METHYLENE CHLORIDE

(CAS Reg. No. 75-09-2)

CH_2Cl_2

INTERIM ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

For
NAS/COT Subcommittee for AEGLS

2009

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. AEGL-2 and AEGL-3 levels, and AEGL-1 levels as appropriate, will be developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. It is believed that the recommended exposure levels are applicable to the general population including infants and children, and other individuals who may be sensitive or susceptible. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm or mg/m3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing odor, taste, and sensory irritation, or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL level, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL level. Although the AEGL values represent threshold levels for the general public, including sensitive subpopulations, it is recognized that certain individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL level.

TABLE OF CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

Methylene chloride (or dichloromethane: DCM) is a clear colorless, highly volatile liquid with a sweet-pleasant odor, although the odor has also been described as penetrating ether-like. Case studies indicate that the odor may not provide a sufficient warning signal. The substance is non-flammable and not explosive. DCM is predominantly produced by the so-called Stauffer process. It is used as a solvent in paint strippers and removers, as a propellant in aerosols, as an extraction solvent for food (e.g., decaffeination of coffee), as a process solvent in the manufacture of drugs, pharmaceuticals, and film coatings, as a metal cleaning and finishing solvent, in electronics manufacturing, and as an agent in urethane foam blowing. USA production was 229,000 tons in 1988, whereas the total production in Western Europe ranged from 331,500 tons in 1986 to 254,200 tons in 1991.

Human data indicate that the main cause of death following DCM exposure by inhalation is related to CNS effects. These effects include loss of consciousness and respiratory depression, resulting in irreversible coma, hypoxia and death. The organ most often affected in fatal accidents is the brain, followed by the lungs and heart. DCM biotransformation can give rise to the formation of CO leading to COHb. Human case reports revealed that COHb levels of up to 40% have been measured after exposure to DCM, however no quantitative relation with airborne DCM concentrations can be made. The effects most frequently described are CNS-related; cardiotoxic effects were found in a few cases.

There are a number of human volunteer studies addressing CNS-effects resulting from DCM exposure. No signs of eye, nose, or throat irritation were reported at exposure concentrations inducing slight CNS-effects (Stewart *et al*. 1972). Also in occupational settings complaints reported appeared to be relatively mild following a 15-min exposure up to 1700 ppm or an 8-h TWA exposure up to 969 ppm (Moynihan-Fradkin 2001). The experimental studies on neurobehavioral effects in humans exposed to DCM showed that specific sensitive endpoints are affected within a concentration range of 195 to 751 ppm (Winneke and Fodor 1976; Fodor and Winneke 1971; Winneke 1974). These responses were not always consistent and lack a clear concentration-response relation. A separate study indicated that a 2-h exposure to 250 ppm, a 1.5-h exposure to 500 ppm, a 1-h exposure to 750 ppm, and a 0.5-h exposure to 1000 ppm can be conservatively regarded as NOAELs for specific neurobehavioral tests (reaction time, short-term memory) (Gamberale *et al*. 1975). The subjects' perception of their own condition in this study was slightly better under DCM exposure compared to control conditions.

The available epidemiological studies gave no definitive information on a relationship of DCM and neurobehavioral or neuropsychological functions and do not support an increased risk for cancer or for ischemic heart disease. International organizations considered the available epidemiological data inadequate for drawing any firm conclusions with regard to human cancer risk. Human genotoxicity data were absent. A few limited studies available showed no effects of DCM exposure on semen quality.

The animal experiments confirm the toxicity as observed in humans. The predominant effect in animals of a single exposure to DCM is CNS-depression. No large interspecies differences in response appear to be present. Clear signs of anesthesia or narcosis start to occur at concentrations between 5000 and 10,000 ppm (within 1 h of exposure to 10,000 ppm). The cardiovascular effects of inhalation exposure to DCM were studied in monkeys, dogs, and mice. Statistically significant effects were noted in dogs at 25,000 ppm, but not at 10,000 ppm. The only statistically significant effect observed in monkeys at 25,000 ppm was a decreased aortic blood pressure. Sensitization to epinephrine was observed in 1/5 mice exposed to 20,000 ppm. No clear teratogenic or adverse developmental effects were observed in rats at exposure levels up to 4500 ppm. A 2-generation study in rats exposed to DCM concentrations of up to 1500 ppm revealed no exposure-related changes. As to genotoxicity, DCM is mutagenic in prokaryotic microorganisms but predominantly negative in eukaryotic systems and in UDS tests in mammalian systems. *In vivo* tests are positive in $B6C3F_1$ mice, but not in rats or hamsters. Carcinogenicity studies with respiratory exposure to DCM were negative in hamsters. An increased

incidence of benign mammary gland tumors was observed in rats. In mice, increased incidences of hepatocellular and alveolar/bronchiolar neoplasms were found.

Sufficient data are available on the lethality of DCM in experimental animals. The concentration-response relation for lethality is very steep, with an increase in mortality from 0 to 100% within a twofold increase in exposure concentration. The data for mice and rats are comparable; lethality appears to occur at lower concentrations in guinea pigs than in mice and rats. Death is generally preceded by CNS effects. A review of highest non-lethal and lowest lethal concentrations suggests that no mortality will occur in experimental animals below 10,000 ppm.

Two toxic endpoints are of importance for setting AEGL-values, CNS-depression caused by the concentration of the parent compound in brain and the formation of COHb from the CO metabolite. An intermediate in the latter pathway is formyl chloride; this substance can be metabolized to CO or, through conjugation with GSH, finally to CO_2. The responsible GST isozyme is most probable GSTT1, for which occurrence of polymorphism has been described in humans. It has been estimated that about 20% of the USA population lack this enzyme, which will result to higher COHb levels in these subjects. In setting AEGLs for DCM, additional considerations included the fact that CNS-effects occur soon after the onset of exposure while peak levels of COHb can be reached hours after cessation of exposure; and that the metabolic pathway for CO is saturable (saturation occurs at about 500 ppm.

It was expected beforehand that the toxic endpoint of interest would change over an exposure range of 10 min to 8 hours. The AEGL-values for the shorter exposure durations would be triggered by the CNS-effects whereas the formation of COHb would determine the AEGL-values for longer exposure durations. Apart from some human case reports no quantitative data are available on effects attributed to COHb resulting from DCM exposure. The maximum COHb levels were taken from the corresponding AEGL-values for exposure to CO (NAC/AEGL draft TSD on CO). The sensitive subpopulation for CO exposure consisted of patients with severe cardiovascular disease; the AEGL-2 values for CO were set at a maximum additional COHb level of 4% and the AEGL-3 level at a maximum COHb of about 15%.

PBPK-modeling was considered necessary to set adequate AEGLs. Two published and validated models (Andersen *et al.* 1991; Reitz *et al.* 1997) were combined to obtain one PBPK-model that adequately predicts both the DCM concentration in brain and the COHb level.

The AEGL-1 is based on the observation in humans by Stewart *et al.* (1972) that exposure concentrations of 868 and 986 ppm (n=3) may lead to light-headedness and difficulties in enunciation. These effects were absent at a 1-h exposure to 514 (n=3) or 515 ppm (n=8). The concentration of 514 ppm is used as point of departure for AEGL-1. These effects could be attributed to the DCM concentration in the brain rather than to CO. The human brain concentration of DCM following a 1-h exposure to 514 ppm was calculated to be 0.063 mM, using the human PBPK-model. Since susceptibility for gross CNS-depressing effects do not vary by more than a factor 2-3, an intraspecies uncertainty factor of 3 is considered sufficient, resulting in a maximum target concentration of DCM in the human brain of 0.021 mM. Starting from this maximum brain concentration of 0.021 mM, AEGL-1 values were calculated using the human PBPK-model. Because the calculated AEGL-1 values at 4- and 8-h (160 and 140 ppm, respectively) are at or above the corresponding AEGL-2 values, no AEGL-1 for these time periods can be recommended.

Several experimental studies with volunteers have addressed neurobehavioral endpoints that are sensitive subtle effects that may be indicative of more severe effects at higher exposure concentrations but are actually not AEGL-2 effects in themselves. No effects on reaction time, short-term memory, or numerical ability were observed in humans exposed for 4 subsequent 30-min periods to 250, 500, 750, and 1000 ppm DCM, respectively (Gamberale *et al.* 1975). Winneke and Fodor (Fodor and Winneke, 1971; Winneke, 1974, 1982) reported decreased performances in an Auditory Vigilance Test and a Critical Flicker Frequency test in subjects exposed to 317, 470, or 751 ppm DCM

for up to 230 min. However, their results were not always consistent and no clear concentration-response relation was present. The effects are indicative of subtle changes which are neither irreversible nor will cause a serious impairment of escape, and, therefore, are regarded as sub AEGL-2 effects. Since no data were available that adequately addresses AEGL-2 endpoints, the highest concentration of 751 ppm (for 230 min) was used as point of departure for AEGL-2 (CNS-effects). Further, with respect to the COHb formation, the AEGL-2 values for DCM should not lead to additional COHb levels of more than 4%. Using the human PBPK-model, the DCM concentration in brain equivalent to a 230-min exposure to 751 ppm was estimated to be 0.137 mM. Since susceptibility for gross CNS-depressing effects do not vary by more than a factor 2-3, an intraspecies uncertainty factor of 3 would normally have been used. However, in this case the CNS-effects observed at 751 ppm are very mild and occur at any exposure that is far below that which would cause effects that would impair the ability to escape. Therefore, the intraspecies uncertainty factor was reduced to 1.The human PBPK-model was used to calculate the AEGL-2 values resulting in a maximum brain concentration of 0.137 mM for an exposure of 10 and 30 minutes. For longer durations of exposure, the PBPK-model shows that the formation of COHb for non-conjugators (subjects lacking GSTT1) is the more important endpoint. Therefore, the AEGL-2 values for 60-, 240-, and 480-min are based on a maximum additional COHb level of 4%. The AEGL-2 values for CNS-related effects are considered to be in compliance with the relevant experimental human data, including data from volunteers exposed under physical exertion.

No human data that adequately address the level of effects defined by AEGL-3 were retrieved. Evaluation of mortality due to CNS-related effects will be based on animal mortality data. Exposure below 10,000 ppm does not result in mortality in several animal species. A 4-h exposure to 11,000 ppm at which no mortality was observed in rats (Haskell Laboratory, 1982) is regarded to be an appropriate point of departure. A maximum target DCM concentration in rat brain of 3.01 mM was calculated for this exposure using the PBPK-model for the rat. An interspecies factor of 1 is considered to be sufficient since the differences in susceptibility regarding mortality between species appear to be very small and because a human PBPK-model is used to calculate the external human exposure. An intraspecies uncertainty factor of 3 is considered to be sufficient since the susceptibility for CNS-depressing effects does not vary by more than a factor 2-3 in the human population. Application of an overall UF of 3 results in a maximum target DCM concentration in human brain of 1.0 mM. The human PBPK-model was subsequently used to calculate the AEGL-3 values for DCM for the endpoint of CNS-depression. The human PBPK-model was also used to calculate the concentration-time curves associated with a maximum additional COHb level of 15 % in non-conjugators. The toxic endpoint of interest changes between 4 and 5 hours of exposure from CNS-depression to COHb-formation for non-conjugators. Therefore, the 8-hour AEGL-3 value is based on the formation of COHb.

Summary of Proposed Values for Methylene Chloride (ppm)

Classification	Exposure duration					
	10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**	**Endpoint (Reference)**
AEGL-1 (Nondisabling)						
- CNS effects	**290 ppm (1000 mg/m^3)**	**230 ppm (810 mg/m^3)**	**200 ppm (710 mg/m^3)**	**NR**	**NR**	No effect level for light-headedness, difficulties in enunciation in humans (Stewart *et al*. 1972)
AEGL-2 (Disabling)						
- CNS effects	**1700 ppm (6000 mg/m^3)**	**1200 ppm (4200 mg/m^3)**	1000 ppm	740 ppm	650 ppm	Absence of AEGL-2 related CNS-effects in humans (Winneke, 1974)

- COHb (non-conjugators)	4600 ppm	1400 ppm	**560 ppm (2000 mg/m^3)**	**100 ppm (350 mg/m^3)**	**60 ppm (210 mg/m^3)**	Maximum of 4% COHb (NAC/AEGL draft TSD on CO)
AEGL-3 (Lethal)						
- CNS effects	**12,000 ppm (42,000 mg/m^3)**	**8500 ppm (30,000 mg/m^3)**	**6900 ppm (24,000 mg/m^3)**	**4900 ppm (17,000 mg/m^3)**	4200 ppm	No mortality in rats (Haskell Laboratory, 1982)
- COHb (non-conjugators)	160,000 ppm	52,000 ppm	25,000 ppm	5300 ppm	**2100 ppm (7400 mg/m^3)**	Maximum of 15% COHb (NAC/AEGL draft TSD on CO)

NR: Not recommended since these values would be higher than the corresponding AEGL-2 values.
The AEGL-values are given for individual endpoints; the final AEGL-values are presented in bold.

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1. INTRODUCTION

Table 1 summarizes the physicochemical properties of methylene chloride (dichloromethane, DCM). DCM is a clear colorless, highly volatile liquid with a sweet-pleasant odor (ATSDR, 2000), although the odor has also been described as penetrating ether-like (WHO, 1996). The substance is non-flammable and not explosive. Pure dry DCM is a very stable compound. Liquid DCM was concluded to be moderately irritant to the skin and eyes, but not corrosive. However, in the presence of water, it undergoes very slow hydrolysis to yield small quantities of hydrogen chloride. Commercial DCM is usually stabilized with 0.005-0.2% methanol, ethanol, amylene, cyclohexane, or *t*-butylamine (WHO, 1996).

TABLE 1. Chemical and Physical Data

Parameter	Value	Reference
Synonyms	DCM, dichloromethane, methane dichloride, methylene dichloride, methylene bichloride	WHO, 1996
Chemical formula	CH_2Cl_2	ATSDR, 2000
Molecular weight	84.93	ATSDR, 2000; Merck, 1996; Lide, 1999
CAS Reg. No.	75-09-2	ATSDR, 2000
Physical state	liquid	ATSDR, 2000
Color	colorless	ATSDR, 2000
Solubility in water	13.0 to 20.0 g/L (at 20°C) 16,700 mg/L (at 25°C)	WHO, 1996; ATSDR, 2000 ATSDR, 2000
Vapor pressure	349 mm Hg (at 20°C) 435 mm Hg (at 25°C) 500 mm Hg (at 30°C) 465 to 475 hPa (at 20°C) 709 hPa (at 30°C)	ATSDR, 2000 NLM, 2002 ATSDR, 2000 IUCLID, 2000 IUCLID, 2000
Vapor density (air =1)	2.93	ATSDR, 2000; Lewis, 1999; WHO, 1996
Liquid density (water =1)	1.33479 g/mL (d^{15}_4) 1.3255 g/mL (d^{20}_4) 1.30777 g/mL (d^{30}_4)	Merck, 1996 Merck, 1996 Merck, 1996
Melting point	-94.9 to -96.7 °C	Lide, 1999; ATSDR, 2000; Lewis, 1999
Boiling point	39.75 °C	Merck, 1996
Odor	Sweet, pleasant Penetrating ether-like	ATSDR, 2000 WHO, 1996
Flammability	Nonflammable	ATSDR, 2000
Explosive	Not explosive	ATSDR, 2000
Conversion factors (20°C, 1.013 hPa)	1 mg/m^3 = 0.28 ppm 1 ppm = 3.53 mg/m^3	WHO, 1996

DCM is predominantly produced by the so-called Stauffer process. First methanol is reacted with hydrogen chloride to yield methyl chloride, which is then reacted with chlorine to DCM (WHO, 1996). An older approach is formation of DCM by a direct reaction of excess methane with chlorine (NLM, 2002; Rossberg, 2001).

DCM is used as a solvent in paint strippers and removers, as a propellant in aerosols, as an extraction solvent for food (e.g., decaffeination of coffee), as a process solvent in the manufacture of drugs, pharmaceuticals, and film coatings, as a metal cleaning and finishing solvent, in electronics manufacturing, and as an agent in urethane foam blowing (ATSDR, 2000; NLM, 2002). USA production was 229,000 tons in 1988, whereas the total production in Western Europe ranged from 331,500 tons in 1986 to 254,200 tons in 1991. The use of DCM in Western Europe had declined to 150,000 tons/year in 1992 (WHO, 1996). The estimated use pattern of DCM in the United States in 1995 was 40% in paint strippers, 13% in metal degreasing, 10% in chemical processing, and 6% in the production of pharmaceuticals, and 6% as urethane blowing agent. Remaining uses were less than 5% (IARC, 1999). Because of increasing concern and/or more strict legislation its use in consumer products has declined (WHO, 1996; ATSDR, 2000).

Because DCM evaporates easily, the greatest potential for exposure is through inhalation. Mean outdoor air concentrations of up to approximately 11 ppb have been reported, with incidental maximum values of about 200 ppb. The use of paint strippers or aerosol cans containing DCM is a frequent source of exposure (ATSDR, 2000).

2. HUMAN TOXICITY DATA

Prior to discussing the toxicity data for DCM, a few remarks necessary for a proper understanding have to be made. It is noted that the biotransformation of DCM to carbon monoxide (CO) and the subsequent formation of carboxyhemoglobin (COHb) was first reported by Stewart *et al.* (1972). Before this date DCM was considered to be inert and not to be metabolized. Hence, all work published before 1972 was not primarily aimed at COHb-formation and related topics and the occurrence of COHb-related health effects may have been overlooked. Further, some cases were found unconscious with skin contact to liquid DCM or were postmortem "exposed" to DCM vapor for a few hours. A recent study by Takeshita *et al.* (2000) showed that postmortem uptake of DCM vapor may occur. Rats exposed to an atmosphere saturated with DCM died within 15-25 min. Animals (one per sacrifice time point) were left dead in the exposure room for 0.5, 1, 2, 5, 10, or 20 h, or placed under gentle ventilation. The carcasses of rats killed by barbiturates were treated in a similar way. The DCM tissue concentrations in the carcasses of the rats killed by DCM exposure or by barbiturates and remaining under DCM "exposure" increased significantly during the 20-h postmortem "exposure" and showed similar concentrations at the end of the 20-h postmortem period.

2.1. Acute Lethality

2.1.1. Case Reports

A large number of lethal inhalation exposures to DCM have been reported, only three report actual measurements of DCM concentrations in air.

A 27-year-old male was found slumped over a tank containing paint-stripper (77% DCM, 18% methanol) 20 to 30 min after he was last seen alive. His head and trunk were in the tank and his arms in the solvent. He was taken to hospital in cardio-respiratory arrest, and could not be resuscitated. Autopsy showed bilateral pulmonary congestion and edema. Microhemorrhagic changes were seen in the lungs together with a significant increase in pigmented macrophages in the alveoli and around the bronchioles. The liver showed slightly increased consistency and size. Further, mild portal inflammation, dilated centrilobular veins, and acute congestion were noted. The cause of death was assigned to asphyxia secondary to inhalation of fumes from a cleaning agent (DCM).

Air samples were taken after the accident and analyzed for DCM. DCM air samples (0.5 to 2.5 L) were collected in activated charcoal tubes. The concentrations were >140,000 mg/m^3 (>39,200 ppm) approximately 5 to 10 cm from the solvent, 89,474 mg/m^3 (25,053 ppm) at 25 cm above the solvent, 4789 mg/m^3 (1341 ppm) at the brim of the tank (75 cm from solvent surface), and 243 and 390 mg/m^3 (68 and 109 ppm) at the level of the upper airways of a worker standing upright (solvent at rest and on stirring), respectively. Statements from his workmates indicated that the subject probably had been very close to the surface with his head. DCM concentrations in blood and pulmonary exudate were 140 and 540 µg/mL, respectively; his COHb level was 3% (Zarrabeitia *et al.*, 2001).

Novak and Hain (1990) described two separate cases who apparently had collapsed over an immersion tank containing paint stripper (solution of DCM and methanol). The first case (a 19-year old male) was found dead with arms and forehead submerged in the solvent. Cause of death was given as suffocation due to inhalation of toxic solvents. Blood concentration for methanol was 2.4 mg/mL and for DCM 0.4 mg/mL; blood analysis revealed no COHb.

The second case (21-year-old male) was found unconscious with head and shoulders submerged in the solvent (65-85% DCM, 6-12% methanol, 6-12% toluene, monoethanolamine). The man was resuscitated and taken to hospital. He remained comatose and died after 7 d. Blood methanol concentration upon admission was 0.2 mg/mL, COHb was 3.6%. Reenactment air sampling revealed estimations of the solvent concentrations at the time of the accident. Air was collected on 150-mg activated charcoal tubes at 1.0 L/min. Seven 6-10-min samples (with two charcoal tubes in series for the samples closest to the source) and one 10-min and one 55-min breathing zone sample (on two charcoal tubes) were collected. The immersion tank was about 71 cm deep and filled halfway with stripping fluid. DCM, toluene, and methanol concentrations approximately 10 cm above the solvent surface in the middle of the tank were 1711, 89, and ≥ 771 ppm, respectively. These concentrations at the top edge of the tank were 64, 6, and ≥ 44 ppm, respectively; while at a horizontal distance of approximately 76 cm from the tank edge breathing zone air samples revealed concentrations for the three solvents of 100, 3, and ≥ 124 ppm (55-min samples) and 313, 13 ppm, and not analyzed (10-min sample), respectively. However, considering all the available data it is considered highly unlikely that a concentration of 1711 ppm would have caused loss of consciousness and death. Further, the reported air concentrations just above the solvent surface are very low compared to those reported above by Zarrabeitia *et al.* (2001).

Two men, aged 50 and 55 years, were found dead in a well in a building at about 2 meter below ground level (Manno *et al.*, 1989, 1992). They had been burying barrels containing mixed solvents and solid waste from a nearby plant for a few hours during the morning. They were found in the evening. From thanatology data, death was estimated to have occurred in the early afternoon, approximately 24 h before autopsy. DCM concentrations in air samples collected near the well soon after the discovery were 1.8 and 10.7 g/m^3 (504 and 2996 ppm, respectively). DCM concentrations in air sampled the following morning was 582.5 g/m^3 (163,100 ppm) at the bottom of the well and 72.9 g/m^3 (20,412 ppm) where the bodies were found. The method of air sampling was not specified. Concentrations of other solvents (1,2-dichloroethane, 1,1,1-trichloroethane, and styrene) were much lower, up to a few g/m^3 for the first two solvents. Blood DCM concentrations at autopsy were 572 µg/mL and 601 µg/mL for the two subjects. COHb levels were 30%, with a total Hb content of 150 mg/mL blood. The narcotic effect of DCM was concluded to have been responsible for the loss of consciousness and the respiratory depression, resulting in irreversible coma, hypoxia and death. The COHb levels were not considered to be lethal *per se.* It was hypothesized that besides CNS depression, the formation of formaldehyde, formic acid, and carbon dioxide could have led to systemic hypoxia, cardio-respiratory failure, and finally death.

DCM concentrations were calculated afterwards in two reports. A fatal case of occupational DCM poisoning occurred in a plant where an employee using a paint stripper (75% DCM) was probably exposed to a DCM concentration of up to 100,000 ppm (Tay *et al.*, 1995). Two employees

(20 and 40-years of age) were found dead when removing the original surface finish of a squash court with a stripper containing more than 80% DCM. They were found dead approximately 2 h and 20 min after they had started. It was not known whether they had stayed in the squash room during this entire period or had returned after spreading the floor stripper. It was calculated afterwards from the amount of stripper used, the room volume, and the physico-chemical characteristics of DCM that the DCM concentrations in the court would have been above 53,000 ppm (Fairfax, 1996). No further details were reported.

In addition to the above cited cases, nearly all other reports on fatal DCM exposure by inhalation deal with occupational exposure (Moskowitz and Shapiro, 1952; Winek *et al.*, 1981; Hall and Rumack, 1990; Leikin *et al*., 1990; Logemann and Van der Smissen, 1991; Kim *et al.*, 1996; Goullé *et al.*, 1999; Takeshita *et al.*, 2000; Fechner *et al.*, 2001); only two fatal cases as a result of consumer exposure have been reported. It concerned a 13-year-old boy using a commercially available paint remover containing DCM, toluene, methanol, ethanol, mineral spirit, methyl ethyl ketone, and n-methylpyrimidol tetraethylammonium phosphate (Bonventre *et al.*, 1977) and a 66-year-old man (Stewart and Hake, 1976). Postmortem determination of DCM concentrations in blood and tissues have been performed in a few cases which are summarized in Table 2. Exposure conditions were unknown in all these cases, and a combination of respiratory and skin uptake may have been present since a few cases came into skin contact with liquid DCM after they lost consciousness.

The main cause of death following DCM exposure by inhalation is related to CNS effects. DCM exposure has been described to result in loss of consciousness and respiratory depression, resulting in irreversible coma, hypoxia, and death (Manno *et al.*, 1989, 1992).

Autopsy revealed that the organ most often affected is the brain, followed by the lungs and heart. Changes observed in these organs generally included congestion and edema while lungs and/or heart additionally showed petechiae in a few cases.

Especially at exposure to high concentrations in which death occurs within a relatively short time it is unlikely that the formation of CO will have resulted in life-threatening levels of COHb (see Chapter 4). Although in specific cases in which death was attributed to solvent-induced narcosis changes in cardiac rhythm have been described (Leikin *et al.*, 1990), only one fatal case was reported to be related to a myocardial infarction without any signs of reported CNS-depression (Stewart and Hake, 1976). A 66-year-old man had chosen furniture refinishing as a hobby soon after retirement for which he used paint stripper containing 80% DCM. He had been working in the basement for 3 h, one hour after leaving the basement he experienced the onset of chest pain, which was diagnosed as an acute anterior myocardial infarction. He had no prior history of heart disease. Two weeks following discharge he again worked for 3 h in the basement using the varnish remover. He was hospitalized with an acute myocardial infarction now complicated by cardiogenic shock, dysrhythmia, and heart failure. He survived and 6 m after discharge he returned to his basement to complete the paint stripping operation. After 2 h he experienced chest pain, collapsed and died. There were no signs of CNS-depression reported.

Upon examination of two cases (of which one fatal) Gerritsen and Buschmann (1960) studied the production of phosgene out of DCM in ill-ventilated rooms heated by kerosene stoves. Painted wooden surfaces treated with approximately 50 g of paint remover (containing 92% solvent, predominantly DCM) were placed in a 6 m^3 cupboard. Within 12 min phosgene concentrations of up to 128 ppm were measured.

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Table 2. Overview of DCM concentrations in blood and tissues and of COHb levels determined in fatal cases (all males)

Subjects	Autopsy (hours postmortem)	DCM Blood (µg/mL)	COHb (%)	Brain (µg/g)	Liver (µg/g)	Heart (µg/g)	Kidney (µg/g)	Lungs (µg/g)	Remarks	Cause of death	References
50, 55-y	±24h	572, 601	30	--	--	--	--	--		Narcosis; respiratory depression	Manno *et al.*, 1992
51-y	≤24h	252	3.0	75	56	30	59	26		Not specified	Kim *et al.*, 1996
22-y	36 h	4590-5240 (heart blood)	--	--	7280	--	--	--	Skin exposure	--	Fechner *et al.*, 2001
13-y	unknown	510	3.0	248	144	--	--	--		Narcosis	Bonventre *et al.*, 1977
29-y	--	155 (serum)	6	109	35	--	58	40		CNS-depression	Leikin *et al.*, 1990
32-y	4 days	55 (serum)	Increased up to 8%	0	--	--	--	--		CNS-depression	Leikin *et al.*, 1990
29-y	--	773 (when found dead) 513-544 (at autopsy)	≤4	291-548	226-328	223-259	--	--		CNS-depression	Logemann and Van der Smissen, 1991
47-y	unknown	150	≤1	122	44	--	15	20	Skin exposure	Narcosis	Goullé *et al.*, 1999
40-y	>20	1660	13	87	130	199	71	103			Takeshita *et al.* 2000

3

2.2. Nonlethal Toxicity

2.2.1. Case Reports

The odor threshold for DCM appears to be in the range of 160-620 ppm (ATSDR, 2000).

A large number of nonfatal cases have been reported, but clear exposure data were absent in all reports. Some cases are workers who were accidentally exposed to high concentrations of DCM. Although it is not always specifically stated, it can be concluded in some cases from their profession that they may have been exposed previously to DCM.

The most frequently described effects are CNS-related only, in a few cases cardiotoxic effects (evidenced by ECG changes) are reported. Effects on lung, liver, or kidney are incidentally reported as primary signs of DCM toxicity. It is noted that in some cases high COHb levels up to 40% are measured without serious complaints (Langehennig *et al.*, 1976). The most important cases are described in more detail. Cases from accidental occupational exposure are discussed separately because these cases will most often have a history of DCM exposure and co-exposure to other solvents may have been present.

Use as an anesthetic

DCM has been used in the 1920s as an anesthetic under the name "Solaesthin", it was reported that it can lead to narcosis within 30 min at concentrations of about 2 Vol.%. DCM was considered to be suitable to induce light narcosis and not full narcosis because the narcotic dose appeared to be very close to the toxic dose (Hellwig, 1922; Flury and Zernik, 1931; Winneke, 1974). Hellwig (1922) reported that the use of in total 50 g DCM during a 3-h surgical procedure induced a satisfactory light narcosis and did not result in complaints afterwards. A total of 50 g DCM inhaled in 3 h is equivalent with a 3-h exposure to 7000-9333 ppm (assuming a respiratory volume of 1.5-2 m^3 in 3 h). An average amount of 20 mL (26.6 g) DCM was used in 1950 as an obstetric analgesic in 44 cases (Grasset and Gauthier, 1950). The women themselves could regulate when to inhale DCM through a mask, inhalation of DCM was predominantly during contractions. The average duration for dilatation was about 3-4 h with an additional 15-45 min for the expulsion.

Single accidental consumer exposure

A 20-year-old woman developed nausea and severe, throbbing headache while using a paint remover in a poorly ventilated, unheated room. After about an hour, she left the room and lost consciousness shortly thereafter. Her symptoms were further relatively mild, despite a COHb level of 50% on admission. Chest radiograph and ECG were normal. She was treated with 60% oxygen, and after 12 h the COHb level had decreased to 12% (Fagin *et al.*, 1980).

Langehennig *et al.* (1976) reported COHb levels in two nonsmoking volunteers of up to 40% in the morning after a 6-h exposure to paint remover containing DCM and up to 33% after a 3-h exposure. Despite these high COHb levels no complaints or symptoms were reported.

A 39-year-old nonsmoking woman and three brothers, aged 4, 5, and 9 years were accidentally exposed to self-defense spray gas (49% DCM, 0.8% o-chlorobenzylidene malononitrile). Effects reported included signs of moderately painful irritative conjunctivitis, slight euphoria, nausea, and headache with sporadic dizziness. The physical examination did not reveal important effects. COHb levels of the four victims ranged from 12-20% (Dueñas *et al.*, 2000).

A 25-year-old nonsmoking male had cleaned his computer with DCM for 6 to 8 h (Rudge, 1990). The following morning he sought medical attention with a severe headache and nausea, he had vomited several times. He also noted incoordination. Neurological examination and examination of, amongst others, liver functions and heart revealed no changes. His initial COHb level was 20.1%.

A 35-year-old nonsmoking male was exposed to DCM while removing paint from floor tiles in a poorly ventilated area (McGirr *et al.*, 1990; abstract only). It was not stated whether the exposure was occupational. The solvent contained approximately 80% DCM. After 30 min he felt lightheaded and

had chest discomfort and an irregular pulse, and was brought to an emergency department. An ECG showed atrial fibrillation; his COHb was 11%. The patient had no cardiac history. The authors considered the COHb level not high enough to cause the arrhythmia; the mechanism was reported to be probably caused by sensitization of the myocardium by DCM.

Two security guards who tried to remove two victims (see 2.1.1) of DCM poisoning from a small washroom also complained of symptoms (Leikin *et al.*, 1990). One guard (38-year-old male) had performed mouth-to-mouth resuscitation on one of the victims. He complained of nausea, three episodes of nonbloody emesis, and subsequent light-headedness. His COHb level was 5%, and did not rise. The second security guard (24-year-old female) was asymptomatic until approximately 1 h after exposure when she developed slight dizziness, nausea and belching, all of which resolved over 20 min. Both victims complained of headaches and nausea at a 2-week follow-up.

Single accidental consumer (mixed) exposure

A 52-year-old woman had been painting her house outdoors with spray paint containing 31% DCM. She experienced shortness of breath, nausea, weakness, left-sided paresthesia, and flashbacks for 4 d before going to hospital. Her COHb level was 11.7%, which decreased to 3.9% after several hours of O_2 treatment. No clear conclusions can be drawn since co-exposure to other organic solvents may have occurred and she smoked 1 pack of cigarettes per day (Nager and O'Connor, 1998).

A 14-year-old boy who had intentionally inhaled vapors of a solvent mixture (60% DCM, 30% trichloroethylene, 10% isopropanol) suffered from clear CNS-effects. His COHb was 13% 4 h after exposure, an ECG showed no important changes (Sturman *et al.*, 1985).

Accidental occupational exposure

Bakinson and Jones (1985) evaluated cases of industrial gassing reported to the occupational health authorities in the United Kingdom over the period 1961-1980. Accidents were to be reported if mortality occurred or if a subject was unable to work for more than 3 days. A total of 33 cases related to DCM exposure (concentrations were not reported) were identified, of which one fatal (see 2.1.1). It was reported that among the cases of DCM poisoning no substantial evidence for hepatorenal or cardiac effects was found. The principal effects were CNS-related.

Four painters removing paint in a closed room using paint remover containing 96% DCM became faint, giddy, and complained of loss of interest in things (Collier, 1936). The men had been exposed to lead for 5-14 years. One man (42-yr male, painter for 13 yr) had irregular but severe pains in legs and arms, precordial pain, attacks of rapid heart beating, rapid pulse, headache, vertigo, difficulties to read, and shortness of breath and great fatigue on exertion. A second man (45-yr male, painter for 20 y) complained of drowsiness, irritability, and headache due to his work and a definite tingling in hands and feet after working with paint remover. Their condition improved within a few weeks without work.

Two men (40 and 50 years of age) were accidentally exposed at work to unknown DCM concentrations (Benzon *et al.*, 1978; Strande, 1978 (including reply by Benzon and Brunner)). One of them had lost consciousness. The ECG of the second patient showed a left anterior fascicular block and sinus bradycardia; his COHb level was 11% on admission. The next day the ECG had worsened showing a complete right bundle branch block, left anterior fascicular block, and nonspecific ST-T wave changes. The COHb level at that time was 6%. Later, serial ECGs showed no further changes.

A 32-year-old man was found unconscious after spraying DCM-containing (ca: 25% by weight) paint for several hours in a semiclosed area (Rioux and Myers, 1989). He reported nausea, vomiting, confusion, and blurring of vision. ECGs were within normal limits and a chest radiograph revealed no abnormalities. The patient was unable to perform neuropsychological tests (a Carbon Monoxide Neuropsychological Screening Battery). His COHb level on admission was 5.4 % but despite hyperbaric oxygen treatment for 46 min continued to rise to 13.0% for the next 12 h. Nine days

after discharge, neuropsychological tests for dysphasia and visual spatial function were in the dysfunctional range. After a third hyperbaric oxygen treatment the results of a repetition of the neuropsychological tests were improved. Similar effects were observed in a second case (27-year-old male) who became unconscious while rescuing his colleague, case no 1.

Workers removing a surface finish in a completely sealed squash court applied stripper (>80% DCM) to the floor and spread it with mops. They complained of burning in the groin area, light-headedness, and feeling very "buzzed". It was calculated afterwards from the amount of stripper used, the room volume, and the specific gravity and molecular weight for DCM, assuming full and even mixing in the room with no loss, that the DCM concentrations in the court would have been between 9500 and 19,000 ppm (Fairfax, 1996).

Three cases have been described with evidence of liver injury after exposure to DCM (Miller *et al.*, 1985; Puurunen and Sotaniemi, 1985; Cordes *et al.*, 1988). Two cases had dermal as well as inhalation exposure. Besides complaints of irritation, dizziness and nausea elevated levels of serum ALAT, ASAT, AP, or LDH activities have been reported in these cases, although not always consistent.

Some cases with chronic occupational exposure to DCM have been reported (Weiβ, 1967; Barrowcliff and Knell, 1979; Tariot, 1983; Linger and Sigrist, 1994; Mahmud and Kales, 1999). One case also had clear co-exposure to other organic solvents (Mahmud and Kales, 1999). The most predominant effects were CNS-related including headache, nausea, memory difficulties, concentration difficulties, and hallucinations. One case reported pain near the heart (Weiβ, 1967). Mahmud and Kales (1999) reported a COHb level of 21% for their case.

2.2.2. Experimental Studies

Eleven subjects exposed to 100 ppm DCM for 2 (n=5) or 4 h (n=5) or to 200 ppm for 2 h (n=7) performed a pegboard test (two different colors and sizes) combined with simultaneously adding three single-digit numerals presented at 5-second intervals. The day-to-day variation in concentration was 5 to 10%. The test was performed immediately at the start of exposure, and after 60 and 100 min (2-h exposures), and additionally after 2 and 3h for the 4-h exposure. No further details were given but the statement that no changes were observed (DiVincenzo *et al.*, 1972).

Stewart *et al.* (1972) performed four experiments in eleven nonsmoking male volunteers (age: 23-43 years). Experiments included 60 min exposure to 213 ppm (n=1), 2 h to 986 ppm (n=3), 1 h to 514 ppm immediately followed by 1 h to 868 ppm (n=3), 1 h to 515 ppm (n=8) (all mean analytical concentrations).

