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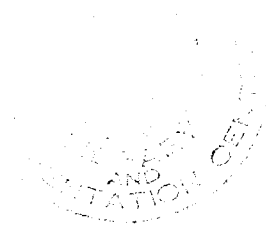
Environmental Health Criteria 32

METHYLENE CHLORIDE

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CHLORIDE

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The WHO Task Group for the Environmental Health Criteria for Methylene Chloride met in Brussels from 19 to 22 September, 1983. Professor A. Lafontaine opened the meeting and welcomed the participants on behalf of the host government, and Dr M. Mercier, Manager, IPCS, on behalf of the heads of the three IPCS co-sponsoring organizations (ILO/WHO/UNEP). The Group reviewed and revised the second draft criteria document and made an evaluation of the health risks of exposure to methylene chloride.

The efforts of Dr G.J. Van Esch and Dr T. Vermeire, who were responsible for the preparation of the draft, and of all who helped in the preparation and the finalization of the document are gratefully acknowledged.

* * *

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PREFACE

A partly-new approach to develop more concise Environmental Health Criteria documents has been adopted with this issue. While the document is based on a comprehensive search of the available, original, scientific literature, only key references have been cited. A detailed data profile and a legal file on methylene chloride can be obtained from the International Register of Potentially Toxic Chemicals, Palais des Nations, 1211 Geneva 10, Switzerland (Telephone No. 988400 - 985850).

The document focuses on describing and evaluating the risks of methylene chloride for human health and the environment.

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Manager, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

1. SUMMARY

Methylene chloride (dichloromethane) is widely used as a multi-purpose solvent and paint remover. The assessment of its toxicity can be complicated by the presence of stabilizers and other solvents, frequently found in commercial products. Methylene chloride can be measured by gas chromatographic techniques at minimum concentrations of approximately $0.02 \mu\text{g}/\text{m}^3$ in air and $0.1 \mu\text{g}/\text{litre}$ in water. Exposure to methylene chloride can be roughly estimated by the determination of its levels in blood or expired air. Exposure to methylene chloride will result in elevated carboxyhaemoglobin levels in blood, which can be measured. However, blood carboxyhaemoglobin levels can give a false picture of exposure when either exercise or smoking is involved.

High concentrations have been measured in industrial indoor environments and during the use of methylene chloride as a paint remover. The general population is exposed to much lower levels of the solvent in ambient air, drinking-water, and food.

About 80% of the world production of methylene chloride is estimated to be released into the atmosphere, but photodegradation takes place at a rate that makes accumulation in the atmosphere unlikely. Initial products are phosgene and carbon monoxide, which are transformed into carbon dioxide and hydrochloric acid. In surface water, volatilization is the major process of removal, hydrolysis and photodegradation being insignificant. The solvent is readily biodegradable, aerobically. Bioaccumulation seems unlikely in the environment. The behaviour of the compound in soil has yet to be determined.

The major route of human exposure is through inhalation. Methylene chloride vapour is rapidly absorbed via the lungs and the gastrointestinal tract, uptake being directly proportional to exposure. It also increases with exercise and with the amount of body fat. The absorbed compound, which is distributed to all body tissues, crosses the placenta and blood-brain barrier. Absorption of liquid methylene chloride via the skin is slow. At current exposure levels, most of the methylene chloride taken up is metabolized to carbon monoxide and probably carbon dioxide, mainly in the liver, kidneys, and lungs. With high exposures, the microsomal cytochrome P-450 enzyme system becomes saturated and some partitioning of unmetabolized methylene chloride may occur in fat. Even at low exposure levels, carboxyhaemoglobin levels in the blood can be sustained for many hours after exposure, because of delayed conversion of methylene chloride from fat.

The toxicity of methylene chloride may be influenced by factors such as exposure to exogenous carbon monoxide, obesity, and an increased workload. The predominant effects of methylene chloride on human beings are elevated carboxyhaemoglobin saturation of the blood and central nervous system depression. The normal levels of blood carboxyhaemoglobin in man are exceeded in non-smoking, sedentary individuals after inhalation exposure to a methylene chloride concentration of 400 mg/m^3 for 7.5 h. The lowest-observed adverse acute effect level, for inhalation exposure of non-smoking, healthy individuals, was approximately 694 mg/m^3 with 1.5 - 3 h of exposure. Some neurobehavioural changes were observed at this exposure level. Two cases of permanent damage to the central nervous system in high, long-term occupational exposures (5 years at 2290 - 12 500 mg/m^3 and 3 years at 1735 - 3470 mg/m^3 , respectively) have been reported. In spite of many reports of fatty degeneration in the liver and tubular degeneration in the kidneys of animals, there is no clear evidence of liver or kidney damage in human beings.

The vapour is moderately irritating to the eyes and respiratory tract while the liquid is irritating to the skin. Patients with heart disease may be at increased risk if exposed to high levels of methylene chloride; this may particularly occur during the use of paint removers.

Results of in vitro studies showed that methylene chloride was weakly mutagenic in bacteria and fungi. Some mutagenic effects were also observed in Drosophila melanogaster, but the results of most tests on mammalian somatic cells, including human cells, were negative.

In 2 inhalation studies on rats, the incidence of benign mammary tumours was not increased in exposed male or female rats compared with controls, but the total number of mammary tumours in treated animals increased in a dose-related manner. In the study, an increase in salivary gland region sarcomas was found in male rats. In Golden Syrian hamsters, no significant increases in tumour incidences were found. In a drinking-water study with rats and mice, no significant increases in tumour incidences were found, while an increased incidence of foci or areas of altered liver cells was observed.

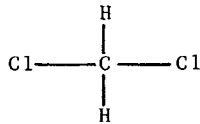
In 2 human epidemiological mortality studies, there was no excess mortality due to cancer compared with control populations. Animal experimental data and human epidemiological data are inadequate for assessing whether or not methylene chloride should be considered carcinogenic for animals and man.

There is only limited evidence that methylene chloride is teratogenic in animals.

2. PROPERTIES AND ANALYTICAL METHODS

2.1 Chemical and Physical Properties of Methylene Chloride

Methylene chloride (CH_2Cl_2) is nonflammable and nonexplosive when mixed with air. It hydrolyses very slowly in the presence of moisture. It also reacts with hydroxyl radicals. No appreciable decomposition occurs at room temperature when the dry compound comes into contact with common metals. Phosgene and hydrochloric acid are formed by contact with hot surfaces or flames.



CAS registry number: 75-09-2

RTECS registry number: PA 8050000

Common synonyms: DCM, dichloromethane, methane dichloride, methylene bichloride, methylene dichloride, methylenum chloratum

Trade names: Aerothene MM, Freon 30, Narkotil, Solaesthin, Solmethine

Some physical data on methylene chloride

physical state	liquid
colour	colourless
odour	ethereal
relative molecular mass	84.93
melting point	-95 °C
boiling point	40 °C
water solubility	20 g/litre, 20 °C
log n-octanol-water partition coefficient	1.25
density	1.33 g/ml, 20 °C
relative vapour density	2.93
vapour pressure	46.52 kPa (349 mm Hg) at 20 °C
surface tension	28.12 dyne/cm at 20 °C
flame limits	0.5-2.3 g/litre in oxygen 0.5-0.8 g/litre in air

Conversion factors for methylene chloride and carbon monoxide:

methylene chloride	1 ppm = 3.47 mg/m ³ air
carbon monoxide	1 ppm = 1.14 mg/m ³ air

2.2 Analytical Methods

A summary of methods for the sampling and determination of methylene chloride in air, water, sediments, food, breath, blood, and urine is presented in Table 1.

