation, the concentration of radioactive material in the liver, brain and fatty tissue fell by 25,75 and 90% respectively (Carlsson and Hultengren,1975). Di Vincenzo and Hamilton (1975) found the highest specific activities in the liver, kidneys and adrenal glands of rats. In healthy human males exposed to 750 ppm for 1 h while working at an intensity of 50 Watts, the uptake correlated with the body fat content (Engström and Bjurström, 1977). Methylene chloride was detectable in the maternal blood, and the cord blood of human neonates, in the general population. (Dowty and Laseter, 1976).

Excretion after inhalation. The elimination of methylene chloride in rats follows a 2-compartment model (Withey and Collins, 1980). In man and rats, over 90% of the absorbed material is eliminated unchanged in alveolar air, and a small amount is found in the urine. The concentration in alveolar air drops very rapidly after exposure, and the rate of excretion is considerably lower after 30 min. Most exposed subjects have traces of methylene chloride in their alveolar air 18 h or more after exposure (Fodor and Winneke, 1970).

4.2. Metabolism

It is now established that methylene chloride is slowly but steadily metabolised to carbon monoxide and dioxide. The concentration of carboxyhaemoglobin (COHb) increases during and after exposure. The metabolism of some of the inhaled methylene chloride to carboxyhaemoglobin has been shown to be governed by the equation below, established on the basis of pharmokinetic studies on male and female human volunteers (Peterson, 1978).

$$\Delta = \frac{0.0842(D.C_i)^{0.72}}{(t+220)^{0.775}}$$

where Δ = % increase in COHb
C_i = inhaled concentration, ppm
D = exposure duration, minutes
t = post-exposure time, minutes

Another study on volunteers confirmed the validity of this equation in the range of 50 to 200 ppm (Di Vincenzo and Kaplan,1981).

Six healthy male volunteers were exposed to 100 and 350 ppm of methylene chloride during each of two 6 h exposure periods. Methylene chloride and carboxyhaemoglobin levels in blood, and methylene chloride and carbon monoxide levels in expired air, were measured during exposure and for 24 h thereafter. The results indicated a saturable metabolism of methylene chloride to carbon monoxide. The authors derived a pharmacokinetic model for predicting the extent of methylene chloride metabolism and the production of carboxyhaemoglobin following exposure in laboratory animals

and men (McKenna et al.,1979). The conclusion that methylene chloride metabolism is a saturable process was further substantiated in rats (McKenna et al.,1982).

In the rat, COHb is not formed in the blood but via the liver microsomal fraction (Hogan et al., 1976). Methylene chloride is dehalogenated in vitro by rat liver cytosol fraction to formaldehyde and inorganic chloride. Glutathione is the necessary co-factor. Administration of an enzyme inducer such as phenobarbital, or repeated administration of methylene chloride, did not affect this process (Ahmed and Anders, 1976). In the rat, formaldehyde is not a product of metabolism in vivo (Di Vincenzo and Hamilton, 1975).

5. Mutagenicity

Methylene chloride induced point mutations and frameshift mutations in the Salmonella typhimurium reverse-mutation assay according to Ames. The administration of rat-liver homogenate did not appear to be essential, although it slightly increased the number of mutations (Jongen et al., 1978, 1982; Gocke et al., 1981; Dow Chemical Co., 1981). Further experiments have shown that bacterial metabolism contributes to the direct mutagenic effect of methylene chloride (Green, 1983).

Chromosome aberrations were observed in cultured Chinese hamster ovary cells (Thilagar and Kumaroo, 1983). Cytogenetic aberrations in bone marrow from male and female rats did not increase, compared to their respective controls, after 6 months of repeated exposure by inhalation to 500, 1,500 or 3,500 ppm (Burek et al., 1980). A micronucleus test in mice (2 doses of up to 1,700 mg/kgbw injected i.p.) was negative, and a BASC (Müller-5, Bar white Apricot SCute test) on Drosophila (recessive lethals) was marginally positive (Gocke et al., 1981). Methylene chloride was negative in the sex-linked recessive lethal test in Drosophila (Abrahamson and Valencia, 1980). A marginal increase in sister chromatid exchange was induced by methylene chloride in Chinese hamster V79 cells (Jongen et al.,1981) but not in Chinese hamster ovary cells (Thilagar and Kumaroo, 1983).

Methylene chloride did not induce unscheduled DNA synthesis in V79 cells, human fibroblasts in vitro (Jongen et al., 1981), or rat hepatocytes (Andrae and Wolff, 1983). DNA synthesis was inhibited but the effect was not clearly

dose-dependent and could not be interpreted to indicate a DNA-damaging action (Jongen et al., 1981). Methylene chloride did not covalently bind to hepatocytic DNA in vitro (Cunningham et al.,1981) or in vivo (Green, 1983).