Exposure of one subject to 213 ppm for 60 min did not result in subjective symptoms. His COHb level increased from 0.4% pre-exposure, via 1.75% at the end of exposure to a maximum of 2.4% 3 h postexposure. Two out of three subjects exposed to 986 ppm for 2 h reported light-headedness after 1 h of exposure, one of them noted difficulty in enunciating clearly. The severity of the effects remained constant for the remainder of the exposure but cleared within 5 min postexposure. No eye, nose, or throat irritation was reported. The odor was reported to be moderately strong but not particularly objectionable. Altered Visual Evoked Responses (VER) during exposure were observed in these subjects. At the end of exposure, augmentation of the early components of the VER was evident in all three subjects, the visual responses shifted towards control levels 1 h after exposure. COHb level increased to a mean of 10.1% (15% in one subject) 1 h after exposure, and was still elevated 17 h postexposure (3.9%). Because the COHb level continued to rise after exposure but the light-headedness and the speech difficulties disappeared within 5 min postexposure, these effects were probably related to CNS-effects caused by DCM itself rather than to CO-formation. The same probably holds for the altered VER. Exposure of the same three subjects to 514 ppm for 1 h continuously followed by a 1-h exposure to 868 ppm caused no subjective complaints in the first hour, but one subject complained of light-headedness within 15 min in the second hour of exposure. Peak COHb

levels were reached 1 to 3 h postexposure (4 to 8.5%). VER alterations were present already after 1 h of exposure. Eight volunteers exposed to 515 ppm for 1 h showed no complaints. COHb levels increased to 2.6% and 3.4% at the end and 1 h after exposure, respectively.

Based on these observations (absence of subjective complaints) it can be concluded that no CNS-depression was observed at exposure to 514 ppm for 1 h; this exposure can, therefore, be used as point of departure for AEGL-1.

Winneke and Fodor (Winneke and Fodor, 1976; Fodor and Winneke, 1971; Winneke, 1974) reported a series of tests performed in female volunteers (age: mostly 20-30-years) exposed to DCM, i.e. auditory vigilance task (AVT), Critical Flicker Frequency (CFF), and a battery of psychomotor tests. The CFF determination is considered to reflect cortical activity or alertness. Initially, CFF (monocular determination of flicker threshold) was determined by ascending and descending flickerlight presentations (Winneke and Fodor, 1976). In later experiments binocular determinations of CFF were done by descending presentation mode only, since ascendingly determined values were found to be rather insensitive (Winneke, 1974). (Based on the description of the experimental settings it appeared that the experiments described by Winneke and Fodor in 1976 were performed prior to those published at a previous date). The CFF results were plotted as decrement from the value obtained at the end of the 30-min starting period; mean scores (percentage of signals missed) per time-block were plotted for AVT performance. The interval between experimental settings was 7 d. An overall summary of the results is given.

Three DCM concentrations were tested, 300, 500, and 800 ppm; actual concentrations were 317 (n=12), 470 (n=14), and 751 ppm (n=6), respectively (Winneke,1974). Volunteers were exposed to one or two concentrations. All subjects performed the test also under conditions of nonexposure, the combined results (n=20) served as control for the separate exposure groups. The experimental groups partly overlap and the results are sometimes combinations of different experiments. The exposure most often lasted for 230 min. During the first half hour the DCM concentration increased to the target concentration followed by four equal 50-min test time-blocks consisting of 45 min AVT (three 15-min tests) and 5-min CFF. A 5-min CFF test was performed at the end of the starting-up period to obtain a baseline value. An additional experiment was performed with volunteers exposed to 50 or 100 ppm CO.

The series of experiments by Winneke and coworkers indicate that exposure to actual DCM concentrations of 317 to 751 ppm may affect AVT and/or CFF. These effects are, however, not considered to cause a serious impairment of escape, and are regarded as sub AEGL-2 effects. Furthermore, the results are not consistent and a clear dose-response relation is absent. For instance, the performance showed a V-shaped curve with the worst performance during the third test time-block for the 300 and 800 ppm exposure groups. A statistically significant response at a given exposure was sometimes observed in only one of two series of experiments. The outcome of the AVT performance also appears to be subject to large fluctuations, the performance under control conditions for the CO exposures were comparable to those for the DCM exposure groups. As to CFF, results for the 300 and 500 ppm exposure groups were comparable. Although the results for the 800 ppm exposure group were markedly depressed in one experiment, no statistically significant decrease in CFF ($p>0.1$) could be observed in a separate experiment with 18 females exposed to 800 ppm. Subjects exposed to 50 or 100 ppm CO showed no CFF-depression.

Psychomotor performance was tested in a number of tests in 18 female volunteers exposed to 800 ppm DCM, the tests were performed during the third and fourth time-block. A statistically significantly decreased performance was observed in tapping speed (with and without hand-eye coordination), in reaction time tests, and in precision tests (purdue hand precision test). However, these differences were rather small, ranging from approximately 3 to 9% (Winneke, 1982). No changes in performance in any test were observed for subjects exposed to 50 or 100 ppm CO.

In a well performed study Putz *et al.* (1979) exposed 12 nonsmoking volunteers (6 males and 6 females, age: 18-40 y) to control air (6 ppm CO), an actual average DCM concentration of 195 ppm, or to an actual average concentration of 76 ppm CO for 4 h. The concentrations of DCM and CO were expected to result in COHb levels of approximately 5% during the 4^{th} h of exposure. The three exposures were on three separated days with a 7-d interval. CO concentration in expired alveolar air increased rapidly in the first 2 h with a leveling off after the third hour (45-47 ppm for CO exposure, 50 ppm for DCM exposure). Control COHb levels were approximately 1.5%, mean COHb levels of 4.85% and 5.1% were measured after 4 h of exposure to CO and DCM, respectively. The exposure period was divided into three 80 min blocks each consisting of a 16-min dual task test (hand-eye coordination (tracking control), response to peripheral light stimuli), a 30-min AVT, 6 min breath sampling, and a recurrence of the 16-min dual task test. The remaining time was for rest. Statistically, significantly diminished responses were present after approximately 2 hours of exposure for hand-eye coordination, peripheral light response time, and AVT. The performance for the first two parameters were worse after 4 hours of exposure to DCM than after CO exposure.

Gamberale *et al.* (1975) exposed 14 healthy male volunteers (20-30 y). The subjects were divided into two groups, one group was first exposed to DCM and 7 d later to control air, while the second group was exposed in the reverse order. Subjects were exposed through a breathing valve for four continuous 30-min periods to nominal concentrations of 870, 1740, 2600, and 3470 mg/m^3 DCM (250, 500, 750, and 1000 ppm according to Gamberale *et al.*), respectively. DCM concentration was measured every third minute and the taste and smell of DCM was disguised by menthol. Information on each subject's perception of conditions was assessed by a questionnaire immediately after the exposure. A significant difference in favor of the DCM exposure was present for the sum of the 6 variables. Four performance tests were carried out in the final 20 min of each exposure period, i.e. numerical ability, short-term memory, and two simple reaction time tests. No effects of DCM exposure on the outcome of the four performance tests were observed. It can be conservatively concluded that a 2-h exposure to 250 ppm, a 1.5-h exposure to 500 ppm, a 1-h exposure to 750 ppm, and a 0.5-h exposure to 1000 ppm are NOAELs for the effects studied.

Sixteen healthy male volunteers (aged 19-21 y) were exposed to DCM for 60 min in a double-blind experiment (Kožená *et al.*, 1990). DCM concentration increased in ten geometrical steps from zero up to 720 ppm. Each concentration was maintained for 5 min (starting with 0 ppm) while the last period lasted 10 min. Blood DCM concentration (estimated from a graph) increased up to 40 µmol/L (3.4 µg/mL), and decreased to circa 5.5 µmol/L (0.5 µg/mL) approximately 50 min after cessation of exposure. COHb concentrations (estimated from a graph) were 2% and 4% at the end of the exposure period and 50 min postexposure, respectively. Vigilant performance (discriminate reactions to weak auditory stimuli) and subjective feelings (sleepiness, fatigue, mood changes) before, during exposure, and during the second hour after exposure did not differ from a sham-exposed control group (n=42). The odor of solvent was reported to be sufficiently masked.

Cherry *et al.* (1983) evaluated the neurotoxicity of DCM in 56 male workers exposed to a mixture of DCM and methanol (9:1). The men worked a rapidly rotating shift and were tested either during a morning, afternoon, or night shift (approximately equal numbers per shift). DCM concentrations were measured on a subgroup of workers using individual pumps sampling onto charcoal tubes (no further details) and ranged from 28 to 173 ppm. A group of 36 workers with no exposure to solvents served as controls. The men were tested at the beginning and at the end of the shift. No differences between the two groups were observed for a simple reaction time test and a digit symbol substitution test. Further, the men were asked to rate themselves on the dimensions of sleepiness, physical tiredness, mental tiredness, and general good health. Lower scores for exposed workers on sleepiness, physical tiredness, and mental tiredness were found only during the morning shift.

The reports described in this section do not reveal any clear effect of DCM exposure up to 751 ppm on neurobehavioral parameters. Further, it is remarked that the effects described are not considered to cause a serious impairment of escape and are, therefore, regarded as sub-AEGL-2 effects.

2.2.3. Occupational / Epidemiological Studies

OSHA monitored DCM exposure during DCM-based gluing operations in two facilities (Moynihan-Fradkin, 2001). Workers fused picture frames to a cardboard backing with a 99% DCM adhesive. The workers did not use any personal protection equipment. Personal air samples were collected for the measurement of 8-h TWA values and short-term-exposures (15-min TWA) according to OSHA procedures. In both facilities interviewed workers complained of DCM exposure symptoms including headaches, dermatitis, and skin cracking (no further details given). The 8-h TWA in the two facilities ranged from 89-143 ppm and 41-969 ppm, respectively. The short-term exposure values (15-min TWA) ranged from 170-240 ppm and from 140-1700 ppm for the two facilities, respectively. It can be concluded from these data that a single 8-h TWA exposure concentration of up to 969 ppm DCM or a single 15-min TWA of up to 1700 ppm can be tolerated without causing any serious adverse health effects that could prevent the workers from doing their job. Hence, it may be concluded that single exposure to these DCM concentrations will not lead to serious effects that could impair escape.

Only epidemiological studies specifically aiming at detrimental effects in humans primarily exposed to DCM are considered. A number of occupational epidemiological studies have been published on mortality due to DCM exposure with specific emphasis on carcinogenicity, cardiotoxicity (ischemic heart disease), and neurotoxicity. The studies reveal no clear consistent cause of death related to DCM exposure. The description of the cohort studies is mainly focused on the most recently published results if follow-up over the years has been reported in subsequent publications.

One of the largest cohort studies concerns the Kodak cohort with between 20 and 50 years of follow-up (Friedlander *et al.*, 1978; Hearne and Friedlander, 1981; Hearne *et al.*, 1987, 1990; Hearne and Pifer, 1999). A specific objective of study was to determine whether mortality from ischemic heart disease was increased in the population exposed to DCM. Regular DCM exposure started in the mid-1940s. Personal and spot samples of air (no further details) over the years 1959-1975 revealed average TWA concentrations of 33-119 ppm DCM with individual maximum values of 250 ppm (in 1975) but up to 350 ppm in the past (in 1959) (Friedlander *et al.*, 1978). In subsequent reports, the cohort was further followed up to December 31, 1994 and cumulative exposure estimates (ppm-years) were assessed based on job histories abstracted from company personnel records and industrial hygiene personal-monitoring samples (Hearne *et al.*, 1987, 1990; Hearne and Pifer, 1999). The exposure analysis was based on more than 1200 area and task-specific samples (usually breathing zone samples) collected between 1945 and 1986 as well as more than 900 full-shift personal samples (1980-1986). Early samples were collected in activated charcoal tubes. Since 1980 full-shift exposures were characterized by personal sampling with diffuse vapor monitoring badges. Person-years were counted after the employee had completed one year of service. Eight-hour TWA exposure estimates were up to 114 ppm for the jobs associated with highest DCM exposure, with peak exposures of 5000 to 10,000 ppm occurring up to four times per day for 50-80% of the workdays (Hearne *et al.*, 1987). TWA exposure estimates appeared to be lower in more recent years (Hearne and Pifer, 1999).

No association between DCM exposure and an increased mortality (total death or individual causes) were observed in the earlier reports; the SMR was rather statistically significantly decreased for total deaths (67.9), circulatory diseases (72.7), and malignant neoplasms (56.0). In the more recent analyses a total of 337 deaths was recorded. Statistical significance was only found for a decreased mortality due to diseases of the circulatory system and for all causes of death. Similar results were obtained with analyses in a differently defined, but overlapping cohort of men hired since 1945 and who had worked at least one year between 1945 and 1970 (n=1311) (Hearne *et al.*, 1987, 1990; Hearne and Pifer, 1999). Besides a positive trend of mortality from leukemia with ppm-years no clear relationships with the extent of exposure (ppm-years nor with the duration of exposure years) were found. The cases of leukemia originated from different cell types and three had a history of benzene

exposure. Mortality was generally lower than expected for most causes. It is noted that no adjustments for confounding factors, e.g. tobacco consumption, were made and that co-exposure to low concentrations of other chemicals (1,2-dichloropropane, 1,2-dichloroethane, acetone, methanol) was present.

A second large cohort study was performed in a plant manufacturing cellulose triacetate in which process DCM was used (Ott *et al.*, 1983; Lanes *et al.*, 1990, 1993). Potential exposure also included methanol and acetone. Based on analyses of more than 500 air samples (both air and personal samples) collected during 3.5 months, three exposure groups were discerned, a low, moderate, and high DCM exposure group with median TWA exposures of 140 (range 60-350 ppm), 280 (range 50-470 ppm), and 475 ppm (range 210-690 ppm), respectively. Acetone exposure in the three exposure groups was 1080, 110, and 110 ppm, respectively. Methanol concentrations were approximately 10% of the DCM concentration. In a reference plant with comparable activities but without DCM exposure, a low, moderate, and high acetone exposure group was defined. A total of 1271 employees with at least 3 months of DCM exposure between 1954 and 1977 and 948 employees from the reference plant were investigated. The United States population was used to calculate SMRs for both plants. No detrimental effects due to DCM exposure were found. The plant ceased production in 1986. Lanes *et al.* (1990, 1993) extended the follow-up through 1990. A total of 172 deaths were identified. The local population of York County, South Carolina, was used as control population since it was the county of residence for 95% of the cohort. No statistically significant excess or deficit mortality was observed for any cause of death.

Soden (1993) studied a subgroup of 150 employees from the cohort of Lanes *et al.* primarily focused on the cardiac, neurologic, and, hepatic systems by means of a health questionnaire focussed on complaints of chest discomfort, irregular heartbeat, severe headaches, numbness or tingling in the extremities, loss of memory, dizziness. A control group of 260 employees was selected from a plant without DCM exposure. No differences in responses to these questions were observed between the two groups. Further, no differences were found in blood analyses for ALAT, ASAT, bilirubin concentration, and hematocrit.

Gibbs *et al.* (1996) studied a cohort in a cellulose triacetate fiber manufacturing plant comparable to that investigated by Ott *et al.* and Lanes *et al.* The cohort consisted of 3211 employees who had worked at the plant for at least 3 months, but focussed on the group with highest exposure (836 males, 146 females; exposure concentration: 350-700 ppm). No details were given on exposure measurements. Calculation of SMRs was based on the mortality rates of the local population. A statistically significant deficit mortality was found in men of the high exposure group only for all causes combined (255 vs. 308.97), all malignancies combined (57 vs. 75.61), and cancer of the bronchus, trachea, and lung (15 vs. 27.34).

Tomenson *et al.* (1997) conducted a retrospective cohort mortality study at a cellulose triacetate film producing plant in the UK. A group of 1473 men had worked in jobs that entailed DCM exposure; the remaining group of 312 workers were included as non-exposed workers and analyzed separately. Mortality statistics for England and Wales were predominantly used for comparison. Exposure estimates (ppm-years) were based on job characteristics and results from area and personal monitoring data. Individual estimates of cumulative exposure was possible for 1034 (70%) of the exposed workers. The average 8-h TWA concentration was 19 ppm, but ranged from 73 to 165 ppm for specific tasks. A total of 287 deaths were observed for the exposed group and of 47 deaths for the non-exposed group. A statistically significant deficit mortality was observed for all causes of death (SMR: 74), all malignant neoplasms (65), cancer of the digestive system (64), cancer of the bronchus, trachea, and lung (48), cerebrovascular disease (50), and non-malignant respiratory disease (57). A statistically significantly lower number of deaths was also observed in the non-exposed group for all causes of death and for cancer of the bronchus, trachea, and lung. Division of the exposed group into three subgroups with increasing cumulative exposure revealed no significant dose-response relation.

Mortality due to ischemic heart disease was slightly higher in exposed workers than in non-exposed workers.

Death-certificate-based case-control studies were performed focussed on exposure to selected chemicals, including DCM, and breast cancer (Cantor *et al.*, 1995), CNS cancer (Cocco *et al.*, 1999), or astrocytic brain cancers (Heinemann *et al.*, 1994). However, due to methodological limitations (e.g. crude exposure estimates) no clear conclusions can be drawn.

2.3. Developmental / Reproductive Toxicity

No human data on developmental or reproductive toxicity after acute exposure were found.

In a limited study Kelly (1988) examined 34 men with respiratory (3.3-154.4 ppm (average 68 ppm)) and dermal exposure to DCM and primary complaints of CNS dysfunction within a period of four years. Among these men, 8 complained of testicular, epididymal, or lower abdominal (prostatic) pain and had clinical histories consistent with infertility. On genital examination four men had tender testes and two had testes atrophy. Four men provided semen samples which showed reduced sperm counts (motile sperm count lower than 20 million/mL) and increased abnormal morphology (up to 50%). However, co-exposure to other organic solvents (primarily styrene) was present and the men had a history of repeated exposures to DCM.

Wells et al (1989) evaluated sperm concentration in semen of furniture strippers who had DCM exposure at least three consecutive months immediately prior to recruitment. Eleven out of 14 eligible men (age: 26-61; 7 nonsmoking, 4 smoking) participated. The mean DCM exposure was 122 ppm (range: 15-366 ppm) and COHb levels were 3.9% (2.2-5.9%) and 10.2% (8.1-13.5%) for nonsmoking and smoking subjects, respectively. No effects of DCM exposure on semen count or quality were found.

Bell *et al.* (1991) found no statistically significant association between birth-weight and environmental exposure to DCM due to emissions from manufacturing processes. However, the estimated exposure concentrations were extremely low with an estimate of 50 $\mu g/m^3$ (14 ppb) for the "high" exposure group.

2.4. Genotoxicity

Thier *et al.* (1998) and Bogaards *et al.* (1993) reported the existence of polymorphism in the θ-class isozymes, which are responsible for the biotransformation through the GST-pathway (section 4.3). Groups of non-conjugators, low-conjugators, and high-conjugators could be distinguished. Thier *et al.*, (1991) incubated blood samples of 10 volunteers with ^{14}C-DCM. Five of these subjects possessed the ability to conjugate methyl halides in erythrocytes with glutathione (GSH) (conjugators) or not (non-conjugators). Only a minimum of the radioactivity was found in erythrocyte cytoplasm or membranes. Minor radioactivity was found in lymphocytes in all subjects. In non-conjugators, hardly any radioactivity was found in low and high molecular weight fractions of blood plasma, whereas the radioactivity increased over incubation time in both the low molecular weight fraction (up to 30% of the radioactivity) and high molecular weight fraction (5%). SCEs were 30 to 60% increased in peripheral blood lymphocytes obtained from non-conjugators (n=5) and incubated with DCM for 2 h, whereas no increase in SCEs was observed with samples from conjugators (Hallier *et al.*, 1993). These results are for the moment difficult to interpret.

No further human genotoxicity data were found with inhalation exposure to DCM.

2.5. Carcinogenicity

The epidemiologic studies focussed on among others the carcinogenic potential of DCM have been summarized in 2.2.3. Based on the available data, IARC (1999) concluded that "for no type of

cancer was there a sufficiently consistent elevation of risk across studies to make a causal interpretation credible". It was concluded that there was inadequate evidence in humans for the carcinogenicity of DCM. Based on both the animal data (section 3.5) and the human data IARC concluded that DCM is possibly carcinogenic to humans (Group 2B).

EPA last revised the carcinogenicity assessment for lifetime inhalation exposure to DCM on 02/01/95 (IRIS, 2002). DCM was classified as a probable human carcinogen, classification B2. This classification was "based on inadequate human data and sufficient evidence of carcinogenicity in animals. EPA is planning to reevaluate potential human risks associated with inhalation exposure (ATSDR, 2000) but DCM has been removed from the IRIS agenda for 2002 (EPA, 2002).

The WHO considered the available epidemiological studies inadequate for drawing any firm conclusions with regard to human cancer risk. It was stated that the carcinogenic potency of DCM in man is expected to be low (WHO, 1996).

As to carcinogenicity DCM has been classified within the EU as a Category 3 substance: "Substances which cause concern for man owing to possible carcinogenic effects but in respect to which the available information is not adequate for making a satisfactory assessment. There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2. Category 2 is assigned to substances "which should be considered as if they are carcinogenic to man".

2.6. Summary

Although the odor threshold is within the range of 160-620 ppm it is noted that two men who had lost consciousness could not remember having detected the odor of DCM. The odor may therefore not provide a sufficient warning signal for suddenly acute high detrimental DCM exposures with a fast build-up of the concentration.

DCM has been used in the 1920s and for sometimes afterwards for its anesthetic or analgesic properties. It was reported that it can lead to narcosis within 30 min at concentrations of about 2 Vol.%. Exposure to an estimated concentration of 7000-9333 ppm for 3-h induced a light narcosis satisfactory for surgical procedures. The narcotic dose appeared to be very close to the toxic dose. An average amount of 26.6 g DCM has been satisfactorily used as an obstetric analgesic.

The main cause of death following DCM exposure by inhalation is related to CNS effects. These effects include loss of consciousness and respiratory depression, resulting in irreversible coma, hypoxia and death. The organ most often affected in fatal accidents is the brain, followed by the lungs and heart. Especially at exposure to high concentrations in which death occurs within a relatively short time it is unlikely that the formation of CO will have resulted in life-threatening levels of COHb. Only one fatal case was reported to be related to a myocardial infarction without any signs of reported CNS-depression. Also in nonfatal cases the effect most frequently described are CNS-related only; in a few cases cardiotoxic effects (evidenced by ECG changes) are reported. Effects on lung, liver, or kidney are incidentally reported as primary signs of DCM toxicity. It is noted that in some cases high COHb levels up to 40% are measured without serious complaints. The reported COHb levels could not be linked to effects in a dose-related way in any of these cases.

No signs of eye, nose, or throat irritation were reported during a 2-h exposure to 986 ppm; the odor was reported to be moderately strong but not objectionable. In experiments with volunteers altered Visual Evoked Responses were observed after a 1-h exposure to 514 ppm DCM, but no subjective complaints like light-headedness were reported. Light-headedness and difficulties with enunciation were reported after 1 h of a 2-h exposure to 986 ppm. Also in occupational settings complaints reported appeared to be relatively mild following a 15-min exposure up to 1700 ppm or an 8 h TWA exposure up to 969 ppm. The experimental studies on neurobehavioral effects of DCM exposures showed that specific sensitive endpoints are affected within a concentration range of 195 to 751 ppm. The responses were not always consistent and no clear concentration-response effect was

observed. The effects observed are indicative of subtle changes which are neither irreversible nor will cause a serious impairment of escape. Therefore, these effects are regarded as sub-AEGL-2 effects. A separate study indicated that a 2-h exposure to 250 ppm , a 1.5-h exposure to 500 ppm, a 1-h exposure to 750 ppm, and a 0.5-h exposure to 1000 ppm can be conservatively regarded as NOAELs for specific neurobehavioral tests (reaction time, short-term memory). The subjects' perception of their own condition in this study was slightly better under DCM exposure compared to control conditions.

The available epidemiological studies gave no definite information on a relationship of DCM and neurobehavioral or neuropsychological functions and do not support an increased risk for cancer or for ischemic heart disease. International organizations considered the available epidemiological data inadequate for drawing any firm conclusions with regard to human cancer risk. Human genotoxicity data were absent.

The few limited studies available showed no effects of DCM exposure on semen quality.

3. ANIMAL TOXICITY DATA

3.1. Acute lethality

3.1.1. Dogs

A group of at least five Beagle dogs was exposed to DCM via tracheal cannulation (Von Oettingen *et al.*, 1950). The anesthetized dogs survived a 7-h exposure to 15,000 ppm showing only very little signs of toxicity. No further details on actual exposure concentrations were given. Maximum blood levels were 0.54 mmol/100 mL (459 µg/mL).

3.1.2. Rats

Groups of six male or female rats were exposed for 15 min to various DCM concentrations (no further details) (Clark and Tinston, 1982). An LC_{50} of 57,000 ppm (95% confidence interval: 45,000-75,000 ppm) was calculated. Death was reported to occur always during exposure. Recovery from a non-lethal exposure was rapid and the rats appeared normal within 10 min postexposure. Slight ataxia, loss of righting effects, loss of movement, narcosis, shallow respiration preceded death.

Rats exposed to an atmosphere saturated with DCM died within 15-25 min (Takeshita *et al.*, 2000).

F344/N rats (5/sex/group) were exposed to target concentrations of DCM of 15,500, 16,500, 16,800, 17,250, 18,500, or 19,000 ppm for 4 h, with a 14-d observation period (NTP, 1986). Three groups were exposed to 19,000 ppm, making a total of 8 exposure groups for each sex. Actual concentrations were not given. Mortality for male rats was 1/5, 0/5, 1/5, 2/5, 2/5, 3/5, 0/5, and 3/5 for the 8 groups, respectively, and mortality for female rats was 0/5, 0/5, 0/5, 0/5, 1/5, 2/5, 0/5, and 1/5, respectively. No meaningful LC_{50} could be determined. No compound-related effects were observed at necropsy.

Groups of 4 male Chr-CD rats were exposed to nominal DCM concentrations of 10,000, 15,000, or 21,531 ppm for 4 h (Haskell Laboratory, 1964). Death rates were 0/4, 1/4, and 2/4 for the three exposure groups, respectively. One rat of the highest dose group died 1.5 h postexposure, the other two rats died during exposure.

Groups of 6 male albino Crl:CD rats were exposed to mean (±SD) concentrations of 9900 (1500), 11,000 (1300), 14,000 (1200), 14,000 (1100), 15,000 (1300), or 18,000 (3200) ppm DCM for 4 h and held under observation for 14 d. The actual exposure concentrations ranged considerably, up to ±30%, with the highest exposure concentration ranging from 14,000-29,000 ppm. Mortality was 0/6, 0/6, 2/6, 2/6, 3/6, and 6/6 for the respective exposure concentrations (Haskell Laboratory, 1982). All

deaths occurred during exposure. Animals exhibited labored breathing, reduced response to sound, and spasms and convulsions followed by no movement. At higher exposure concentrations surviving animals exhibited decreased muscle tone and lethargy or no movement when removed from exposure.

Groups of 12 male Sprague-Dawley rats were exposed to various DCM concentrations ranging between approximately 12,000 and 16,000 ppm (estimated from a graph) for 6 h with an observation period of 14 d (Bonnet *et al.*, 1980). Within this range mortality ranged from 1/12 to 9/12, with deaths occurring during or within 24 h after exposure. An LC_{50} of 14,992 ppm (95% confidence limits: 14,585-15,745 ppm) was determined. The dose-response curve did not show a consistent increase in response with a sometimes lesser mortality at higher concentrations. Animals were hypotonic, sleepy, and showed ptosis and trembles. No macroscopic lesions were observed in the liver, lung, and kidneys of surviving animals.

3.1.3. Mice

Groups of ten male CD-1 mice were exposed to nominal concentrations of 1, 2, 3, 4, or 5 vol% (10,000, 20,000, 30,000, 40,000, or 50,000 ppm) DCM for 20 min (Aviado *et al.*, 1977). No details were given on actual exposure concentrations. Mortality rates were 0, 2, 6, 9, and 10 out of ten, respectively (observation time was unclear, but was maximally 24 h). The calculated LC_{50} was 26,710 ppm.

White mice (unspecified strain and number) were exposed for 2 h to DCM (Lazarew, 1929). The lowest concentration at which the animals lay on their sides, showed loss of reflexes, and died were found to be 30-35 g/m^3 (8400-9800 ppm), 35 g/m^3 (9800 ppm), and 50 g/m^3 (14,000 ppm), respectively. No details were given on actual exposure concentrations, but it is noted that they are initial concentrations in a closed system .

Groups of 5 male and 5 female $B6C3F_1$ mice were exposed to actual DCM concentrations of 15,975, 16,356, 16,948, 17,175, 18,035, 18,670, 19,271, and 20,398 ppm for 4 h, with a 14-d observation period (NTP, 1986). Mortality for male mice was 0/5, 0/5, 0/5, 4/5, 2/5, 4/5, 4/5, and 5/5, respectively, and mortality for female mice was 0/5, 0/5, 0/5, 3/5, 3/5, 1/5, 1/5, and 3/5, respectively. The LC_{50} for male mice was 17,703 (95% confidence limit range, 16,163-18,505). No meaningful LC_{50} could be determined for female mice. No compound-related effects were observed at necropsy.

Groups of 20 female OF_1 mice were exposed to DCM concentrations ranging between approximately 10,000 and 18,000 ppm (estimated from a graph) for 6 h with an observation period of 14 d (Gradisky *et al.*, 1978). An LC_{50} of 14,155 ppm (95% confidence limits: 13,691-14,535) was determined. No further details were given on actual exposure concentrations.

A 6-h LC_{50} of 16,100 ppm DCM was reported for male mice; animals were held for observation during 18 h postexposure (Scott *et al.*, 1979; abstract only).

Groups of 20 white Swiss mice were exposed to actual DCM concentrations of 11,485, 13,730, 13,126, and 15,400 ppm for 7 h (Svirbely *et al.*, 1947). A steep dose-response was observed with death rates of 0/20, 2/20, 4/20, and 18/20, respectively. All deaths occurred within 1 h postexposure, with one additional death (exposed to 13,126 ppm) occurring within 17 h postexposure. The mice showed symptoms of restlessness, muscular twitchings, uncoordinated movements, labored respiration, and narcosis.

Female Swiss Webster white mice were exposed to 13,500 ppm DCM (±7%) (Gehring, 1968). This concentration was chosen on the expectation that 50% of the animals would be killed between 9-12 h of exposure. The time to induce anesthesia in 50% (of 20) animals was 128 min, with the first animal anesthetized after 50 min. The time to mortality was studied in an additional group of 40 female mice. Mortality occurred between 400 and 800 min of exposure, with an LT_{50} (the time at which 50% of the animals (n=40) was killed) of 640 min (95% confidence interval: 622-658 min).

One white mouse (unspecified sex) died immediately after a 30-min exposure to approximately 17,000 ppm DCM. A second white mouse exposed for 82 min died within 5 d, while a third mouse survived a 63-min exposure for 4 d, whereafter the animal was killed (Müller, 1925). No deaths were observed in three white mice exposed for circa 60 min to approximately 14,000 ppm DCM.

3.1.4. Guinea pigs

Balmer *et al.* (1976) exposed Hartley male guinea pigs to DCM for 6 h with an observation period of 18 h postexposure. Mortality was 0/5, 3/20, 3/10, 4/10, 7/10, and 5/5 for exposure concentrations of 5000, 8700, 10,600, 11,000, 13,100, and 16,000 ppm, respectively. No details were given on actual exposure concentrations. The LC_{50} was 11,600 ppm with 95 % confidence limits of 10,500 and 12,800 ppm. For comparison, during simultaneous experiments the COHb levels in guinea pigs exposed for 6 h to 560, 5000, or 11,100 ppm DCM were 14.3, 16.3, and 17.6 %, respectively, as compared to 5.9 % for controls.

Nuckolls (1933) exposed groups of 3 guinea pigs for 5, 30, 60, or 120 min to a low (0.8-1.0 Vol%), medium (2.0-2.4 Vol%; groups of 2 guinea pigs) or high concentration ((5.0-5.4 Vol% at either low or high humidity) of DCM, control groups were included. The surviving animals were observed for ten days. No deaths were observed at the low (8000-10,000 ppm) and medium (20,000-24,000 ppm) exposure concentrations. Animals exposed to the high concentration (50,000-54,000 ppm at 24% or 72% humidity) lost co-ordination within 3-4 min of exposure and were unable to stand. All animals survived 5 min of exposure but one animal exposed for 30 min at high humidity died 35 min after exposure. Lungs, kidneys, and the heart were most severely affected. Two animals exposed for 1 hour at low humidity died after 35 min and 5 days, respectively, whereas all animals exposed at high humidity died almost immediately after exposure. Both at low and high humidity animals intended to be exposed for 2 hours died during the second exposure hour.

3.1.5. Summary

A summary of LC_{50} values is presented in Table 3.

TABLE 3. Summary of Acute Lethal Inhalation Data in Laboratory Animals

Species	Concentration (ppm)	Exposure Time	Effect	Reference
Rat	57,000	15 min	LC_{50}	Clark and Tinston, 1982
Rat	14,992	6 h	LC_{50}	Bonnet *et al.*, 1980
Mouse	26,710	20 min	LC_{50}	Aviado *et al.*, 1977
Mouse, male	17,703	4 h	LC_{50}	NTP, 1986
Mouse	14,155	6 h	LC_{50}	Gradisky *et al.*, 1978
Mouse	16,100	6 h	LC_{50}	Scott *et al.*, 1979
Mouse	16,189	7 h	LC_{50}	Svirbely *et al.*, 1947
Guinea pig	11,600	6 h	LC_{50}	Balmer *et al.*, 1976

The dose-response relation for lethality is very steep, with an increase in mortality from 0 to 100% within a twofold increase in exposure concentration. The data for mice and rats are comparable, lethality appears to occur at lower concentrations in guinea pigs than in mice and rats following 6 h of exposure to DCM. The comparable results for mice and rats are difficult to explain from a mechanistic point of view since the kinetics between these two species clearly differ at relatively high concentrations. Death is generally preceded by CNS effects that may be related to DCM concentration in brain. However, DCM concentration in blood was approximately 3 to 4 times higher in rats

compared to mice during exposure to 4000 ppm DCM for up to 6 h (Green, 1986), due to a higher metabolic rate in mice at high exposure concentrations (see chapter 4).

3.2. Nonlethal Toxicity

3.2.1. Nonhuman Primates

Groups of three anesthetized Rhesus monkeys (with trachea cannulation) were exposed for 5 min to 2.5 or 5 % v/v (25,000 or 50,000 ppm) DCM (Belej *et al.*, 1974). No further details were given on actual exposure concentrations. A dose-related increase in heart rate was observed, although statistically significant only at the higher exposure group (+11.8%). The myocardial contractility was dose-related depressed but did not reach statistical significance. Further, aortic blood pressure was dose-related decreased by 6.1 and 16.6% for the low and high exposure group, respectively. This decrease was statistically significant for both groups. No effects were observed on the left atrial pressure and pulmonary arterial pressure.

The same group of investigators studied pulmonary and circulatory functions in Rhesus monkeys following the same procedure and with the same DCM exposures (Aviado and Smith, 1975). Effects on aortic blood pressure and heart rate were similar to that observed by Belej *et al.* (1974). With respect to respiratory functions, pulmonary resistance was decreased by 32 % in the 5 % v/v exposure group although not statistically significant. No differences in respiratory minute volume with non-exposed controls were found, but pulmonary compliance was statistically significantly increased by 20.6% in the 5 % v/v exposure group.

Heppel *et al.* (1944) exposed two female monkeys (unspecified strain) to a mean actual concentration of 33.8 g/m^3 (SD:1.4 g/m^3) DCM (9464 ppm) for 5 d/w for about 7 weeks. Exposure on day one lasted 6 h whereas on all other days exposure was for 4 h. The animals showed a decreased activity during the 1st h and incoordination and difficulties in getting up from a lying position during the 2nd h of exposure. At the end of the 4th h of exposure the animals lay prostrate with barely perceptible respiration. These effects were less severe during the last two weeks of exposure. Histopathological examination of the heart, lungs, liver, spleen and kidneys revealed no alterations.

3.2.2. Dogs

Hemodynamic effects of DCM were studied in groups of 5 anesthetized adult mongrel dogs. Dogs were artificially ventilated via an endotracheal cannula and several parameters of cardiac functions (e.g. pulmonary arterial pressure, atrial pressure, ventricular pressure, heart rate, stroke volume) were studied (Aviado *et al.*, 1977). Each dog was exposed to nominal concentrations of 0.5, 1.0, 2.5, and 5 vol% (5,000, 10,000, 25,000, and 50,000 ppm) via the respirator for 5 min; each exposure immediately following the preceding one. No further details were given on actual exposure concentrations. Although for some parameters a dose-response was observed, statistically significant differences from control values were only observed in the 25,000 and 50,000 ppm exposure groups. The mean left arterial pressure, the left ventricular end-diastolic pressure, and systemic vascular resistance were increased, while mean pulmonary arterial flow, stroke volume, and stroke work were decreased. Exposure to paint remover under similar conditions (90.2 % DCM, 4.2 % methanol, 3.2 % isopropanol, and 2.4 % toluene) induced more or less comparable results. Although DCM itself was considered to be arrhythmogenic this was not the case with the paint remover.

Unanesthetized beagle dogs (number and sex unspecified) were exposed for 5 min to varying concentrations (no further details) of DCM under restrain (Clark and Tinston, 1982). A preexposure injection of adrenaline was made during air inhalation; after 10 min the animals were exposed for 5 min and received a challenge injection of adrenaline during the last 10 sec of the exposure period. Cardiac sensitization was deemed to have occurred when ventricular tachycardia of ventricular fibrillation resulted from the challenge injection. The EC_{50} for cardiac sensitization to adrenaline was 25,000 ppm (95% confidence interval: 19,000-34,000 ppm).