Table 1. Sampling, preparation, analysis

Medium	Specification	Sampling method	Analytical method	Detection limit	Comments	References
air	occupational	on charcoal desorption with carbon disulfide	gas chromatography with flame ionization detection	10 µg per sample (1 litre sample)	recommended range 350 - 10 400 mg/m ³ (1 litre sample)	White et al. (1970) NIOSH (1984)
air	occupational		direct reading detector tube		a non-specific cheap method to estimate exposure	Saltzman (1972)
air	occupational		infra-red spectroscopy		continuous monitoring and breath analysis	Baretta et al. (1969)
air	occupational		photodetection	3-6 mg/m ³ (for CCl ₄)	suitable for continuous monitoring when methylene chloride is the only contaminant	Nelson & Shapiro (1971)
air	ambient		gas chromatography and mass spectrometry	0.017 µg/m ³	direct analysis	Grimsrud & Rasmussen (1975)

Table 1 (contd).

Medium	Specification	Sampling method	Analytical method	Detection limit	Comments	References
water	drinking-water		gas chromatography with electrolyte conductivity detection	0.1 µg/litre	sparging, trapping in line on gas chromatography column	Nicholson et al. (1977)
water	drinking-water		gas chromatography and mass spectrometry	0.2 µg/litre	direct aqueous injection, diglycerol as liquid phase on precolumn	Fujii (1977)
sediment		cooling, extraction with pentane	gas chromatography with flame ionization detection	0.1-1 mg/litre	also used for drinking-water analysis	Dietz & Traud (1973)
food		extraction by vacuum distillation washing	gas chromatography with electron capture detection	1 mg/kg	analysis of spice oleoresins	Page & Kennedy (1975)
blood urine breath			gas chromatography with flame-ionization detection	0.7 mg/m ³	head-space analysis (blood, urine), direct analysis (breath)	Di Vincenzo et al (1971)

3. PRODUCTION, USES, DISPOSAL OF WASTES; ENVIRONMENTAL TRANSPORT AND DISTRIBUTION

3.1 Production, Uses, Disposal of Wastes

3.1.1 Production levels and processes

World production in 1980 amounted to 570 kilotonnes of which 270 kilotonnes were produced in Western Europe (CEFIC, 1983). In the USA, production increased from 180 kilotonnes in 1971 (Gordon, 1976) to 254 kilotonnes in 1980 (IRPTC; 1984) and 269 kilotonnes in 1981 (USITC, 1982). In Japan, 35 kilotonnes were produced in 1980 (IRPTC, 1984).

Two processes are important: the chlorination of methyl chloride, obtained from the reaction of methanol and hydrogen chloride, and the direct chlorination of methane (IARC, 1979).

Additives may include 0.0001 - 1% of stabilizers such as: amines, 4-cresol, hydroquinone, methanol, 2-methylbut-2-ene, 1-naphthol, nitromethane + 1,4 dioxane, phenol, resorcinol, and thymol.

3.1.2 Uses

Methylene chloride is used as: a solvent, a blowing agent for polyurethane, a component of paint remover, a degreasing solvent, and as a propellant in aerosols such as insecticides, hair sprays, shampoos, and paints. Methylene chloride is being increasingly used as a replacement for fluorocarbons in aerosols. As a solvent, it is used in pharmaceutical applications, in the manufacture of photographic and synthetic fibres, and, as an extraction solvent, for naturally-occurring, heat-sensitive substances such as edible fats, cocoa, butter, caffeine, and beer flavouring in hops. It is also used as a component in fire-extinguishing products, as an insecticidal fumigant for grains, and as a coolant and refrigerant (Gordon, 1976; IARC, 1979).

3.1.3 Disposal of wastes

Methylene chloride can be destroyed by incineration, sometimes after adsorption by activated carbon. End products are carbon dioxide, water, and hydrochloric acid, which can be recovered (Gordon, 1976).

3.2 Environmental Transport and Distribution

Approximately 80% of the world production of methylene chloride is emitted into the atmosphere during its use as a

solvent, and in paint removers, aerosols, solvent degreasers, and fumigants. Minor losses occur during production and shipping. In the USA alone, emissions in 1975 amounted to 177 kilotonnes (Gordon, 1976). Volatilization also appears to be the major process by which methylene chloride is lost from water (Dilling et al., 1975). Under field conditions, half-lives of 33 and 38 days were estimated for river water (Zoeteman et al., 1980).

Once in the troposphere, hydroxyl radicals can attack the compound yielding mainly carbon dioxide and hydrogen chloride and minor quantities of carbon monoxide and phosgene (Pearson & McConnell, 1975; Cox et al., 1976; Spence et al., 1976). Phosgene readily hydrolyses to hydrochloric acid and carbon dioxide. Cox et al. (1976) estimated a lifetime for methylene chloride in the troposphere of 0.3 years, with respect to oxidation by hydroxyl radicals. Photodegradation and hydrolysis in water do not seem to take place to any significant extent (Dilling et al., 1975). Methylene chloride is absorbed on dry bentonite clay and peat moss but not significantly on limestone and silica sand (Dilling et al., 1975).

The available reports show that methylene chloride is readily biodegradable. The compound was rapidly degraded aerobically by microorganisms from settled domestic waste water containing methylene chloride concentrations of 5 and 10 mg/litre (Tabak et al., 1981). After adaptation, sewage microorganisms and a Pseudomonas species were found to degrade methylene chloride aerobically and to use it for growth at concentrations below 425 mg/litre (Brunner et al., 1980; Rittman & McCarty, 1980). Similar results were obtained by Stucki et al. (1981) using hyphomicrobium species. Methylene chloride was dehalogenated by aerobic microorganisms from municipal activated sludge yielding carbon dioxide and chloride, after adaptation only. The compound was toxic above a concentration of 1000 mg/litre (Klechka, 1982).

4. ENVIRONMENTAL LEVELS AND EXPOSURES

It can be seen from the uses and the physical and chemical properties of methylene chloride that the main route of human exposure is through vapour inhalation, sometimes accompanied by direct skin and eye contact, both at the place of work and at home. Much lower levels of human exposure can occur through inhalation of methylene chloride in ambient air and through its ingestion via drinking-water, food, and beverages.

4.1 Air

The background concentration of methylene chloride at surface level at 40 °N latitude was found to be about 0.12 $\mu\text{g}/\text{m}^3$ by Cox et al., (1976) and 0.17 $\mu\text{g}/\text{m}^3$ by Singh et al., (1982). In the air of 7 cities in the USA during 24-h sampling periods, concentrations ranged between 0.17 and 196.75 $\mu\text{g}/\text{m}^3$, while average concentrations varied from 1.35 to 6.76 $\mu\text{g}/\text{m}^3$ (Singh et al., 1982). The highest detected concentrations in drinking-water have been less than 5 $\mu\text{g}/\text{litre}$ (Saunders et al., 1975; Fujii, 1977; US National Academy of Science, 1977).

4.2 Water

Few reports contain data concerning the occurrence of methylene chloride in natural waters. In a survey in the USA, 8% of finished-water supplies tested contained methylene chloride, but only 1% of the raw-water supplies (US National Academy of Sciences, 1977). Water from a sewage treatment plant contained a methylene chloride concentration of 8.2 $\mu\text{g}/\text{litre}$ before treatment, 2.9 $\mu\text{g}/\text{litre}$ after treatment but before chlorination, and 3.4 $\mu\text{g}/\text{litre}$ after chlorination (Bellar et al., 1974). These results show that methylene chloride is formed during the chlorination of water. Concentrations of 1 - 2 $\mu\text{g}/\text{litre}$ (Bauer, 1978) and 5 $\mu\text{g}/\text{litre}$ (Zoeteman et al., 1980) were reported at the same point in the river Rhine.

4.3 Food

One report is available describing the occurrence of the extractant methylene chloride in 15 out of 17 spice-oleoresins at levels between 1 and 83 mg/kg wet weight (Page & Kennedy, 1975).