Jongen et al. (1981) observed no forward mutation to 6-thioguanine-resistance in V79 cells or Chinese hamster ovary cells. They found conflicting results in yeast. Methylene chloride induced forward mutation in mouse lymphoma cells (Litton Bionetics, 1977). A cell-transformation assay with BALB/C-3T3 mouse embryo cells gave negative results (A.D. Little Inc., 1980).

6. Reproductive Toxicity

Wistar rats fed about 2.8 mg/kgbw (125 ppm) per day in their drinking water, for 91 days, were mated and sacrificed at the end of the exposure period. No significant adverse effects on reproduction were noted (Bornmann and Loeser, 1967).

Sprague-Dawley rats and Swiss-Webster mice were exposed to 1,250 ppm of methylene chloride in air for 7 h/d on days 6-15 of gestation. No significant maternal, embryonal (including teratogenic) or foetal toxicity was observed in either species (Schwetz et al., 1975).

Female rats were exposed by inhalation, at a concentration of 4,500 ppm, 3 weeks before and/or during the first 17 days of gestation. The authors reported a low degree of maternal- and embryo-toxicity but no teratogenic effect (Hardin and Manson, 1980).

E. ECOTOXICOLOGICAL DATA

For a review , see Edwards et al.(1982).

1. Environmental Distribution

The uses of methylene chloride are such that most of the product evaporates into the atmosphere.

Estimated yearly atmospheric emissions for 1973 were about 190,000 t in the United States and 350,000 t worldwide. In 1976, the figures were 200,000 t in

the US and 400,000 t worldwide (Anon, 1976-a; Anon, 1976-b; EPA, 1975; A.D. Little Inc., 1975). In 1980, emission was around 510-520,000 t/y based on estimated production (CEFIC, 1982).

There are no known natural sources, and little information is available on the background atmospheric concentrations in regions remote from human activity. A series of 13 measurements made in Wiltshire, UK (Cox et al., 1976) gave a mean value of 35 ppt*. It was not detected in the air over rural areas in the southeastern part of the state of Washington (USA), by an analytical method whose detection limit was 5 ppt (Grimsrud and Rasmussen, 1975).

Almost no data were found on the concentrations of methylene chloride in surface waters, the oceans or the tissues of living organisms. Concentrations recorded in the Rhine (LWA, 1978, 1980, 1981) were 15 ppb in 1978 and below 1 ppb, the limit of detection, in 1980 and 1981.

2. Degradation

2.1. Breakdown of methylene chloride in the atmosphere.

The most likely atmospheric breakdown pathway is reaction with OH radicals naturally present in the troposphere. As methylene chloride does not absorb in the visible light region (>290 nm), direct photolysis in the atmosphere is improbable (Dilling et al., 1976). From the rate constant for the initiating reaction for breakdown by OH radicals (Cox et al., 1976; Davis et al., 1976 a,b; Howard and Evenson, 1976; Perry et al., 1976; Edwards et al., 1982) and the (varying) concentration of these radicals in the atmosphere (Crutzen, 1974, 1975; Levy, 1974), the atmospheric lifetime of methylene chloride has been calculated to be in the range of 4 to 18 months. Carbon monoxide and hydrogen chloride are the major breakdown products found experimentally.

As methylene chloride has a very low reactivity towards ozone it will

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* 1 ppt = 10^{-12} by volume.

not contribute significantly to photo-chemical smog formation (Derwent et al., 1976). It was therefore exempted by the Environmental Protection Agency from air pollution control regulations (Clean Air Act of 1970 and Amendment of August 1977).

From lifetime values of methylene chloride in the atmosphere it is estimated that 2.5 per cent of the compound released at ground level (conc. 35 ppt: Cox et al.,1976) may reach the stratosphere (Derwent et al.,1976). No significant effects on ozone levels are therefore expected.

2.2. Breakdown of methylene chloride in the hydrosphere.

In theory, methylene chloride can reach rivers and oceans by two routes: directly in aqueous effluents, and indirectly from the atmosphere by dissolution in the ocean and in rainwater. However, it has been shown (Dilling et al., 1975; Dilling, 1977) that evaporation from water is rapid.