A group of five or more Beagle dogs was exposed to 15,000 ppm DCM for 7 h via tracheal cannulation (Von Oettingen *et al.*, 1950). No further details on actual exposure concentrations were given. Maximum blood levels were 0.54 mmols/100 mL (459 µg/mL). The effects of exposure on several parameters of cardiac function were presented in figures. Blood pressure was hardly affected, while venous pressure dropped after 1 h of exposure and increased thereafter. Heart rate was decreased during exposure while DCM caused considerable fluctuations in respiration rate and, hence, the minute volume.

Exposure to 6100 ppm DCM for 6 h induced light narcoses in dogs after 2 h (Flury and Zernik, 1931). No details were given on actual exposure concentrations. Animals recovered from the narcosis soon after the end of exposure.

Repeated exposure

Heppel *et al.* (1944) exposed six dogs (1 male and 5 females; unspecified strain) to an actual DCM concentration of 17 g/m^3 (SD: 2 g/m^3) (4760 ppm) for 7 h/d, 5 d/w up to 6 m. No ill effects were noted. Exposure of four female dogs to 33.8 g/m^3 (SD: 1.4 g/m^3) (9464 ppm) for 6 h on day one and for 4 h on the subsequent days was terminated after 6 exposure days. Within a few minutes of exposure they appeared to go into stages of excitement, bit one another, and knocked their heads against the sides of the cage. Frothing appeared about their mouths. A rapid improvement occurred after cessation of the exposure.

Beagle dogs (3/sex/group) were exposed to 0 or 5000 ppm (±3%) DCM for 6 h/d, 7 d/w for 90 d (Leuschner *et al.*, 1984). DCM induced slight sedation and dogs exhibited slight erythema of the conjunctivae, but it was not reported whether this started already on day 1. The erythema lasted for 10 h after each exposure. No further clinical, hematological, histopathological effects or alteration of heart functions were observed.

3.2.3. Rats

The arrhythmogenic properties of DCM were tested in an open-chest preparation of male Wistar rats (Scholz *et al.*, 1991). DCM was infused and after a 30-min stabilization period a regional myocardial ischemia was produced by occlusion of the left descending coronary artery for 5 min followed by a reperfusion period of 10 min. DCM concentrations in arterial blood ranged approximately from 1.04-1.14 µmol/mL (88-97 µg/mL). The incidence of atrioventricular block was markedly increased by DCM-infusion and the PR-interval of the ECG was prolonged.

Ciuchta *et al.* (1979) exposed groups of five to six Sprague-Dawley rats to DCM for 1 h. Peak COHb levels were determined at 2 h after exposure. No further details were given on actual exposure concentrations. Peak COHb levels were 1.3%, 3.2%, and 9.4% following a 1-h exposure to 50, 500, or 5000 ppm, respectively. A clear effect of age (as determined by weight, i.e. 200 g rats versus 450 g rats) on the increase in COHb levels (above control levels) was noted. The increase in COHb levels was twice as high in older rats (ca. 6 vs. 3%) 2 h after a 1-h exposure to 500 ppm DCM and approximately 3-times higher (ca. 12 vs. 4%) 4 h after a 1-h exposure to 5000 ppm. Following exposure to 5000 ppm COHb levels returned to control values approximately 8 h after exposure.

Male Wistar rats were exposed to 250,000 ppm for 20 s, and cytochrome c oxidase activity was determined at 20, 45, 90 and 180 min postexposure in brain, liver, kidney, muscle, lung, and heart (Lehnebach *et al.*, 1995). No further details on the actual exposure concentration were given. This exposure scenario resulted in peak COHb levels of 3-4% 2-h after exposure. Cytochrome c oxidase activity was decreased by 40-50% in brain, liver, kidney and muscle 20 min postexposure. Only cytochrome c oxidase activity in muscle was decreased 45 and 90 min after exposure. Pretreatment with DDTC (diethyldithiocarbamate), a cytochrome P4502E1 inhibitor, prevented the inhibition of cytochrome c oxidase by DCM. This indicates that the inhibition is through a metabolite formed by the mixed function oxidase-pathway, probably CO (see section 4.1.2).

The influence of DCM exposure on sleeping behavior was studied in groups of approximately five female albino rats (Ivan-Wistar strain) exposed to 500, 1000, or 3000 ppm DCM for 24 h (Fodor and Winneke, 1971). No further details on actual exposure concentrations were given. During the 24-h period the total length of sleep was slightly dose-related increased in exposed rats, though not statistically significant. A statistically significant suppression of REM-sleep as well as an increase in time between two successive REM-periods was observed at 1000 and 3000 ppm.

Groups of six male or female Alderley Park rats were exposed for 10 min to varying DCM concentrations (Clark and Tinston, 1982). The animals were observed for CNS-effects, i.e. ataxia and loss of righting reflex. The EC_{50} was 9000 ppm (95% confidence interval: 7000-12,000 ppm). No further details are given.

Frantík *et al.* (1994) exposed male albino SPF rats (0.5-1 year of age; number not specified) for 4 h to various DCM concentrations. The effect studied was shortening of the tonic extension of the hindlimbs in rat and lengthening of the latency of extension in mice following a short electrical impulse. The concentration inducing a 30% response was calculated to be 1980 ppm.

One group of nine female rats was exposed in a random order to a concentration of 15,000 ppm of nine solvents among which was DCM (Schumacher and Grandjean, 1960). No further details on the actual exposure concentration were given. A 1-d interval was assured between exposures. Paralysis of the hind limbs was observed after 519 s on average. After another 1318 s animals did not respond anymore to a 100 V electric shock. Hereafter the exposure was stopped and the animals responded to electric shocks again about 211 s after cessation of exposure.

The influence of a 2-h exposure to 40 g/m^3 DCM (11,200 ppm) on the biosynthesis of ascorbic acid was studied in rats (Ulanova and Yanovskaya, 1959). Animals were killed 1 or 24 h after exposure. All animals showed an impaired coordination, dragging of limbs, lying on their sides, and narcosis; recovery was complete within 40-50 min postexposure. One hour after exposure the ascorbic acid content was increased in liver (by 70%), small intestine (25%), spleen (40%), brain (29%), kidneys (20%), and heart (30%), but not in lungs and adrenals as compared to an unexposed control group of 20 rats. Differences were not tested for statistical significance. Twenty-four hours postexposure these levels were still slightly elevated in most tissues.

Sprague-Dawley rats (5/sex/group) were exposed to target concentrations of 0, 1500, 3500, or 8000 ppm DCM for 6 h/d for 1 day (Landry *et al.*, 1981). Mean actual concentrations were within 5% of the targeted concentrations. Animals were necropsied 2 h after exposure. No adverse effects were noted in the 1500 and 3500 ppm exposure groups. Animals exposed to 8000 ppm exhibited a number of effects during exposure, including increased activity, tremors, staggering, and gasping. These effects were diminished within 0.5 h postexposure. A slight increase (10%) in relative liver weights was noticed in both sexes. All rats showed swellings of the skin and subcutaneous tissues around the external nares; these swellings were due to edema and acute inflammation. Acute inflammation and/or hemorrhages were observed in the cervical lymph nodes in several rats. Further, lymphoid necrosis of the thymus and some lymph nodes was reported for some rats.

Groups of ten male F344 rats were exposed to mean analytical DCM concentrations of 0, 1910, or 3910 ppm for 6 h (Hext *et al.*, 1986). Animals were killed 1 d postexposure and lungs and liver were histopathologically examined. No clinical or histopathological effects were observed in rats during and after exposure.

The acute effects of DCM on the spontaneous EEG and evoked potentials (EPs) were studied by exposing twelve adult male F344 rats to actual mean concentrations of 5200, 10,100, or 15,100 ppm for 1 h (Rebert, *et al.*, 1989). Each rat was exposed to each concentration (one-week interval) and served as its own control. EPs were quantified by measuring peak latencies and peak-to-peak amplitudes. FEPs and SEPs were recorded every 5 min during the first 45 min of exposure. A general

test battery of electrophysiological tests (including spontaneous EEG) was administered after 25 and 50 min of exposure, and at 5, 30, and 60 postexposure. Auditory EPs, FEPs, and SEPs (latencies and amplitudes of specific peaks) were affected at all exposure concentrations, although not always clearly concentration-related. The several sensory systems were not always affected in the same way. The main effect of DCM on the EEG was to increase the power in the 8-12 Hz and 12-16 Hz frequency bands in a concentration- and time-related way. The COHb level in arterial blood at the end of the 1-h exposure to 15,100 ppm DCM was 7.1%.

Other routes of exposure

The effects of single oral or ip administration of DCM on specific organs have been investigated. The effects reported included renal toxicity (Kluwe *et al.*, 1982; Marzotko and Pankow, 1987; 1988) and a dose-response related decreased nerve conduction velocity (Pankow *et al.*, 1979; Glatzel *et al.*, 1987). Pankow *et al.* (1979) reported decreased nerve conduction velocities for DCM blood concentration ranging from 2.3-27.6 mg/mL.

Repeated exposure

A few data on repeated exposures are presented to provide either data on effects at the first day of exposure or information on DCM concentrations related to mortality.

Heppel *et al.* (1944) exposed 21 rats (15 male and 6 female; unspecified strain) to 17 g/m^3 (SD: 2g/m^3) (4760 ppm) DCM for 7 h/d, 5 d/w up to 6 m. No signs of narcosis were seen. One female rat died shortly after giving birth on the 22nd exposure day. Sixteen rats (9 males, 7 females) were exposed to 33.8 g/m^3 (SD: 1.4 g/m^3) (9464 ppm) for 5 d/w for 7.5 weeks. Exposure on day one lasted 6 h whereas on all other days exposure was for 4 h. The rats showed signs of narcosis within the first 30 min of exposure and at he end of the fourth hour they were prostrate with very depressed respiration. Two rats died after 33 and 38 exposure days, respectively. In a separate series of experiments five rats were exposed to 5000 ppm DCM (no details on actual concentration) for 1.5 h (Heppel and Neal, 1944). Five exposure days were alternated with exposure-free days. The 1-h running activity was measured on every day, on exposure days running activity was measured twice: during the last hour of exposure and starting 30 min after exposure. The running activity during exposure was markedly decreased. The postexposure activity was higher than during exposure but lower than at days of nonexposure. The overall mean running activity on nonexposure days was 576 cage revolutions per hour, compared with 59 revolutions per hour on exposure days.

Sprague-Dawley (20/sex/group) were exposed to 0 or 10,000 ppm (±3%) DCM for 6 h/d, 7 d/w for 90 d (Leuschner *et al.*, 1984). DCM caused slight erythema lasting for 1 to 10 h after each exposure period. No further clinical, hematological, or histopathological alterations were observed.

Groups of five F344/N rats per sex were exposed to target DCM concentrations ranging from 1625 to 16,000 ppm for 6 h/d, 5 d/w on 11 d over a 18-d period (NTP, 1986). No further details on actual concentrations were reported. Necropsy was performed on all animals, but no histological examination of tissues was performed. No effects were observed at exposure concentrations up to 3250 ppm. Mortality was 4/5 for males and 5/5 for females exposed at 16,000 ppm, while 1 male and 1 female rat exposed at 13,000 ppm died (time of death was not given). Groups of ten F344/N rats per sex were exposed to target DCM concentrations ranging from 525 to 8400 ppm for 6 h/d, 5 d/w for 13 weeks (NTP, 1986). The deaths of one male and one female in the highest exposure group were attributed to DCM exposure. No effects were observed in animals exposed to DCM concentrations up to 2100 ppm.

3.2.4. Mice

Female Swiss Webster white mice were exposed to 13,500 (±7%) ppm DCM (Gehring, 1968). This concentration was chosen on the expectation that 50% of the animals would be killed between 9-12 h of exposure. The time to induce anesthesia in 50% (of 20) animals was 128 min, with the first animal anesthetized after 50 min.

Aranyi *et al.* (1986) studied the effect of DCM exposure on the lung host defenses in female CD1 mice. Five groups of approximately 30 mice (140 mice in total) were exposed to 50 or 100 ppm DCM for 3 h and simultaneously challenged with an aerosol of viable *Streptococcus zooepidemicus*. No details were given on actual exposure concentrations. Mortality (as recorded over a 14-d observation period) was statistically significant increased after a 3-h exposure to 100 ppm DCM as compared to control groups (no DCM exposure). A 3-h exposure to 50 ppm DCM either one or on five consecutive days did not increase mortality. However, it is noted that mortality in the two control groups was rather high. In addition, bacterial clearance was determined in groups of 18 mice simultaneously exposed to aerosols containing viable ^{35}S-*Klebsiella pneumonia*. The rate at which bacteria were destroyed was assessed at 3-h after exposure, and was statistically significantly lower after a single 3-h exposure to 100 ppm DCM as compared to controls, but no differences were observed after single or repeated 3-h exposure to 50 ppm DCM.

The induction of cardiac arrhythmia by DCM was studied in Male Swiss mice (Aviado and Belej, 1974). Anesthetized mice were exposed to nominal concentrations of 20 or 40 % v/v DCM with or without an iv epinephrine injection. No details were given on actual exposure concentrations. Exposure to DCM lasted for 6 min and epinephrine was administered after 2 min of exposure. Exposure to 20 % v/v DCM did not induce arrhythmia's in 5 exposed mice, whereas a 2nd degree block was observed in 3/5 mice exposed to 40 % v/v. Exposure in combination with administration of epinephrine caused a 1st degree block in 1/5 mice exposed to 20 % v/v DCM and in all three mice exposed to 40 % v/v.

Male Swiss Webster mice (3-, 5-, or 8-weeks of age) were trained to avoid a grid where they received a foot shock (passive-avoidance conditioning task) (Alexeeff and Kilgore, 1983). Thereafter, they were exposed to 168.1 mg DCM/L (47,068 ppm) until loss of their righting reflex (usually 20 s). Animals appeared fully recovered in approximately 5-10 min. Mice were tested on possibility to recall the task 1, 2, or 4 days after exposure. Each exposure group consisted of about 15 mice with a control group of 20 mice for each exposure group. The percentage of mice recalling the task was statistically significantly lower in the 3-week old mice on the 3rd day of testing. For the 5-week old mice the exposure group performed significantly less than the control group only at day 1 postexposure, but this difference was not statistically significant. The results for the 8-week old mice were inconsistent.

Three-week old mice (n=15) were placed on a heated surface to test the analgesic activity of DCM. The time of contact with the surface to the time of pain sensation was recorded. The mouse was considered to be in an analgesic state if no reactions were observed for 30 s. No differences were observed between DCM exposed mice and control mice.

Frantík *et al.* (1994) exposed female mice (H strain; 2-4 m of age; number not specified) for 2 h to various DCM concentrations. The effect studied was lengthening of the latency of extension following a short electrical impulse. The concentration inducing a 30% response was calculated to be 3980 ppm in mice.

Groups of 24 male NMRI mice were exposed to 400, 550, 600, and 750 ppm, and groups of 13 male mice were exposed to 850, 1100, 2200, and 2500 ppm DCM for 1 h from 23.00 to 24.00 hour (Kjellstrand *et al.*, 1985). No details were given on actual exposure concentrations. Motor activity was recorded. The highest concentration at which no effect on motor activity was observed was 600 ppm. At higher concentrations the motor activity was increased at the onset of exposure and decreased after exposure to below pre-exposure activity levels. In control animals motor activity remained on a more or less constant level between 22.00 and 2.00 hours.

White mice (unspecified sex) were exposed to nominal DCM concentrations of 24 g/m^3 (6720 ppm) DCM for 122 min (n=1), to 49 g/m^3 (13,720 ppm) for about 60 min (n=3), or to approximately 62 g/m^3 (17,360 ppm) for 30 to 82 min (n=3) (Müller, 1925). No details were given on actual exposure

concentrations. No sign of anesthesia was observed at 24 g/m^3, while at higher concentrations mice were anesthetized after 5-15 min.

Mice (strain, sex, and number not specified) lay on their sides after 6-11 min of exposure to 18,000 ppm DCM, or after 8-15 min of exposure to 14,500 ppm, or 4.5 h of exposure to 5800 ppm. This effect was not observed in mice exposed to 7000 ppm for 2 h. Deep narcosis was observed at the end of a 6-h exposure to 5800 ppm, recovery occurred 2-3 h after exposure (Flury and Zernik, 1931).

Groups of 20 female ICR mice were continuously exposed to 5000 ppm DCM (SD: 170 ppm) for 1, 4, 8, or 12 h and for 1, 2, 3, 4, 6, or 7 d and sacrificed immediately after exposure (Weinstein *et al.*, 1972). The study was especially focussed on liver effects. Heart, lung, spleen, pancreas, intestine, and kidney appeared normal upon microscopic examination. Principal initial clinical signs were increased activity while at 24 h of exposure spontaneous activity had decreased dramatically and the mice appeared lethargic. Livers of mice exposed for up to 8 h showed loss of glycogen, but no other changes. Definite changes were present in centrilobular hepatocytes after 12 h of exposure, consisting of changes in the rough endoplasmatic reticulum (RER) and to a lesser extent in the smooth endoplasmatic reticulum (SER). A breakdown of polysomes and detachment of many ribosomal particles from the RER was noted. Further, an onset of "balloon degeneration" (coalescence of ER membranes into large vacuoles) was also observed. Other effects noted are increase of lipid droplets (size and number), glycogen depletion, and mitochondrial changes. Liver triglycerides increased linearly over the first three days of exposure to a peak level 12-fold above control levels. After the third day the triglyceride levels decreased rapidly until a plateau level of 2-3 times the control level was reached at day 6. Some of these changes may also be attributed to nutritional factors since exposed animals ate less and lost body weight during exposure as compared to controls. The effects reached its severest extent after two to three days of exposure. Hereafter some improvement occurred although differences were still present compared to livers of control animals after 7 d of exposure.

Groups of ten male B6C3F$_1$ mice were exposed to mean actual DCM concentrations of 0, 2010, or 3960 ppm DCM for 6 h/d for one day (Hext *et al.*, 1986). Animals were killed one day postexposure. The effects in mice deviated from those in rats (as described in section 3.2.3). Mice of the high exposure group were slightly hyperactive during the first three hours of exposure and were subdued for the remaining hours. A decreased liver weight was noted and light and electron microscopic examination (performed on 5 animals) revealed an increase in the number of myelin whorls present in the bile cannuli of centrilobular hepatocytes in the 3960 ppm exposure group. Effects on the lungs were more extensive with vacuolation, pyknosis and swelling of the endoplasmatic reticulum of the non-ciliated (Clara) cell of the bronchiolar epithelium at both exposure concentrations. A considerable loss of cilia was noted in the ciliated bronchiolar cells of some of the mice of both exposure groups. Alveolar type II cells showed pale enlarged mitochondria, predominantly in the high exposure group. It is noted that these effects were less severe in mice exposed for 10 d to the same concentrations.

The results of Hext *et al.* (1986) were confirmed by Foster *et al.* (1992), who exposed male B6C3F$_1$ mice to 4000 ppm DCM, 6 h/d, 5 d/w, for up to 13 weeks. No details on the actual concentration were given. Acute Clara cell damage (vacuolization in the majority of the cells) was observed after one exposure day, which resolved after 5 d of exposure. A similar pattern was observed during the second week of exposure. The severity of the lesion decreased over the study period. No effects on lung cytosolic GST-metabolism of DCM was observed, but the activity of cytochromes P450 IIB 1 and 2 was inversely related to the degree of damage. NPSH levels were increased in the lung. The number of bronchiolar cells labeled by tritiated thymidine was approximately doubled after one exposure day. Type II alveolar epithelial cells remained unaffected. In additional studies, male B6C3F$_1$ mice were exposed to 0, 125, 250, 500, 1000, 2000, or 4000 ppm DCM for 6 h and killed 18 h postexposure, (Foster *et al.*, 1994). Clara cell damage was present at concentrations of 2000 ppm and higher. Cytochrome P450 inhibition by piperonyl butoxide reduced the number of vacuolated cells from 26.3 to 2.4% in mice exposed to 2000 ppm; glutathione depletion had no effect. This indicates

that a metabolite formed via the microsomal pathway may be responsible for the lesions. NPSH levels were slightly increased at concentrations of 250 ppm and higher. The incorporation of tritiated thymidine into isolated Clara cells was increased at DCM concentrations of 1000 ppm and higher, indicative of an increased level of DNA synthesis.

The RD_{50} for DCM for a 30-min exposure appeared to be above 1000 ppm in male Swiss-Webster mice (Stadler and Kennedy, 1996).

Repeated exposure

Groups of five $B6C3F_1$ mice per sex were exposed to target DCM concentrations ranging from 1625 to 16,000 ppm for 6 h/d, 5 d/w on 11 d over a 18-d period (NTP, 1986). No details on the actual concentrations were given. Necropsy was performed on all animals, but no histological examination of tissues was performed. No effects were observed at exposure concentrations up to 6500 ppm. All mice exposed to 16,000 ppm and 3/5 male and 4/5 female mice exposed at 13,000 ppm died.

Groups of ten $B6C3F_1$ mice per sex were exposed to target DCM concentrations ranging from 525 to 8400 ppm for 6 h/d, 5 d/w for 13 weeks (NTP, 1986). No details on the actual concentrations were given. Complete necropsy was performed on all animals, and a complete histological examination was performed on high dose animals and controls, and on animals from lower dose groups were appropriate. No effects were observed in mice exposed to DCM concentrations up to 2100 ppm. The deaths of 4/10 male and 2/10 female mice in the highest exposure group were attributed to DCM exposure. A reduced body weight gain (although not statistically significant) was reported for female mice exposed to 8400 ppm. Centrilobular hydropic degeneration was observed in 3/10 males and 8/10 females exposed at 8400 ppm and in 9/10 females exposed at 4200 ppm. A decreased liver lipid:liver weight was noted in female mice exposed at 8400 ppm.

3.2.5. Guinea pigs

Nuckolls (1933) exposed groups of 3 guinea pigs for 5, 30, 60, or 120 min to a low (0.8-1.0 Vol%), medium (2.0-2.4 Vol%; groups of 2 guinea pigs) or high concentration ((5.0-5.4 Vol% at either low or high humidity) of DCM, control groups were included. The surviving animals were observed for ten days. No deaths were observed at the low and medium exposure concentrations. At the low exposure concentration (8000-10,000 ppm) tremors and irregular breathings increased with exposure duration but all animals recovered after exposure. At the medium concentration (20,000-24,000 ppm) more severe signs of toxicity were observed, animals were semiconscious and barely able to stand at the end of the 30-min exposure. One animal exposed for two hours was sacrificed one hour postexposure. The lungs, liver and kidneys showed a pale appearance, no other histopathological signs were observed. The effects reported for the two high dose groups (50,000-54,000 ppm at 24% or 72% humidity) were very similar. Animals lost co-ordination within 3-4 min of exposure and were unable to stand. All animals survived 5 min of exposure but one animal exposed for 30 min at high humidity died 35 min after exposure. Lungs, kidneys, and the heart were most severely affected. Two animals exposed for 1 hour at low humidity died after 35 min and 5 days, respectively, whereas all animals exposed at high humidity died almost immediately after exposure. Both at low and high humidity animals intended to be exposed for 2 hours died during the second exposure hour.

Balmer *et al.* (1976) exposed groups of five Hartley male guinea pigs to DCM for 6 hours per day for one or five consecutive days. Single exposure concentrations were 560, 5000, 11,100 ppm, the DCM concentration in repeated exposure varied between 552 and 679 ppm. The lung, liver, kidney and spleen were examined for gross pathological changes; the first three organs were also histologically examined. At the start of the single exposure to 11,100 ppm the animals exhibited continuous motion of both fore and hind limbs while lying on their side or back. These effects were not observed at lower concentrations. Two out of five animals died at 11,100 ppm. COHb levels (in blood taken from the right side of the heart) were 14.3, 16.3, and 17.6 % at the three exposure concentrations respectively, as compared to 5.9% for controls. This may indicate saturation in the formation of COHb between exposure concentrations of 560 to 11,100 ppm. It is remarked that the COHb level after exposure to

552-679 ppm for 5 days did not differ from control values. Plasma triglyceride levels were unaffected in DCM exposed animals, but hepatic triglyceride levels were statistically significantly increased after single exposure to 5000 and 11,100 ppm (concentration-related). No effects on spleen and kidneys were found. Congestion and hemorrhage was noted in 3/5 animals exposed to 11,100 ppm. As to the liver, fatty changes were observed in all guinea pigs exposed to 11,100 ppm.

Morris *et al.* (1979) studied the effect of a single 6-h exposure to 5200 ppm (SEM: 100 ppm) DCM on the liver in 10 male Hartley guinea pigs. A control group was exposed to air for 6 hours. Animals were killed immediately after exposure. Animals exposed to DCM showed a 2.5-fold increase in hepatic triglycerides, but a 2.8-fold decrease in serum triglycerides. It was suggested that DCM inhibited the secretion of triglycerides into the serum, and hereby causing a fatty liver. Hepatic protein synthesis studied during the final hour of exposure by ip injection of radiolabelled leucine was not affected. No morphological changes in hepatocytes were found.

Exposure to 6100 ppm DCM for 6 h induced light narcoses in guinea pigs after 2-2.5 h (Flury and Zernik, 1931). No details were given on actual exposure concentrations. Animals recovered from the narcosis soon after the end of exposure.

Repeated exposure

Heppel *et al.* (1944) exposed 14 male guinea pigs (unspecified strain) to 17 g/m^3 (SD: 2g/m^3) (4760 ppm) DCM for 7 h/d, 5 d/w up to 3 or 6 months. Three animals died after 35, 90, and 96 exposure days, respectively. Extensive pneumonia associated with moderate centrilobular fatty degeneration of the liver was reported in these animals. No deaths occurred among 12 guinea pigs (11 male and 1 female) exposed to 33.8 g/m^3 (SD: 1.4 g/m^3) (9464 ppm) for 5 d/w for 8 weeks. Exposure on day one lasted 6 h whereas on all other days exposure was for 4 hours. A few animals showed mild incoordination.

3.2.6. Rabbits

Exposure to 6100 ppm DCM for 6 hours induced light narcoses in rabbits after 45 min (Flury and Zernik, 1931). No details were given on actual exposure concentrations. Animals appeared to recover from the narcosis soon after the end of exposure but were dead after 24 hours.

Repeated exposure

Heppel *et al.* (1944) exposed 4 rabbits (2 male and 2 female; unspecified strain) to 17 g/m^3 (SD: 2g/m^3) (4760 ppm) DCM for 7 h/d, 5 d/w up to 6 months. No signs of narcosis were seen. Histopathological examination of the heart, lungs, liver, spleen and kidneys revealed no alterations. Five male rabbits were exposed to 33.8 g/m^3 (SD: 1.4 g/m^3) (9464 ppm) for 5 d/w for 7.5 weeks. Exposure on day one lasted 6 hours whereas on all other days exposure was for 4 hours. Three rabbits died after 1, 12, and 22 exposure days, respectively. Two of these rabbits (the rabbit that died after one exposure day was not examined) showed pulmonary congestion and edema with focal necrosis, and splenic congestion. The animals showed signs of narcosis during exposure; no evidence of mucous membrane irritation was observed.

3.2.7. Summary

A summary of relevant nonlethal inhalation data in laboratory animals is given in Table 4.

Table 4. Summary of relevant nonlethal inhalation data in laboratory animals (emphasis on one-day exposure)

Species	Concentration (ppm)	Exposure time	Effect	Reference
Monkey (n=2)	9464	6 h	Side laying after 4 h	Heppel *et al.*, 1944
Dog (n=6)	9464	6 h	Excitement within min, arousal	Heppel *et al.*, 1944
Rat (n=5)	1000	24 h	Depression of REM-sleep during exposure	Fodor and Winneke, 1971
Rat (n=?)	1980	4 h	EC_{30} for shortening of tonic extension of the hindlimbs/ lengthening of latency of extension	Frantík *et al.*, 1994
Rats (n=21)	4760	6 h	No signs of narcosis	Heppel *et al.*, 1944
Rats (n=5)	5000	1.5 h	Decreased running activity	Heppel and Neal, 1944
Rat (n=6)	9000	10 min	EC_{50} for ataxia	Clark and Tinston, 1982
Rats (n=16)	9464	6 h	Signs of narcosis within 30 min, side lying after 4 h	Heppel *et al.*, 1944
Rats (n=20)	10,000	6h/d,7d/w,90d	No clinical, hematological, histopathological changes	Leuschner *et al.*, 1984
Rat (n=20)	11,200	2 h	Increasing CNS-effects, incl. narcosis	Ulanova and Yanovskaya, 1959
Rat (n=9)	15,000	519 s	Hind limb paralysis	Schumacher and Grandjean, 1960
Mouse (n=?)	3980	2 h	EC_{30} for shortening of tonic extension of the hindlimbs/ lengthening of latency of extension	Frantík *et al.*, 1994
Mouse (n=10)	4000	6 h	Hyperactive followed by subdued appearance, decreased liver weight, lung effects (damaged Clara cells)	Hext *et al.*, 1986; Foster *et al.*, 1992
Mouse (n=20)	13,500	50 min	First animal with anesthesia	Gehring, 1968
Guinea pig (n=5)	5000	6 h	No CNS-effects, increased hepatic triglyceride level	Balmer *et al.*, 1976
Guinea pig (n=10)	5200	6 h	Increased hepatic and serum triglycerides	Morris *et al.*, 1979
Rabbit (n=4)	4760	7h/d,5d/w,6m	No signs of narcosis, no histopathological effects	Heppel *et al.*, 1944

3.3. Developmental/Reproductive Toxicity

CO is known to readily cross the placenta and is reported to be eliminated at a slower rate than from the maternal circulation. The developing fetus may be more susceptible to CO than the mother. Hence, a risk may also be present following exposure to DCM (Bentur *et al.*, 1994).

Anders and Sunram (1984) showed that DCM can cross the placenta. Pregnant Sprague-Dawley rats were exposed to 500 ppm DCM for 1 hour on gestation day 21, and killed immediately thereafter. DCM concentrations were 176 and 115 nmol/mL in maternal and fetal blood (approximately

15 and 9.8 µg/mL), respectively. The respective blood CO concentrations were comparable, 167 and 160 nmol/mL (4.7 and 4.5 µg/mL).

A group of 13 pregnant Swiss Webster mice and a group of 19 pregnant Sprague-Dawley rats were exposed to 1225 ppm DCM for 7 h/d on gestation day 6-15 (Schwetz *et al.*, 1975). Concurrent exposure of non-pregnant animals revealed COHb levels of 9-10% in rats and 10-12% in mice, respectively. Pregnant animals were killed on gestation day 21 (rats) or 18 (mice). A significant increase was observed in maternal body weight (11-15%) (mice only) and absolute but not relative liver weight in both species. No effects were seen on the number of implantation sites/litter, the number of live fetuses per litter, the incidence of fetal resorptions, the sex ratio, fetal body weight, and fetal length in either species. Among litters of rats, the incidence of lumbar ribs or spurs was significantly decreased as compared to controls (4/19 litters affected versus 10/30 for controls). The incidence of delayed ossification of sternebrae was reported to be statistically significantly increased but this was not substantiated by the presented incidences (5/19 litters versus 9/30 litters for controls). As to mice, a significant number of litters contained pups with a single extra center of ossification in the sternum (6/12 litters affected versus 0/26 litters for controls). Microscopic examination of sagittal sections of whole fetuses revealed no exposure-related effects.

The teratogenicity and behavioral toxicity in offspring of DCM were studied in Long-Evans rats (Hardin and Manson, 1980; Bornschein *et al.*, 1980). Female rats were exposed either before and during gestation (group A), only before gestation (group B), or only during gestation (group C). A control group (group D) was exposed to filtered air. Exposure to 4500 ppm DCM was for 6 h/d and for 7 d/w. Pregestational exposure was for approximately 3 weeks, while exposure during gestation lasted until day 17; the dams were sacrificed on gestation day 21. The number of litters used for the teratogenicity study were 18, 18, and 16 for the three exposure groups, respectively and 21 for the control group. Ten additional litters per treatment group were used for the neurobehavioral testing.

COHb levels were measured in one rat from group C and three rats from group A on gestation day 17, and in one rat of group A on gestation day 1. COHb levels ranged from 7.2 to 10.1%. No effects were observed on maternal body weight. No differences in maternal toxicity or embryotoxicity were observed between groups A and C, indicating that pregestational exposure did not increase the incidence of maternal toxicity or embryotoxicity. Dams exposed during gestation (groups A and C) had statistically significantly increased absolute and relative liver weights. Fetal body weight in these groups was decreased only in the study by Hardin and Manson (1980), but not in the study by Bornschein *et al.* (1980). No effects were seen on the number of implantation sites/litter, the number of life fetuses per litter, the incidence of fetal resorptions, and the sex ratio. No significant differences were found in soft tissue or skeletal anomalies between the groups.

As to behavioral effects, all litters were culled to 4 males and 4 females. Pups were weaned at 21 days of age at which point litter size was further reduced to 2 males and 2 females by random selection. All pups from five litters per group were tested for reactivity to transfer from the home litter to a new environment at 5 and 10 days of age, while one male and one female pup per litter was tested in a photocell activity cage at 15 days of age. The two males per litter were subsequently placed in running wheels for 9 weeks (45 – 108 days of age). Two weeks after removal from the running wheels all animals (4 months of age) were tested to acquire an avoidance response in a jump-up escape/avoidance chamber. Finally, at 5 months of age the short-term exploratory behavior of individual male and female rats were assessed by recording activity counts per 5 min during a 90-min stay in a new environment. The activity tests showed no consistent detrimental effect of DCM exposure prior to and/or during gestation (Bornschein *et al.*, 1980).

Groups of 20 Swiss-Webster male mice were exposed to 0, 100, 150, or 200 ppm DCM for 2 h/d, 5 d/w for 6 weeks (Raje *et al.*, 1988). Mating started 2 days after exposure and was allowed for two weeks or until successful mating. Females were killed on gestation day 17. Histopathological examination of the testes of the males did not reveal any microscopic changes. The number of

successful matings was 18/19, 19/20, 16/20, and 16/20 for the control group and the 100, 150, and 200 ppm exposure groups respectively. No differences between the groups were observed in number of implants/litter, in number of live fetuses per litter or the percentage dead or resorbed per litter.

Groups of 30 male and 30 female F344 rats were exposed to 0, 100, 500, or 1500 ppm DCM for 6h/d for 5d/w (excluding holidays) in a two-generation study (Nitschke *et al.*, 1988b). P_0 animals were exposed for 14 weeks prior to mating (1 male and 1 female) starting at an age of 7 weeks. F_1 offspring was weaned at 4 weeks of age and exposed for 17 weeks until mating. Dams were not exposed from gestation day 21 through the fourth day postpartum. Exposure of P_0 and P_1 male and female adult rats continued until the animals were euthanized. No effects of DCM exposure on demeanor or physical appearance of P_0 animals were observed. The fertility and gestation survival indices, litter size, and F_1 pup weights were unaffected by DCM exposure. Gross examination of P_0 and F_1 animals and histopathologic examination of F_1 animals revealed no exposure-related lesions. Similarly, no effects attributable to DCM exposure were found upon examination of the P_1 and F_2 animals.

The effects of DCM on the developing embryo were tested *in vitro* by Brown-Woodward *et al.* (1998). Rat embryos obtained from Sprague-Dawley rats on day 10 of pregnancy were cultured with different concentrations of DCM for 40 hours. Due to changing the gaseous medium of the culture bottles after 16 and 24 h, DCM concentrations decreased by approximately 50-70% at both time points. Endpoint studied were development of yolk sac blood vessels, fully dorsally convex, heart beat, crown-rump length, somite number, and protein content (µg/embryo). DCM affected crown-rump length, somite number, and protein content in a dose-dependent way starting from a concentration of 6.54 µmol/mL (555 µg/mL), with 3.46 µmol/mL (294 µg/mL) being a NOAEL. From comparison with blood concentrations in cases and experiments it was noted by the authors that similar blood DCM concentrations would only occur in humans exposed to (near-)lethal concentrations of DCM.

In summary, no clear teratogenic or adverse developmental effects were observed in rats at exposure levels up to 4500 ppm. A 2-generation study in rats exposed to DCM concentrations of up to 1500 ppm revealed no exposure-related changes.

3.4. Genotoxicity

The genotoxicity of DCM has been evaluated by several organizations (WHO, 1996; IARC, 1999; ATSDR, 2000). A summary of the main results is presented, see IARC (1999) for more details (IARC, 1999: tables 8 and 9) and individual references.

DCM appears to be positive in a number of assays with different strains of *S. typhimurium*, with and without exogenous metabolic activation, in *E. coli*, and in *S. cerevisiae*. DCM did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*. DNA-protein cross links were induced *in vitro* in hepatocytes of male $B6C3F_1$ mice but not in hepatocytes of F344 rats, Syrian hamsters, or in human hepatocytes with functional GSTT1 genes. DNA-protein cross-links also occurred in CHO cells with or without metabolic activation.

DCM induced DNA single-strand breaks in hepatocytes of AP rats and of $B6C3F_1$ mice, but not of Syrian hamsters *in vitro*. In addition, DNA single-strand breaks were also induced in $B6C3F_1$ Clara cells, DNA damage was decreased in the presence of a glutathione-depleting agent. When tested without metabolic activation, DCM did not induce UDS or *hprt* locus gene mutations in Chinese hamster lung V79 cells, but a slight increase in SCEs was reported. DCM was mutagenic in CHO cells at the *hprt* locus only with metabolic activation, but equivocal results were found in a mouse lymphoma assay. DCM did not induce SCEs in hamster ovary CHO cells, but induced chromosomal aberrations in one of two studies. Positive results were observed in tests with human lymphocytes (SCE) and with lymphoblastoid cells, but not in human primary hepatocytes and AH fibroblasts (all tests without metabolic activation).