4.4 Occupational Exposure

Methylene chloride exposure was investigated in a variety of jobs in the USA including: servicing of diesel engines, spray-painting of booths, plastic tank construction, ski manufacture, cleaning foam heads, and cleaning nozzles in plastic manufacture. The concentrations ranged from below the detection limit to 257 mg/m³ air. In a chemical plant, an 8 h, time-weighted average exposure was measured of 3040 mg/m³ with an exposure range from below the detection limit to 19 150 mg/m³ (NIOSH, 1976). In cellulose-acetate-fibre-producing plants in Czechoslovakia and the USA, methylene chloride concentrations in air in a total of 335 samples ranged from 100 to 17 000 mg/m³ (Kuzelova & Vlasak, 1966; NIOSH, 1976). The median 8-h, time-weighted average concentrations in another plant producing cellulose acetate fibre in the USA, ranged from 280 to 1650 mg/m³ (Ott et al., 1983). In a beauty salon, where methylene chloride exposure stemmed from its use as an aerosol propellant in sprays, daily mean background concentrations were below 6.9 mg/m³, while peak concentrations of 451 mg/m³ were reached, directly after spraying coiffures (Hoffman, 1973).

4.5 Controlled Exposure

Methylene chloride is widely used at home in paint removers and aerosol sprays. Most paint-stripping formulations contain about 80% by weight of methylene chloride, often in combination with methanol. Breathing zone concentrations were measured during the use of a paint remover under controlled conditions with normal ventilation, (70 m³/h). The maximum concentration was 4430 mg/m³ during the 3 h studies, the averages, in various experiments, ranging between 2270 and 2730 mg/m³ (Stewart & Hake, 1976).

In a detailed study on exposure levels during the use of paint removers, the time-weighted averages in a room without ventilation varied between 460 and 2980 mg/m³ during the first 6 h following application. Grab samples showed levels of up to 3410 mg/m³, 30 min after application. In studies with the door open, the levels were between 60 and 490 mg/m³ (Otson et al., 1981).

5. CHEMOBIOKINETICS AND METABOLISM

5.1 Absorption

5.1.1 Animal studies

From the moment of application, dermal absorption of liquid methylene chloride in mice increased linearly with time at a rate of 0.1 mg per cm² per min (Tsuruta, 1975). Rapid absorption occurred in rats after both oral ingestion and inhalation of methylene chloride. A steady state plasma concentration, attained after 2 h of vapour exposure, was not proportional to the exposure level at low concentrations (McKenna et al., 1982). Almost directly after oral application, peak concentrations of methylene chloride could be detected in expired air (McKenna & Zempel, 1981). In rats, methylene chloride readily passed the placenta (Anders & Sunram, 1982) and the blood-brain barrier (e.g., Fodor & Winneke, 1971).

5.1.2 Human studies

Dermal exposure of volunteers to liquid methylene chloride resulted in maximum levels of the compound in expired air, 30 min after exposure (Stewart & Dodd, 1964). After 0.5 - 8 h of inhalation exposure to concentrations ranging from 173 to 1740 mg/m³, blood and expired air concentrations and thus, total uptake of methylene chloride, were found to be directly proportional to the magnitude of exposure, in sedentary individuals. The concentration in blood increased gradually and appeared to be slowly reaching a plateau over 8 h. In the lungs, the uptake was rapid and remained almost constant after 1 h of exposure. The concentration in expired air at the end of the respiration cycle was 2.3 - 2.8 times lower than that in inspired air. Repeated exposures did not result in higher blood or expired-air levels. The uptake varied between 55 and 75% of the total exposure in sedentary individuals. The absolute uptake increased during exercise, but uptake relative to total exposure decreased (DiVincenzo et al., 1972; Astrand et al., 1975; DiVincenzo & Kaplan, 1981a,b). In another study on human volunteers, the uptake of methylene chloride, at steady-state with respect to expired air within 2.5 - 3 h in 6-h exposures, seemed to deviate from a linear increase when the exposure level was above 690 - 870 mg/m³ (McKenna et al., 1980). The absolute uptake in a 1-h exposure to 2600 mg/m³ was proportional to the body weight and to the amount of body fat. The uptake in relation to total exposure was almost the same for both slim and obese persons,

reflecting an increase in respiratory volume with body weight (Engström & Bjurström, 1977). Methylene chloride was found to cross the placenta (Vosovaja et al., 1974) and the blood-brain barrier (e.g., Pütz et al., 1976).

5.2 Distribution

5.2.1 Animal studies

Forty-eight hours after a single oral ingestion of labelled methylene chloride at 1 mg/kg body weight or inhalation during 6 h at 170 mg/m³ by rats, the percentages of radioactivity in the skin and carcass were 7.5% and 30% of the body burden, respectively.

At higher exposures, retention was less. Unmetabolized methylene chloride was not detected. In rats and mice, most radioactivity was retained in the liver, kidneys, and lung (Bergman, 1979; McKenna & Zempel, 1981; McKenna et al., 1982). Body autoradiography in mice also indicated metabolites in tissues with a high rate of protein synthesis such as the pancreas, thymus, and salivary glands (Bergman, 1979). Directly after short vapour exposures, the highest amounts of volatile radioactivity, which probably represented unmetabolized methylene chloride and were found in the adipose tissue, brain, and blood, diminished rapidly within 2 h (Carlsson & Hultengren, 1975; Bergman, 1979). After repeated exposure of rats to methylene chloride concentrations in air of 1735 mg/m³ and 3470 mg/m³ during a 2-week period, the concentration of the compound in perirenal fat increased, while that in the brain decreased. Exposure to a time-weighted average concentration of 3470 mg/m³, involving short intervals at high concentrations, resulted in greater accumulation than exposure to a constant concentration of 3470 mg/m³ (Savolainen et al., 1981).

In liver microsomes of rat, methylene chloride was found to be bound to lipids and proteins, but only after metabolic activation in the presence of NADPH and oxygen. Pretreatment of rats with phenobarbital increased the binding (Anders et al., 1977). In whole liver cells, this binding was enhanced by oxygen and decreased by phenobarbital pretreatment or glutathione depletion. Nucleic acids were not alkylated (Cunningham et al., 1981). In vivo binding of labelled metabolites was observed in rat liver. Radioactivity was mainly found in the acid-soluble and protein fractions and, to a lesser extent in the lipid and nucleic acids fractions. The labelling pattern was similar to that of formaldehyde (Reynolds & Yee, 1967).

5.2.2. Human studies

The average concentration of methylene chloride in the adipose tissue of obese men was found to decline from 10.2 to 1.6 mg/kg, between 1 and 22 h after a single, 1-h exposure to 2600 mg/m³. The concentration was not measured just after exposure. Even though obese subjects had lower concentrations of methylene chloride in adipose tissue than slim subjects, the former had a greater fraction of the uptake in their adipose tissue (Engström & Bjurström, 1977).

5.3 Metabolic Transformation

5.3.1 Animal studies

The metabolism of methylene chloride was found to be a saturable process. Forty-eight hours after inhalation, 55% of the uptake was expired unchanged at 4920 mg/m³, 30% at 1700 mg/m³, and 5% at 170 mg/m³, respectively (McKenna et al., 1982).

In these studies, the major metabolites were carbon monoxide and carbon dioxide found in expired air. This was also observed in other studies with rats, mice, and rabbits (e.g., Kubic et al., 1974; Roth et al., 1975; McKenna & Zempel, 1981). McKenna et al (1982) reported that, after inhalation exposure, about 60% of all metabolites represented these 2 compounds at all exposures, without a clear predominance of either one of them. After oral application, this value was about 80% (McKenna & Zempel, 1981). The balance was mostly retained as unidentified metabolites in the carcass and skin, while small quantities of unidentified metabolites were recovered in the urine and faeces.