Experiments with aerated water containing 1 mg/kg of methylene chloride in sealed glass tubes have shown that hydrolysis is slow (Dilling et al., 1975). In the dark at 25°C, the half-life was found to be about 1.5 years. Similar results were obtained when samples were exposed to normal daylight, outdoors, for one year under ambient temperature cycles, i.e. -20 to + 40°C (Fells and Moelwyn-Hughes, 1958).

Methylene chloride completely biodegrades in the static-culture flask screening test during 7 days incubation. 25% was lost by volatilisation at 25°C (Tabak et al.,1981). Its biodegradability by municipal activated sludge was investigated by Klecka (1981) who found that 100 mg/l of C^{14}-labelled methylene chloride disappeared within 10 hours. After 50 hours incubation, 65% of the parent compound was recovered as C^{14}-labelled CO_2.

3. Toxicity to Aquatic Organisms.

Data are summarised in Table 1 below.

Table 1

Toxicity to Aquatic Organisms

Species	Conditions	LC_{50} $mg.l^{-1}$	References
Daphnia Magna	24 h 48 h	2,270 310	Bringmann and Kuehn,1977 Leblanc, 1980
Golden Orfe	static test	237; 626	Juhnke and Luedemann,1978
Fathead Minnow	96 h static 96 h flow through	310 193	Alexander et al.,1978 Alexander et al.,1978
Bluegill	96 h	220	Buccafusco et al.,1981
Sheepshead Minnow	96 h	330	Heitmuller et al.,1981
Various amphibian species	from fertilisation through 4 days posthatching	18 to 32	Birge et al., 1980
Algae	50% inhibition of photosynthesis at 1500 $mg.l^{-1}$	non toxic	Hutchinson et al., 1980

Although only acute ecotoxicity data are available, they indicate that methylene chloride will pose no toxic hazard to fish and aquatic invertebrates, in situations other than local accidental discharge, because concentrations in environmental waters are likely to be orders of magnitude lower than the acutely toxic concentrations.

4. Bioaccumulation

The logarithm of the calculated n-octanol/water partition coefficient (log Pow) is 1.51 suggesting that methylene chloride will not significantly bioaccumulate in aquatic organisms (Koenemann, 1979).

F. SUMMARY AND CONCLUSIONS

Liquid methylene chloride is painful and irritating when confined on human skin, but it is not a skin sensitiser. Transient corneal burns may result from contact with the eye.

The most common route of human exposure is by inhalation, and gross over-exposure by this route depresses the central nervous system. No clinical effects have been found in workers exposed to concentrations below 100 ppm, the most widely adopted occupational exposure limit.

The acute toxicity to animals is low. Exposure to rather high concentrations induces hepatotoxic effects in animals. In man and animals methylene chloride is biotransformed to give carbon monoxide and hydrogen chloride by the major, saturable metabolic process. Pharmacokinetic models can be used to predict the extent of increase in carboxyhaemoglobin following exposure.

Methylene chloride has no adverse effects on reproduction in rats.

Long-term carcinogenicity studies have been performed by the inhalation route on hamsters and rats, and by administration to rats in drinking water. No tumourigenic effects were found in hamsters exposed to up to 3500 ppm of methylene chloride in air. Male rats similarly exposed at 3,500 ppm (23% of the LC_{50}) developed sarcomas in and around the salivary glands, but the interpretation of this finding is confused by the occurrence of a viral infection of these glands early in the treatment. Female rats showed an increase in benign mammary tumours at and above concentrations of 500 ppm, but the strain of rat used has a high spontaneous incidence of these benign tumours. In the drinking water study, rats showed no carcinogenic response at levels as high as 250 mg/kgbw.

Several mortality studies on exposed workers indicated that they had no higher risk of dying from cancer than did control populations.

Methylene chloride is mutagenic to bacteria, probably because of interference by bacterial metabolism. In view of the negative results in numerous short-term tests (especially the in vivo tests) with a variety of different end-points, methylene chloride cannot be considered as a confirmed mutagen.

From the results of the long-term carcinogenicity bioassays, the overall profile of the substance in short term tests, and the negative epidemiological results, it is concluded that methylene chloride is not likely to induce malignant tumours in man under the normal conditions of occupational exposure.

Methylene chloride can be regarded as non-toxic to aquatic species at concentrations likely to be encountered in the aquatic environment. Because of its high evaporation rate, low octanol/water partition coefficient and rapid biodegradability, the compound is not expected to be present in significant concentrations in the aquatic environment, or to accumulate significantly in aquatic species.

Methylene chloride does not seem likely to contribute to the formation of photo-chemical smog in the air or to affect the ozone layer in the stratosphere, despite the fact that it has a calculated atmospheric lifetime of between 4 and 18 months.

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