As to *in vivo* tests with inhalation exposure, most studies revealed negative or equivocal results (Table 5); positive or equivocal results were only obtained with mice, not with rats or hamsters. The exposure concentrations were generally 2000 – 8000 ppm for one or more days; the endpoints studied were DNA single-strand breaks, DNA-protein cross links, UDS, SCE, chromosomal aberrations, and bone marrow micronucleus tests. DNA-protein cross links increased in a concentration-dependent way in livers from $B6C3F_1$ mice exposed to concentrations of approximately 145, 495, 1550, 2600, and 4000 ppm DCM, 6 h/d for 3 days (Casanova *et al.*, 1996). With respect to single (one-day) inhalation exposure positive results were observed as to DNA single-strand breaks in mouse liver and lung, but not in rat liver and lung. Pretreatment with a glutathione-depleting agent decreased the amount of DNA damage to control levels in mice. Further, DCM induced DNA-protein cross-links in mouse liver (498 ppm and higher, 6 h/d for 2 days), but neither in mouse lung nor in Syrian hamster liver or lung (up to 3923 ppm, 6 h/d for 2 days). As to other routes of exposure, single administration (po, ip, sc) of doses up to 5000 mg/kg bw did not induce SCE or chromosomal aberrations in mice, and an ip dose of 1720 mg/kg was negative in a micronucleus assay. A single oral dose of 1275 mg/kg bw induced DNA single-strand breaks in livers of CD rats, but a dose of 1000 mg/kg did not induce UDS in hepatocytes from F344 rats.

With respect to the possible mechanism of the genotoxicity of DCM the role of the GST-pathway has been studied. Cells depleted of glutathione decreased the mutagenicity of DCM, whereas expression of a rat GST increased the mutagenicity in *S. typhimurium*. Cytosol fractions but not microsomal fractions supported the bioactivation of DCM. It was suggested that the mutagenicity and the carcinogenicity in mice of DCM was linked to the metabolism of DCM by the GST-pathway (IARC, 1999).

The WHO (WHO, 1996) concluded that DCM is mutagenic in prokaryotic microorganisms with or without metabolic activation. In eukaryotic systems results are predominantly negative. *In vitro* gene mutation assays and tests for UDS in mammalian cells were uniformly negative. Positive results were obtained with *in vitro* chromosomal aberration assays whereas tests for SCE induction were negative or revealed equivocal results. Positive responses in *in vivo* test systems were restricted to tests using $B6C3F_1$ mice.

Table 5. Genetic effects of DCM after *in vivo* inhalation exposure (partially obtained from IARC, 1999)

Species	Tissue	Exposure (HID or LED)[a]	Endpoint[b]	Results[c]
$B6C3F_1$/CrlBr mouse	liver	146 ppm; 6h/d, 3d	DPX	-
$B6C3F_1$/CrlBr mouse	liver	498 ppm; 6h/d, 3d	DPX	+
$B6C3F_1$/CrlBr mouse	liver	4000 ppm; 6h/d, 3d	DPX	+
$B6C3F_1$ mouse	liver	4831 ppm; 6h	DNA ss	+
$B6C3F_1$ mouse	liver	4000 ppm; 6h	DNA ss	+ (decreased results with GSH depleting agent)
$B6C3F_1$ mouse	liver	4000 ppm; 6h	UDS	-
$B6C3F_1$/CrlBr mouse	lung	4000 ppm; 6h/d, 3d	DPX	-
$B6C3F_1$ mouse	lung	2000 ppm; 3h	DNA ss	+ (decreased results with GSH depleting agent)
$B6C3F_1$ mouse	lung	2000 ppm; 6h/d, 5d/w, 12w	SCE	+
$B6C3F_1$ mouse	lung	8000 ppm; 6h/d, 5d/w, 2w	CA	(+)
$B6C3F_1$ mouse	erythrocytes	2000 ppm; 6h/d, 5d/w, 12w	MN	(+)
$B6C3F_1$ mouse	bone marrow	8000 ppm; 6h/d, 5d/w, 2w	CA	(+)
AP rat	liver	4727 ppm; 6h	DNA ss	-
AP rat	lung	4000 ppm; 3h	DNA ss	-
F344 rat	hepatocytes	4000 ppm; 6h	UDS	-
Sprague-Dawley rat	bone marrow	3500 ppm; 6h/d, 5d/w, 2y	CA	-

Syrian hamster	liver	4000 ppm; 6h/d, 3d	DPX	-
Syrian hamster	liver	3923 ppm; 6h/d, 3d	DPX	-
Syrian hamster	lung	4000 ppm; 6h/d, 3d	DPX	-

a) HID: highest ineffective dose, LED: lowest effective dose
b) DPX: DNA-protein cross-links; DNA ss: DNA single-strand breaks; UDS: unscheduled DNA synthesis; CA: chromosomal aberrations; MN: micronucleus
c) +: positive results; (+): equivocal results; -: negative results

The EPA (IRIS, 2002) stated that DCM was mutagenic in *S. typhimurium* and produced mitotic recombination in yeast. Tests with cultured mammalian cells were concluded to be generally negative, but DCM had been shown to transform rat embryo cells and to enhance viral transformation of Syrian hamster embryo cells.

3.5. Chronic Toxicity/Carcinogenicity

Several carcinogenicity studies with different species have been performed. The main results of these studies are briefly reported and are limited to carcinogenic effects related to DCM exposure.

Rats

Groups of approximately 95 Sprague-Dawley rats per sex were exposed to 0, 500, 1500, or 3500 ppm DCM for 6 h/d, 5 d/w for 2 years (Burek *et al.*, 1984). Additional groups were exposed for interim kills at 6, 12, 15, or 18 months of exposure. Mortality was only significantly increased in female rats exposed to 3500 ppm. Although the number of tumor-bearing female rats was not increased, the total number of benign mammary tumors was concentration-related increased in female rats. In male rats sarcomas were found in the ventral midcervical region, in and around the salivary gland, incidences were 1/92, 0/95, 5/95, and 11/97 for the controls and the 500, 1500, and 3500 ppm exposure groups, respectively. They were considered to originate from within the salivary gland.

In an additional investigation the same group of workers exposed groups of 70 Sprague-Dawley rats per sex to 50, 200, or 500 ppm DCM for 6 h/d, 5 d/w for 2 years (Nitschke *et al.*, 1988a). Additional subgroups for interim kill after 6, 12, 15, or 18 months were incorporated. No effects of exposure on mortality was observed. With respect to neoplasms, the only exposure-related increase was for mammary tumors. Although the number of animals with mammary gland neoplasms was not increased, the number of benign mammary tumors per tumor-bearing rat was increased for female rats exposed at 500 ppm (1.8, 2.1, 2.0, and 2.2 for the control, 50, 200, and 500 ppm exposure groups, respectively).

Groups of 50 male and 50 female F344/N rats were exposed to 0, 1000, 2000, or 4000 ppm DCM for 6 h/d, 5 d/w, for 102 weeks (NTP, 1986). Survival was statistically significantly lower than controls in female rats exposed at 4000 ppm. Survival was low in all groups of male rats (including controls). A significant positive trend in mammary gland fibroadenomas and adenomas or fibroadenomas (combined) was observed both in male and female rats. Adenomas were observed in one male and one female rat exposed at 4000 ppm. The incidences of fibroadenomas was significantly higher in males and females of the 4000 ppm exposure group as compared to controls (males: 4/50 versus 0/50; females: 22/50 versus 5/50). A positive trend in neoplastic nodules in the liver was observed in female rats, but the incidence in the 4000 ppm exposure group did not differ significantly with that in controls. Further, a significant positive trend in male rats was observed in the incidence of integumentary system tumors in the area of the mammary chain. Other clearly increased incidences attributed to DCM exposure were found for mononuclear cell leukemia (females) and squamous metaplasia of the nasal cavity (females). As to the mononuclear cell leukemia, the incidence was unusually high in all groups of male rats (including controls), which may have contributed to the high mortality. Based on the increased incidences of the benign neoplasms of the mammary gland it was concluded that there was some evidence of carcinogenicity of DCM for male rats and clear evidence for carcinogenicity for female rats.

Mice

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 2000 or 4000 ppm DCM for 6 h/d, 5 d/w, for 102 weeks (NTP, 1986). Survival was statistically significantly lower than controls in both male exposure groups and in the high exposure group of female mice. Clearly increased incidences of alveolar/bronchiolar adenomas and carcinomas were observed in both sexes. Incidences of alveolar/bronchiolar adenomas and carcinomas combined in male mice were 5/50, 27/50, and 40/50 for controls and the low and high dose groups, respectively; these incidences for female mice were 3/50, 30/48, and 41/48, respectively. In addition, the number of animals bearing multiple tumors was also increased. The incidence of hepatocellular adenomas and carcinomas was statistically significantly increased in the high dose groups for both sexes, while the incidence hepatocellular carcinomas was increased also in females of the low dose group. Further, increased incidences were observed for hemangiosarcomas, 5 of the 6 tumors in the high dose group were hemangiosarcomas of the liver. Based on the increased incidences of alveolar/bronchiolar and of hepatocellular neoplasms it was concluded that there was clear evidence of carcinogenicity of DCM for male and female B6C3F$_1$ mice.

Kari *et al.* (1993) exposed female B6C3F$_1$ mice to 2000 ppm DCM 6 h/d, 5 d/w according to varying exposure regimens. Groups of 67 or 68 rats were exposed for 26, 52, or 78 weeks and observed for the remaining duration of a 104-week period. The exposure periods were scheduled both in the earliest and in the latest time periods of the 104-week study period. Additionally, one group was exposed for 104 weeks, and one unexposed group served as controls. Further, interim kill groups (20 rats exposed from the onset of the study, 10 control rats) were scheduled after 26, 52, and 78 weeks of exposure. Both for liver and lung neoplasms clearly increased incidences and total number of adenomas and carcinomas (combined) were observed after 2 y of exposure. Generally, tumor incidences were higher when animals were exposed in early life.

Hamsters

Groups of approximately 91-94 Golden Syrian hamsters per sex were exposed to 0, 500, 1500, or 3500 ppm DCM for 6 h/d, 5 d/w for 2 y (Burek *et al.*, 1984). Additional groups were exposed for interim kills at 6, 12, or 18 m of exposure. No exposure-related mortality or effect on tumor incidence were observed.

The epidemiologic studies focussed on among others the carcinogenic potential of DCM have been summarized in 2.2.3. Based on the available data, IARC concluded that “for no type of cancer was there a sufficiently consistent elevation of risk across studies to make a causal interpretation credible”. It was concluded that there was inadequate evidence in humans for the carcinogenicity of DCM. Based on both the animal data (section 3.5) and the human data IARC (1999) concluded that DCM is possibly carcinogenic to humans (Group 2B).

EPA last revised the carcinogenicity assessment for lifetime inhalation exposure to DCM on 02/01/95 (IRIS, 2002). DCM was classified as a probable human carcinogen, classification B2. This classification was “based on inadequate human data and sufficient evidence of carcinogenicity in animals; increased incidence of hepatocellular neoplasms and alveolar/bronchiolar neoplasms in male and female mice, and increased incidence of benign mammary tumors in both sexes of rats, salivary gland sarcomas in male rats and leukemia in female rats. This classification is supported by some positive genotoxicity data, although results in mammalian systems are generally negative.”

The inhalation Unit Risk was $4.7 * 10^{-7}$ µg/m^3, calculated by the linearized multistage procedure (IRIS, 2002). The data on female mice (combined adenomas and carcinomas) obtained from the NTP inhalation study (NTP, 1986) were used for the calculation of the inhalation Unit Risk. Information on pharmacokinetics and metabolism of DCM was incorporated. The internal dose estimates were based on the metabolism by the GST-pathway, as estimated by the model of Andersen *et al.* (1987). A correction for interspecies differences in sensitivity was applied by using the surface correction factor. The air concentrations at the risk levels of 1 in 10^{-4}, 1 in 10^{-5}, or 1 in 10^{-6} were calculated to be 200 µg/m^3 (56 ppb), 20 µg/m^3 (5.6 ppb) , and 2 µg/m^3 (0.56 ppb), respectively. It was

remarked that the unit risk should not be used if the air concentration exceeds $2 * 10^4$ µg/m^3, since the unit risk may differ from that stated above this concentration. It was therefore stated that the presented unit risk might not be applicable to acute, high exposures. EPA is planning to reevaluate potential human risks associated with inhalation exposure (ATSDR, 2000) but DCM has been removed from the IRIS agenda for 2002 (EPA, 2002).

The WHO concluded that "the pharmacokinetic of methylene chloride and the response seen in B6C3F$_1$ mice suggest that this species is a poor model on which to base human hazard assessment to methylene chloride". It was concluded that "the mechanism of mammary tumour formation in the rat is probably related to the effect of methylene chloride on prolactin levels in this species". The available epidemiological studies were considered inadequate for drawing any firm conclusions with regard to human cancer risk. It was stated that the carcinogenic potency of DCM in man is expected to be low (WHO, 1996).

As to carcinogenicity DCM has been classified within the EU as a Category 3 substance: "Substances which cause concern for man owing to possible carcinogenic effects but in respect to which the available information is not adequate for making a satisfactory assessment. There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2." (Category 2 is assigned to substances "which should be considered as if they are carcinogenic to man".

3.6. Summary

A review of highest non-lethal and lowest lethal concentrations are given in Table 6. In addition, although Bonnet *et al.* (1980) do not provide actual exposure concentrations it can be estimated from a graph that 1/12 rats exposed for 6 h to a concentration just below 13,000 ppm died.

The concentration-response curve for mortality is very steep. Mortality increased from 0 to 100% within an increase in exposure of 50 to 100%. No large species differences appeared to be present. In general, mortality due to DCM does not occur below 10,000 ppm for up to 7 h. One rabbit died after a 6 h exposure to 9464 ppm DCM but was not further examined. No further deaths occurred until the 12th exposure day. Further, 3/20 guinea pigs died after a 6 h exposure to 8700 ppm, but in another study no deaths were observed in 12 guinea pigs exposed to 9464 ppm DCM (6 h on day 1, and 4 h on subsequent days) for up to 38 exposure days.

The cardiovascular effects of inhalation exposure to DCM were studied in monkeys, dogs, and mice. Statistically significant effects were noted in dogs at 25,000 ppm, but not at 10,000 ppm. The only statistically significant effect observed in monkeys at 25,000 ppm was a decreased aortic blood pressure. Sensitization to epinephrine was observed in 1/5 mice exposed to 20,000 ppm.

The predominant effect of a single exposure to DCM is CNS-depression (see Table 3 for summary). No large interspecies differences in response appear to be present. Clear signs of anesthesia or narcosis start to occur at concentrations between 5000 and 10,000 ppm (within 1 h of exposure to 10,000 ppm), these effects may be preceded by periods of excitement at the onset of exposure. These effects are not observed at concentrations of up to 5000 ppm for 6 h. Effects observed at DCM concentrations below 5000 ppm include an EC_{30} for shortening of tonic extension of the hind limbs of 1980 ppm for 4 h in rats and of 3980 ppm for 2 h in mice. Mice exposed to 4000 ppm were slightly hyperactive during the first three hours of exposure and subdued for the remaining hours. Further, 1.5 h of exposure to 5000 ppm decreased the running activity in rats. Effects on enzyme activities in specific sections of the brain have been observed in rats upon repeated exposure to 1000 ppm. A 1-h exposure to 5200 ppm and higher DCM induced changes on evoked potentials in rats but not always in a concentration-related way.

Table 6. Summary of the highest non-lethal and lowest lethal data in laboratory animals

Species	Exposure Time	Non-lethality data		Lethality data		Reference
		Concentration (ppm)	Effect	Concentration (ppm)	Effect	
Single exposures						
Rat, male	1 h	15,100	0/12	--	--	Rebert *et al.*, 1989
Rat, unknown sex	2 h	11,200	0/20	--	--	Ulanova and Yanovskaya, 1959
Rat, male	4 h	10,000	0/4	15,000	1/4	Haskell Laboratory, 1964
Rat, male	4 h	11,000[a]	0/6	14,000[b]	2/6	Haskell Laboratory, 1982
Rat, male	4 h 4 h 4 h	16,500	0/5	15,500 16,800	1/5 1/5	NTP, 1986
Rat, female	4 h	17,250	0/5	18,500	1/5	NTP, 1986
Mouse	20 min	10,000	0/10	20,000	2/10	Aviado *et al.*, 1977
Mouse	82 min	17,360	0/3	--	--	Müller, 1925
Mouse, male	4 h	16,948	0/5	17,175	4/5	NTP, 1986
Mouse, female	4 h	16,948	0/5	17,175	3/5	NTP, 1986
Mouse	7 h	12,795	0/20	15,293	2/20	Svirbely *et al.*, 1947
Rabbit	20 min	11,520	0/4	--	--	Roth *et al.*, 1975
Guinea pig	30 min	20,000-24,000	0/3	50,000-54,000	1/6	Nuckolls 1933
Guinea pig	1 h	20,000-24,000	0/3	50,000-54,000	5/6	Nuckolls 1933
Guinea pig	2 h	20,000-24,000	0/3	50,000-54,000	6/6	Nuckolls 1933
Guinea pig	6 h	5000	0/5	8700	3/20	Balmer *et al.*, 1976
Repeated exposures						
Monkey, female	36-37 exposures[c]	9464	0/2	--	--	Heppel *et al.*, 1944
Dog, female	6 exposures[c]	9464	0/4	--	--	Heppel *et al.*, 1944
Rat, (m + f)	38 exposures[c]	--	--	9464	2/16 (>33d)	Heppel *et al.*, 1944
Rat, (10m, 10f)	6h/d, 7d/w, 13w	10,000	0/20	--	--	Leuschner *et al.*, 1984
Rabbits	≥37 exposures[c]	4760	0/4	9464	3/5 (1, 12, 22d)	Heppel *et al.*, 1944
Guinea pig (11m, 1f)	38 exposures[c]	9464	0/12	--	--	Heppel *et al.*, 1944

a) mean concentration, range: 9300-17,000 ppm;
b) mean concentration, range: 12,000-16,000 ppm;
c) exposure was for 6 h on day 1, and 4 h on subsequent days

Further DCM exposure affected the liver in mice (6 h to 4000 ppm) and guinea pigs (6 h to 5000 ppm; increased triglyceride level) and the lungs in mice (6 h to 4000 ppm; Clara cell damage, some recovery appears to occur in repeated exposure).

No clear teratogenic or adverse developmental effects were observed in rats at exposure levels up to 4500 ppm. A 2-generation study in rats exposed to DCM concentrations of up to 1500 ppm revealed no exposure-related changes.

As to genotoxicity, DCM is mutagenic in prokaryotic microorganisms but predominantly negative in eukaryotic systems and in UDS tests in mammalian systems. *In vivo* tests are positive in $B6C3F_1$ mice, but not in rats or hamsters.

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The predominant effect of a single exposure to DCM is CNS-depression (see Table 3 for summary). No large interspecies differences in response appear to be present. Clear signs of anesthesia or narcosis start to occur at concentrations between 5000 and 10,000 ppm (within 1 h of exposure to 10,000 ppm), these effects may be preceded by periods of excitement at the onset of exposure. These effects are not observed at concentrations of up to 5000 ppm for 6 h. Effects observed at DCM concentrations below 5000 ppm include an EC_{30} for shortening of tonic extension of the hind limbs of 1980 ppm for 4 h in rats and of 3980 ppm for 2 h in mice. Mice exposed to 4000 ppm were slightly hyperactive during the first three hours of exposure and subdued for the remaining hours. Further, 1.5 h of exposure to 5000 ppm decreased the running activity in rats. Effects on enzyme activities in specific sections of the brain have been observed in rats upon repeated exposure to 1000 ppm. A 1-h exposure to 5200 ppm and higher DCM induced changes on evoked potentials in rats but not always in a concentration-related way.

No clear teratogenic or adverse developmental effects were observed in rats at exposure levels up to 4500 ppm. A 2-generation study in rats exposed to DCM concentrations of up to 1500 ppm revealed no exposure-related changes.

As to genotoxicity, DCM is mutagenic in prokaryotic microorganisms but predominantly negative in eukaryotic systems and in UDS tests in mammalian systems. *In vivo* tests are positive in $B6C3F_1$ mice, but not in rats or hamsters.

Carcinogenicity studies with respiratory exposure to DCM were negative in hamsters. An increased incidence of benign mammary gland tumors was observed in rats. In mice, increased incidences of hepatocellular and alveolar/bronchiolar neoplasms were found.

4. SPECIAL CONSIDERATIONS

4.1. Toxicokinetics

4.1.1. Introduction

For a good interpretation and understanding of the human and animal kinetic data, a brief summary of the biotransformation is presented as introduction.

Following inhalation exposure to DCM the biotransformation of DCM occurs via two routes. Up to DCM exposure concentrations of approximately 300-500 ppm the predominant biotransformation route is oxidation by the Mixed Function Oxidase system (MFO-pathway) to formyl chloride. The P450 enzyme involved is most likely P4502E1. This route finally leads to the formation of CO and subsequently to COHb. Formyl chloride may also give rise to formation of CO_2 through conjugation with GSH, but most (about 70%) of the formyl chloride appears to be converted to CO. The second pathway is through direct conjugation of DCM with GSH leading via chloromethyl glutathione and formaldehyde to CO_2 (GST-pathway). This pathway only becomes of importance at relatively high exposure concentrations when the MFO-pathway is saturated. The responsible GST isozyme is most probable GSTT1.

Qualitatively, the biotransformation of DCM is comparable between species. The main difference between species is in the rate of the GST-pathway which appears to be an order of magnitude higher in mice compared to other species.

4.1.2. Human data

The kinetics of DCM in humans have been intensively studied in experimental settings with exposure concentrations ranging from 50 to 500 ppm. The exposure duration varied from 30 min to 7.5 hours. A summary of the most important data is followed by a more detailed description of the individual studies.

Absorption

The pulmonary uptake of DCM ranges roughly from 40 to 60% (Gamberale *et al.*, 1975; Stewart *et al.*, 1976; Andersen *et al.*, 1991), but may be up to 70% during the first minutes of exposure (Riley *et al.*, 1966). The uptake decreases with exposure duration and exposure concentration (Stewart *et al.*, 1976; Peterson, 1978). A steady-state absorption rate is generally achieved within 2 h of exposure up to 200 ppm (DiVincenzo *et al.*, 1972; Divincenzo and Kaplan, 1981a). Although retention decreases under conditions of physical exertion, the absolute amount taken up will be 2- to 4-fold higher compared with exposure under conditions of rest (Åstrand *et al.*, 1975; Divincenzo and Kaplan, 1981b). The amount of DCM taken up after a 1-hour exposure to 750 ppm under exertion appeared to be positively correlated with the amount of body fat (Engström and Bjurström, 1977).

DCM concentrations in blood

The DCM concentration in blood linearly increases with exposure concentration at relatively low concentrations (50 to 200 ppm), and reaches about 2 µg/mL after a 2-hour exposure to 200 ppm. Peak concentrations were 0.35 and 0.85 µg/mL during exposure to 50 and 100 ppm, respectively. ((DiVincenzo *et al.*, 1972; DiVincenzo and Kaplan, 1981a). A more than linear increase in blood DCM concentration was noted when volunteers were exposed to 100 or 350 ppm indicating saturation of metabolism. Maximal blood DCM concentrations of 1.11 and 5.92 µg/mL in male volunteers were reached within 3 hours of exposure to 100 and 350 ppm DCM, respectively (Anderson *et al.*, 1991). DCM concentrations in blood followed the increased uptake of DCM under physical exertion (Åstrand *et al.*, 1975; Divincenzo and Kaplan, 1981b). A 2-h exposure to 500 ppm under conditions of increasing physical exertion resulted in an end-exposure blood DCM concentration of 15 µg/mL (Åstrand *et al.*, 1975).

DiVincenzo and Kaplan (1981a) exposed groups of 4 to 6 volunteers (age:21 to 42 years) to 50, 100, 150, or 200 ppm DCM for 7.5 hours, with a half-hour break after 4 hours. DCM concentrations in blood stabilized between the 2nd and 4th h of exposure for the two lowest exposure groups. DCM blood concentration in the two highest exposure groups were lower at 6 h of exposure compared to the 4th h of exposure, due to the half-hour break. Since the DCM blood concentrations reached peak values at the end of the exposure period (1.2 and 1.9 µg/mL for the 150 and 200 ppm exposure group, respectively), it cannot be determined whether the DCM concentration in blood would have reached a plateau value during exposure to 150 or 200 ppm for up to 7.5 h. Blood concentrations of DCM decreased to less than 0.1 µg/mL at 2 h postexposure for the 50 ppm exposure group, at 4 h postexposure for the 100 and 150 ppm exposure group, and at 6 h for the 200 ppm exposure group.

COHb levels in blood

Due to saturation of the metabolic pathway leading to the formation of CO and hence COHb (see below under biotransformation) at DCM exposure levels of about 500 ppm and higher CO will still be formed after exposure and the COHb level reaches peak values sometimes hours after exposure has ceased. A summary of the COHb levels measured in human volunteers is given in Table 7.

COHb levels will increase with increasing exposure concentration and increasing exposure duration. A saturation of CO formation at exposure concentrations of 350-500 ppm is indicated. For instance, the COHb levels during a 6-hour exposure to 350 ppm were less than 50% higher than during exposure to 100 ppm despite the much higher blood DCM concentrations (Andersen *et al.*, 1991). COHb levels of 4 to 5% are reached after a 4-h exposure to 200 ppm (DiVincenzo and Kaplan, 1981a) or a 7.5-h exposure to 100 ppm (Fodor and Roscovanu, 1976). At higher exposure concentrations COHb levels may further increase with individual levels of 15% (Stewart *et al.*, 1972), 20% (Fodor and Roscovanu, 1976) and higher. COHb levels will reach peak values after cessation of exposure when exposed to relatively high DCM exposure concentrations (above 250 ppm) and/or for a relatively short exposure duration (1 h). Exercise doubled the peak COHb levels in three healthy males exposed to 100 ppm for 7.5 hours. Depending on the exposure concentration and duration, COHb levels may be elevated for hours after cessation of exposure.

Table 7. COHb levels in human volunteers following single exposure to DCM.

Exposure conditions	Peak COHb level		Remarks	Reference
	%	Timepoint after onset of exposure (hours)		
50 ppm; 16 hours	3.3	12	- COHb levels estimated from a graph	Fodor and Roscovanu (1976)
100 ppm; 7.5 hours	4	8	- 0.5 hour lunch break - no blood samples after exposure	
500 ppm; 7.5 hours	12	8		
50 ppm; 7.5 hours	1.5	-	- blood was sampled only 1-h postexposure; - COHb levels estimated from a graph	Peterson, 1978
100 ppm; 7.5 hours	3	-		
250 ppm; 1 hour	1.5	-		
250 ppm; 3 hours	3	-		
250 ppm; 7.5 hours	7	-		
500 ppm; 7.5 hours	10	-		
100 ppm; 6 hours	5.5	5	Sampling times a.o. 3, 5, 8, 12 hours	Andersen *et al.* 1991
350 ppm; 6 hours	9	8		
50 ppm; 7.5 hours	1.9	8	- next blood sample at 2 hours postexposure - 0.5 hour break after 4 hours	DiVincenzo and Kaplan, 1981a
100 ppm; 7.5 hours	3.4	8		
150 ppm; 7.5 hours	5.3	8		
200 ppm; 7.5 hours	6.8	8		
986 pm; 2 hours	7-15	3		Stewart *et al.*, 1972

Postexposure excretionn of DCM and CO

After a 7.5-h exposure to DCM concentrations up to 200 ppm up to 30% of the absorbed amount of DCM may be exhaled as CO. The CO concentration in exhaled air was up to 36 ppm when exposed to 350 ppm for 6 hours (Andersen *et al.*, 1991). The CO concentration in end tidal air did not stabilize during exposure and reached peak values of 9, 12, 21, and 30 ppm in subjects exposed to 50, 100, 150, and 200 ppm, respectively. The decrease in CO concentration after cessation of exposure was rather slow and reached pre-exposure concentrations after approximately 16 h for the two lowest exposure groups and after 24 h for the two highest exposure groups. About 24 to 32% of the absorbed amount of DCM was excreted as CO in end tidal air (DiVincenzo and Kaplan, 1981a). CO excretion as percentage of absorbed amount of DCM was increased in exercising subjects to 28-39%, compared to 25% under sedentary conditions (DiVincenzo and Kaplan, 1981b).

The DCM concentrations in exhaled breath were approximately twofold higher during and after 200 ppm exposure compared to 100 ppm exposure values. Subjects who alternately exercised and rested showed significantly higher DCM concentrations in exhaled breath at the end of the exercise period than at rest. Postexposure concentration very rapidly decreased in a biphasic way (DiVincenzo *et al.*, 1972). DCM concentrations in end tidal air were directly proportional to the exposure concentration during and for 2 h postexposure. DCM levels in exhaled air stabilized after 1 to 2 h of exposure, with peak values of approximately 20, 40, 60, and 85 ppm for subjects exposed to 50, 100, 150 and 200 ppm, respectively. The DCM concentration rapidly decreased after exposure and was less than 0.1 ppm at 6 h postexposure for the three lowest exposure groups, and 1 ppm at 16 h postexposure for the 200 ppm exposure group. Postexposure excretion of DCM was less than 5% of the amount absorbed (DiVincenzo and Kaplan, 1981a). The decrease in DCM concentration in exhaled air occurred more slowly with increasing exposure duration and concentration. Following a 6-hour exposure to 100 ppm the DCM concentration decreased from 46 to 12 ppm in five minutes, whereas after exposure to 350 ppm the DCM concentration decreased from 202 to 81 ppm after five minutes and to 39 ppm after 30 minutes (Andersen *et al.*, 1991). The DCM concentration in alveolar air had decreased to less than 3 ppm at 3 h postexposure in subjects exposed for 7.5 hours to 50 and 100 ppm, and to 5 and 15 ppm 3 h after a 7.5-h exposure to 250 and 500 ppm, respectively (Stewart *et al.*, 1976; Peterson, 1978). DCM may still be detectable in exhaled air at 16 hours postexposure (Andersen *et al.*, 1991; Stewart *et al.*, 1976; Peterson, 1978).

Twenty-four-hour urinary DCM excretion (sampled postexposure) ranged from 18.6 to 26.8 µg (n=4) and from 63.3 to 106.7 µg (n=7) for 2-h exposure to 100 and 200 ppm under resting conditions, respectively. Approximately 70 to 100% was excreted with the first urine sample collected within 30 min postexposure (DiVincenzo *et al.*, 1972).

Biotransformation

The biotransformation scheme of DCM is presented in Figure 1, section 4.1.3. The MFO-pathway leading to the formation of CO and subsequently COHb is saturable and saturation starts to occur at exposure concentrations of about 300-500 ppm. This would indicate that COHb levels in humans will reach a maximum when the metabolic rate is at maximum. It is noted that a large interindividual variation may be present. For instance, Stewart *et al.* (1972) found maximum COHb levels ranging from about 7-15% in 3 volunteers exposed to 986 ppm DCM for 2 h. Andersen *et al.* (1991), when trying to fit his PBPK-model to these data, assumed a 5-fold difference in V_{max} for this pathway between the individuals in order to obtain proper individual fits. Further, despite the fact that this pathway becomes saturated, very high levels of up to 50% COHb have been reported in specific cases (section 2.2.1).

The P450 isozyme involved in the MFO-pathway of DCM metabolism is P4502E1. It is well known that this enzyme is easily inducible by many substances with a low-molecular weight, among which ethanol. Considerable interindividual differences in activity of P4502E1 may be present (Snawder and Lipscomb, 2000). Polymorphism of *CYP2E1* (the gene corresponding to the enzyme

P4502E1) has been described although its functional significance is unclear. It has been stated however that variability in P4502E1 activity may be dominated by environmental and other factors that regulate *CYP2E1* rather than genetic polymorphism of *CYP2E1* itself (Haber *et al.* 2002). Induction of P4502E1 would lead to an increased formation of CO, and hence of COHb.

The GST involved in the biotransformation of DCM has been identified to be a θ class GST (GSTT1-1) (Bogaards *et al.*, 1993; Mainwaring *et al.*, 1996a, 1996b; Sherratt *et al.*, 1997). A polymorphism in GSTT1 has been well characterized. Bogaards *et al.* (1993) studied the rate of cytosolic metabolism of DCM in 22 human liver samples by measuring the rate of formaldehyde formation. The results pointed to the existence of three distinct subpopulations differing in activity towards DCM. Three samples showed no activity, 11 samples showed an activity ranging form 0.20 to 0.41 nmol/min/mg protein, and 8 samples showed an activity of 0.82 to 1.23 nmol/min/mg protein. The difference between the low-conjugators and high conjugators was statistically significant ($p<0.001$). In liver samples that showed expression of α-, μ-, and π-class subunits, no activity towards DCM was observed. Metabolism was therefore most likely to be catalyzed by θ-class GSTs. The existence of three human subpopulations was also confirmed by Hallier *et al.* (1994), who tested formaldehyde formation in hemolysates of 13 subjects (6 non-conjugators and 7 conjugators).

Thier *et al.* (1998) studied the activity of GSTT1-1 (rate of formaldehyde formation) in liver and kidney cytosol of, among others, humans (25 liver samples and 13 kidney samples), and in human erythrocytes (9 samples). The blood samples were drawn from nine kidney donors. A distinction could be made between non-conjugators (NC), low-conjugators (LC), and high-conjugators (HC) with measured liver cytosolic activities of 0.62 nmol/min/mg protein for LC (n=11) and 1.60 nmol/min/mg protein for HC (n=12); no activity was detected in NC (n=2).

In contrast to other species the cytosolic activity in human kidney samples was higher than in liver samples. The activities were 3.05 nmol/min/mg protein for HC (n=4) and 1.38 nmol/min/mg protein for LC (n=4); no activity was detected in NC (n=1). The activity in human erythrocytes was twice as high in HC (n=3) as compared to LC (n=5), while NC showed no activity (n=1). Pemble *et al.* (1994) reported polymorphism for GSTT1 in humans and estimated that about 40% of the human population to be a non-conjugator, i.e. not able to conjugate halomethanes like DCM with GSH. Haber *et al.* (2002) presented a summary table of population distributions of GSTT genotypes. Non-conjugators accounted for approximately 20% of the Caucasian, African American, and Hispanic population, but up to 60% in the Asian American population. The U.S. average was estimated to be 20%.

Additional studies

Stewart and Hake (1976) exposed human volunteers (19-47 y) in an experimental setting of application of paint remover (80% DCM, 20% methanol) and stripping for 3 hours. Two subjects were exposed in each setting, of which one was actively stripping while the other remained sedentary. DCM concentrations and COHb levels were monitored under three different ventilation rates of the room, two experiments were performed under the conditions of the lowest ventilation rate. Mean breathing zone DCM concentrations at a low ventilation rate were 654 and 788 ppm for the two experiments, with upper values of 1278 ppm. COHb levels peaked at 4 hours postexposure and were 9.1 and 6.9% for the active subjects in the two experiments, and 6.0 and 5.9% for the sedentary subjects. COHb levels were still slightly elevated 21 hours postexposure (1.8 to 3.8%). At higher ventilation rates, breathing zone DCM concentrations were lower: 368 (upper limit of 576 ppm) and 216 (379) ppm for the mid and high ventilation rate settings, respectively. COHb levels peaked sooner at 1-2 h postexposure and decreased faster. Peak COHb levels were approximately 7 and 4% for the mid and high ventilation rate settings, respectively,

Three groups of four subjects (23-49 y of age; one group of smokers) were exposed to DCM in an experimental session (Stevenson *et al.*, 1978). Subjects were exposed for 13 min and subsequently remained in a solvent-free waiting room for 4 h followed by a second 13-min exposure period. During the exposure period the subjects sprayed two paint cans (containing 29% DCM) until empty. Two controls for each group remained in the solvent-free waiting room during the experimental period. The

26-min average DCM concentration (two exposure periods combined) was 450 ppm. Highest DCM blood concentrations were found after the first exposure. Preexposure COHb levels averaged 2.8% and 5.5%in nonsmoking and smoking subjects, respectively; these values increased to 3.7% and 8.5% 3 hours after the second exposure. Smoking was allowed during the test period, and appeared to have a greater effect on COHb levels than DCM exposure.

Ghittori *et al.* (1993) sampled air from the breathing zone of 20 workers (age: 27-59 y) from a pharmaceutical factory. Urine was sampled at the start of the shift and after 4 h. Mean air concentrations during this 4-h period was 50.3 mg/m^3 (14 ppm) (range: 3.4-200.8 mg/m^3; 1.0-56 ppm). Preshift urine samples contained less than 10 µg/L, while mean DCM concentrations in urine sampled after 4 h of work was 190.8 µg/L (range: 4.2-787.5 µg/L). The mean CO concentration in alveolar air sampled after 4 h was 10.5 ppm (4-22 ppm). A good correlation (r=0.90) was found between urinary DCM concentration and DCM concentration in breathing zone air. A correlation between airborne DCM concentration and CO concentration in exhaled air was only present for the group of non-smoking subjects (n=8).

Blood samples were drawn from a group of 136 DCM exposed workers (66 nonsmokers) and analyzed for COHb (Ott *et al.*, 1983). Blood and alveolar air were sampled immediately preceding and following a shift. Personal monitoring for DCM exposure was performed during the shift. TWA DCM exposure concentrations ranged from 0 up to 900 ppm. Only the best fit between DCM exposure concentration (up to 500 ppm) and CO concentration in alveolar air or COHb were graphically presented. The COHb levels following exposure appeared to level off at concentrations of about 300 ppm, possibly indicating saturation of metabolism. Exposure to 500 ppm was associated with a COHb of about 9% in nonsmokers, and up to 13% in smokers. The CO concentration in exhaled air showed a pattern similar to the COHb level. Exposure to 500 ppm was associated with CO concentrations of 40 ppm and 60 ppm for nonsmokers and smokers, respectively.