Rats inhaling a low dose of labelled methylene chloride in a closed rebreathing system excreted 47% of the administered label as carbon monoxide and 29% as carbon dioxide. No radioactivity was detected in the carcass. The initial rate of carbon monoxide production was constant at all exposures; this also points to a saturable enzymatic conversion (Rodkey & Collison, 1977a,b).

Endogenous carbon monoxide production following exposure to methylene chloride leads to accumulation of carboxyhaemoglobin in blood. In a study on rats, McKenna et al. (1982) measured steady-state carboxyhaemoglobin levels of 3 and 10 - 13% of saturation, respectively, after 1 h of exposure to 173 mg/m³ and after 2.5 - 3 h of exposure to 1730 and 5200 mg/m³. The carboxyhaemoglobin levels in the blood of rats rose with increasing exposure until a plateau was reached at about 12% of saturation (Fodor et al., 1973). This was also found during long-term exposures (Burek et al., 1984).

5.3.1.1 Enzyme pathway

Two pathways that have been proposed on the basis of in vitro experiments are:

(a) The conversion of methylene chloride to carbon monoxide by the hepatic microsomal cytochrome P-450 1-dependent mixed function oxidase system (Kubic & Anders, 1975, 1978; Jongen et al., 1982). It is proposed that the metabolism starts by a rate-limiting oxygen insertion, followed by rearrangement to formyl chloride, which decomposes to carbon monoxide. The formyl chloride may be involved in macromolecular binding. Phenobarbital pretreatment increases binding to cytochrome P-450 and the rate of conversion in vitro, but not in vivo (Haun et al., 1972; Kubic et al., 1974; Roth et al., 1975).

(b) The conversion of methylene chloride to formaldehyde, formic acid, and chloride by the hepatic cytosol fraction (Ahmed & Anders, 1976, 1978; Jongen et al., 1982). In this conversion, it is proposed that binding of glutathione to methylene chloride is followed by hydrolysis via glutathione transferase (EC 2.5.1.18). Carbon dioxide will be the main product. The resulting S-hydroxymethyl glutathione may yield formaldehyde or formic acid. Formic acid may inhibit cytochrome c oxidase (EC 1.9.3.1) (Nicholls, 1975).

5.3.2 Human studies

DiVincenzo & Kaplan (1981a,b) exposed volunteers for 7.5 h to concentrations of methylene chloride up to 694 mg/m³. Less than 5% of the uptake was excreted unchanged via the lungs, while 30% of the metabolized methylene chloride was converted to carbon monoxide. It was suggested that the rest was converted to carbon dioxide. Exercise increased both the biotransformation to carbon monoxide and the blood carboxyhaemoglobin levels. An increased workload did not further elevate the carboxyhaemoglobin levels, because of increased excretion of carbon monoxide. Smoking had a additive effect on the carboxyhaemoglobin values, as was found with carbon monoxide exposure by Fodor et al. (1973).

Blood carboxyhaemoglobin levels increased in direct proportion to the level and the duration of exposure up to 694 mg/m³. Thereafter, a plateau seemed to be reached. Peak carboxyhaemoglobin saturation was reached, either at the end of the exposure or, at high uptakes, shortly afterwards. Nonsmoking subjects have control values between 0.4 and 2.0% of saturation, while smokers generally have control values between 2 and 8%. An 8-h exposure of non-smokers to a methylene chloride concentration of 350 mg/m³ appeared

equivalent to an 8-h exposure to a carbon monoxide concentration of 43 mg/m³, leading to a carboxyhaemoglobin level of 5% of saturation (Stewart et al., 1972; Fodor & Roscavanu, 1976; Stewart & Hake, 1976; DiVincenzo & Kaplan, 1981a). During repeated exposures to methylene chloride over 5 working days, carboxyhaemoglobin levels did not show any, or only a slight increase, compared with levels following single exposures, and returned to pre-exposure levels over the weekend. Occupational exposure of non-smokers to a time-weighted average of 114 mg/m³ resulted in carboxyhaemoglobin levels of between 0.8 and 2.5% of saturation (DiVincenzo & Kaplan, 1981a; Fodor & Roscavanu, 1976). Residual elevated carboxyhaemoglobin levels associated with, and proportional to, the level of previous-day exposure to methylene chloride was found both in smoking and non-smoking industrial workers, in equal measure. In this epidemiological study, the dose-related increases in the carboxyhaemoglobin levels and alveolar carbon monoxide concentration were correlated with a decrease in the oxygen half-saturation pressure. Sex, race, and age of the subjects were found to be unimportant in predicting carboxyhaemoglobin levels in contrast to smoking and time of venipuncture (Ott et al., 1983). In the blood of workers exposed to methylene chloride concentrations ranging from 552 to 760 mg/m³, but not to carbon monoxide, the carboxyhaemoglobin levels rose to 8.3% after 8 h and dropped to base-line values of about 4.5% at the start of the new work day (Ratney et al., 1974). Kuzelova & Vlasak (1966) detected formic acid in the urine of workers exposed to methylene chloride for long periods.

5.4 Excretion

5.4.1 Animal studies

The concentrations of methylene chloride in the blood and expired air of rats and dogs, after exposure to methylene chloride, declined exponentially and were directly proportional to the level of exposure (DiVincenzo et al., 1972; McKenna & Zempel, 1981; McKenna et al., 1982). In rats, the excretion from blood after inhalation was resolved in a fast and a slow first order process with half-lives of respectively 2 and 15 min. The fast and slow processes in the excretion in expired air of rats, after oral ingestion, had half-lives of 13 and 46 min. After a high oral dose of 50 mg/kg body weight, the concentration of methylene chloride in the expired air was constant for 1 h and then declined. The excretion of carbon dioxide and carbon monoxide after oral intake and inhalation also followed 2 first order processes

with half-lives of 1.4 - 2.7 h for the first 24 h after exposure and of 6.7 - 17.3 h, thereafter.

The disappearance of carboxyhaemoglobin from the blood was exponential with a half-life of 23 - 35 min. At high exposures, blood carboxyhaemoglobin levels remained elevated for 60 - 90 min following exposure (McKenna & Zempel, 1981; McKenna et al., 1982).

5.4.2 Human studies

After exposure, expired air and blood concentrations of methylene chloride measured were directly proportional to the concentration of the vapour. Elimination of methylene chloride occurred rapidly, mainly through expiration. An initially rapid phase of elimination had a half-life of less than 1 min. The elimination of carbon monoxide in the expired air and of carboxyhaemoglobin from blood was more gradual and returned to pre-exposure values about 24 h after exposure to up to 694 mg/m³ for 7.5 h. The half-lives for the observed 2 phases of elimination were reported to be 1.5 h and 10 - 15 h, respectively (McKenna et al., 1980; DiVincenzo & Kaplan, 1981b). Pulmonary excretion of carbon monoxide increased when exposure was continued with exercise. Excretion of methylene chloride via the urine was negligible (DiVincenzo & Kaplan, 1981b). In contrast, Stewart et al. (1976) reported a biological half-life following exogenous carbon monoxide exposure of only 5 h. Moreover, after short exposures to 1740 mg/m³, blood carboxyhaemoglobin levels continued to rise for several hours (Astrand et al., 1975). These data suggest a delayed conversion of methylene chloride from fat (Engström & Bjurström, 1977). Methylene chloride has been found in breast milk (Vosovaja et al., 1974).

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

A summary of studies on the acute toxicity of methylene chloride in aquatic organisms is presented in Table 2.

No experimental bioconcentration factor was available, but the low log *n*-octanol-water partition coefficient of 1.25 (Hansch et al., 1975) and the rather high water solubility suggest that bioaccumulation is very limited. A bioconcentration factor in fish of 5 can be calculated according to the method of Veith et al. (1980).