Soden *et al.* (1996) compared DCM concentrations with COHb levels in workers exposed to DCM. The individual employees wore sampling pumps for a full 8-h shift. COHb was measured in blood samples drawn at the end of the same workshift in which exposure monitoring of DCM had occurred. In total 631 combinations were available, 410 samples for nonsmokers and 221 for smokers (at least once per day). COHb levels showed a dose-response to DCM exposure for nonsmokers only. Since detailed smoking habits were not available analyses were mainly restricted to nonsmokers. Ambient DCM concentrations for this group ranged from 1 to 159 ppm (8-h TWA) while COHb levels were up to 5.8%. A slight correlation (r=0.58) was calculated between these two parameters but the scatterplot revealed a large interindividual variation in COHb. Aspects that were not accounted for were that the COHb level may not have peaked at the end of shift, but sooner or later, and individual variation in biotransformation. Further, co-exposure existed for methanol and acetone, both compounds are known modulators of P4502E1, the enzyme considered responsible for the biotransformation pathway leading to CO formation.

4.1.3. Animal data

A large number of data are available on kinetics in several animal species. Qualitatively, the differences between species in biotransformation are relatively small and comparable to that in humans. Many experiments were aimed at elucidating the observed differences in tumor response between species. The main difference appeared to be in the rate of metabolism through the GST-pathway, which is an order of magnitude higher in mice compared to other species. Because of this and in addition to the fact that the mouse appeared to be the most susceptible species in carcinogenic response to DCM exposures, the carcinogenicity potency of DCM was related to this pathway. Therefore, most of the studies focussed on differences between species in this pathway.

In this section only data that provides information in addition to what is described for humans or what is of importance for explanation of interspecies differences will be presented.

Nonhuman primates

Groups of 4 Cynomolgus monkeys were exposed to 4600 ppm for 1 h or 1000 ppm for 3 h (Ciuchta *et al.*, 1979). No further details were given on actual exposure measurements.

COHb levels (approximately 4.5% above control values) peaked at 2 h after the 1-h exposure, and did not return to control levels within 24 h postexposure. Following the 3-h exposure a peak increase in COHb level above control values (almost 7%) was observed immediately after exposure, after which a steady decline was observed.

Dog

Groups of 6-year-old fasted male beagle dogs (n=3-5) were exposed for 2 h to 100, 200, 500, or 1000 ppm, or to 100 ppm for 4 h (DiVincenzo *et al.*, 1972). The DCM concentration in postexposure breath was directly proportional to the exposure concentration, and was significantly higher in dogs than in humans (human data described above). The ratio of the DCM concentration in exhaled air of dogs and humans increased from 1.6 (5 min postexposure) to 8.3 at 4 h postexposure. This ratio was similar for a 2-h exposure to 100 and 200 ppm. Combined with a higher half-life of DCM in blood observed for dogs this may indicate that dogs eliminate DCM at a slower rate than humans.

Rat

Saturation of the MFO appears to occur in rats at a concentration of 300 to 500 ppm DCM (e.g. McKenna *et al*., 1982; Gargas *et al.*, 1986; Wirkner *et al.*, 1997). The maximum COHb level in rats generally appeared to be about 15% for DCM concentrations up to 4000 ppm; this level may already be reached at an exposure concentration of 500 ppm (McKenna *et al.*, 1982; Green *et al.*, 1986b; Carlson and Kim, 1986). The increase in COHb levels during and elimination after exposure to DCM was reported to be faster in rats than in simultaneously exposed men. Peak COHb levels were twice as high in rats compared to men after a 3-h exposure to 200 ppm DCM (Fodor *et al.*, 1973; Fodor and Roscovanu, 1976).

Groups of Sprague-Dawley rats with a total weight of approximately 1 kg were exposed to DCM in a closed rebreathing system (Rodkey and Collison, 1977a, 1977b). The total dose ranged from 0.082 to 0.793 mmol/kg [^{14}C] DCM. An average of 47 % of the radiolabel was recovered as ^{14}CO and 29 % as $^{14}CO_2$. No radioactivity was recovered in spleen, lung, adipose tissue, brain, or blood after exposure. The initial rate of CO production was similar in all groups, the rate of production leveled off after approximately 2-3 hours in the lower exposure groups but increased slightly in the highest exposure group over a 10.5-h exposure period. At the highest exposure group 62% of the DCM was converted to CO at the end of the exposure period; a for rats unusual high COHb level of 44% at the end of exposure was reported.

Groups of 3 male Sprague-Dawley rats were exposed to 50, 500, or 1500 ppm [^{14}C] DCM for 6 h (McKenna *et al.*, 1982). Steady-state concentrations of DCM in whole blood were reached within 2 h of exposure, and were 0.22 µg/mL and 39.53 µg/mL for the 50 and 1500 ppm exposure groups, respectively. Elimination from plasma was biphasic for the two higher exposure groups with half-lives of 2 and 15 min, respectively. COHb levels reached a steady-state of 3% within 1 h of exposure to 50 ppm. No significant difference in COHb levels were present between the two higher exposure groups. A steady-state COHb level of about 10 to 13% was reached within 2.5 to 3 h of exposure which lasted until approximately 1 to 1.5 h postexposure. Body burden was calculated from the total amount of radioactivity recovered during the first 48 h after exposure. The fate of [^{14}C] DCM was calculated as percentage of this body burden. Almost 60% (DCM: 5%; CO: 27%; CO_2: 26%) of the radioactivity was expired after exposure to 50 ppm DCM. Approximately 9% was excreted in urine (no volatile compounds) and 23% was found in the carcass of which most was in the liver, followed by kidneys and lungs. The percentage ^{14}C expired increased with increasing exposure level to almost 80% (DCM: 55%; CO: 14%; CO_2: 10%). However, it should be remarked that the body burden and the excretion of metabolites were underestimated because of sampling only after exposure. The excretion of ^{14}C during exposure was neglected. This may have obfuscated the results which were confirmed by Reitz et al

(1986, abstract only) who reported that 60-70% of the total metabolites was excreted during a 6-h exposure of male $B6C3F_1$ mice to 50 or 1500 ppm ^{14}C-DCM, and 30-40% in the 18-h directly following exposure.

Groups of 3 male F344 rats were exposed to 0, 500, 1000, 2000, or 4000 ppm ^{14}C-DCM for up to 6 h (Green *et al.*, 1986b). The groups were sacrificed at regular intervals during and after exposure. No formic acid was detected in blood samples. Blood DCM concentrations increased disproportionally when the exposure increased from 500 to 1000 ppm DCM from approximately 10 to 60 µg/mL. At higher concentrations, blood DCM concentrations increased linearly with exposure to approximately 120 and 240 µg/mL for the 2000 and 4000 ppm exposure groups, respectively. The AUCs showed a similar pattern. After a fast increase during the first 2-3 h the COHb levels increased slowly, peak levels of approximately 15, 13, 12, and 11% for the 500, 1000, 2000, and 4000 ppm exposure groups, respectively, were reached at the end of the exposure. In the 4000 ppm exposure group COHb could be detected up to 7 h after exposure; the postexposure DCM expiration decreased slowly and DCM was detectable in exhaled breath up to 8 h postexposure. $^{14}CO_2$ and ^{14}CO could be detected in exhaled breath for almost 9 and 11 h after cessation of exposure, respectively. The $^{14}CO_2$ concentrations remained approximately constant during this period, whereas the ^{14}CO slowly increased during the first 6 h postexposure and decreased thereafter. These results clearly differed from those observed in male $B6C3F_1$ mice (see below).

Groups of 5 male Sprague-Dawley rats were exposed to 200, 500, or 1000 ppm for 8 or 12 h (Kim and Carlson, 1986). COHb levels were approximately similar at the end of exposure (between 8 and 10%) in the two lowest exposure groups but somewhat higher in the 1000 ppm exposure group (13-14%). COHb levels following 12 h of exposure were somewhat higher. However, in a separate experiment the COHb level increased to approximately 16% after exposure to 500 ppm. None of the differences were statistically significant, however. Following exposure to 1000 ppm the half-life of DCM in blood was 20 min, irrespective of the exposure duration (8 or 12 h).

Blood DCM concentrations and COHb levels were measured in groups of 5 male Sprague-Dawley rats exposed for 8 h to 500 or 1000 ppm DCM under sedentary conditions or under forced exercise in a rotating cage (Carlson and Kim, 1986). Exercise had no effect on end-exposure DCM blood concentrations and COHb levels in the 500 ppm exposure group, but in the 1000 ppm exposure group end-exposure blood DCM concentrations and COHb were statistically significantly lower in the exercised groups.

Groups of three male F344 rats were exposed to 200 or 1014 ppm DCM for 4 h (Andersen *et al.*, 1991). At the end of the exposure to 1014 ppm DCM a blood DCM concentration of 60 µg/mL was reached. Maximum COHb levels (about 8%) were comparable at both concentrations but COHb levels were still near the maximum value 1 h after exposure to 1014 ppm while COHb levels decreased rapidly after exposure to 200 ppm. When rats were exposed to 5159 ppm for 30 min the COHb level reached a peak level occurred at about 1.5 h postexposure and maintained at a maximum level until approximately 3 h postexposure.

Anders and Sunram (1984) showed that DCM can cross the placenta. Pregnant Sprague-Dawley rats were exposed to 500 ppm DCM or 22 ppm CO for 1 hour on gestation day 21, and killed immediately thereafter. Following DCM exposure DCM concentrations were 176 and 115 nmol/mL in maternal and fetal blood (approximately 15 and 9.8 µg/mL), respectively. The respective blood CO concentrations were 167 and 160 nmol/mL (4.7 and 4.5 µg/mL). Exposure to 22 ppm CO resulted in CO concentrations in maternal and fetal blood of 140 and 157 nmol/mL (3.9 and 4.4 µg/mL), respectively.

Mice

Groups of 6 male $B6C3F_1$ mice were exposed to 0, 500, 1000, 2000, or 4000 ppm ^{14}C-DCM for up to 6 h (Green *et al.*, 1986b). The groups were sacrificed at regular intervals during and after

exposure. No formic acid was detected in blood samples. Blood DCM concentrations were more variable within each exposure group than observed in rats (see above) and were lower than in rats at all concentrations. The AUCs were in the proportion of 0.2, 1.0, 1.3, and 2.1 for the four exposure groups, respectively. After a fast increase during the first 1-2 hours the COHb levels remained more or less constant. In the 500 ppm exposure group a peak level of approximately 17% was observed at the end of the 6-hour exposure, whereas for the other exposure groups peak levels were reached after approximately 5 hours of exposure (circa 14, 13, and 8.5% for the 1000, 2000, and 4000 ppm exposure groups, respectively). COHb levels had decreased to undetectable levels 1.5 h postexposure. Postexposure ^{14}C-DCM and $^{14}CO_2$ exhalation following 4000 ppm DCM decreased fast, DCM was undetectable within 2 h postexposure while $^{14}CO_2$ was undetectable after 5 h. ^{14}CO concentration in exhaled air increased the first hour postexposure and decreased rapidly thereafter.

The distribution of DCM was studied by whole-body autoradiography in NMRI-mice exposed for 10 min to ^{14}C-DCM, and sacrificed at regular intervals (Bergman, 1979). A total of 10 µl DCM was added to the inhalation apparatus and evaporated by gentle heating. Immediately after exposure DCM was evenly and rapidly distributed, predominantly in the white matter of the brain, body fat, blood, liver, and kidney. The radioactivity in fatty tissues, blood, and brain was attributed to volatile compounds, which could not be detected anymore at 30-60 min postexposure. From 30 min postexposure radioactivity was also registered in tissues with a rapid cell turnover. Elimination studies were performed in four mice exposed under similar conditions. Of the absorbed dose, 63% was excreted as unchanged DCM, 4.6% as CO_2, and 0.6% as CO during 8 h following exposure. About 1% was excreted in urine. At 8 h postexposure highest levels of radioactivity were detected in the liver followed by the kidneys and the lungs.

Groups of 5 male Swiss-Webster mice were exposed to 200, 500, or 1000 ppm DCM for 8 or 12 h (Kim and Carlson, 1986). Postexposure COHb levels were similar for 8 and 12 h of exposure. Exposure to 500 or 1000 ppm resulted in COHb levels twice as high as did exposure to 200 ppm, 16% versus 8%, respectively.

Rabbits

A group of four male New Zealand rabbits was exposed for 20 min to actual concentrations of 1270, 1770, 4480, 8010, and 11,520 ppm DCM over a 4-week period (Roth *et al.*, 1975). The maximum increase in COHb was concentration dependent and ranged from circa 5% at the lowest concentration to approximately 12 % at the highest concentration. COHb levels peaked after circa 2-3 hours for all exposure concentrations. In a separate experiment, the COHb level increased during the first 2 to 3 hours of a 4-hour exposure to 7320 ppm DCM, and leveled of at the end of exposure.

Hamsters

The pharmacokinetics of ^{14}C DCM was studied in groups of 4 male Syrian Golden hamsters exposed to 50 or 1500 ppm DCM for 6 h (Schumann *et al.*, 1983). Animals were placed in metabolism cages for 48 h immediately following exposure. Hence, the amount taken up will be underestimated and the results will not reflect metabolism during exposure. Percentages are relative to the total amount of radioactivity recovered after exposure. After exposure to 50 ppm 3.2%, 23.8%, and 40.6% was excreted as unchanged DCM, CO_2, and CO respectively. Following exposure to 1500 ppm these values were 26.9, 21.7, and 26.8% respectively. These values differ considerably from those reported for rats (McKenna *et al.*, 1982). Postexposure exhalation of CO and CO_2 was far less in rats, while rats excreted a higher percentage as unchanged DCM following exposure to 1500 ppm. The percentage of radioactivity recovered from urine, feces, and skin was approximately similar for both exposure groups, only the amount recovered from carcass was lower for the 1500 ppm exposure group (10.8 vs. 17.8%). The majority of the excreted radioactivity was recovered during the first 12 h postexposure.

Biotransformation

Anders and coworkers have intensively studied the metabolism of dihalomethanes, among which was DCM (e.g. Kubic *et al.*, 1974; Kubic and Anders, 1975, 1978; Anders *et al.*, 1977; Ahmed

and Anders, 1978). More recently, Gargas *et al.* (1986) and Andersen *et al.* (1987) studied the metabolism in more detail both by further experimentation and by modeling. A proposed biotransformation scheme is presented in figure 1.

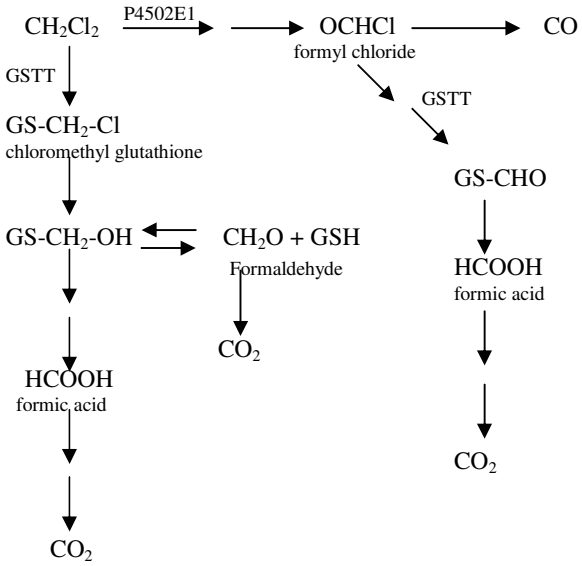

Figure 1. Biotransformation scheme of DCM (modified after Gargas et al., 1986).

It was shown that DCM is metabolized by two major pathways. One is a microsomal oxidation process (by cytochrome P450) and the other is a glutathione-dependent cytosolic pathway. The former leads to the formation of formyl chloride, which in its turn can either result in the formation of CO and, hence, give rise to COHb formation or can react with GSH to yield CO_2. Below levels of saturation approximately 70% of the formyl chloride is considered to decompose to CO. The cytosolic pathway can result in chloromethyl glutathione as an intermediate and finally lead to CO_2. Formaldehyde may also be detected as an intermediate. The reactive intermediates are considered to be formyl chloride in the microsomal or MFO-pathway and chloromethyl glutathione in the cytosolic or GST-pathway.

The enzymes in the MFO-pathway have a higher affinity for DCM than the GST-enzymes but this pathway is saturable. In experimental animals saturation of the microsomal pathway appears to occur between air concentrations of 200-500 ppm DCM, based on a plateauing of the COHb level above these concentrations. At higher concentrations the carcinogenic effects of DCM are considered to result from the reactive metabolites derived from the GST-pathway, e.g. chloromethyl glutathione.

Comparisons of *in vitro* metabolism by the GST- and the MFO-pathways were made for mouse, rat, hamster, and human liver samples, and for mouse, rat, and hamster lung samples (Green *et al.*, 1986a). Metabolism by the GST-pathway was studied by the rate of formaldehyde formation, whereas the rate of CO formation was used as indicator for the MFO-pathway. The rate of hepatic metabolism of DCM by the MFO-pathway was highest in hamster and mouse and much lower in human and rat liver samples. It was remarked that although the cytochrome P450 content of mouse liver was approximately three times higher than in the lung (nmol P450/mg protein), the rate of MFO metabolism was higher in mouse lung. Rats showed low MFO metabolism both in liver and in lung tissue samples. With respect to the GST-pathway, the highest rate of formaldehyde formation was found in mouse liver, followed by rat liver. No significantly time- or substrate-dependent increase in formaldehyde was found for human and hamster liver samples. As to lung samples, significant formation of formaldehyde could only be detected in mouse lung, but not in hamster or rat lung samples (see also section 4.3.2).

Male B6C3F$_1$/CrlBR mice and male Syrian golden hamsters were exposed for 3 days (6 h/d) to DCM concentrations ranging from circa 150 to 4000 ppm (Casanova *et al.*, 1996). DCM was labeled on the 3rd exposure day. Metabolic incorporation of ^{14}C into lung and liver DNA was studied. In the hamster, the amount of ^{14}C incorporated into liver and lung DNA were similar. Incorporation was detectable at a DCM concentration of 498 ppm, but did not increase with increasing concentrations. In the mouse, the amount of ^{14}C incorporated into liver DNA was detectable at an exposure concentration of 146 ppm and rose to the level observed with hamsters at 1553 ppm. However, the amount of ^{14}C incorporated in lung DNA increased 27-fold over the concentration range tested. ^{14}C incorporation into liver DNA in mice exposed for 1 d was similar to that for mice exposed for 3 d. However, at exposure concentrations of 2647 ppm and higher, ^{14}C incorporation into lung DNA was approximately 4-fold higher in mice exposed for 3 d compared to 1-d exposed mice. This indicates that the increase in ^{14}C incorporation in mice lung DNA is largely due to an increase in DNA synthesis as a result of DCM exposure. Green *et al.* (1988) exposed male F344 rats and male B6C3F$_1$ mice to 4000 ppm ^{14}C DCM for 3 hours, during the 3rd hour the exposure concentration decreased to 3000 ppm due to consumption of the DCM. Lung and liver DNA was isolated 6, 12, and 24 h after the onset of exposure. Covalent binding to hepatic protein was also measured. Radioactivity in hepatic and pulmonary DNA was approximately 2-3 fold higher in mice compared to rats. In rats, radioactivity in pulmonary DNA was comparable to that in hepatic DNA, but in mice radioactivity in pulmonary DNA was higher than in hepatic DNA. The results of these and additional studies with iv administered ^{14}C formate indicated incorporation of the carbon atom of DCM into DNA via the C-1 pool. No evidence for direct alkylation of DNA by DCM was found in either rats or mice. As to hepatic protein the results indicated covalent binding of DCM or its metabolites in addition to incorporation via the C-1 pool.

Induction of P4502E1 and its effect on the biotransformation of DCM has been subject of several studies. Administration of pyrazole 15 min before onset of exposure to 510 ppm DCM for 12 h decreased the COHb level by more than 50% and increased the DCM concentration in blood by 50% in rats (Kim and Carlson, 1986). The half-life of both the blood DCM concentration and the COHb level were increased by pyrazole treatment. In another study groups of 5-6 male Wistar rats were exposed for 4 h to 100, 500, or 2500 ppm DCM (Wirkner *et al.*, 1997). COHb levels were up to 5% and 10% for the 100 and 500 ppm exposure groups, respectively. Pretreatment with ethanol (4, 12, or 36 weeks) only slightly increased the mean COHb level at all DCM concentrations. The individual animal data show that the intraspecies variation in COHb level was greater following ethanol pretreatment with only about half of the animals in each exposure group showing clearly increased COHb levels. Ethanol pretreatment was reported to decrease blood DCM concentrations after a 4-h exposure to DCM. Ottenwälder *et al.* (1989) reported that pretreatment with the cytochrome P450 inhibitors pyrazole or dithiocarb significantly decreased the uptake of DCM in male B6C3F$_1$ mice exposed to initial concentrations of 1000 or 3000 ppm in a closed chamber.

Saturation of the MFO-pathway following DCM administration by gavage appears to occur in rats and mice at a dose of approximately 100 mg/kg (Kirschman *et al.*, 1986), although others suggested saturation of metabolism to occur already at 50 mg/kg in rats (McKenna *et al.*, 1981). Pre-administration of a P4502E1-inducer generally leads to an increased metabolic rate of the MFO-pathway, whereas simultaneous administration often results in (sometimes complete) inhibition of the CO formation. The latter is due to the fact that the P4502E1 inducers often are substrates themselves for the enzyme, which results in competitive inhibition. Chemical substances showing this effect on the metabolism of DCM are for example benzene, toluene, o- or m-xylene, p-xylene (Pankow *et al.*, 1991), ethanol (Glatzel *et al.*, 1987), isoniazid and acetone (Pankow and Hoffmann, 1989), isonicotinic acid hydrazide (Pankow *et al.*, 1988), and acetylsalicylic acid (Pankow *et al.*, 1994).

4.2. Mechanism of Toxicity

Cardiotoxicity

DCM inhibited the Ca^{2+} dynamics in isolated cardiac myocytes obtained from 2- to 4-day old rats in a dose-dependent way (Hoffman *et al.*, 1996). Oral administration of DCM to anesthetized male

Wistar rats induced a negative inotropic and a negative chronotropic effect on the heart. The former effects were observed at venous blood concentrations between 0.98 to 1.6 mM DCM (83-136 µg/mL). No effects were observed on systolic blood pressure and no arrhythmic events were noted. Toraason *et al.* (1992) reported that DCM inhibited cell communications in isolated cardiac myocytes obtained from 2- to 4-day old Sprague-Dawley rats by a blockade of gap junctions, as measured by inhibition of cell-to-cell transfer of Lucifer yellow). The EC_{50} (the concentration inhibiting intercellular communication in 50% of the cells) was 21.05 mM (1788 µg/mL). A 100% recovery was observed upon washout of the medium. A blockade of junctional channels increases electrical resistance in the myocardium and may predispose the heart to arrhythmia.

Carcinogenicity

Since the publication of the carcinogenicity studies with rats, mice, and hamsters an extensive research program has been carried out to explain the observed differences in the carcinogenic response between the species. Since the differences in the GST-pathway between the species predominantly becomes evident at high exposure concentrations (2000-4000 ppm) above the saturation level for the MFO-pathway and because no increased tumor incidences have been observed at relatively low doses, the carcinogenic potential was considered to be related to the GST-pathway. The studies focused on genotoxicity, metabolism and pharmacokinetics, mode of action, and extrapolation of animal data to humans. Green (1997) summarized the studies sponsored by industry. The MFO-pathway appeared to be more or less similar between the species but specific lung damage (bronchiolar Clara cells) appeared to occur in the mouse. GST activity towards DCM was an order of magnitude greater in mice than in rats, hamsters, or human liver samples. The major GST enzyme involved in the metabolism of DCM was GSTT1-1. In contrast to rats and humans high concentrations of GSTT1 mRNA were found in specific parts of the mouse liver (around the central vein) and predominantly in the nucleus. More recent data indicated that the GSTT1-1 activity in hepatocytes is much higher in mice than in rats or humans. (Mainwaring *et al.*, 1996a, 1998; Schröder *et al.*, 1996; Sherratt *et al.*, 2002). Green (1997) concluded that DCM is a species specific genotoxicant and carcinogen. Hence the mouse would be an inappropriate model for human health risk assessment. However, this hypothesis was debated because the data on the subcellular distribution of the GSTT1 mRNA did not support the view that the GSTT1 enzyme itself is localized in the nucleus of mouse but not in human hepatocytes (Liteplo *et al.*, 1998). This was further supported by the fact that addition of mouse hepatic cytosol containing GSTT1 to *in vitro* incubations of intact Chinese hamster ovary cells increased single-strand DNA breaks and the frequency of HPRT mutations.

4.3. Other Relevant Information

4.3.1. Irritation

Adult female New Zealand white rabbits were used in several series of experiments to study the ophthalmic toxicity of DCM (Ballantyne *et al.*, 1976). Animals were examined for eye irritation, in vivo measurements of corneal thickness, intra-ocular tension, and histopathological evaluation of eye lesions. Fifteen rabbits were exposed to 1750 or 17,500 mg DCM/m^3 (490 or 4900 ppm) for 10 min. No details on actual concentration were given. Three rabbits were examined for eye irritation, six for corneal thickness, and six for measurement of intraocular tension. Eye irritation was assessed at 10 min, 1, 6, and 24 h, and thereafter daily for 2 weeks. No signs of eye irritation were seen in rabbits exposed to DCM vapor. The increase in corneal thickness was much smaller compared to instillation of liquid DCM. The maximum increase was observed 30 min postexposure and was 5 and 13% for the 490 and 4900 ppm exposure group, respectively. The effects had returned to normal values within 6 h for the 490 ppm exposure group and within 1-2 days for the 4900 ppm exposure group. Peak increases in ocular tension, measured after 10 min, were 11 and 18% for the low and high dose group, respectively; tensions returned to control values by 2 days.

4.3.2. PBPK-modeling

Based on the kinetic data it was anticipated beforehand that specific problems were to be encountered in the process of derivation of AEGL-values for DCM:

- two different toxicity endpoints are relevant for acute exposure
  - CNS-depression, related to the DCM concentration in brain,
  - COHb formation, via biotransformation to CO,
- CNS-effects occur soon after the onset of exposure,
- peak levels of COHb can be reached hours after cessation of exposure,
- the metabolic pathway for CO is saturable,
- polymorphism of GSTT1 is present in humans, more CO will be formed in non-conjugators leading to higher COHb levels.

It was expected beforehand that the toxic endpoint of interest would change over an exposure range of 10 min to 8 hours. The AEGL-values for the shorter exposure durations would be triggered by the CNS-effects whereas the formation of COHb would determine the longer exposure durations. Further, saturation of the MFO-pathway occurs at about 500 ppm, which has to be accounted for in the extrapolation from high to low doses. In addition, no data are available on effects attributed to COHb resulting from DCM exposure. Therefore, the DCM concentrations in environmental air had to be calculated from the predetermined maximum COHb levels that have been set for exposure to CO itself (4% COHb for AEGL-2 and approximately 15% for AEGL-3). PBPK-modeling was considered to be the most appropriate if not the only way to tackle these problems adequately.

Several publications deal with the development of PBPK-models for DCM accounting for both biotransformation pathways and applicable for different routes of exposure and for different species (Gargas *et al.*, 1986; Reitz *et al.*, 1989; Andersen *et al.*, 1987, 1991). More recently, several other models have been developed based on the work of Andersen *et al.* and Reitz *et al.* ATSDR has summarized and evaluated most of these models including, among others, a model developed by Casanova *et al.* (1996) for DNA-protein crosslink formation in mouse liver (ATSDR 2000). Most of these models focus on metabolites formed by the GST-route that is associated with the carcinogenicity of DCM. Formaldehyde is considered to be the proximate metabolite for this endpoint. However, since these models are of little importance within the present context it suffices to refer to the ATSDR-report on methylene chloride for further information. More recently, the impact of the GSTT1 polymorphism on the carcinogenic risk of DCM has been studied using PBPK-modeling (El-Masri *et al* 1999; Jonsson and Johanson 2001; Jonsson *et al*. 2001).

The toxic endpoints of interest in AEGL-setting for DCM are CNS-depression associated with the DCM concentration in brain and the COHb formation. For this purpose, the most usable PBPK-models are the ones published by Andersen *et al*. (1991), focussing on the formation of COHb, and by Reitz *et al* (1997) who included a separate brain compartment to estimate the DCM concentration in brain. These models are discussed into more detail.

Initially, the model developed by Andersen *et al.* (1987) made use of allometric scaling relationships to extrapolate the metabolic rate of DCM in mice to the metabolic rate in humans. Subsequently, Reitz *et al.* (1988, 1989) extended the model by using *in vitro* data on MFO and GST metabolism of DCM obtained with human liver samples from four individuals. Originally these models focussed on the metabolites formed through the GST-pathway since the carcinogenic potential was considered to be related to this pathway. In 1991, Andersen *et al.* published a model specifically designed to estimate the formation of CO and subsequently COHb through the MFO-pathway for both rat and humans.

Dankovic and Bailer (1994) also used the 1987 model developed by Andersen *et al.* and modified by Reitz *et al.* (1989) to study the impact of exercise and interindividual variation in metabolic rates on dose estimates for DCM. Physiological parameters associated with exercise conditions (e.g., alveolar ventilation, cardiac output, tissue perfusion rates) were varied. The individual metabolic parameters (V_{max} and K_m) for the MFO-pathway and the first-order rate constant for the GST-pathway (Reitz *et al.*, 1988, 1989) were used instead of the average values to study interindividual variation. The K_m and the V_{max} for the MFO-pathway ranged 3-fold and 8.5-fold, respectively. The

first-order rate constant for the GST-pathway were comparable in liver samples from three subjects, but no GST-activity towards DCM was found for one liver sample. An 8-h exposure to 25 ppm was modeled at rest and with light exercise (alveolar ventilation rate of 17.4 L/min versus 7 L/min at rest) using average values for metabolic rate parameters. In addition, an 8-h exposure under light exercise using individual parameters for metabolic rate parameters was modeled. Comparison of the exposure at rest and under light exercise showed that light exercise increased the area under time/concentration curve for DCM in the liver (AUCL), and the amount of metabolites formed by the MFO- and the GST-pathway in the liver approximately twofold. Using the individual values for the metabolic rate parameters showed a 10-fold difference in the AUCL, the lowest value found for the liver sample that showed the lowest K_m but highest V_{max}. The amount of metabolites formed by the GST-pathway was also considerably lower (more than 5-fold) for this liver sample, despite a comparable first-order rate constant as two other liver samples. Using the average value for the GST first-order rate constant instead of the individual values had no significant effect on the AUCL or the amount of metabolites formed through the MFO-pathway under the modeled exposure conditions.

More recently, Reitz *et al.* (1997) further developed this model to derive, among others, an acute oral MRL starting form inhalation data (route-to-route extrapolation) through a contract with the ATSDR. The dose-metrics used for acute exposure hereby were peak concentrations in brain based on the study by Winneke (1974). The PBPK-model was further used to extrapolate the 4-h LOAEL of 300 ppm derived from the Winneke study (Winneke, 1974) to a 24-h LOAEL of 60 ppm, also with the peak DCM concentration in brain as dose surrogate. Further, the rat developmental inhalation study of Schwetz *et al.* (1975) (with 1250 ppm considered by ATSDR as a less serious LOAEL) was used as a basis to derive an oral equivalent dose for humans for developmental effects. The PBPK-model was used to estimate the fetal concentration in pregnant rats exposed to 1250 ppm, and to calculate the equivalent oral dose for humans.

For the purpose of AEGL-setting for DCM the models of Andersen *et al.* (1991) and Reitz *et al*. (1997) were combined. The basic model structure and more details on the validity and applicability of the used model are described into more detail in Appendix B. In brief, the model of Andersen *et al.* (1991) was chosen as basis and the Reitz *et al.* (1997) model was used to incorporate the brain compartment into the Andersen model. In this way both dosemetrics (COHb level and DCM concentration in brain) can be estimated within one PBPK-model. The final model was validated against the original data of the individual models and was found to produce curves for COHb formation and DCM concentration in brain which were similar to those published by Andersen *et al.* (1991) and Reitz *et al.* (1997), respectively. The existence of polymorphism in the GST-pathway (conjugators versus non-conjugators) was accounted for as follows. The GST-route was switched off for non-conjugators resulting in a 100% biotransformation of formyl chloride to CO.

4.3.3. Species Variability

As pointed out previously, clear interspecies differences in metabolic rate exist, predominantly in the rate of the GST-pathway that is much higher in mice hepatocytes compared with other species. Reitz *et al.* (1989) reported *in vitro* GST-activities assayed at a DCM concentration of 40 mM of approximately 26, 7, and 1 nmol product formed/min/mg protein in samples of liver cytosol of mouse, rat, and hamster respectively. The cytosol preparation from one human liver did not show any activity while for three others the rate was approximately 3 nmol product formed/min/mg protein. The liver MFO for these species were less active in the order hamster>mouse>rat~human.

Thier *et al.* (1998) studied the activity of GSTT1-1 (rate of formaldehyde formation) in liver and kidney cytosol of F344 rats (5 per sex), $B6C3F_1$ mice (5 per sex), Syrian golden hamsters (3 per sex), and humans (25 liver samples and 13 kidney samples), and in human erythrocytes (9 samples). The blood samples were drawn from nine kidney donors. In man, a distinction could be made between non-conjugators (NC), low-conjugators (LC), and high-conjugators (HC). As to liver cytosolic activity, a statistically significant higher activity was observed for female mice compared with male mice (29.7 versus 18.2 nmol/min/mg protein, respectively). The next highest activity was found in rats (3.71

nmol/min/mg protein), followed by HC (1.60 nmol/min/mg protein; n=12), LC (0.62 nmol/min/mg protein; n=11), and hamsters (0.27 nmol/min/mg protein), no activity was detected in NC (n=2). In contrast to the other species, the cytosolic activity in human kidney samples was higher than in liver samples. The cytosolic activity in kidney samples was highest in female (3.88 nmol/min/mg protein) and male mice (3.19 nmol/min/mg protein), followed by HC (3.05 nmol/min/mg protein, n=4), rat (1.71 nmol/min/mg protein), LC (1.38 nmol/min/mg protein; n=4), and hamster (0.25 nmol/min/mg protein), no activity was detected in NC (n=1). The activity in human erythrocytes was twice as high in HC (n=3) as compared with LC (n=5), while NC showed no activity (n=1).

Green et al (1986b) observed clear differences in kinetics between male $B6C3F_1$ mice and male F344 rats exposed to 0, 500, 1000, 2000, or 4000 ppm DCM. Blood DCM concentrations were approximately 2- to 3-fold higher in rats at all concentrations. COHb levels after exposure and the amount of DCM, ^{14}C-CO_2, and ^{14}C-CO expired after exposure (in mg or mg equivalents expired per kg body weight) decreased much faster in mice than in rats. The authors concluded that this was due to a lower rate of metabolism and hence a higher deposition of DCM in tissues in the rat during exposure which was slowly released after exposure. At the end of 6 h exposure to 4000 ppm, the amount of ^{14}C-CO_2 exhaled (mg equivalents DCM/kg bw) by mice was almost 10-fold higher than by rats, which is in good agreement with the *in vitro* comparison of the rates of metabolism by the cytosolic pathway in rat and mouse liver (Green *et al.*, 1986a).

4.3.4. Susceptible Subpopulations

Interindividual variability in activity of biotransformation enzymes

As described in section 4.1.2, the P450 isozyme involved in the MFO-pathway of DCM metabolism is P4502E1. Considerable interindividual differences in activity of P4502E1 may be present (Snawder and Lipscomb, 2000). Although CYP2E1 polymorphism has been described, its functional significance is unclear and it has been suggested that variability in P4502E1 activity may be dominated by environmental and other factors that regulate *CYP2E1* rather than genetic polymorphism of *CYP2E1* itself (Haber *et al.* 2002). Many substances with a low-molecular weight can easily induce this enzyme that will result in an increased rate of metabolism for DCM, although simultaneous exposure may decrease the metabolic rate due to competitive inhibition. A higher P4502E1 activity would lead to an increased formation of CO, and hence of COHb, but simultaneously to lower tissue concentrations of DCM itself. This might be illustrated with the results of Stewart *et al.* (1972). They found maximum COHb levels ranging from about 7-15% in 3 volunteers exposed to 986 ppm DCM for 2 h. Andersen *et al.* (1991), when trying to fit his PBPK-model to these data, assumed a 5-fold difference in V_{max} for the MFO-pathway between the individuals in order to obtain proper individual fits. However, the net result of the induction of P4502E1 on the biotransformation of DCM and the consequences for the DCM-induced toxicity is too complex to predict and will be accounted for by the usual intraspecies factor.

The GST involved in the biotransformation of DCM is a θ class GST (GSTT1-1) (Bogaards *et al.*, 1993; Mainwaring *et al.*, 1996a, 1996b; Sherratt *et al.*, 1997). A polymorphism for this enzyme has been well-characterized in humans. A distinction could be made between non-conjugators who lack the GSTT1-1 enzyme, low-conjugators (heterozygotes who have one positive and one null allele), and high-conjugators (homozygotes) (Bogaards *et al.*, 1993; Hallier *et al.*, 1994; Thier *et al.*, 1998). Especially at concentrations above the saturation level for the MFO-pathway it is to be expected that non-conjugators will show higher tissue levels of the parent compound DCM than conjugators. Further, since about 30% of the formyl chloride is estimated to be conjugated through GST (figure 1) the yield of CO, and thus of COHb, from formyl chloride may be increased in non-conjugators. Gargas *et al.* (1986) showed that the COHb level was increased in DCM exposed rats after pretreatment with a GSH-depletor.

Pemble *et al.* (1994) reported an estimate of about 40% of the human population to be a non-conjugator, i.e. not able to conjugate halomethanes like DCM with GSH. Haber *et al.* (2002) presented a summary table of population distributions of GSTT genotypes. Non-conjugators accounted for

approximately 20% of the Caucasian, African American, and Hispanic population, but up to 60% in the Asian American population. The U.S. average was estimated to be 20%.

Age

A few animal studies suggested some age-related differences in susceptibility towards DCM. Oral LD_{50} values were determined in newborn rats (1-2 d old), immature rats (14-d old), young adult rats (80-160 g), and older adult rats (300-470 g) (Kimura *et al.*, 1971). For the former two groups male Sprague-Dawley rats (n=6) were used, for the latter two groups rats of both sexes (6-12 rats) were exposed. LD_{50}s were <1.0 mL/kg, 1.8 mL/kg, 1.6 mL/kg, and 2.3 mL/kg for the four groups, respectively. The LD_{50}s for the latter three groups were not statistically significantly different.

Male Swiss Webster mice (3-, 5-, or 8-weeks of age) were trained to avoid a grid where they received a foot shock (passive-avoidance conditioning task) (Alexeeff and Kilgore, 1983). Thereafter, they were exposed to 168.1 mg/L (47,068 ppm) until loss of their righting reflex (usually 20 s). Animals appeared fully recovered in approximately 5-10 min. Mice were tested on possibility to recall the task 1, 2, or 4 d after exposure. Each exposure group consisted of about 15 mice with a control group of 20 mice for each exposure group. The percentage of mice recalling the task was statistically significantly lower in the 3-week old mice on the 3 d of testing. For the 5-week old mice the exposure group performed significantly less than the control group only at day one postexposure, but this difference was not statistically significant. The results for the 8-week old mice were inconsistent.

Older rats (as determined by weight) showed a 2- to 3-fold higher increase in COHb level as compared to younger rats following exposure to 500 or 5000 ppm (Ciuchta *et al.*, 1979).

These data point at a not fully developed MFO-pathway and a higher susceptibility towards DCM toxicity in (very) young rats. However, the data are too limited to draw any clear conclusion.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

There is no evidence that DCM causes eye or respiratory irritation in humans. Volunteers exposed to 986 ppm DCM for 2 h reported no signs of eye, nose, or throat irritation. The odor was present but not considered objectionable (Stewart *et al.*, 1972). Although the odor threshold is reported to be within the range of 160-620 ppm two men who had lost consciousness could not remember having detected the smell (Moskowitz and Shapiro, 1952). Therefore, odor may not be a proper warning signal for this substance, especially in case of a rapid build-up of the concentration. Light-headedness and difficulties with enunciation were reported after 1 h of exposure to 986 ppm or within 15 min of exposure to 868 ppm directly following a 1-h exposure to 514 ppm. No complaints were reported during a 1-h exposure to 514 ppm (n=3) or 515 ppm (n=8). Gamberale *et al.* (1975) reported that the subjects' assessment of their own well-being was slightly better under DCM exposure than under control conditions of nonexposure. Subjects were exposed to 4 subsequent 30-min exposure periods to 250, 500, 750, and 1000 ppm, respectively. The available occupational data do not provide quantitative information on exposure in relation to AEGL-1 effects.

5.2. Summary of Animal Data Relevant to AEGL-1

The animal data related to AEGL-1 effects are scarce. No signs of eye irritation were seen in rabbits exposed to 490 and 4900 ppm DCM for 10 min. A slight increase in corneal thickness was observed (Ballantyne *et al.*, 1976). Five male rabbits exposed to 33.8 g/m^3 (SD: 1.4 g/m^3) (9464 ppm) for 5 d/w for 7.5 weeks (6 h on day one, 4 h/d on subsequent days) showed no signs of mucous membrane irritation (Heppel *et al.*, 1944).

5.3. Derivation of AEGL-1

The AEGL-1 is based on the observation by Stewart *et al.* (1972) that exposure concentrations of 868 and 986 ppm may lead to light-headedness and difficulties in enunciation. These effects were absent at a 1-h exposure to 514 ppm. The latter concentration is therefore used as point of departure for AEGL-1. Since these effects disappeared within 5 min postexposure whereas the COHb level increased postexposure for at least another hour they were attributed to the DCM concentration in the brain rather than to CO.

The human brain concentration following a 1-h exposure to 514 ppm was calculated to be 0.063 mM, using the human PBPK-model (see Appendix B). Because human data are taken as point of departure an interspecies factor is not necessary. Since susceptibility for gross CNS-depressing effects do not vary by more than a factor 2-3 an intraspecies uncertainty factor of 3 is considered sufficient, resulting in a maximum target concentration of DCM in the human brain of 0.021 mM. The human PBPK-model was subsequently used to calculate the DCM concentrations in environmental air for exposure of up to 8 hours that will result in a maximum brain concentration of 0.021 mM (Figure 2). The DCM concentrations for the AEGL-1 exposure times from 10 min to 8 hours were thus derived with the PBPK-model.

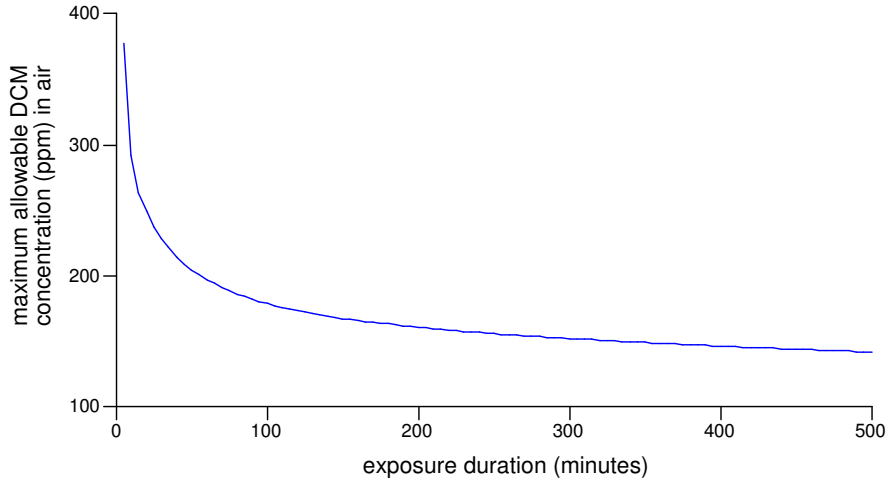

Figure 2. Maximum allowable DCM concentration in ambient air equivalent to a maximum target DCM concentration of 0.021 mM in human brain.

The AEGL-1 values are presented in Table 8. However, because the AEGL-1 values at 4- and 8-h (160 and 140 ppm, respectively) are at or above the corresponding AEGL-2 values (section 6.3), no AEGL-1 for these time periods can be proposed.

For the purpose of comparison, the values using the default approach (UF=3, n=3 or 1) would be: 10 min: 310 ppm; 30 min: 210 ppm; 1 hour: 170 ppm; 4 hours: 42 ppm; 8 hour: 21 ppm.

TABLE 8. AEGL-1 Values for methylene chloride

10-minute	30-minute	1-hour	4-hour	8-hour
290 ppm (1000 mg/m^3)	**230 ppm (810 mg/m^3)**	**200 ppm (710 mg/m^3)**	**NR**	**NR**

NR: Not recommended since these values would be higher than the corresponding AEGL-2 values.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Two different endpoints are of importance for a proper assessment of adequate AEGL-2 values for DCM, the formation of COHb, which is the end result of biotransformation of DCM through the MFO-pathway, and the CNS-depression which can be related to the brain concentration of the parent compound.

No adequate data are available to directly assess a relationship between DCM exposure and effects related to COHb levels. However, AEGL-2 values for CO itself have been based on the COHb level. For CO, the AEGL-2 values were based on cardiovascular effects in patients with coronary artery disease, which constitute the most susceptible subpopulation (EPA, 2001). Based on this endpoint a maximum COHb level of 4% was chosen as point of departure for CO. It was mentioned that at this level, patients with coronary artery disease may experience a reduced time until onset of angina (chest pain) during physical exertion. It was further stated that an exposure level of 4% COHb is unlikely to cause a significant increase in the frequency of exercise-induced arrhythmias. An exposure level of 4% COHb was also considered protective of acute neurotoxic effects in children, such as syncopes, headache, nausea, dizziness, and dyspnea. An intraspecies UF of 1 was considered adequate for CO because the values are based on observations in the most susceptible human subpopulation (EPA, 2001). The maximum level of 4% COHb is therefore also used as predetermined point of departure for setting AEGL-2 values for DCM.

The second relevant endpoint for DCM is CNS-depression. The human data provide only limited information on exposure concentrations of DCM in relation to clear AEGL-2 effects. The use of in total 50 g DCM during a 3-h surgical procedure (equivalent to an average estimated 3-h exposure to 7000-9333 ppm exposure) induced a satisfactory light narcosis and did not result in complaints afterwards (Hellwig, 1922). An average amount of 26.6 g DCM was used in 1950 as an obstetric analgesic in 44 cases (Grasset and Gauthier, 1950). The women could regulate DCM inhalation themselves. The average duration for dilatation was about 3-4 h with an additional 15-45 min for the expulsion.

Further, several experimental studies with volunteers have addressed neurobehavioral endpoints that are sensitive subtle effects that may be indicative of more severe effects at higher exposure concentrations but are actually no AEGL-2 effects in themselves. Gamberale *et al.* (1975) observed no effects on reaction time, short-term memory, or numerical ability in subjects exposed for 4 subsequent 30-min periods to 250, 500, 750, and 1000 ppm DCM, respectively. It can be conservatively concluded that a 2-h exposure to 250 ppm, a 1.5-h exposure to 500 ppm, a 1-h exposure to 750 ppm, and a 0.5-h exposure to 1000 ppm are NOAELs for the effects studied. Putz *et al.* (1979) observed that a 4-h exposure to 195 ppm DCM causes some decreased performance on AVT and an increase in reaction time to a peripheral light stimulus. Winneke and Fodor (Fodor and Winneke, 1971; Winneke, 1974, 1982) reported decreased performances in AVT and CFF in subjects exposed to 317, 470, or 751 ppm DCM for up to 230 min. However, the results were not always consistent and no clear concentration-response relation was present. Exposure to 751 ppm also induced a diminished performance in some additional tests, but the deviations from control values were small, ranging from approximately 3 to 9%. The effects observed are not considered to be severe enough to cause a serious impairment of escape, and, therefore, are regarded as sub AEGL-2 effects. In addition, the physical performance of volunteers exposed under physical exertion, appeared not to be seriously impaired when exposed to 500 ppm for 2 h (work load up to 150 W) or to 750 ppm for 1 h (work load of 50 W) (Åstrand *et al.*, 1975; Engström and Bjurström, 1977). Exposure under physical exertion may lead to a 2- to 4-fold higher uptake than under sedentary conditions (DiVincenzo and Kaplan, 1981b). Finally, workers occupationally exposed to a 15-min TWA concentration of up to 1700 ppm or to an 8-h TWA exposure of up to 969 ppm in an occupational setting apparently reported only relatively mild symptoms such as headache (Moynihan-Fradkin, 2001). Obviously they were not hampered to function properly in their jobs under these exposure conditions.

In summary, no human exposure data are available concerning clear AEGL-2 effects. The endpoints studied in experiments with volunteers are considered to be sub AEGL-2 effects and do not seriously impair escape.

6.2. Summary of Animal Data Relevant to AEGL-2

Animal data relevant to AEGL-2 predominantly concern CNS-effects. No large interspecies differences in response appear to be present. Exposure to 5000 ppm for up to 6 h did not result in narcosis or anesthesia in several animal species (Table 4). Exposure to 9464 ppm and 13,500 ppm caused narcosis within 1 h in rats (Heppel *et al.*, 1944) and mice (Gehring, 1986), respectively. A 1.5-h exposure to 5000 ppm significantly decreased the running activity in rats (Heppel and Neal, 1944). Clark and Tinston (1982) calculated an EC_{50} of 9000 ppm (95% confidence interval: 7000-12,000 ppm) for ataxia and loss of righting reflex in rats for a 10-min exposure to DCM. Exposure to 15,000 ppm caused hindlimb paralysis after 519 s in rats (Schumacher and Grandjean, 1960). Mice exposed to 4000 ppm for 6 h were slightly hyperactive during the first hours of exposure but subdued during the second part of the exposure (Hext *et al.*, 1986). The shortening of tonic extension of the hind limbs in rats (EC_{30}: 1980 ppm for 4 h) and the lengthening of the latency of extension in mice (EC_{30}: 3980 ppm for 2 h) are considered to be sub AEGL-2 effects.

6.3. Derivation of AEGL-2

The human data are considered adequate for the derivation of AEGL-2 values, although data on clear AEGL-2 effects are limited. It could be estimated that a 3-h exposure to approximately 7000-9333 ppm induced light narcosis without any complaints afterwards. In the absence of more adequate data the highest concentration-time combination tested in the experimental volunteer studies (751 ppm for 230 min) is regarded as an appropriate point of departure for the derivation of AEGL-2 values for CNS-effects. Further, the AEGL-2 values for DCM that are based on the formation of COHb have to be in compliance with the AEGL-2 values for CO that are set at a maximum COHb level of 4%. As for CO, this COHb level of 4% is considered to be additional to the background COHb value. Therefore, DCM exposure should not lead to an increase in the COHb level of more than 4%.

The human PBPK-model (Appendix B) was used to calculate the concentration-time curves for DCM exposure resulting in a maximum COHb level of 4 % in both conjugators and non-conjugators (Figure 3 and Table 9). The DCM concentrations in environmental air leading to an increase of 4% in the COHb level are about twofold higher in conjugators as compared to non-conjugators.

As to the CNS-effects, Reitz *et al.* (1997) showed that the appropriate dosemetric for the CFF effects as found by Winneke (1974) was DCM concentration in brain rather than the AUC for DCM in brain. In their derivation of a 24-h MRL, the ATSDR also used the DCM concentration in the brain as the dosemetric. It is assumed that this will also be applicable to other neurobehavioral effects. The DCM concentration in brain equivalent to a 230-min exposure to 751 ppm was estimated to be 0.137 mM using the human PBPK-model (see Appendix B). Because human data are taken as starting point an interspecies UF is not necessary. According to section 2.5.3.4 of the SOP for developing AEGLs (NRC, 2001) several topics are relevant for consideration when assessing an intraspecies UF. The toxic effects of DCM studied in the relevant experiments are less severe than those defined for AEGL-2. Since susceptibility for gross CNS-depressing effects do not vary by more than a factor 2-3, an intraspecies uncertainty factor of 3 would normally have been used. However, in this case the CNS-effects observed at 751 ppm are very mild and occur at any exposure that is far below that which would cause effects that would impair the ability to escape. Therefore, the intraspecies uncertainty factor was reduced to 1. Furthermore, taking all the relevant data (section 6.1) into consideration application of an intraspecies factor of >1 would lead to CNS-based AEGL-2 values that would conflict with these data. Therefore, application of an intraspecies UF of 1 is considered sufficient resulting in a maximum target concentration of DCM in human brain of 0.137 mM. The human PBPK-model was subsequently used

to calculate the DCM concentrations in environmental air for exposure of up to 8 hours that will result in a maximum brain concentration of 0.137 mM (Figure 3). The toxic endpoint of interest changes from CNS-depression to COHb-formation between 30 and 60 min of exposure for non-conjugators. For exposure durations longer than 30 min, the PBPK-model shows that the formation of COHb for non-conjugators (subjects lacking GSTT1) is the more important endpoint. Since the amount of absorbed DCM that is metabolized to CO is relatively small the presence or absence of GSTT1 has little or no significant influence on the brain concentration of DCM.

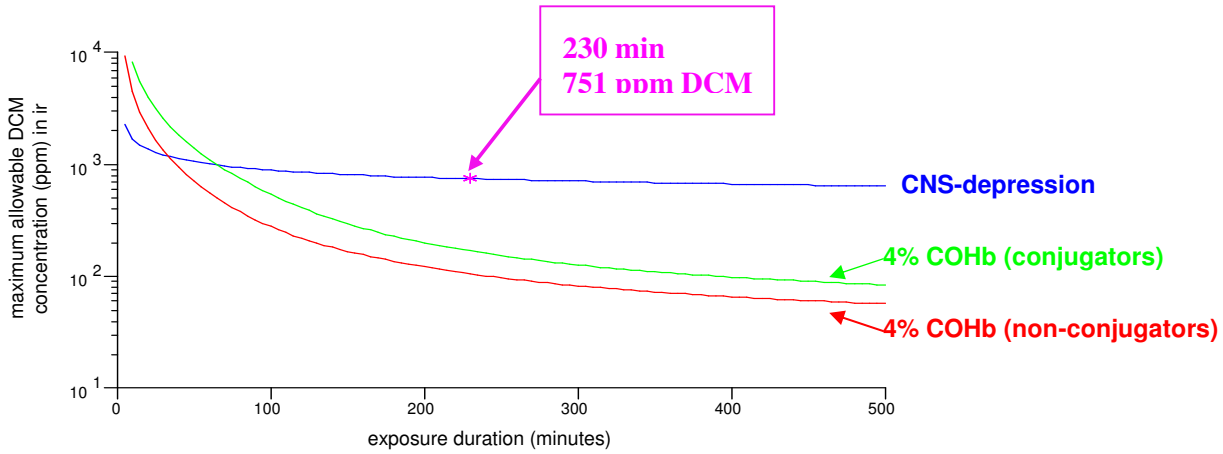

Figure 3. Maximum allowable DCM concentrations in ambient air equivalent to a maximum target DCM concentration of 0.137 mM in human brain based on an exposure regimen of 230 minutes to 751 ppm DCM (blue, upper flat line; asterisk: reference exposure regimen), or to a maximum COHb level of 4% (green, curved upper line: conjugators; red, curved lower line: non-conjugators).

The DCM concentrations for the AEGL-2 exposure times from 10 min to 8 hours were thus derived with the PBPK-model for both endpoints. The DCM concentrations for the relevant time periods for the endpoint of CNS-effects are presented in Table 9. These AEGL-2 values for CNS-related effects are considered to be in compliance with the relevant experimental human data. No evidence of impairment of physical performance was observed at exposures of 15 min to 1700 ppm, of 8 h to 969 ppm, of 2 h to 500 ppm, or of 1 h to 750 ppm. These values are also far below the concentration reported to induce light narcosis in surgical procedures (3-h exposure to ≥7000 ppm).

The results of the obtained AEGL-2 values for both endpoints (COHb formation for conjugators and non-conjugators, DCM concentration in brain) are compared and for each time point the lowest value is chosen as AEGL-2 value. The AEGL-2 values for the 10- and 30-min time periods are based on the CNS-effects, whereas the values for the 1-, 4- and 8-h time periods are based on a maximum additional COHb level of 4%. Non-conjugators accounted for approximately 20% of the Caucasian, African American, and Hispanic population, but up to 60% in the Asian-American population. The U.S. average was estimated to be 20% (Haber *et al.* 2002). The controlling endpoint for COHb formation is therefore a maximum increase of 4% in non-conjugators. Table 9 summarizes the AEGL-2 values for DCM for the individual endpoints and provides the proposed values. It is noted that the sensitive subpopulation for the endpoint of COHb formation consists of non-conjugators with severe coronary artery disease.

TABLE 9. AEGL-2 Values for methylene chloride

Endpoint	10-minute	30-minute	1-hour	4-hour	8-hour
CNS-effects	*1700 ppm*	*1200 ppm*	1000 ppm	740 ppm	650 ppm
COHb level					
- *conjugators*	8400 ppm	2600 ppm	1100 ppm	160 ppm	85 ppm
- *non-conjugators*	4600 ppm	1400 ppm	*560 ppm*	*100 ppm*	*60 ppm*
AEGL-2 values	**1700 ppm (6000 mg/m^3)**	**1200 ppm (4200 mg/m^3)**	**560 ppm (2000 mg/m^3)**	**100 ppm (350 mg/m^3)**	**60 ppm (210 mg/m^3)**

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Relevant human data are only very limited available. With respect to CNS-related mortality it has been described that a patient underwent a surgical procedure with an estimated 3-h exposure to 7000-9333 ppm without complaints afterwards (Hellwig, 1922). Further, DCM is metabolized to CO and hence, DCM exposure leads to the formation of COHb. This endpoint will be covered by deriving AEGL-3 values that will be in compliance with the AEGL-3 values for CO. For CO, the AEGL-3 values were based on observations in humans (EPA, 2001). The available case reports for CO exposure were not considered an adequate basis for the derivation of AEGL-3 values because of uncertainties in the end-of-exposure COHb levels and the insufficient characterization of the exposure conditions. No severe or life-threatening symptoms were observed in healthy subjects exposed to a COHb level of 40-56%. A maximum exposure of 40% COHb at the end of exposure was used as basis to calculate exposure concentrations in air. An intraspecies UF of 3 was applied to the calculated CO concentrations in air. The derived air concentrations corresponded to a COHb level of approximately 15%. This level is considered to be additional to the background COHb value. Therefore, DCM exposure should not lead to an increase in the COHb level of more than 15%.

The human PBPK-model (Appendix B) was used to calculate the concentration-time curves for DCM exposure resulting in a maximum COHb level of 15 % in both conjugators and non-conjugators. However, due to saturation of the MFO-pathway leading to the formation of CO, the rate of CO-production in conjugators is not sufficiently high to reach an increase of 15% in the COHb level.

7.2. Summary of Animal Data Relevant to AEGL-3

No large differences appear to be present between animal species in mortality response to DCM exposure. A steep concentration-response relation is present with mortality increasing from 0 to 100% within a 1.5- to 2-fold increase in concentration. Generally no deaths occur at exposure concentrations below 10,000 ppm for up to 7 h. Balmer *et al.* (1976) reported deaths in 3/20 guinea pigs exposed to 8700 ppm for 6 h. However Heppel *et al.* (1944) observed no deaths in 12 guinea pigs exposed to 9464 ppm (6 h on day 1, 4 h/d on subsequent days, 5d/w) for up to 38 exposure days. Heppel *et al.* (1944) reported the death of 1/5 rabbits after one day of exposure (6 h) to 9464 ppm, the rabbit was not subjected to further examination. Additional deaths occurred on exposure days 12 and 22, while 2 rabbits survived ≥37 exposure days. All other tested animal species (2 monkeys, 4 dogs, 12 guinea pigs, and 16 rats) appeared to tolerate the exposures rather well; only 2 rats died after 33 and 38 exposures, respectively. Hence, it is doubted that the death of the rabbit after one day of exposure can be attributed to DCM. Apart from these data the lowest single exposure causing death in animals (2/6 rats) is 4 h of exposure to a mean concentration of 14,000 ppm (range: 12,000-16,000 ppm); no deaths were observed after exposure to a mean concentration of 11,000 ppm (range: 9300-17,000 ppm) (Haskell Laboratory, 1982) (see Table 6).

7.3. Derivation of AEGL-3

Human mortality may occur as a result of the CNS-depression leading to narcosis, coma and finally death or to a cardiac arrest as a result of COHb formation. Both types of effects have been described as cause of death in fatal DCM exposures although CNS-related effects appear to be the most frequent cause of death. AEGL-3 values will be derived for both causes and ultimately combined to derive the final AEGL-3 values for DCM.

No adequate data are available to directly assess a relationship between DCM exposure and effects related to COHb levels. However, AEGL-3 values for CO are equivalent to approximately 15% COHb. This level of 15% COHb is therefore also used as predetermined point of departure for setting AEGL-3 values for DCM. The human PBPK-model (Appendix B) was used to calculate the concentration-time curves for DCM exposure resulting in a maximum COHb level of 15 % in both conjugators and non-conjugators. However, due to saturation of the MFO-pathway leading to the formation of CO, the rate of CO-production in conjugators is not sufficiently high to reach an increase of 15% in the COHb level. The DCM concentrations in environmental air leading to an increase of 15% in the COHb level in non-conjugators are presented in Figure 4 and Table 10.

Regarding mortality due to CNS-depression the following data are relevant. A patient underwent a surgical procedure with an estimated 3-h exposure to 7000-9333 ppm without complaints afterwards. However, this does not provide sufficient data to serve as a basis for AEGL-3. In animal studies, CNS-depression, finally leading to narcosis, generally preceded death. This was also the case in fatal DCM exposures in humans. No adequate human data are available and evaluation of mortality due to CNS-related effects will be based on animal mortality data. Considering all the relevant mortality data (Table 6), the 4-h exposure to 11,000 ppm at which no mortality was observed in rats (Haskell Laboratory, 1982) is regarded to be an appropriate point of departure for the derivation of AEGL-3 values, despite the large variation in exposure concentrations. Deaths due to CNS-depression occur above exposure concentrations at which the MFO-pathway (CO-formation) is saturated. It is, therefore, concluded that the brain concentration of DCM itself, rather than CO formation, is an appropriate dosemetric for CNS-related mortality. Starting from the 4-h exposure concentration of 11,000 ppm as a nonlethal exposure in rats, a maximum target DCM concentration in rat brain of 3.01 mM was calculated with the PBPK-model for the rat. An interspecies factor of 1 is considered to be sufficient since the differences in susceptibility regarding mortality between species appear to be very small and because a human PBPK-model is used to calculate the external exposure concentrations, thereby discounting the pharmacokinetic differences between rat and human. An intraspecies uncertainty factor of 3 is considered to be sufficient since the susceptibility for CNS-depressing effects does not vary by more than a factor 2-3 in the human population. Application of an overall UF of 3 results in a maximum target DCM concentration in human brain of 1.0 mM. The human PBPK-model was subsequently used to calculate the DCM concentrations in environmental air for exposure of up to 8 hours that will result in a maximum brain concentration of 1.0 mM (Figure 4). The toxic endpoint of interest changes between 4 and 5 hours of exposure from CNS-depression to COHb-formation for non-conjugators.

The DCM concentrations for the AEGL-3 exposure times from 10 min to 8 hours were thus derived with the PBPK-model for both endpoints. The DCM concentrations for the relevant time periods for mortality due to CNS-depression are presented in Table 10. These values appear to be in reasonable agreement with the available mortality data in humans and experimental animals. It has been reported that a 3-h exposure to an average concentration of about 7000-9333 ppm was used during a surgical procedure and did not result in mortality. However, data are available for only one case and the narcotic dose appears to be close to the toxic dose.

Table 10 summarizes the AEGL-3 values for DCM for the individual endpoints and provides the proposed values. It is noted that the sensitive subpopulation for the endpoint of COHb formation consists of non-conjugators with severe coronary artery disease.

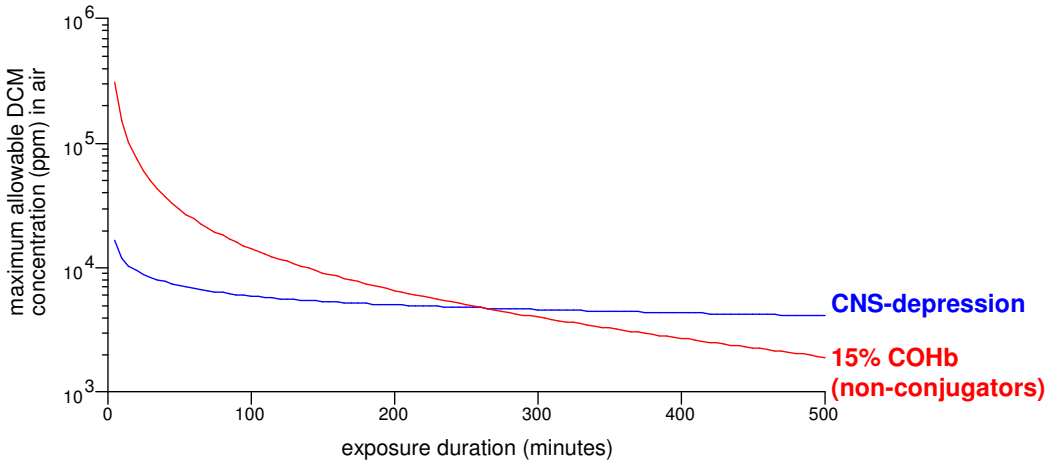

Figure 4. Maximum allowable DCM concentrations in ambient air equivalent to a maximum target DCM concentration of 1.0 mM in human brain (blue, flat line; based on an exposure regimen of 4 hours to 11,000 ppm DCM in rats, total UF =3) or to a maximum additional COHb level of 15% (red, curved line: non-conjugators).

TABLE 10. AEGL-3 Values for methylene chloride

Endpoint	10-minute	30-minute	1-hour	4-hour	8-hour
CNS-effects	*12,000 ppm*	*8500 ppm*	*6900 ppm*	*4900 ppm*	4200 ppm
COHb level					
- *conjugators*	--	--	--	--	--
- *non-conjugators*	160,000 ppm	52,000 ppm	25,000 ppm	5300 ppm	*2100 ppm*
AEGL-3 values	**12,000 ppm (42,000 mg/m^3)**	**8500 ppm (30,000 mg/m^3)**	**6900 ppm (24,000 mg/m^3)**	**4900 ppm (17,000 mg/m^3)**	**2100 ppm (7400 mg/m^3)**

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

A summary of the AEGL-values is presented in Table 11.

TABLE 11. Summary of AEGL Values

Classification	Exposure duration				
	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-1 (Nondisabling)					
- CNS effects	**290 ppm**	**230 ppm**	**200 ppm**	**NR**	**NR**
AEGL-2 (Disabling)					
- CNS effects	**1700 ppm**	**1200 ppm**	1000 ppm	740 ppm	650 ppm
- COHb (non-conjugators)	4600 ppm	1400 ppm	**560 ppm**	**100 ppm**	**60 ppm**
AEGL-3 (Lethal)					
- CNS effects	**12,000 ppm**	**8500 ppm**	**6900 ppm**	**4900 ppm**	4200 ppm
- COHb (non-conjugators)	160,000 ppm	52,000 ppm	25,000 ppm	5300 ppm	**2100 ppm**

NR: Not recommended since these values would be above the corresponding AEGL-2 values.
The AEGL-values are given for individual endpoints; the final values are presented in bold.

8.2. Comparison with Other Standards and Guidelines

TABLE 12. Extant Standards and Guidelines for Methylene Chloride

Guideline	Exposure Duration					
	10 minute	15 minute	30 minute	1 hour	4 hour	8 hour
AEGL-1	290 ppm		230 ppm	200 ppm	NR	NR
AEGL-2	1700 ppm		1200 ppm	560 ppm	100 ppm	60 ppm
AEGL-3	12,000 ppm		8500 ppm	6900 ppm	4900 ppm	2100 ppm
ERPG-1 (AIHA)[a]				200 ppm		
ERPG-2 (AIHA)				750 ppm		
ERPG-3 (AIHA)				4000 ppm		
IDLH (NIOSH)[b]				2300 ppm		
REL-STEL (NIOSH)[c]				--[i]		
PEL-TWA (OSHA)[d]						25 ppm
PEL-STEL (OSHA)[e]		125 ppm				
TLV-TWA						50 ppm

(ACGIH)[f]						
MAK (Germany)[g]						--[j]
MAC (The Netherlands)[h]		500 ppm				100 ppm

NR: Not recommended since these values would be above the corresponding AEGL-2 values.

[a]**ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association** (AIHA 1994)
The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.
The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual=s ability to take protection action.
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to one hour without experiencing or developing life-threatening health effects.

[b]**IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 199?) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

[c]**NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH 1977) is defined analogous to the ACGIH-TLV-TWA.

[d]**OSHA PEL-TWA (Occupational Safety and Health Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA 19??) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[e]**OSHA PEL-STEL (Permissible Exposure Limits - Short Term Exposure Limit)** (OSHA 199?) is defined analogous to the ACGIH-TLV-STEL.

[f]**ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH 1991) is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[g]**MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration])** (Deutsche Forschungsgemeinschaft [German Research Association] 2003) is defined analogous to the ACGIH-TLV-TWA.

[h]**MAC (Maximaal Aanvaarde Concentratie [Maximal Accepted Concentration])** (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000) is defined analogous to the ACGIH-TLV-TWA.

[i]Carcinogen, lowest feasible concentration recommended.

[j]No MAK value or Technical Exposure Limit has been set.

8.3. Data Quality and Research Needs

The data quality is considered sufficient for the derivation of AEGLs. No further research needs are identified.

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APPENDIX A: Derivation of AEGL Values

Derivation of AEGL-1

Key Study:	Stewart *et al.*, 1972
Toxicity endpoint:	No subjective complaints were noted in humans at exposure to 514 ppm (n=3) or 515 ppm (n=8) for 1 h, whereas exposure to 868 ppm immediately following the exposure to 514 ppm or a 2 h exposure to 986 ppm resulted in light-headedness and/or difficulties with enunciation. The exposure of 514 ppm for 1 h was chosen as point of departure for AEGL-1. The effects disappeared soon after exposure while the COHb level still increased. Therefore, these effects were related to the DCM concentration in brain rather than to the formation of CO. The human brain concentration following a 1-h exposure to 514 ppm was calculated to be 0.063 mM, using the human PBPK-model (see Appendix B).
Time scaling:	A PBPK-model was used to calculate exposure concentrations for the relevant time periods.
Uncertainty factors:	Since the mechanism of action will not vary greatly between individuals an intraspecies factor of 3 is considered sufficient. Hence, point of departure is the maximum target DCM concentration in human brain of (0.063/3 =) 0.021 mM.
Modifying factor:	none
Calculations:	
10-minute AEGL-1	290 ppm (1000 mg/m^3)
30-minute AEGL-1	230 ppm (810 mg/m^3)
1-hour AEGL-1	200 ppm (710 mg/m^3)
4-hour AEGL-1	NR, the calculated value is higher than the corresponding AEGL-2 value.
8-hour AEGL-1	NR, the calculated value is higher than the corresponding AEGL-2 value.

Derivation of AEGL-2

Key Studies:	Winneke (1974); TSD on Carbon monoxide
Toxicity endpoints:	a) CNS-effects: sub AEGL-2 effects (CFF, AVT) were observed in humans at an exposure range of 195-751 ppm for up to 4 h. No AEGL-2 related effects were observed in humans at exposure to 751 ppm for 230 min. Point of departure is a maximum target DCM concentration in human brain of 0.137 mM at this exposure concentration, as calculated by a PBPK-model. b) COHb formation: AEGL-2 was based on a maximum additional COHb level of 4% in humans (non-conjugators).
Time scaling:	A PBPK-model was used to calculate exposure concentrations for the relevant time periods for both types of effects. For each time period the lowest value is chosen as AEGL-2 value. The toxic endpoint of interest in non-conjugators changes from CNS-effects to COHb-formation between 30- and 60-min of exposure.
Uncertainty factors:	a) An intraspecies factor of 1 is considered sufficient since the toxic effects studied are less severe than those defined for AEGL-2 and application of a factor greater than 1 will result in AEGL-2 values that conflict with the available human data. Hence, point of departure is the maximum target DCM concentration in human brain of 0.137 mM. b) COHb formation: similar to point of departure for carbon monoxide.
Modifying factor:	none
Calculations:	
10-minute AEGL-2	CNS-effects: 1700 ppm (6000 mg/m^3)
30-minute AEGL-2	CNS-effects: 1200 ppm (4200 mg/m^3)
1-hour AEGL-2	COHb formation: 560 ppm (2000 mg/m^3)
4-hour AEGL-2	COHb formation: 100 ppm (350 mg/m^3)
8-hour AEGL-2	COHb formation: 60 ppm (210 mg/m^3)

Derivation of AEGL-3

Key Studies:	Haskell Laboratory, 1982; TSD on Carbon monoxide
Toxicity endpoint:	a) CNS-effects: No mortality was observed in rats at exposure to 11,000 ppm for 4 h. Mortality is attributed to be finally caused by CNS-depression. Point of departure is a maximum target DCM concentration in rat brain of 3.01 mM, as estimated by a PBPK-model. b) COHb formation: AEGL-3 was based on a maximum additional COHb level of approximately 15% in humans (non-conjugators).
Time scaling	A PBPK-model was used to calculate exposure concentrations for the relevant time periods for both types of effects. For each time period the lowest value is chosen as AEGL-3 value. The toxic endpoint of interest in non-conjugators changes from CNS-effects to COHb-formation between 4- and 5-hours of exposure.
Uncertainty factors:	a) An interspecies factor of 1 is considered sufficient because differences in susceptibility between species appear to be small and because a human PBPK-model is used to calculate the AEGL-3 values. Since susceptibility for CNS-depressing effects will not vary by more than a factor 2-3, an intraspecies factor of 3 is considered sufficient for mortality related to CNS-depression. Hence, point of departure is the maximum target DCM concentration in human brain of (3.01/3 =) 1.0 mM. b) COHb formation: similar to point of departure for carbon monoxide.
Modifying factor:	none
Calculations:	
10-minute AEGL-3	CNS-effects: 12,000 ppm (42,000 mg/m^3)
30-minute AEGL-3	CNS-effects: 8500 ppm (30,000 mg/m^3)
1-hour AEGL-3	CNS-effects: 6900 ppm (24,000 mg/m^3)
4-hour AEGL-3	CNS-effects: 4900 ppm (17,000 mg/m^3)
8-hour AEGL-3	COHb formation: 2100 ppm (7400 mg/m^3)

APPENDIX B: PBPK-modeling for calculating DCM concentration in brain and the formation of COHb and the exposure concentrations

1. INTRODUCTION

In the process of derivation of AEGL-values for dichloromethane (DCM) specific problems have to be encountered, predominantly arising from its biotransformation pathways (Figure 1):

- Two different toxicity endpoints are relevant for acute exposure:
  - CNS-depression, related to the brain concentration of DCM itself,
  - COHb formation, via biotransformation to carbon monoxide (CO).
- CNS-effects occur soon after the onset of exposure.
- Peak levels of COHb can be reached hours after cessation of exposure.
- Saturable metabolic pathway for CO.
- Polymorphism of GSTT1 is present in humans. More CO will be formed in non-conjugators leading to higher COHb levels.