Table 2. Acute aquatic toxicity

Organism	Description	t(°C)	pH	Dissolved oxygen (mg/litre)	Hardness (mgCaCO ₃ /litre)	Flow/l stat	Parameter	Concentration mg/litre	Reference
algae	<u>Chlorella vul-garis</u> , <u>Chlamydomonas angulosa</u>	19	6.5				3-h EC50	27 000 and 17 400 respectively	Hutchinson et al. (1978) ¹
crustacea	water flea (<u>Daphnia magna</u>)	22	7.4-9.4	6.5-9.1	173	stat	48-h LC50 no-observed adverse-effect level	220 68	Le Blanc (1980) ²
fish	fathead minnow (<u>Pimephales promelas</u>)	12	7.8-8.0	>5.0		stat	96-h LC50	310	Alexander et al. (1978) ³
fish	fathead minnow, (<u>Pimephales promelas</u>)	12	7.8-8.0	>5.0		flow	96-h LC50 96-h EC50	193 99	Alexander et al. (1978) ³

Table 2 (contd).

Organism	Description	t(°C)	pH	Dissolved oxygen (mg/litre)	Hardness (mgCaCO ₃ /litre)	Flow/l stat	Parameter	Concentration mg/litre	Reference
fish	bluegill sunfish (<u>Lepomis macrochirus</u>)	21-23	7.9-6.5	9.7-3.0	32-48	stat	96-h LC50	220	Buccafusco et al. (1981) ²
fish	sheepshead minnow (<u>Cyprinodon variegatus</u>)	25-31				stat	96-h LC50 no-observed adverse- effect level	330 130	Heitmuller et al. (1981) ⁴

Notes

- 1) Flow through or static method.
- 2) EC50 for growth inhibition by determination of ¹⁴C0₂ uptake, no analysis for methylene chloride reported.
- 3) 15 daphnias/concentration, < 24 h of age, no analysis for methylene chloride reported.
- 4) Dechlorinated, sterilized lake water, analysis for methylene chloride by gas chromatography.
- 5) 10 juvenile fish/concentration, deionized reconstituted water; no aeration, no analysis for methylene chloride reported.
- 6) 10 juvenile fish/concentration, sea water with salinity of 1.0 - 3.1‰, no aeration, no analysis for methylene chloride reported.

7. EFFECTS ON ANIMALS

7.1 Short-Term Exposures

In short-term exposure studies, effects on organs after inhalation of methylene chloride are mainly limited to the liver, kidneys, and heart but central nervous system depression also occurs. There are not sufficient data to indicate definitely the effects after oral and dermal exposure. Acute mortality data are shown in Table 3.

Table 3. Acute mortality after oral intake or inhalation of methylene chloride

Species	Route	Vehicle	Parameter studied	Value	Reference
rat	oral	none	LD ₅₀	3000 mg/kg body weight	Kimura et al. (1971)
rat	inhalation	-	2-h LC ₅₀	79 000 mg/m ³	Kashin et al. (1980)
rat	inhalation	-	6-h LC ₅₀	52 000 mg/m ³	Bonnet et al. (1980)
mouse	inhalation	-	7-h LC ₅₀	56 230 mg/m ³	Svirbely et al. (1947)
mouse	inhalation	-	6-h LC ₅₀	49 100 mg/m ³	Gradiski et al. (1978)
mouse	inhalation	-	2-h LC ₅₀	51 500 mg/m ³	Kashin et al. (1980)
dog	oral	mucilage of acacia	LD ₅₀	3000mg/kg body weight	Barsoum & Saad (1934)
guinea-pig	inhalation	-	6-h LC ₅₀	40 200 mg/m ³	Balmer et al. (1976)

The slope of the regression line giving the probability units of the percentage mortality as a function of the logarithm of the concentration is rather steep for both rats and mice, the difference between the LC₁₀ and the LC₉₀ being less than 14 000 mg/m³ (Gradiski et al., 1978; Bonnet et al., 1980).

7.1.1 Inhalation exposure

No macroscopic lesions were found in rats at the 6-h LC₅₀ of 52 000 mg/m³ (Bonnet et al., 1980). After 6 h of exposure to 17 350 mg/m³, the concentration of triglycerides was increased in the liver of guinea-pigs, and reduced in the serum (Balmer et al., 1976; Morris et al., 1979). Histopathological liver changes, consisting of the appearance of lipid droplets, were first seen in guinea-pigs at 18 000 mg/m³ (Morris et al., 1979). Slight to moderate vacuolization in the liver was seen after 6 h at 38 520 mg/m³. In addition, lungs showed congestion and haemorrhage; behavioural changes were also noted (Balmer et al., 1976). Heppel et al. (1944) did not find organ lesions related to exposure at 17 350 mg/m³ in studies on dogs, monkeys, rats, rabbits, and guinea-pigs, with the exception of moderate centrilobular fatty degeneration of the liver and pneumonia in 3 out of 14 guinea-pigs. At 34 700 mg/m³, dogs also showed fatty degeneration.

After continuous exposure to methylene chloride concentrations of 87 and 347 mg/m³ for 100 days, slight cytoplasmatic vacuolization with positive fat stains were noted in livers of rats as well as tubular degeneration in kidneys. Similar changes and a decrease in the microsomal cytochrome P-450 content were found in the livers of mice exposed to a concentration of 347 mg/m³ (Haun et al., 1972).

Similar changes in the liver were found in dogs and monkeys exposed continuously to a methylene chloride concentration of 3470 mg/m³. The dogs also showed vacuolar changes in the renal tubules. After 4 weeks, they exhibited abnormal haematology, increased activities of serum glutamic pyruvic transaminase (SGPT) (EC 2.6.1.2), isocitric dehydrogenase (EC 1.1.1.41), and bromosulphthalein (BSP) retention. Additional effects at 17 350 mg/m³ were oedema of the brain in dogs and encephalomalacia in monkeys (Haun et al., 1972).

The effects on the mouse liver were studied microscopically by Weinstein & Diamond (1972) and Weinstein et al. (1972) during continuous exposures. At 347 mg/m³, fatty infiltration, vacuolization, and enlarged nuclei persisted up to the end of the 10-week exposure, while an increase in triglycerides concentration was reversible. At 17 350 mg/m³, body weights fell, and relative liver weights increased up to the end of the 168-h exposure. Fatty infiltration, an increase in the triglycerides concentration, and hydropic degeneration of the endoplasmic reticulum gradually disappeared. Protein synthesis was depressed. Necrosis was observed in a few hepatocytes.

No consistent increase of the total liver microsomal concentration of cytochrome P-450 was found after repeated exposure of rats, but the metabolic activity of liver microsomal enzymes increased in vitro and in vivo (Norpoth et al., 1974; Toftgard et al., 1982; Kurppa & Vainio, 1981).

Cardiac effects such as arrhythmia, tachycardia, and hypotension was found in monkeys and rabbits exposed for 1 - 5 min to levels of methylene chloride exceeding 60 000 mg/m³ (Belej et al., 1974; Taylor et al., 1976).

Central nervous system depression was noted in dogs, monkeys, rats, rabbits, and guinea-pigs during each daily session of repeated exposure to a methylene chloride concentration of 34 700 mg/m³ for 7 h/day, 5 days per week, for 6 months. All animals became inactive, some time after initial excitement (Heppel et al., 1944). Mice continuously exposed to a level of 17 350 mg/m³ showed decreased activity, and water and food intake, and changes in appearance, which disappeared after 168 h (Weinstein & Diamond, 1972).

Central nervous system depression resulting in reversible narcosis occurred in dogs, mice, and guinea-pigs after 2 - 6 h of exposure to levels of methylene chloride between 13 900 and 20 800 mg/m³ (Flury & Zernick, 1931). During exposure for 1.5 h to 17 350 mg/m³, rats showed decreased running activity (Heppel & Neal, 1944). The sleep-wakefulness patterns were disturbed in rats from a level of 3470 mg/m³ upwards, as shown mainly by a reduction in Rapid-Eye Movement sleep (Fodor & Winneke, 1971).

7.1.2 Oral exposure

A single oral dose of 1000 mg/kg body weight resulted in a decreased cytochrome P-450 content in liver microsomes of rats (Moody et al., 1981).

Rats, receiving methylene chloride in the drinking-water at a concentration of 125 mg/litre for 13 weeks, did not show any effects on behaviour, body weight, haematology, urinalysis, blood glucose, plasma-free fatty acids, and the estrus cycle (Bornmann & Loeser, 1967).

7.1.3 Intraperitoneal exposure

One intraperitoneal injection of methylene chloride at 510 mg/kg body weight in rats, slowed down the sciatic motor conduction velocity by 11%, and gave rise to a carboxy-haemoglobin level of 6.8% of saturation (Pankow et al., 1979).

7.1.4 Effects on the eye and skin

Duprat et al. (1976) and Ballantyne et al. (1976) exposed rabbits once to 0.5 ml of methylene chloride by ocular instillation. Moderate to severe changes were seen in the conjunctiva, together with increased corneal thickness and intra-ocular tension. All effects were reversible. Vapour exposure of the eyes caused slight increases in corneal thickness and intra-ocular tension.

Application of methylene chloride to the skin of rabbits caused severe erythema and oedema with necrosis and acanthosis (Duprat et al., 1976).

7.2 Long-Term Exposure and Carcinogenicity

7.2.1 Inhalation exposure

Groups of 129 male and 129 female Sprague Dawley rats and 107 - 109 male and 107 - 109 female Golden Syrian hamsters were exposed to methylene chloride (99% pure) at 0, 1730, 5200, and 12 100 mg/m³ for 2 years, 6 h/day and 5 days per week (Burek et al., 1984). The survival rate of high-exposure female rats was reduced. Slight exposure-related effects consistent with fatty infiltration were seen in the livers of both sexes at all exposures. Mean corpuscular volume, mean corpuscular haemoglobin, and carboxyhaemoglobin were increased in both sexes at all exposures. An increased, dose-related incidence of salivary gland region sarcomas was observed in males at the 2 highest exposures (the authors assume that the effect might have been due to the combination of viral infection and methylene chloride exposure). The total number of benign mammary tumours in the rats increased in a dose-related manner in both sexes, most pronouncedly in females. It was noted that the Sprague-Dawley rats used in this study have a very high incidence of spontaneous mammary tumours. This incidence was not increased in exposed rats compared to controls.

In hamsters, elevated haematocrit, haemoglobin levels (both dose-related), mean corpuscular volume, mean corpuscular haemoglobin, and carboxyhaemoglobin were found in both sexes. There was no significant increase in the incidence of tumours.

Groups of 90 male and 90 female Sprague Dawley rats were exposed to 99.5% pure methylene chloride at 0, 173, 694, and 1730 mg/m³ for 20 (male) or 24 (female) months, 6 h per day and 5 days per week. Two additional groups of 30 female rats were each exposed for 6 months to 1730 mg/m³ followed by 6 months without exposure and vice versa.

There was an increase in the incidence of foci of altered hepatocytes at 1730 mg/m³, in surviving females, and at 694

and 1730 mg/m³, in surviving males. When these liver alterations in interim kills and end kills were combined, no increase was found. Hepatocellular vacuolization was noted in males and females receiving high doses and multinucleated hepatocytes in females receiving high doses. The number of female rats with benign mammary tumours was not increased, but the total number of mammary tumours in the female rats was increased at 1730 mg/m³ (Nitschke et al., 1983).

7.2.2 Oral exposure

Groups of 85 male Fischer 344 rats received 99% pure methylene chloride in the drinking-water for 24 months at levels of 6, 52, 125, and 235 mg/kg body weight per day. Groups of 85 female rats received 6, 58, 136, and 263 mg/kg body weight per day. Duplicate control groups comprised a total of 135 rats of each sex. A high dose recovery group was only treated for 18 months.

Slight reductions in weight gain, water intake, and food consumption were found at the 2 highest doses. Survival was not affected. Dose-related increases were noted in mean haematocrit, haemoglobin levels, and red blood cell counts at the 3 highest doses. Decreases in serum alkaline phosphatase (EC 3.1.3.1) activity in males and in creatinine, blood urea nitrogen, serum-protein, and cholesterol in both sexes were also dose-related.

At the 2 highest doses, a dose-related increased incidence of fatty livers was reversible. At all levels, except the lowest, the incidence of foci or areas of altered hepatocytes was increased in a dose-related manner.

In the liver of females, a total of 0, 1, 2, 1 and 4 neoplastic nodules were observed at 0, 6, 58, 136, and 263 mg/kg, respectively, while 2 hepatocellular carcinomas were observed at both 58 and 263 mg/kg, against none in the control groups. This incidence of carcinoma was within the range of historical control values. In treated males, these incidences were comparable to those of the controls. No earlier onset of nodules or carcinoma was observed (NCA, 1982),

In the same project, groups of 100 - 200 male and 50 - 100 female B6C3F1 mice received food grade methylene chloride in the drinking-water for 24 months at levels of approximately 60, 125, 175, and 235 mg/kg body weight per day. Duplicate control groups comprised a total of 125 male and 100 female mice.

The survival of the treated female mice was better than that of the controls. At the highest dose level in both sexes, the leukocyte count was increased at week 52, but not at the end of the study.

A very slightly increased incidence of hepatocellular adenomas and carcinomas, alone or combined, was found in the treated males. There was no dose relation. The only significant increase was found for carcinomas in males at 235 mg/kg, which was nevertheless very close to the average historical incidence for B6C3F1 mice in the performing laboratory. A slightly increased incidence of Harderian gland neoplasms were observed in males at 125 and 235 mg/kg. The significance of this finding is unclear (NCA, 1983).

At the time of the evaluation of this document, the results of an inhalation study in progress in the US National Institute of Environmental Health Sciences were not available (NTP, 1984).

7.3 Mutagenicity

The numbers of revertants of Salmonella typhimurium TA98, TA100, and TA1535 were increased 3- to 7-fold in a dose-related manner, when plates were exposed to the vapour of methylene chloride of undisclosed purity at levels up to 200 g/m³ air. Metabolic activation by either induced rat liver S9 fraction, cytosol fraction, or microsomal fraction increased the mutagenicity (Simmon et al., 1977; Jongen et al., 1978, 1982; Nestmann et al., 1980, 1981; Gocke et al., 1981). It was shown that the direct mutagenicity of methylene chloride could be attributed to bacterial metabolic pathways similar to those in the rat (Green, 1983).

A dose-related increase in the frequency of gene conversions, mitotic recombinations, and reversions was found for cultures of Saccharomyces cerevisiae strain D7, but not for strains D4 and D3, exposed to methylene chloride of undisclosed purity. Strain D7 contains more cytochrome P-450 than strain D4 and could, perhaps, activate methylene chloride (Simmon et al., 1977; Callen et al., 1980).

No mutagenicity was detected in the recessive lethal test on Drosophila melanogaster fed, or injected with, 1 - 2% methylene chloride (Abrahamson & Valencia, 1980). A 2-fold increase in the number of recessive lethals was found after the feeding of 1 - 5% methylene chloride in 2% dimethylsulfoxide (Gocke et al., 1981).