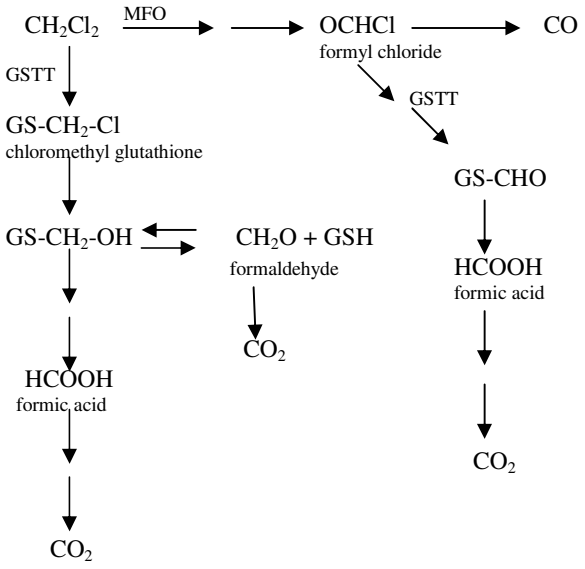

Figure 1. Biotransformation scheme of DCM (modified after Gargas et al., 1986).

It was expected beforehand that the AEGL-values for the shorter exposure durations would be triggered by the CNS-effects whereas the COHb formation would determine the longer exposure durations. There are no data available to determine the intersection of the two curves. In addition, the oxidative pathway leading to the formation of CO becomes saturated at a DCM concentration of about 500 ppm which has to be accounted for in the extrapolation from high to low concentrations.

Modeling was considered to be the most appropriate if not only way to tackle these problems adequately. Several PBPK-models have been described for DCM. The basic model for DCM is the one published by Andersen *et al.* (1987). This model has been used by US EPA to calculate the inhalation Unit Risk for carcinogenic effects of DCM, and has been further updated since for specific purposes. For the purpose of deriving AEGL-values the following steps were made:

- Two previously published PBPK-models were combined:

- Andersen *et al.* (1991), studying the formation of CO and COHb following DCM exposure,
- Reitz *et al.* (1997) who added the brain as a compartment to the PBPK model. This model was especially developed for and used by the ATSDR to derive a 24-h MRL.

➢ Algorithms were developed to derive the time-concentration relation for DCM exposure resulting in predetermined DCM concentrations in brain or peak COHb-levels.

➢ Modeling of non-conjugators was achieved by switching off the glutathione pathway.

These steps are described into more detail in the following sections.

2. BASIC MODEL STRUCTURE

In concordance with earlier models of Volatile Organic Compounds (VOCs) in mammals, Andersen *et al.* (1991) described DCM kinetics in terms of inhalatory uptake in the lungs, blood flow-limited distribution of DCM between a "Richly Perfused Organ"(RPO) compartment, a "Slowly Perfused Organ" (SPO) compartment, a liver and an adipose tissue compartment. DCM metabolism, which is thought to occur predominantly in the liver, occurs via a saturable oxidative (Mixed Function Oxydase (MFO)) pathway and a (first-order) glutathione (GSH) pathway (Figure 1). The MFO pathway yields CO. The produced CO enters the blood where it leads to the formation of carboxyhemoglobin (COHb). Next to formation via DCM metabolism CO enters the blood as a result of heme catabolism and by inhalation of CO from ambient air. Reitz *et al.* (1997) added the brain as a compartment to the PBPK model as developed by Andersen. This extended PBPK model was implemented into the ACSL computer language.

Both a human and a rat model were developed. The ACSL source code and the model parameters for the human model are given in Appendix B-1. Appendices B-2 and B-3 describe the relevant equations related to the blood concentrations of CO and COHb. The ACSL source code for the rat model is given in Appendix B-4. Appendix B-5 describes the algorithm for estimating time-concentration relations based on internal dose-metrics.

A. Human model

Model reproducibility

Andersen *et al*. (1991) extended their original PBPK model for DCM (Andersen *et al*. 1987) to describe the kinetics of CO and COHb. The modeling approach used was based on the Coburn-Forster-Kane (CFK) description of the physiological factors which influence COHb levels in humans (Coburn *et al*. 1965), with an additional element to account for CO arising from the oxidative metabolism of DCM.

Andersen *et al.* (1991) calibrated their model to inhalation experiments with 6 volunteers exposed for 6 hours to 100 and 350 ppm with a 2-week interval. Figures 2 and 3 show the PBPK simulation with the "Andersen" model of the COHb and DCM concentration in venous blood, respectively, together with the experimental data. These simulations are similar to those presented by Andersen *et al.* (1991). The model simulations of Andersen could be reproduced.

Model verifications

The model as described in Appendix B-1 was applied to data obtained from literature in order to study the general applicability of the model. It is noted that due to the natural variation in physiological and kinetic parameters within the human population it cannot be expected that a model that is validated for a specific group of people will precisely predict the results for another group of people. In addition to the natural variation, analytical errors are also a cause of differences in observations between experiments. More of importance is that a model describes the general pattern of the measurements rather than the precise level or concentration.

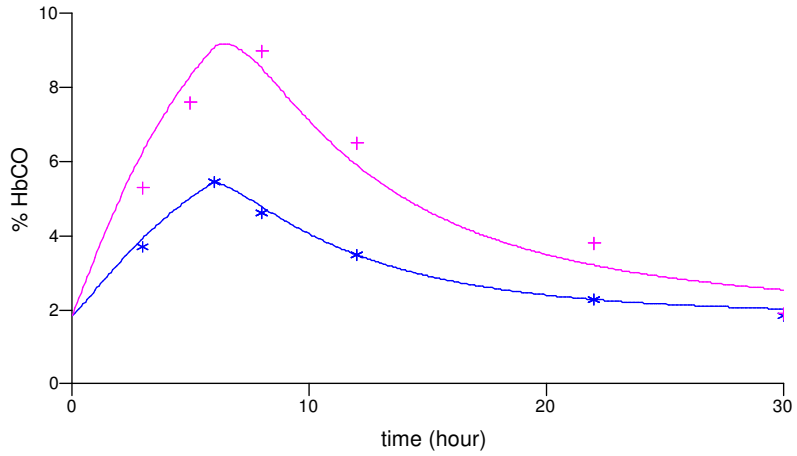

Figure 2. PBPK-simulation of the percentage COHb in blood in humans exposed for 6 hours to 100 ppm (lower line, *) and 350 ppm (upper line, +) DCM. Data represent averages of 6 individuals.

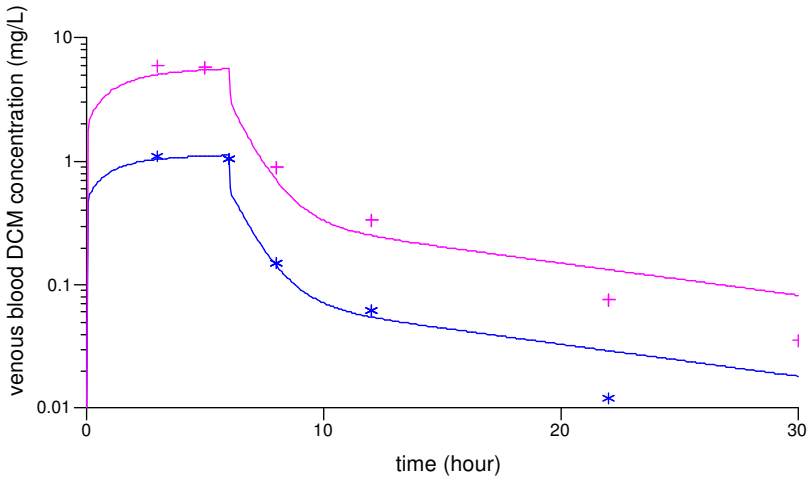

Figure 3. PBPK-simulation of the concentration of DCM in venous blood of humans exposed for 6 hours to 100 ppm (lower line, *) and 350 ppm (upper line, +) DCM. Data represent averages of 6 individuals.

The data obtained by DiVincenzo and Kaplan (1981) were the best described and the most suitable for the present purpose. However, it was noted beforehand that at similar exposure levels DiVincenzo and Kaplan reported lower DCM concentrations and COHb levels than Andersen *et al.* (1991). Eleven male and three female non-smoking volunteers (age: 21 to 42 years) were exposed to 50, 100, 150, or 200 ppm DCM for 7.5 h (groups of 4 to 6 volunteers per exposure concentration). Exposures were interrupted after 4.5 h for a half-hour break. All subjects remained sedentary during and after exposure. Figures 3 and 4 show the measured values for COHb and DCM in blood, respectively, together with the model simulations.

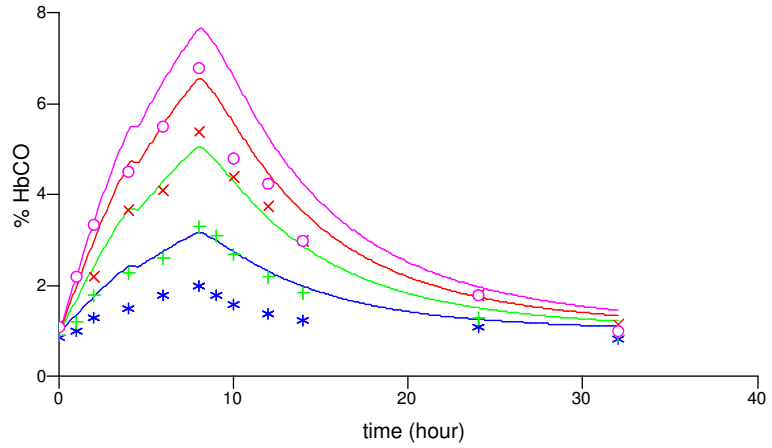

Figure 4. PBPK-simulation of the percentage COHb in blood in humans exposed for 7.5 hours (with a half-hour break after 4.5 hours) to 50 (*), 100 (+), 150 (x), or 200 ppm (o). Data represent averages of 4-6 individuals.

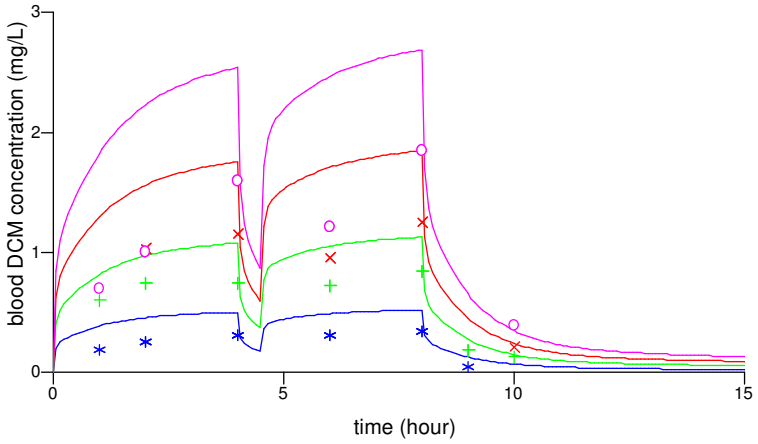

Figure 5. PBPK-simulation of the concentration of DCM in blood in humans exposed for 7.5 hours (with a half-hour break after 4.5 hours) to 50 (*), 100 (+), 150 (x), or 200 ppm (o). Data represent averages of 4-6 individuals.

Figures 4 and 5 show that the PBPK-simulations overestimate the data by about 50% at the most. This is acceptable considering the general variation within the human population, which is often considered to be greater as illustrated by the use of intraspecies factors for kinetics of generally greater than 2. The patterns over time of the DCM concentration in blood and the COHb level are adequately predicted and the time at which the COHb level reaches its peak is properly estimated. DiVincenzo and Kaplan do not provide any physiological details about their volunteers. The physiology and other characteristics of their group of volunteers may have been considerably different from the group studied by Andersen *et al.*

DCM concentration in brain

Through a contract with the ATSDR, Reitz *et al.* (1997) extended the original human model for DCM with a brain compartment to derive, among others, an acute (24-hour) MRL. The PBPK-model was used to extrapolate a 4-h exposure to 300 ppm to a 24-h exposure (i.e. of 60 ppm), using the peak DCM concentration in brain as dose metric. A similar model was previously developed and validated for methyl chloroform, a solvent with comparable characteristics as DCM. It was shown that the methyl chloroform concentration in rat brain could be reliably predicted by a PBPK-model by comparing predicted with observed brain concentrations of methyl chloroform. Reitz *et al.* (1997) present a simulation of the brain concentration of DCM in human brain following a 4-hour exposure to 300 ppm. The same exposure scenario was simulated using our extended model and resulted in a similar prediction of the DCM concentration in brain (Figure 6 to be compared with figure 3 in Reitz *et al.* 1997).

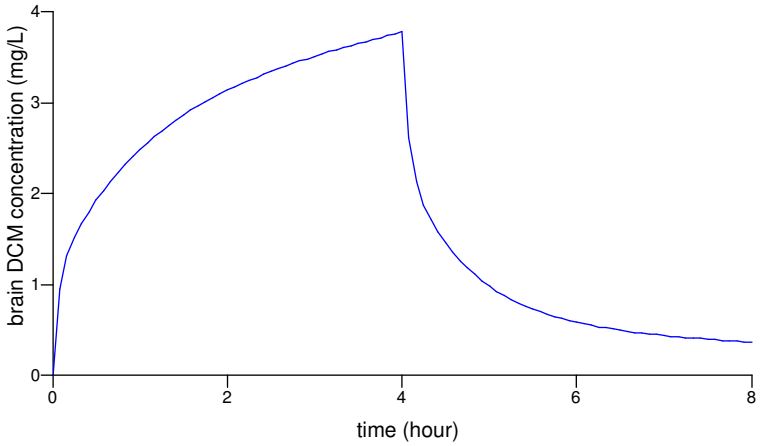

Figure 6. PBPK-simulation of the concentration of DCM in the brain of humans exposed for 4 hours to 300 ppm DCM. Note that the parameter settings were those of Reitz *et al*.

B. Rat model

Model reproducibility

Andersen *et al.* (1991) also developed a similar model for rats. The rat model was validated by simulation of the formation of COHb with different exposure scenarios:

- 5159 ppm DCM for 0.5 hours,
- 200 ppm DCM for 4 hours,
- 1014 ppm DCM for 4 hours.

Similar scenarios were simulated using the "Andersen" rat model as described in Appendix B-4; results are shown in Figures 7, 8, and 9, respectively.

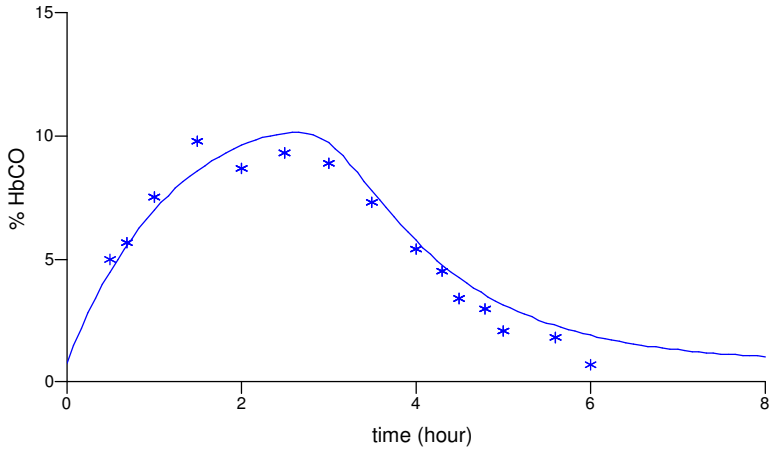

Figure 7. PBPK-simulation of the percentage COHb in blood in rats exposed for 0.5 hours to 5159 ppm. Data (*) represent averages of 3 rats.

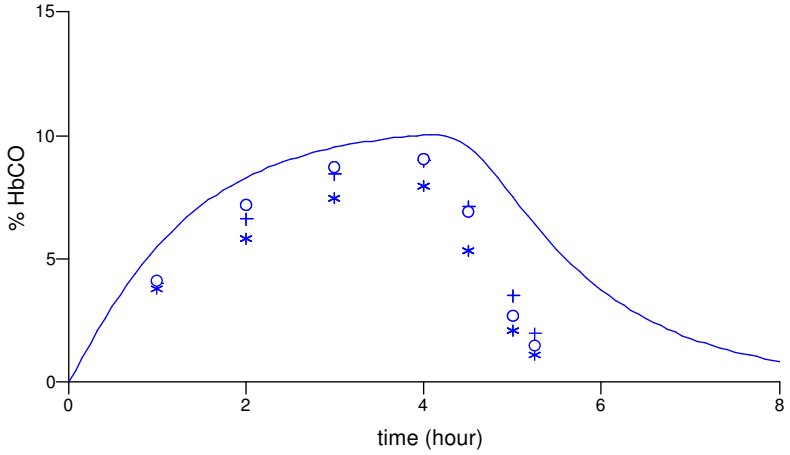

Figure 8. PBPK-simulation of the percentage COHb in blood in rats exposed for 4 hours to 200 ppm. Data (*, +, o) represent individual values for 3 rats.

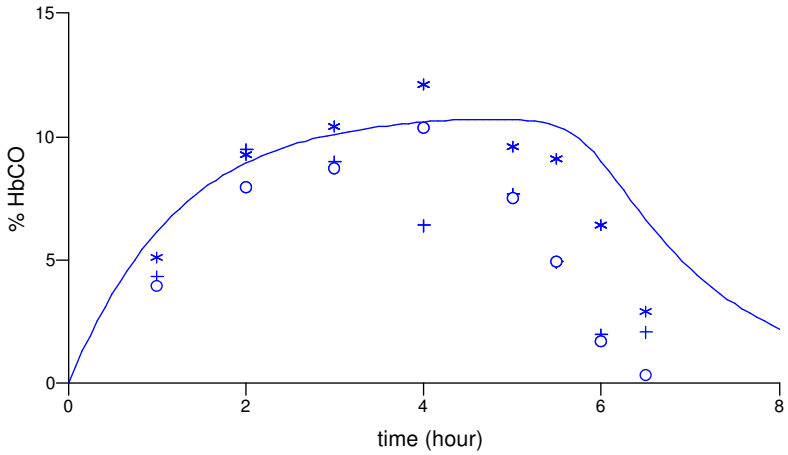

Figure 9. PBPK-simulation of the percentage COHb in blood in rats exposed for 4 hours to 1014 ppm. Data (*, +, o) represent individual values for 3 rats.

Model verifications

The rat model as described in Appendix B-4 was applied to data obtained from literature in order to study the general applicability of the model. The best-described data were obtained form Green *et al*. (1986). Groups of 3 male F344 rats were exposed to 0, 500, 1000, 2000, or 4000 ppm DCM for up to 6 hours. The groups were sacrificed at regular intervals during and after exposure. Figures 10 and 11 show their observations of COHb and DCM in blood, respectively, together with the model simulations.

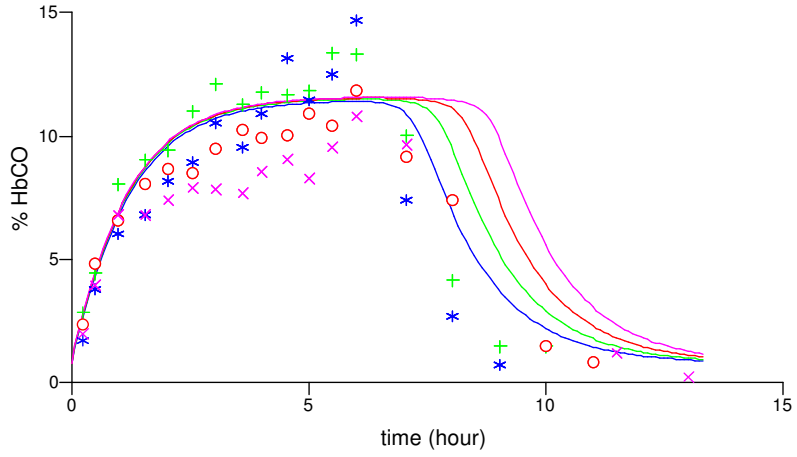

Figure 10. PBPK-simulation of the percentage COHb in blood in rats exposed for 6 hours to 500 (*), 1000 (+), 2000 (o) and 4000 (x) ppm DCM. Data (*, +, o, x) represent average values of 3 rats. Note that the maximum observed COHb level in rats is about 13-14% due to saturation of the biotransformation pathway.

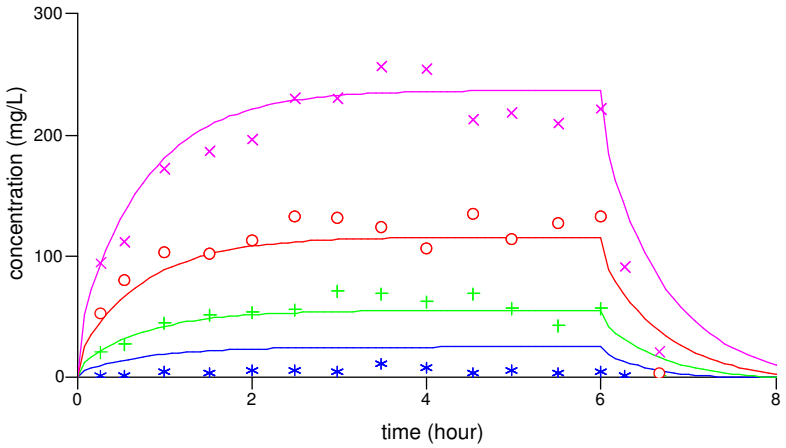

Figure 11. PBPK-simulation of the DCM concentration in blood in rats exposed for 6 hours to 500 (*), 1000 (+), 2000 (o) and 4000 (x) ppm DCM. Data (*, +, o, x) represent average values of 3 rats.

The COHb levels as reported by Green *et al*. (1986) show a relatively large variation. However, it is concluded from these simulations that the rat model adequately predicts the COHb level (including saturation of the CO-forming pathway) and the DCM concentration in the blood of rats in a dose range of 500 to 4000 ppm. The fact that the elimination is somewhat faster than predicted is not of great importance for the present purpose.

C. Conclusions on the PBPK model

Specific problems have to be dealt with in the derivation of AEGL-values for DCM. The modeling approach as described is considered adequate to overcome these problems. The final model, including the general parameter setting, was a combination of models that have been peer reviewed and used for specific risk assessments by US EPA (Andersen *et al*. 1987, 1991) and ATSDR (Reitz *et al*. 1997). Through combining these two basic models both the COHb formation as well as the DCM concentration in brain, as the most appropriate dose metrics, can be simulated with one model. By using the PBPK model, the target tissue concentrations that are associated with adverse health effects can be predicted and the critical tissue dose metrics producing these effects can be determined. Next, the PBPK model will be used to calculate the DCM concentrations in environmental air that will produce the critical target tissue concentration at different exposure durations. This approach will reduce the uncertainties in the derivation of AEGL-values for DCM to a great extent.

4. MODEL APPLICATION

A. Development of algorithms

Algorithms were developed that enable the estimation of the concentration of DCM in ambient air which, for the exposure duration of interest (10-480 min), does not exceed a predetermined concentration of DCM in the brain or a predetermined COHb level additional to background level. DCM concentrations in brain occurred at the end of the exposure period whereas peak COHb levels could occur postexposure. These algorithms are described in Appendix B-5.

B. GSTT1 polymorphism

The GST involved in the biotransformation of DCM is a θ class GST (GSTT1-1). A polymorphism for this enzyme has been well-characterized in humans. A distinction could be made

between non-conjugators who lack the GSTT1-1 enzyme, low-conjugators (heterozygotes who have one positive and one null allele), and high-conjugators (homozygotes). Especially at concentrations above the saturation level for the MFO-pathway it is to be expected that non-conjugators will show higher tissue levels of the parent compound DCM than conjugators. Further, since about 30% of the formyl chloride is estimated to be conjugated through GST (Figure 1) the yield of CO, and thus of COHb, from formyl chloride may be increased in non-conjugators. It has been shown that the COHb level was increased in rats exposed to DCM after pretreatment with a GSH-depletor. GSTT1 activity has been incorporated in the basic model. To simulate a population of non-conjugators the GSTT1 pathway was switched off (kGSH0=0 and YCO=1) giving a 100% conversion of formyl chloride to CO.

C. Parameter setting for the AEGL-derivation

The basic model for the derivation of AEGLs is the one developed by Andersen *et al*. (1991), extended with the brain compartment as applied by Reitz *et al*. (1997). The model parameters have been set after Andersen *et al*. with one exception. The volunteers who were exposed for the validation of the model had an average body weight of 83 kg. This parameter was set at the more generally used value of 70 kg (also used by Reitz *et al*. 1997), and related parameters were adapted accordingly.

No AEGL-1 values were derived for CO. Accordingly, the AEGL-1 values for DCM will be based solely on the DCM concentration in brain. As to AEGL-2 and -3, the appropriate curves for both dose metrics (DCM concentration in brain; COHb) will be determined and plotted in one graph.

5. REFERENCES

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Reitz, R.H., S.M. Hays, and M.L. Gargas. 1997. Addressing priority data needs for methylene chloride with physiologically based pharmacokinetic modeling, Document prepared for the Agency for Toxic Substances and Disease Registry (ATSDR) on behalf of the Halogenated Solvents Industry Alliance (HSIA).

Appendix B-1

ACSL SOURCE CODE FOR HUMAN DCM MODEL

```
PROGRAM DCM
  INTEGER mDCair,nDCair,iDCair
  CONSTANT mDCair=4,nDCair=1
  ARRAY DCair0(1:4),TCair(1:4)
'Physiological constants from ANDERSEN'
'Body weight and relative compartment volumes'
  CONSTANT BW=83.
  CONSTANT vrb=0.059,vrl=.0314,vrr=0.0371,vrf=0.23,vrs=0.62,...
           vrc=0.0194 !vrc void in Andersen because qrc(Andersen)=0
'Alveolar ventilation, Cardiac output and relative compartment flows'
  CONSTANT Qalv0=15.0,Qb0=15.0
  CONSTANT qrl=0.24,qrs=0.19,qrf=0.05,qrr=0.52,...
           qrc=0.0
'Partition coefficients'
  CONSTANT Pba=8.94,Plb=1.46,Prb=0.82,Psb=0.82,Pfb=12.4,...
           Pcb=0.917 !Pcb void in Andersen
'DCM metabolism parameters'
  CONSTANT Vmax0=6.25,KM0=0.75,kGSH0=2.
'CO model parameters'
  CONSTANT DCO0=0.058,PenCO0=0.15,CHBt=10.,YCO=0.71,...
           FelCO=0.85,COairb=2.2,rCO0=1100.,...
           MHald=234.,Atm=760.,Palv=713.,CO2f=0.13,PO2b=100.
'Molecular weights and ppm coversion factor'
  CONSTANT MwCO=28.,MwDC=84.93,ppmcon=24450.
'Initial amounts'
  CONSTANT ADCb0=0.,ADCl0=0.,ADCr0=0.,ADCs0=0.,ADCf0=0.,ADCc0=0.
'Exposure regimen'
  CONSTANT DCair1=100.,DCair2=0.,DCair3=0.,DCair4=0.
  CONSTANT TCair1=360.,TCair2=1800.,TCair3=1800.
  CONSTANT tSTOP=1800.,Cint=5.,Hour=60.

  INITIAL
    Vb=vrb*BW $ Vl=vrl*BW $ Vr=vrr*BW $ Vs=vrs*BW $ Vf=vrf*BW $ Vc=vrc*BW
    Qalv=Qalv0*BW**0.74/Hour $ Qb=Qb0*BW**0.7/Hour
    Ql=qrl*Qb $ Qr=qrr*Qb $ Qs=qrs*Qb $ Qf=qrf*Qb $ Qc=qrc*Qb
    Vmax=Vmax0*(BW**0.7)/(Hour*MwDC) $ KM=KM0/MwDC
    kGSH=kGSH0/(Hour*BW**0.3)
    ADCb=ADCb0 $ ADCl=ADCl0 $ ADCr=ADCr0 $ ADCs=ADCs0 $ ADCf=ADCf0
    ADCc=ADCc0
    DCair0(1)=DCair1 $ DCair0(2)=DCair2 $ DCair0(3)=DCair3 $
DCair0(4)=DCair4
    TCair(1)=TCair1 $ TCair(2)=TCair2 $ TCair(3)=TCair3 $ TCair(4)=tSTOP+1

    iDCair=1 $ DCair=DCair0(1)/ppmcon $ SCHEDULE expose.AT.TCair(1)
    DCO=DCO0*BW**0.92/Hour
    PenCO=PenCO0*BW**0.7/(Hour*MwCO) $ PCOair=COairb*Atm/1000000.
    rCO=rCO0/MwCO
    maxDC=0. $ maxCO=0.
    q=MHald*(PCOair+(1.+Palv*DCO/Qalv)*PenCO/(rCO*DCO))/PO2b
    ACO0=Vb*q*(CHBt/(q+1.)+CO2f/MHald) $ ACO=ACO0 $ AbgCO0=MwCO*ACO0/BW
    CCOt=ACO/Vb
    PROCEDURAL(CHbCOb=CCOt)
      a=MHald*CCOt $ B=MHald $ c=CO2f $ d=CHbt
      x=(a+b*d+c+sqrt((a+b*d+c)**2-4.*a*b*d))/(2.*b)
      CHbCOb=a*d/(b*x)
    END
    Ratbb=100*CHbCOb/CHbt
  END
```

```
DYNAMIC

    DISCRETE expose
      iDCair=iDCair+1
      DCair=DCair0(iDCair)/ppmcon $ SCHEDULE expose.AT.TCair(iDCair)
    END

    DERIVATIVE
      ALGORITHM IALG=2

      CDCl=ADCl/Vl $ CDCr=ADCr/Vr $ CDCs=ADCs/Vs
      CDCf=ADCf/Vf $ CDCc=ADCc/Vc
      CCOt=ACO/Vb
      maxDC=max(maxDC,CDCc)
      PROCEDURAL(CHbCO=CCOt)
        a=MHald*CCOt $ B=MHald $ c=CO2f $ d=CHbt
        x=(a+b*d+c+sqrt((a+b*d+c)**2-4.*a*b*d))/(2.*b)
        CHbCO=a*d/(b*x)
      END
      CHbO2=CHBt-CHbCO
      PCOb=PO2b*CHbCO/(MHald*CHbO2)
      PCOalv=(DCO*PCOb+Qalv*PCOair/Palv)/(DCO+Qalv/Palv)


CDCven=(Ql*CDCl/Plb+Qr*CDCr/Prb+Qs*CDCs/Psb+Qf*CDCf/Pfb+Qc*CDCc/Pcb)/Qb
      CDCb=(Qb*CDCven+Qalv*DCair)/(Qb+Qalv/Pba)
      dADCl=Ql*(CDCb-CDCl/Plb)-Vmax*(CDCl/Plb)/(KM+(CDCl/Plb))-
kGSH*ADCl/Plb
      dADCr=Qr*(CDCb-CDCr/Prb)
      dADCs=Qs*(CDCb-CDCs/Psb)
      dADCf=Qf*(CDCb-CDCf/Pfb)
      dADCc=Qc*(CDCb-CDCc/Pcb)
      dACO=PenCO+YCO*(Vmax*CDCl/Plb)/(KM+CDCl/Plb)-DCO*(PCOb-PCOalv)*rCO

      ADCl=INTEG(dADCl,ADCl0)
      ADCr=INTEG(dADCr,ADCr0) $ ADCs=INTEG(dADCs,ADCs0)
      ADCf=INTEG(dADCf,ADCf0) $ ADCc=INTEG(dADCc,ADCc0)
      ACO=INTEG(dACO,ACO0)

      CDCbmg=MwDC*CDCven $ CDCamg=MwDC*(0.3*DCair+0.7*CDCb/Pba)
      CDCcmg=MwDC*CDCc
      Ratb=100.*CHbCO/CHbt $ maxCO=max(maxCO,Ratb)

      TERMT(t.GE.tSTOP)
    END
  END
END    Ql=qrl*Qb $ Qr=qrr*Qb $ Qs=qrs*Qb $ Qf=qrf*Qb $ Qc=qrc*Qb
    Vmax=Vmax0*(BW**0.7)/(Hour*MwDC) $ KM=KM0/MwDC
    kGSH=kGSH0/(Hour*BW**0.3)
    ADCb=ADCb0 $ ADCl=ADCl0 $ ADCr=ADCr0 $ ADCs=ADCs0 $ ADCf=ADCf0
    ADCc=ADCc0
    DCair=DCAIR0/ppmcon $ SCHEDULE expoff.AT.Texpof
    DCO=DCO0*BW**0.92/Hour
    PenCO=PenCO0*BW**0.7/(Hour*MwCO) $

    "Conversion of ppm to pressure in mm Hg"
    PCOair=COairb*Atm/1000000.

    rCO=rCO0/MwCO
    maxDC=0. $ maxCO=0.

    "Steady state background amount of CO in blood" (see Appendix B-3)
    q=MHald*(PCOair+(1.+Palv*DCO/Qalv)*PenCO/(rCO*DCO))/PO2b
    ACO0=Vb*q*(CHBt/(q+1.)+CO2f/MHald) $ ACO=ACO0 $

  END
```

```
  DYNAMIC

    DISCRETE expoff
      DCair=0.
    END

    DERIVATIVE
      ALGORITHM IALG=2

   "DCM organ concentrations"
      CDCl=ADCl/Vl $ CDCr=ADCr/Vr $ CDCs=ADCs/Vs
      CDCf=ADCf/Vf $ CDCc=ADCc/Vc

      "Total CO blood concentration"
      CCOt=ACO/Vb
      maxDC=max(maxDC,CDCc)

      "Blood HbCO concentration" (see Appendix B-2)
      PROCEDURAL(CHbCO=CCOt)
        a=MHald*CCOt $ b=MHald $ c=CO2f $ d=CHbt
        x=(a+b*d+c+sqrt((a+b*d+c)**2-4.*a*b*d))/(2.*b)
        CHbCO=a*d/(b*x)
      END

   "Mass-balance of Hb"
      CHbO2=CHBt-CHbCO

   "Partial pressure of CO in the blood"
      PCOb=PO2b*CHbCO/(MHald*CHbO2)

      "Partial pressure of CO in alveoli"
      PCOalv=(DCO*PCOb+Qalv*PCOair/Palv)/(DCO+Qalv/Palv)

      "Venous and arterial DCM blood concentration"
      CDCven=(Ql*CDCl/Plb+Qr*CDCr/Prb+Qs*CDCs/Psb+
                          Qf*CDCf/Pfb+Qc*CDCc/Pcb)/Qb
      CDCb=(Qb*CDCven+Qalv*DCair)/(Qb+Qalv/Pba)

      "Organ mass-balance equations"
      dADCl=Ql*(CDCb-CDCl/Plb)-Vmax*(CDCl/Plb)/(KM+(CDCl/Plb))-
            kGSH*ADCl/Plb
      dADCr=Qr*(CDCb-CDCr/Prb)
      dADCs=Qs*(CDCb-CDCs/Psb)
      dADCf=Qf*(CDCb-CDCf/Pfb)
      dADCc=Qc*(CDCb-CDCc/Pcb)

   "CO mass-balance in the body"
      dACO= PenCO+YCO*(Vmax*CDCl/Plb)/(KM+CDCl/Plb)-DCO*(PCOb-PCOalv)*rCO


   "Integration statements"
      ADCl=INTEG(dADCl,ADCl0)
      ADCr=INTEG(dADCr,ADCr0) $ ADCs=INTEG(dADCs,ADCs0)
      ADCf=INTEG(dADCf,ADCf0) $ ADCc=INTEG(dADCc,ADCc0)
      ACO=INTEG(dACO,ACO0)

   "Concentrations in mg/l"
      CDCbmg=MwDC*CDCven $ CDCcmg=MwDC*CDCc

   "Fraction of HbCO in blood"
      Ratb=100.*CHbCO/CHbt $ maxCO=max(maxCO,Ratb)

      TERMT(t.GE.tSTOP)
    END
  END
END
```

Model parameters (A: Andersen etal. 1991; R: Reitz *et al*. 1997)

Parameter	Dimension	Value	Source
Body weight (BW)	Kg	83	A
Relative organ volumes			
Liver (vrl)		0.0314	A
RPO (vrr)		0.0371	A
Adipose tissue (vrf)		0.230	A
Brain (vrc)		0.0194	R
SPO (vrs)		0.592	A
Blood (vrb)		0.059	A
Alveolar ventilation rate (Qalv0)[1]	L/hr	15.0	A
Cardiac output (Qb0)[2]	L/hr	15.0	A
Relative blood flows			
Liver (qrl)		0.24	A
SPO (qrs)		0.19	A
Adipose tissue (qrf)		0.05	A
Brain (qrc)[3]		0.0	
RPO (qrr)		0.52	A
Partition coefficients			
Blood:air (Pba)		8.94	A
Liver:blood (Plb)		1.46	A
RPO:blood (Prb)		0.82	A
SPO:blood (Psb)		0.82	A
Adipose tissue:blood (Pfb)		12.4	A
Brain:blood (Pcb)		0.917	R
MFO-metabolism			
Maximal rate (V_{max0})[4]	Mg/hr	6.25	A
Michaelis Menten constant (K_{m0})	Mg/l	0.75	A
MFO CO-yield factor (YCO)		0.71	A
GSH-metabolism	Hr^{-1}	2.0	A
First-order rate constant (k_{GSH0})			
Lung CO diffusing capacity (D_{CO0})[5]	l/hr/mm Hg	0.058	A
Endogenous CO production (P_{enCO0})[6]	Mg/kg/hr	0.15	A
Hemoglobin concentration ($C_{HB,t}$)	mM	10.0	A
Background CO concentration in air (CO_{airb})	ppm	2.2	A
CO density (rCO0)	Mg/l	1100	A
Haldane coefficient (MHald)		234	A
Atmosheric pressure (Atm)	mm Hg	760	

Atmospheric pressure minus pressure of vapor pressure at 37 ^{o}C (P_{alv})	mm Hg	713	A
Free oxygen concentration in blood ($C_{O2,f}$)	mM	0.13	A
Alveolar capillary oxygen tension ($P_{O2,b}$)	mm Hg	100	A

[1]In concordance with Reitz *et al*. (1997) the alveolar ventilation rate (Q_{alv}) was allometrically scaled as $Q_{alv} = Q_{alv0} \cdot BW^{0.74}$ (l/hr, in the model the alveolar ventilation rate was expressed in l/min). For a body weight of 83 kg the allometric relationship calculates an alveolar ventilation rate of 395 l/hour.