Methylene chloride was not mutagenic in several tests in which mammalian somatic cells, including human cells were used (Gocke et al., 1981; Jongen et al., 1981; Perocco & Prodi, 1981; Andrae & Wolff, 1983; Burek et al., 1984). A weakly positive effect on SCEs was observed in vapour-exposed Chinese hamster V79 cells (Jongen et al., 1981). The same test was negative with and without metabolic activation in a suspension of Chinese hamster ovary cells, while mitotic delays and chromosome aberrations were found (Thilagar & Kumaroo, 1983).

Transformation of vapour-exposed Syrian hamster embryo cells by SA7 adenovirus was enhanced in a dose-related manner (Hatch et al., 1983).

7.4 Effects on Reproduction and Teratogenicity

Rats received methylene chloride in the drinking-water at a level of 125 mg/litre during a period of 13 weeks before mating. No effects were found on the female fertility index, litter size, survival of pups at 4 weeks, and the number of resorptions (Bornmann & Loeser, 1967). Fetuses of 19 rats exposed to a methylene chloride concentration of 4340 mg/m³ air on days 6 - 15 of pregnancy, for 7 h/day, showed an increased incidence of dilated renal pelvis. Fetuses of 12 mice, exposed similarly, showed an increased incidence of extra sternbrae. The maternal weight of mice increased. Rat and mice dams had carboxyhaemoglobin levels as high as 12.5% during exposure (Schwetz et al., 1975). Groups of 18 rats were exposed before and during, or only during 17 days of pregnancy to a methylene chloride concentration of 15 600 mg/m³ air for 6 h/day. Blood carboxyhaemoglobin levels of dams ranged from 7.2 to 10.1% of saturation. Relative and absolute liver weights were increased. Fetal body weights decreased (Hardin & Manson, 1980). In the same study, 4 groups of 10 rats each were exposed under the same conditions and were allowed to deliver their litters for neurobehavioural testing. Changes in the general activity of pups were found from the age of 10 days in both sexes, to 150 days in males only (Bornschein et al., 1980).

Exposure of pregnant rats to methylene chloride may lead to exposure of the fetus to both methylene chloride and carbon monoxide (Anders & Sunram, 1982).

8. EFFECTS ON MAN

8.1 Short-Term Exposures

8.1.1 Controlled studies

Neurobehavioural changes were observed at low exposures. After 1.5 - 3 h of exposure to 694 mg/m³, vigilance disturbance and an impaired combined tracking monitoring performance were found (Putz et al., 1976). The critical flicker frequency, a measure for sensory function, was reduced after 95 min of exposure to 1040 mg/m³ (Fodor & Winneke, 1971). After 4 h of exposure to 2610 mg/m³, psychomotor performance was decreased (Winneke, 1974). Visually evoked response alterations, also a measure of sensory function, were seen after 1 h of exposure to 2400 mg/m³, while exposed subjects experienced lightheadedness. Blood and urine variables, except carboxyhaemoglobin levels, were normal in this study after 1 - 2 h of exposure to levels of methylene chloride between 739 and 3420 mg/m³. No eye, nose, or throat irritation was observed (Stewart et al., 1972). Most neurobehavioural effects observed were less pronounced or absent with carbon monoxide exposures resulting in comparable carboxyhaemoglobin levels (Winneke, 1974; Putz et al., 1976). The odour threshold for methylene chloride is 743 mg/m³ (Leonardos et al., 1969).

8.1.2 Accidental exposures

The increase in blood carboxyhaemoglobin saturation following methylene chloride exposure has already been discussed. The most prominent effect of methylene chloride exposure is a reversible central nervous system (CNS) depression, ultimately resulting in narcosis, for example, after 30 min of exposure to 69 000 mg/m³ (Moskowitz & Shapiro, 1952). High carboxyhaemoglobin levels (up to 50%) have been measured in the blood of unconscious subjects (Fagin et al., 1980).

Signs of CNS-depression, narcosis, irritation of the eyes and respiratory tract, lung oedema, and sometimes death were found after accidental exposures to methylene chloride or paint remover containing this compound (Moskowitz & Shapiro, 1952; Hughès, 1954; Bonventre et al., 1977; Fagin et al., 1980). Three myocardial infarctions in one subject were reported to have followed 3 exposures to a paint remover containing methylene chloride over a period of approximately 8 months. The subject was exposed in a poorly ventilated room, and concentrations may have been very high (Stewart & Hake,

1976). Electrocardiographic changes resembling those after carbon monoxide poisoning were found in an exposed man with a history suggesting ischaemic heart disease (Benzon et al., 1978). Three probable cases of phosgene poisoning occurred after the use of methylene chloride-based paint remover near a source of heat (Gerritsen & Buschmann, 1960; English, 1964).

8.1.3 Effects on the skin and eyes

Several reports already discussed indicate the irritative action of methylene chloride on the eyes and skin.

Slight erythema was found, when methylene chloride-containing, aerosol-spray deodorants were used twice a day for 12 weeks by 75 men and women (Meltzer et al., 1977). On direct contact, methylene chloride caused a burning sensation and pain (Stewart & Dodd, 1964).

8.2 Long-Term Exposure

8.2.1 Occupational exposure

The few reports available deal with small groups of occupationally-exposed subjects. Workers exposed occupationally to a time-weighted average of 114 mg/m³ had carboxyhaemoglobin levels of between 0.8 and 2.5%. No effects were found on clinical chemistry, haematology, or electrocardiograms (DiVincenzo & Kaplan, 1981a). Cherry et al. (1981) did not find any exposure-related, long-term damage in 29 subjects as evidenced by subjective symptoms, neurobehavioural tests, motor nerve conduction velocity, electrocardiograms, and clinical examinations. The men had been exposed for several years to levels of methylene chloride ranging from 260 to 347 mg/m³. Age-matched controls were used. In a study without a control group, neurasthenic disorders and irritation of the eyes and respiratory passages were experienced by half of the 33 workers exposed to methylene chloride for an average of 2 years. Digestive disorders were reported by one-third of these workers. Formic acid was found in the urine. No other deviations were found during the internal, nervous, eye, and laboratory examinations. The methylene chloride concentrations measured varied between 100 and 17 000 mg/m³ (Kuzelova & Vlasak, 1966). Irreversible damage to the central nervous system with acoustic and optical illusions and hallucinations was diagnosed in 1 man, who had been exposed for 5 years to methylene chloride at levels ranged from 2290 to 12 500 mg/m³ (Weiss, 1969). Another man, exposed for 3 years to levels of methylene chloride ranging from 1735 to 3470 mg/m³ showed a bilateral temporal lobe degeneration (Barrowcliff &

Knell, 1979). A case of delirium and seizures was reported of a man who was exposed to methylene chloride during 4 years. The man reported a 12-month history of intermittent headache, nausea, blurred vision, shortness of breath, and transient memory disturbances. Neuropsychological and EEG examinations revealed a dysfunction of the right hemisphere. All symptoms and signs cleared with removal from the workplace (Tariot, 1983). In 46 subjects exposed to methylene chloride concentrations of 6 - 34 mg/m³ for several years, an excess (not significant) of digestive disorders and hypotonia was found over controls, while symptoms of gall bladder pathology and swollen liver were frequent. No details were given concerning drinking or smoking habits (Kashin et al., 1980).

A clinical laboratory evaluation of 266 exposed volunteer workers and 251 reference volunteer workers from two cellulose di- and tri-acetate plants in the USA, taking into account smoking habits, race, sex, age, intensity of exposure, and time of venepuncture, revealed increases in red cell counts, haemoglobin levels and haematocrit among white women exposed to a methylene chloride level of approximately 1650 mg/m³. Carboxyhaemoglobin levels were elevated in all exposed groups at all exposure levels (section 5.3). A dose-related increase was observed in serum bilirubin for exposed subjects of both sexes. A total of 24 exposed male volunteers and 26 reference male volunteers from the above 2 industries were also selected for 24-h electrocardiographic monitoring. Three exposed and 8 reference workers had reported a history of heart disease. Neither increased ventricular or supraventricular ectopic activity nor increased episodic ST-segment depression was found to be associated with methylene chloride exposure (Ott et al., 1983).

In women occupationally exposed to an average methylene chloride concentration of 86 mg/m³, the compound was found in the placenta, fetus, and breast milk (0.07 mg/litre milk average) (Vosovaja et al., 1974).

8.2.2 Mortality studies

When the mortality experience of 334 deceased male industrial workers, who had been exposed to levels of methylene chloride up to 1210 mg/m³, was compared with that of the non-exposed industrial workers and the New York State male populations, no excess age- and cause-specific mortality was found. A total of 751 male workers with exposures for up to 30 years were subsequently followed up for mortality for 13 years. No excess mortality was found compared with 2 internal and 2 external control groups (Friedlander et al., 1978). The data on this cohort have been updated with 4 more years. The mortality was consistent with that of industrial controls and

less than that expected of the New York State controls (Hearne & Friedlander, 1981).

The mortality experience of 1271 male and female workers of a cellulose di- and tri-acetate plant with time-weighted-average exposures to methylene chloride of between 486 and 1648 mg/m³, for a period up to 23 years, was compared with that of corresponding USA populations and a reference cohort of 948 workers in a cellulose diacetate plant, where no methylene chloride was used. The mortality, specified by cause (with a focus on ischaemic heart disease), sex, race, and for each cause, duration of exposure, length of follow-up, and employment status was comparable to that of the USA populations. The mortality rate among white men was higher than that of the reference cohort for the following categories: all causes, diseases of the circulatory system, ischemic heart disease, and all external causes. Observed deaths in the reference group were considerably fewer than the USA experience for each of these categories. According to the authors, the mortality trends for cardiovascular disease can be explained by expected geographical differences (Ott et al., 1983).

9. EVALUATION OF HEALTH RISKS FOR MAN

Human exposure is mainly through inhalation. Absorption via the skin is slow. Methylene chloride is rapidly absorbed via the gastrointestinal tract and crosses the placenta and blood-brain barrier.

Acute effects

The odour threshold for methylene chloride is 743 mg/m³ (Leonardos et al., 1969). The predominant effects of methylene chloride in human beings are central nervous system depression and the production of elevated carboxyhaemoglobin levels in the blood. These effects are reversible. Mild behavioural disturbances (e.g., disturbances of vigilance) have been reported following exposure to 694 mg/m³ of methylene chloride in air for 1.5 - 3 h (Putz et al., 1976) and impairment of psychomotor performance after a 4-h exposure to 2610 mg/m³ (Winneke, 1974). Narcosis occurred following exposure to 69 000 mg/m³ for 30 min (Moskowitz & Shapiro, 1952). Individuals with heart disease may be especially at risk, when exposed to methylene chloride, because of the hypoxia induced.

The minimum observed effect level for short-term inhalation exposure was approximately 690 mg/m³ (Putz et al., 1976).

Chronic effects

The predominant chronic effects in human beings are nervous system depression and an elevated carboxyhaemoglobin saturation of the blood. However, no exposure-related subjective symptoms, neurobehavioural effects, motor nerve conduction velocity changes, electrocardiogram changes, or clinical effects were noted in workers exposed to methylene chloride levels of between 260 - 347 mg/m³, compared with age-matched controls (Cherry et al., 1981).

Methylene chloride has been shown to cross the placenta and has been found to accumulate in fetal tissue and breast milk (Vosovaja et al., 1974). In one study designed to assess teratogenic potential (Schwetz et al., 1975), rats and mice were exposed by inhalation to 4340 mg/m³ during days 6 - 15 of gestation. The results of this study indicated an increased incidence of extra sternbrae in mice as well as a greater incidence of dilated renal pelvis in rats. In another study (Hardin & Manson, 1980), rats were exposed by inhalation to a methylene chloride concentration of 15 600 mg/m³ for 6 h/day, before and/or during 17 days of pregnancy. The

purpose of this study was to ascertain whether exposure before and during gestation has a greater effect on the conceptus than exposure only before gestation. No significant effects were reported, except that the pups of rats exposed during gestation had lower fetal body weights. In the same study, additional groups of rats were similarly exposed and allowed to litter, in order to evaluate the potential for behavioural teratogenic effects (Bornschein et al., 1980). As early as 10 days of age, both male and female pups exhibited treatment-related effects in general activity tests. Changes in the general activity of male pups were still demonstrable 150 days post partum. While results of the previous studies (Schwetz et al., 1975; Hardin & Manson, 1980) tend to indicate that the teratogenic hazard is minimal, the results of the neurobehavioural study of Bornschein et al. (1980) suggest the possibility of delayed behavioural effects.

Only one reproduction study was available, which precludes assessment of a potential reproduction hazard. In this study (Bornmann & Loeser, 1967), no reproductive impairment was found when rats were allowed to mate after receiving 125 mg of methylene chloride per litre of drinking-water for 13 weeks.

Results of in vitro studies showed that methylene chloride was mutagenic in bacteria and fungi. However, most tests on mammalian somatic cells, including human cells, were negative.

In 2 inhalation studies in rats the incidence of benign mammary tumours was elevated neither in male nor in female rats, but the total number of mammary tumours was increased in a dose-related manner (Nitschke et al., 1983; Burek et al., 1984). In the study of Burek et al. (1984), an increase of salivary gland region sarcomas was found in male rats. In Golden Syrian hamsters, no significant increases in tumour incidences were found. In a drinking-water study with rats and mice, no significant increases in tumour incidences were found, while an increased incidence of foci or areas of altered liver cells was observed (NCA, 1982, 1983). In two epidemiological mortality studies, there was no excess mortality due to cancer compared with control populations (Friedlander et al., 1978; Ott et al., 1983). The data are inadequate for assessing whether or not methylene chloride is to be considered carcinogenic for animals and man. According to a previous reevaluation of all available published epidemiological, experimental, and short-term test data, an IARC Working Group concluded that methylene chloride could not be classified as to its carcinogenicity for human beings (IARC, 1982).

A no-observed-adverse-effect level for long-term inhalation exposure was of the order of 260 - 350 mg/m³ (Cherry et al., 1981). A lowest-observed-effect level could not be derived from the available human data.

10. SOME CURRENT REGULATIONS, GUIDELINES, AND STANDARDS

10.1 Occupational Exposure

Maximum allowable concentrations^a are 49 mg/m³ (14 ppm, ceiling value) in the USSR, 250 mg/m³ (70 ppm, TWA) in Sweden, 360 mg/m³ (100 ppm, TWA) in the Federal Republic of Germany, 700 mg/m³ (200 ppm, TWA) in the Netherlands, 360 mg/m³ (100 ppm, TWA) in the USA.

10.2 Food

The Council of Europe (1978) recommends a maximum of 5 mg/kg wet weight in food. The Food and Drug Administration (1977) in the USA allows maxima of 30 mg/kg wet weight in spice oleoresins, 2.2% in hops, and 10 mg/kg wet weight in coffee.

10.3 Storage and Transport

The United Nations Committee of Experts on the Transport of Dangerous Goods (1984) qualifies methylene chloride as a toxic substance (Class 6.1) with minor danger for packing purposes (Packing Group III). Packing methods and a label are recommended. The Inter-Governmental Maritime Consultative Organization^b (1981) also qualifies methylene chloride as a toxic substance (Class 6.1) and recommends packing, stowage, and labelling methods for maritime transport in glass bottles, cans, and drums. It is stressed, that phosgene fumes are formed when methylene chloride is involved in a fire and that stowage should be under shaded conditions away from radiant heat.

The label recommended by both organizations is:



^a Values quoted in national lists.

^b Now the International Maritime Organization.

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