[2]In concordance with Reitz *et al*. (1997) the cardiac output (Q_b) was allometrically scaled as $Q_b = Qb_0 \cdot BW^{0.7}$ (l/hr, in the model the cardiac output was expressed in l/min).
For a body weight of 83 kg the allometric relationship calculates a cardiac output of 331 l/hr.

[3]In concordance with Reitz *et al*. (1997) the relative blood flow to the brain was set at 0.114 with the other relative blood flows adjusted accordingly to calculate the brain concentration of DCM.

[4]In concordance with Andersen *et al*. (1991) the maximal metabolic rate for MFO-dependent DCM metabolism was allometrically scaled as $V_{max} = V_{max0} \cdot BW^{0.7}$ (mg/hr, in the model metabolism is expressed in mmole/min).

[5]In concordance with Andersen *et al*. (1991) the diffusion capacity of CO in the lungs was allometrically scaled as $D_{CO} = D_{CO0} \cdot BW^{0.92}$ (l/hr/mm Hg, in the model diffusion was expressed in l/min/mm Hg).

[6]In concordance with Andersen *et al*. (1991) the endogenous CO production was allometrically scaled as $P_{enCO} = P_{enCO0} \cdot BW^{0.7}$ (mg/kg/hr, in the model endogenous CO production was expressed in mmole/min).

Appendix B-2

TOTAL CONCENTRATION OF CARBON MONOXIDE IN THE BLOOD

Elucidation of the CFK-mathematical model as used by Andersen et al. (1991).
Chemical binding of CO and O_2 to Hb is described by

$$K_{CO}[HbCO]_t = [Hb]_f[CO]_f$$
$$K_{O_2}[HbO_2]_t = [Hb]_f[O_2]_f \quad (1)$$

where [] = concentration in molair, the coefficients *K* denote dissociation constants and the subscript *f* denotes the free concentration. The ratio of these two

$$\frac{K_{O_2}[HbO_2]}{K_{CO}[HbCO]} = M\frac{[HbO_2]}{[HbCO]} = \frac{[O_2]_f}{[CO]_f} \quad (2)$$

where the so called Haldane coefficient $M = K_{O_2} / K_{CO}$. Equation (2) can be reformulated as

$$[CO]_f = \frac{[O_2]_f[HbCO]}{M[HbO_2]} \quad (3)$$

which is basically equation (3) in the Appendix of Andersen *et al*. (1991). However, in the denominator of the right hand side of their equation, erroneously, $[HbCO]$ instead of $[HbO_2]$ has been noted.

Note that the dissociation constants and the Haldane coefficient are expressed in units of molair.

The total concentrations

$$[Hb]_t = [Hb]_f + [HbO_2] + [HbCO]$$
$$[CO]_t = [CO]_f + [HbCO] \quad (4)$$

and the free oxygen concentration $[O_2]_f$ are assumed to be known: the first ($[Hb]_t$)and the third ($[O_2]_f$) are physiologically determined and the second ($[CO]_t$) follows from the differential equation describing disposition of carbon monoxide. For, in equation (4) the contribution of the free fraction of hemoglobin to the total concentration will be neglected henceforth:

$$[Hb]_t = [HbO_2] + [HbCO] \quad (5)$$

Equation (5) is basically equation (5) in Andersen *et al.*.
Substitute from equation (5) $[HbO_2] = [Hb]_t - [HbCO]$ and from equation (4), second line, $[CO]_f = [CO]_t - [HbCO]$ in equation (3):

$$[\mathrm{CO}]_t - [\mathrm{HbCO}] = \frac{[\mathrm{O_2}]_f [\mathrm{HbCO}]}{M\left([\mathrm{Hb}]_t - [\mathrm{HbCO}]\right)} \tag{6}$$

from which

$$[\mathrm{HbCO}] + \frac{[\mathrm{O_2}]_f [\mathrm{HbCO}]}{M\left([\mathrm{Hb}]_t - [\mathrm{HbCO}]\right)} = \left(1 + \frac{[\mathrm{O_2}]_f}{M\left([\mathrm{Hb}]_t - [\mathrm{HbCO}]\right)}\right)[\mathrm{HbCO}] = [\mathrm{CO}]_t \tag{7}$$

or, equivalently,

$$[\mathrm{HbCO}] = \frac{[\mathrm{CO}]_t}{1 + [\mathrm{O_2}]_f / M\left([\mathrm{Hb}]_t - [\mathrm{HbCO}]\right)} \tag{8}$$

This is basically equation (6) in the Appendix of Andersen *et al.* (1991). However, the factor M in the denominator of the second term in the denominator of the right hand side has been omitted in their equation and brackets are placed erroneously: "$(1 + [\mathrm{O_2}]_f)/$" (in our notation) instead of "$1 + [\mathrm{O_2}]_f /$".

Equation (8) is basically a quadratic equation in $[\mathrm{HbCO}]$. Quadratic equations have two roots. A root is admissible when

$$[\mathrm{HbCO}] \le [\mathrm{CO}]_t \text{ and } [\mathrm{HbCO}] \le [\mathrm{Hb}]_t \tag{9}$$

Denoting

$$\begin{aligned} &0 \le x = [\mathrm{HbCO}], \quad 0 \le a = M[\mathrm{CO}]_t, \quad b = M, \\ &0 \le c = [\mathrm{O}]_f, \quad 0 \le d = [\mathrm{Hb}]_t \end{aligned} \tag{10}$$

equation (8) can be reformulated as

$$x = \frac{a}{b + c/(d - x)} \tag{11}$$

from which

$$bx^2 - (a + bd + c)x + ad = 0 \tag{12}$$

and the only admissible root is

$$x = \left(a + bd + c - \sqrt{(a + bd + c)^2 - 4abd}\right)/2b \tag{13}$$

or

$$\begin{aligned} [\mathrm{HbCO}] = \big(&M[\mathrm{CO}]_t + M[\mathrm{Hb}]_t + [\mathrm{O_2}]_f - \\ &\sqrt{\left(M[\mathrm{CO}]_t + M[\mathrm{Hb}]_t + [\mathrm{O_2}]_f\right)^2 - 4M^2[\mathrm{CO}]_t[\mathrm{Hb}]_t}\big)/2M \end{aligned} \tag{14}$$

For this root, $[\mathrm{HbCO}] = 0$ when $[\mathrm{CO}]_t = 0$ and $[\mathrm{HbCO}] \to [\mathrm{Hb}]_t$ when $[\mathrm{CO}]_t \to \infty$, while for the other root $[\mathrm{HbCO}] = M[\mathrm{Hb}]_t + [\mathrm{O}_2]_f$ when $[\mathrm{CO}]_t = 0$ and $[\mathrm{HbCO}] \approx [\mathrm{CO}]_t$ when $[\mathrm{CO}]_t \to \infty$, violating the condition in equation (9).

From the other hand, when the carboxyhaemoglobin concentration is known as a fraction *p* from the total haemoglobin concentration, then the total carbon monooxyde concentration, and thus also the corresponding free concentration can be readily derived from equation (7) to be

$$[\mathrm{CO}]_t = \left(1 + \frac{[\mathrm{O}_2]_f}{M(1-p)[\mathrm{Hb}]_t}\right) \cdot p[\mathrm{Hb}]_t \qquad (15)$$

As shown in the next paragraph equation (15) may be further refined by expressing p in terms of known physico-chemical and physiological constants.

Appendix B-3

BACKGROUND ("STEADY STATE") OF THE TOTAL AMOUNT OF CARBON MONOXIDE IN THE BLOOD

When no DCM is in the ambient air, i.e. neglecting carbon monoxide from DCM metabolism, then for the amount of CO the following mass balance holds:

$$\frac{dA}{dt} = \dot{P} - \rho D(p_b - p_{alv}) \tag{15}$$

where A denotes the amount of CO (grams), $\dot{P}$ the endogenous CO production (gram/hr), ρ CO-density (g/l), D is a diffusion coefficient for CO-gas diffusion over the alveolar walls (l/hr/mm Hg) and p_b, p_{alv} and p_{air} are the partial pressure in blood, alveoles and ambient air (mm Hg), respectively.

In equation (15), the partial pressure of CO in the alveoles is given by

$$p_{alv} = \frac{P_{alv}D}{P_{alv}D + Q_{alv}} p_b + \frac{Q_{alv}}{P_{alv}D + Q_{alv}} p_{air} \tag{16}$$

where P_{alv} is the atmospheric pressure in the alveoles corrected for the saturated vapor pressure at 37^0 C (mm Hg) and Q_{alv} is the alveolar ventilation (l/hr), while the partial pressure in blood is given by

$$p_b = \frac{p_{O_2,b}[HbCO]}{M[HbO_2]} \tag{17}$$

where $p_{O_2,b}$ is the partial pressure of oxygen in blood, $[HbCO]$ the concentration of carboxyhaemoglobin in blood, $[HbO_2]$ the hemoglobin concentration and M the Haldane coefficient, which is the ratio of the dissociation constants of the binding of oxygen and carbon monoxide to free hemoglobin.

From equation (15), one can derive easily that under steady state conditions the partial pressure of CO in blood is determined by the endogenous CO production and the ambient air CO concentration

$$p_b = p_{alv} + \frac{\dot{P}}{\rho D} \tag{18}$$

so that, substituting equation (16) and rearranging

$$p_b = p_{air} + \left(1 + \frac{P_{alv}D}{Q_{alv}}\right) \cdot \frac{\dot{P}}{\rho D} \tag{19}$$

So, from equation (17) it follows that the steady state concentration of carboxyhaemoglobin is hen given by

$$[HbCO] = \frac{M[HbO_2]}{p_{O_2,b}} \cdot \left(p_{air} + \left(1 + \frac{P_{alv} D}{Q_{alv}} \right) \frac{\dot{P}}{\rho D} \right) = q \cdot [HbO_2] \tag{20}$$

where

$$q = \frac{M}{p_{O_2,b}} \cdot \left(p_{air} + \left(1 + \frac{P_{alv} D}{Q_{alv}} \right) \frac{\dot{P}}{\rho D} \right) \tag{21}$$

As

$$[Hb]_t = [HbCO] + [HbO_2] + [Hb]_f \tag{22}$$

is the total hemoglobin concentration, then, neglecting the contribution of the free hemoglobin concentration $[Hb]_f$, it follows that

$$[HbCO] = \frac{q}{q+1} [Hb]_t \tag{23}$$

From the foregoing paragraph it is known that when the carboxyhemoglobin concentration is known as a fraction $[HbCO] = p \cdot [Hb]_t$ of the total hemoglobin concentration, then

$$[CO]_t = \left(1 + \frac{[O_2]_f}{M(1-p)[Hb]_t} \right) \cdot p[Hb]_t \tag{24}$$

where $[O_2]_f$ is the free oxygen concentration in blood.

Substituting $p = q/(q+1)$ from equation (23) in equation (24) and multiplying the result with blood volume V_b, it follows that the steady state total amount of carbon monoxide in blood is given by

$$A = qV_b \cdot \left(\frac{[Hb]_t}{q+1} + \frac{[O_2]_f}{M} \right) \tag{25}$$

Note that equations (7) and (11) indicate that the steady state total amount of carbon monoxide in the blood can be expressed in terms of already used model parameters. It is therefore unnecessary to add this amount as a separate model parameter.

Appendix B-4

ACSL SOURCE CODE FOR RAT DCM MODEL

```
PROGRAM COrat
  CONSTANT BW=0.22
  CONSTANT vrb=0.059,vrl=.04,vrr=0.05,vrf=0.07,vrs=0.75,...
           vrc=0.00696
  CONSTANT Qalv0=15.0,Qb0=15.0
  CONSTANT qrl=0.20,qrs=0.15,qrf=0.09,qrr=0.56,...
           qrc=0.0
  CONSTANT Pba=19.4,Plb=0.732,Prb=0.732,Psb=0.408,Pfb=6.19,...
           Pcb=0.387
  CONSTANT Vmax0=4.0,KM0=0.4,kGSH0=2.
  CONSTANT DCO0=0.060,PenCO0=0.035,CHBt=10.,YCO=0.71,...
           FelCO=1.21,COairb=2.2,rCO0=1100.,...
           MHald=197.,Atm=760.,Palv=713.,CO2f=0.13,PO2b=100.
  CONSTANT MwCO=28.,MwDC=84.93
  CONSTANT ADCb0=0.,ADCl0=0.,ADCr0=0.,ADCs0=0.,ADCf0=0.,ADCc0=0.
  CONSTANT DCair0=5159.,ppmcon=24450.,Texpof=30.
  CONSTANT tSTOP=480.,Cint=5.,Hour=60.

  XERROR ADCL=1.d-10

  INITIAL
    Vb=vrb*BW $ Vl=vrl*BW $ Vr=vrr*BW $ Vs=vrs*BW $ Vf=vrf*BW $ Vc=vrc*BW
    Qalv=Qalv0*BW**0.74/Hour $ Qb=Qb0*BW**0.7/Hour
    Ql=qrl*Qb $ Qr=qrr*Qb $ Qs=qrs*Qb $ Qf=qrf*Qb $ Qc=qrc*Qb
    Vmax=Vmax0*(BW**0.7)/(Hour*MwDC) $ KM=KM0/MwDC
    kGSH=kGSH0/(Hour*BW**0.3)
    ADCb=ADCb0 $ ADCl=ADCl0 $ ADCr=ADCr0 $ ADCs=ADCs0 $ ADCf=ADCf0
    ADCc=ADCc0
    DCair=DCAIR0/ppmcon $ SCHEDULE expoff.AT.Texpof
    DCO=DCO0*BW**0.92/Hour
    PenCO=PenCO0*BW**0.7/(Hour*MwCO) $ PCOair=COairb*Atm/1000000.
    rCO=rCO0/MwCO
    maxDC=0. $ maxCO=0.
    q=MHald*(PCOair+(1.+Palv*DCO/Qalv)*PenCO/(rCO*DCO))/PO2b
    ACO0=Vb*q*(CHBt/(q+1.)+CO2f/MHald) $ ACO=ACO0 $ AbgCO0=MwCO*ACO0/BW
  END

  DYNAMIC

    DISCRETE expoff
      DCair=0.
      CALL RSTART(evolut,.0001)
    END

    DERIVATIVE evolut
      ALGORITHM IALG=2

      CDCl=ADCl/Vl $ CDCr=ADCr/Vr $ CDCs=ADCs/Vs
      CDCf=ADCf/Vf $ CDCc=ADCc/Vc
      CCOt=ACO/Vb
      maxDC=max(maxDC,CDCc)
      PROCEDURAL(CHbCO=CCOt)
        a=MHald*CCOt $ b=MHald $ c=CO2f $ d=CHbt
        x=(a+b*d+c+sqrt((a+b*d+c)**2-4.*a*b*d))/(2.*b)
        CHbCO=a*d/(b*x)
      END
      CHbO2=CHBt-CHbCO
      PCOb=PO2b*CHbCO/(MHald*CHbO2)
      PCOalv=(DCO*PCOb+Qalv*PCOair/Palv)/(DCO+Qalv/Palv)
```

```
1
2
3   CDCven=(Ql*CDCl/Plb+Qr*CDCr/Prb+Qs*CDCs/Psb+Qf*CDCf/Pfb+Qc*CDCc/Pcb)/Qb
4         CDCb=(Qb*CDCven+Qalv*DCair)/(Qb+Qalv/Pba)
5         dADCl=Ql*(CDCb-CDCl/Plb)-Vmax*(CDCl/Plb)/(KM+(CDCl/Plb))-
6   kGSH*ADCl/Plb
7         dADCr=Qr*(CDCb-CDCr/Prb)
8         dADCs=Qs*(CDCb-CDCs/Psb)
9         dADCf=Qf*(CDCb-CDCf/Pfb)
10        dADCc=Qc*(CDCb-CDCc/Pcb)
11        dACO=PenCO+YCO*(Vmax*CDCl/Plb)/(KM+CDCl/Plb)-DCO*(PCOb-PCOalv)*rCO
12
13        ADCl=INTEG(dADCl,ADCl0)
14        ADCr=INTEG(dADCr,ADCr0) $ ADCs=INTEG(dADCs,ADCs0)
15        ADCf=INTEG(dADCf,ADCf0) $ ADCc=INTEG(dADCc,ADCc0)
16        ACO=INTEG(dACO,ACO0)
17        ADCbod=ADCl+ADCr+ADCs+ADCf+ADCc+Vb*CDCb/4.+3*Vb*CDCven/4.
18
19        CDCbmg=MwDC*CDCven $ CDCamg=MwDC*(0.3*DCair+0.7*CDCb/Pba)
20        CDCcmg=MwDC*CDCc
21        Ratb=100.*CHbCO/CHbt $ maxCO=max(maxCO,Ratb)
22
23        TERMT(t.GE.tSTOP)
24      END
25    END
26  END
27
28  prepare /all
29  set wesitg=.false.
30
31  proc brainrat
32  !!set qrl=0.2 qrf=0.07 qrs=0.15 qrc=0.0386 qrr=0.5414
33  end
```

Model parameters (A: Andersen *et al.* 1991; R: Reitz *et al*. 1997)

Parameter	Dimension	Value	Source
Body weight (BW)	Kg	0.22	A
Relative organ volumes			
Liver (vrl)		0.04	A
RPO (vrr)		0.05	A
Adipose tissue (vrf)		0.07	A
Brain (vrc)		0.00696	R
SPO (vrs)		0.75	A
Blood (vrb)		0.059	A
Alveolar ventilation rate (Qalv0)[1]	L/hr	15.0	A
Cardiac output (Qb0)[2]	L/hr	15.0	A
Relative blood flows			
Liver (qrl)		0.20	A
SPO (qrs)		0.15	A
Adipose tissue (qrf)		0.09	A
Brain (qrc)[3]		0.0	
RPO (qrr)		0.56	A
Partition coefficients			
Blood:air (Pba)		19,4	A
Liver:blood (Plb)		0.732	A
RPO:blood (Prb)		0.732	A
SPO:blood (Psb)		0.408	A
Adipose tissue:blood (Pfb)		6.19	A
Brain:blood (Pcb)		0.387	R
MFO-metabolism			
Maximal rate (V_{max0})[4]	Mg/hr	4.0	A
Michaelis Menten constant (K_{m0})	Mg/l	0.4	A
MFO CO-yield factor (YCO)		0.71	
GSH-metabolism	Hr^{-1}	2.0	A
First-order rate constant (k_{GSH0})			
Lung CO diffusing capacity (D_{CO0})[5]	l/hr/mm Hg	0.060	A
Endogenous CO production (P_{enCO0})[6]	Mg/kg/hr	0.035	A
Hemoglobin concentration ($C_{HB,t}$)	mM	10.0	A
Background CO concentration in air (CO_{airb})	ppm	2.2	A
CO density (rCO0)	Mg/l	1100	A
Haldane coefficient (MHald)		197	A
Atmosheric pressure (Atm)	mm Hg	760	

Atmospheric pressure minus pressure of vapor pressure at 37 ^{o}C (P_{alv})	mm Hg	713	A
Free oxygen concentration in blood ($C_{O2,f}$)	mM	0.13	A
Alveolar capillary oxygen tension ($P_{O2,b}$)	mm Hg	100	A

[1]In concordance with Reitz *et al*. (1997) the alveolar ventilation rate (Q_{alv}) was allometrically scaled as $Q_{alv} = Q_{alv0} \cdot BW^{0.74}$ (l/hr, in the model the alveolar ventilation rate was expressed in l/min). For a body weight of 0.22 kg the allometric relationship calculates an alveolar ventilation rate of 4.89 l/hour.

[2]In concordance with Reitz *et al*. (1997) the cardiac output (Q_b) was allometrically scaled as $Q_b = Qb_0 \cdot BW^{0.7}$ (l/hr, in the model the cardiac output was expressed in l/min).
For a body weight of 0.22 kg the allometric relationship calculates a cardiac output of 5.2 l/hr.

[3]In concordance with Reitz *et al*. (1997) the relative blood flow to the brain was set at 0.0386 with the other relative blood flows adjusted accordingly to calculate the brain concentration of DCM.

[4]In concordance with Andersen *et al*. (1991) the maximal metabolic rate for MFO-dependent DCM metabolism was allometrically scaled as $V_{max} = V_{max0} \cdot BW^{0.7}$ (mg/hr, in the model metabolism is expressed in mmole/min).

[5]In concordance with Andersen *et al*. (1991) the diffusion capacity of CO in the lungs was allometrically scaled as $D_{CO} = D_{CO0} \cdot BW^{0.92}$ (l/hr/mm Hg, in the model diffusion was expressed in l/min/mm Hg).

[6]In concordance with Andersen *et al*. (1991) the endogenous CO production was allometrically scaled as $P_{enCO} = P_{enCO0} \cdot BW^{0.7}$ (mg/kg/hr, in the model endogenous CO production was expressed in mmole/min).

Appendix B-5

ALGORITHM DESCRIPTION FOR ESTIMATING TIME-CONCENTRATION RELATIONS BASED ON INTERNAL DOSE-METRICS

The internal dose metrics of interest for DCM are the DCM concentration in brain on the one hand and the COHb level on the other hand. The maximum COHb levels were preset at 4% and 15% for AEGL-2 and -3, respectively, based on the rationale for the AEGL-derivation for CO itself (no AEGL-1 values were derived for CO).

As to the DCM concentration in brain, the question is that given a specific exposure scenario resulting in a specific brain concentration, which DCM exposure level will lead to the same calculated maximum concentration in brain, given some exposure duration. In a more abstract sense, given an exposure duration of *T* minutes, the question is which ambient DCM concentration of *D* ppm will lead to a maximum DCM concentration in brain that equals the norm concentration of *N* mM in brain.

Let the DCM concentration in brain after *T* minutes of exposure to an ambient DCM concentration of D ppm be designated by the function $B(D;T)$, i.e., for each value of the exposure duration *T*, the concentration in brain is a function B of the exposure level. The mathematical problem is to solve for the value D_T of ambient DCM concentration, such that

$$B(D_T;T) = N \tag{26}$$

As we have no analytical expression for the function *B*, which is the result of a numerical solution of a set of differential equations describing DCM kinetics, an analytical expression cannot be found and equation (26) can only be solved by numerical techniques.

The following technique (known as chord Newton-Raphson iterations) is used:
Suppose that $B(D;T) \neq N$, then we want to find Δ such that $B(D+\Delta;T) = N$.
Next, suppose that $B(D+\Delta;T)$ is fairly approximated by its first order Taylor expansion

$$N = B(D+\Delta;T) \approx B(D;T) + \Delta \frac{d}{dD} B(D;T) \tag{27}$$

then

$$\Delta = \frac{N - B(D;T)}{\frac{d}{dD} B(D;T)} \tag{28}$$

As there in neither an analytical expression for $\frac{d}{dD} B(D;T)$, it is approximated by

$$\frac{d}{dD} B(D;T) \approx \frac{B(D;T) - B(D+d;T)}{d} \tag{29}$$

This leads to the following iteration scheme:

Choose some initial value D_0 for the solution D_T and a second, different value D_1.

Once the iterates D_n and D_{n-1} are known, calculate (see equation (29))

$$\frac{d}{dD}B(D_n;T) \approx \frac{B(D_n;T)-B(D_{n-1};T)}{D_n - D_{n-1}} \tag{30}$$

and from this (see equation (28)

$$\Delta_n = \frac{N - B(D_n;T)}{\frac{d}{dD}B(D_n;T)} \tag{31}$$

and a new iterate

$$D_{n+1} = D_n + \Delta_n \tag{32}$$

until

$$|\Delta_n| << D_n \quad \text{or} \quad \frac{N - B(D_n;T)}{N} << 1 \tag{33}$$

i.e., the relative contribution is small compared to the current iterate value or the relative function deviation is small compared to the required norm value.

The same procedure is *mutatis mutandis* applied for finding the ambient DCM concentration (ppm) leading to a 4% or 15% COHb/Hb ratio in blood additional to the background ratio. It should be noted that due to the saturation of COHb formation, maximum level might be reached a long time after the end of exposure (see Figure).

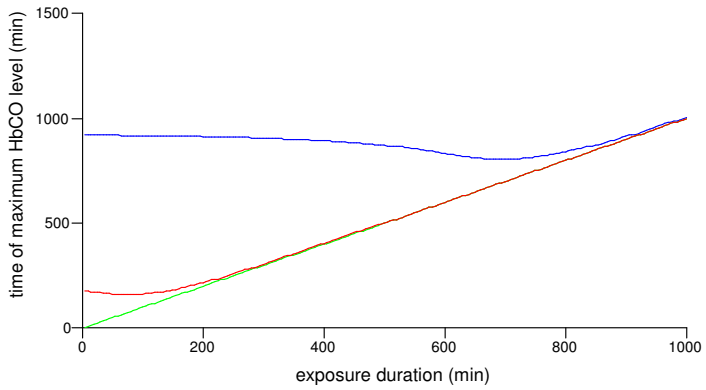
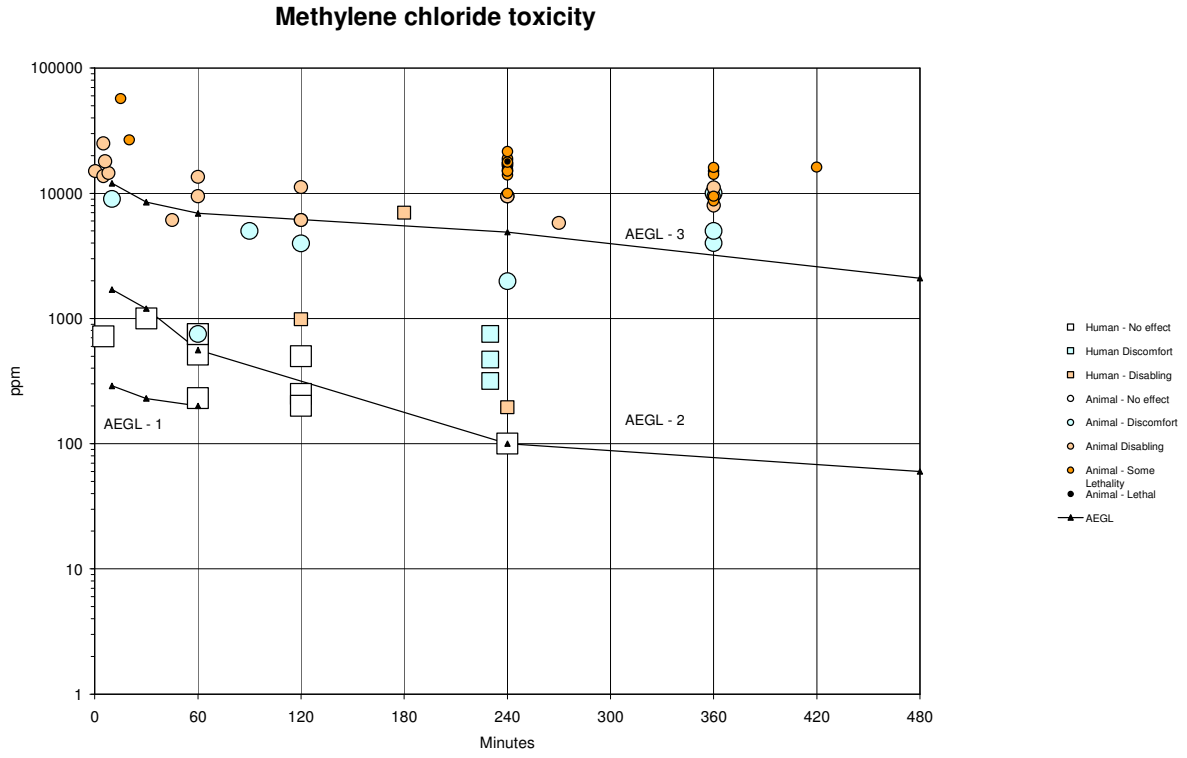


Figure. Time of maximum COHb level as a function of exposure duration (upper line 15% additional COHb, next upper line 4%).

The shorter the exposure duration, the higher the allowed DCM ambient air level, the more saturated COHb formation, the longer it takes for reaching maximum level. Eventually, COHb formation is no longer saturated and time of maximum COHb level approaches exposure duration (lower straight line).

APPENDIX C: Category plot for methylene chloride



APPENDIX D: Carcinogenicity Assessment

EPA classified DCM as a probable human carcinogen, based on the findings that DCM induced hepatocellular neoplasms and alveolar/bronchiolar neoplasms in $B6C3F_1$ mice, an increased incidence in male and female F344 rats, and salivary gland carcinomas in male rats and leukemia in female rats (IRIS, 2002). Other organizations concluded that DCM is a possible carcinogen for humans (IARC, 1999) and that the carcinogenic potency is expected to be low (WHO, 1996). It may be doubted whether the mouse is an appropriate model on which to base the carcinogenic risk to humans.

Considering the specific metabolic pathways for DCM and the specific biotransformation rates for the mouse, linear extrapolation to estimate the human carcinogenic risk will result in erroneous results. EPA calculated the inhalation Unit Risk on the data on female mice (combined adenomas and carcinomas) obtained from the NTP study (NTP, 1986) using a PBPK-model developed by Andersen (1987). Information on pharmacokinetics and metabolism of DCM were incorporated and the internal dose estimates, based on the metabolism by the GST-pathway, were calculated. (The thus calculated unit risk was approximately 9-fold lower than a previous applied dose estimate). A correction for interspecies differences in sensitivity was applied by using the surface correction factor.

The inhalation Unit Risk was $4.7 * 10^{-7}$ per $\mu g/m^3$, calculated by the linearized multistage procedure.

To convert to a level of methylene chloride that would cause a theoretical excess cancer risk of 10^{-4}:
Risk of 1×10^{-4}: $10^{-4} / 4.7 \times 10^{-7}$ $(\mu g/m^3)^{-1}$ = 212.7 $\mu g/m^3$ (round to 0.2 mg/m^3)

To convert a 70 year exposure to a 24 h exposure:
24-hour exposure = C * 25,600 days = 5120 mg/m^3

To account for uncertainty regarding the variability in the stage of the cancer process at which methylene chloride or its metabolites may act, a multistage factor of 6 is applied (NRC, 2001):
5120 mg/m^3 * 1/6 = 853.3 mg/m^3

Therefore, based upon the potential carcinogenicity of methylene chloride when continuous lifetime exposure takes place, an acceptable 24 h exposure would be 853.3 mg/m^3.

If the exposure is limited to a fraction (f) of a 24-hour period, the fractional exposure becomes 1/f x 24 h:
24-hour exposure = 853.3 mg/m^3
8-hour exposure = 2560 mg/m^3 (720 ppm)
4-hour exposure = 5120 mg/m^3 (1400 ppm)
1-hour exposure = 20,500 mg/m^3 (5700 ppm)
30-minute exposure = 41,000 mg/m^3 (11,500 ppm)
10-minute exposure = 123,000 mg/m^3 (34,000 ppm)

For 10^{-5} and 10^{-6} risk levels, the 10^{-4} values are reduced by 10-fold and 100-fold, respectively.

However, these values were calculated using the assumption that the PBPK-model used to derive the Inhalation Unit Risk is linear with increasing exposure to methylene chloride. Such is not the case. EPA (IRIS, 2002) remarked that the unit risk should not be used if the air concentration exceeds $2 * 10^4$ $\mu g/m^3$ (5.6 ppm), since the unit risk may differ from that stated above this concentration. Calculation of a slope factor from the unit risk is considered inappropriate when pharmacokinetic models are used. It was therefore, stated that the presented unit risk might not be applicable to acute, high exposures.

The carcinogenic potential is considered to be related to metabolites of the GST-pathway. At

low concentrations (below 300-500 ppm) the MFO-pathway is the predominant pathway of the biotransformation for DCM. The GST-pathway plays only a minor role at these concentrations and becomes of importance at DCM concentrations in air above the saturation level for the MFO-pathway, which is 300-500 ppm DCM. It is therefore, considered not possible to calculate the exposure concentrations for a single 8-h exposure corresponding to risk levels of 10^{-4}, $10^{-5,}$ and 10^{-6} through the procedure of linear extrapolation as proposed in the Standing Operating Procedures. The same PBPK-model as used to calculate the unit risk may be used to calculate the carcinogenic risk for the appropriate time periods.

APPENDIX E: Derivation Summary for methylene chloride AEGLs

ACUTE EXPOSURE GUIDELINE LEVELS FOR METHYLENE CHLORIDE (CAS Reg. No. 75-09-2) DERIVATION SUMMARY

AEGL-1 VALUES

10 minute	30 minute	1 hour	4 hour	8 hour
290 ppm (1000 mg/m^3)	230 ppm (800 mg/m^3)	200 ppm (710 mg/m^3)	NR	NR

Key Reference: Stewart *et al.* 1972

Test Species/Strain/Number: Groups of 1 to 8 humans

Exposure Route/Concentrations/Durations: Inhalation exposure to 213 (n=1), 515 ppm (n=8) for 1 hour, 514 for 1 hour followed by 1-h to 868 ppm (n=3), or to 986 ppm for 2 hours (n=3).

Effects:

213 ppm No effects
515 ppm No complaints.
514/868 ppm No complaints during exposure to 514 ppm; light-headedness and difficulties with enunciation at 868 ppm.
986 ppm Light-headedness and difficulties with enunciation

Endpoint/Concentration/Rationale: Absence of slight CNS-effects (light-headedness and difficulties with enunciation) at a 1-h exposure to 514 ppm. DCM concentration in brain was calculated with aid of a PBPK-model and used as dose-metric. Point of departure was a maximum DCM concentration in brain of 0.063 mM resulting from a 1-h exposure to 514 ppm.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: Not applicable
Intraspecies: 3

Modifying Factor: None

Animal to Human Dosemetric Adjustment: Not applicable

Time Scaling: PBPK-model was used. Time-scaling is based on maximum DCM concentration in human brain as dosemetric.

Data Adequacy: Sufficient.

NR: Not recommended since these values would be above the corresponding AEGL-2 values.

AEGL-2 VALUES				
10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
1700 ppm (6000 mg/m^3)	1200 ppm (4200 mg/m^3)	560 ppm (2000 mg/m^3)	100 ppm (350 mg/m^3)	60 ppm (210 mg/m^3)

Key Reference: Winneke (1974); TSD on Carbon monoxide.

Test Species/Strain/Number: Humans

Exposure Route/Concentrations/Durations:

a) No AEGL-2 effects in humans exposed to DCM concentrations to 317, 470, or 751 ppm for 4 hours.

b) Maximum COHb level of 4% in humans (TSD on Carbon monoxide).

Effects:

317 ppm (n=12): only sub AEGL -2 (neurobehavioral) effects observed
470 ppm (n=14): only sub AEGL -2 (neurobehavioral) effects observed
751 ppm (n=6): only sub AEGL -2 (neurobehavioral) effects observed

Endpoint/Concentration/Rationale:

a) Absence of AEGL-2 related CNS-effects at a 4-h exposure to 751 ppm. DCM concentration in brain was calculated with aid of a PBPK-model and used as dose-metric. Point of departure was a maximum DCM concentration in brain of 0.137 mM.

b) Maximum COHb level of 4% in humans (from TSD on Carbon monoxide).

Uncertainty Factors/Rationale:

Total uncertainty factor: 1 for CNS-effects; COHb formation based on TSD on Carbon monoxide.
Interspecies: Not applicable
Intraspecies: 1 for CNS-effects; COHb formation based on TSD on Carbon monoxide.

Modifying Factor: None

Animal to Human Dosemetric Adjustment: Not applicable

Time Scaling: PBPK-model was used. Time-scaling is based on maximum DCM concentration in human brain as dosemetric (10- and 30-min AEGL-2), or on COHb formation (1-, 4-, and 8-h exposure).

Data Adequacy: Sufficient

AEGL-3 VALUES				
10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
12,000 ppm (42,000 mg/m^3)	8500 ppm (30,000 mg/m^3)	6900 ppm (24,000 mg/m^3)	4900 ppm (17,000 mg/m^3)	2100 ppm (7400 mg/m^3)

Key Reference: Haskell Laboratory (1982); TSD on Carbon monoxide

Test Species/Strain/Number: Humans for COHb formation (see TSD on Carbon monoxide).

a) Lethality study in rats (deaths due to CNS-effects).

b) Maximum COHb level of 15% in humans (TSD on carbon monoxide).

Exposure Route/Concentrations/Durations:

a) Groups of 6 rats were exposed via inhalation to 9900, 11,000, 14,000, 14,000, 15,000 or 18,000 ppm for 4 hours.

b) Maximum COHb level of 15% in humans (TSD on carbon monoxide).

Effects:

9900 ppm: 0/6 deaths
11,000 ppm: 0/6 deaths
14,000 ppm: 2/6 deaths
14,000 ppm: 2/6 deaths
15,000 ppm: 3/6 deaths
18,000 ppm: 6/6 deaths

Endpoint/Concentration/Rationale:

a) No mortality due to CNS-effects was observed in rats during a 4-hour exposure to 10,000 ppm. DCM concentration in brain was calculated with aid of a PBPK-model and used as dose-metric. Point of departure was a maximum DCM concentration in brain of rats of 3.01 mM.

b) Maximum COHb level of 15% in humans (TSD on carbon monoxide).

Uncertainty Factors/Rationale:

Total uncertainty factor: 3 for mortality due to CNS-effects; COHb formation based on TSD on Carbon monoxide.

Interspecies: 1 for mortality in rats due to CNS-effects (species differences in susceptibility are very small and a human PBPK-model is used).

Intraspecies: 3 for mortality due to CNS-effects; COHb formation based on TSD on Carbon monoxide.

Modifying Factor: None

Animal to Human Dosemetric Adjustment: Via PBPK-modeling.

Time Scaling: PBPK-model was used. Time-scaling is based on maximum DCM concentration in human brain as dosemetric (10-, 30- and 60-min and 4-hour AEGL-3), COHb formation (8-h exposure).

Data Adequacy: Sufficient