Recommendation from the Scientific Committee on Occupational Exposure Limits for methylene chloride (dichloromethane)

Table of Contents

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Recommendation from the Scientific Committee on

Occupational Exposure Limits for

Methylene chloride (dichloromethane)

8 hour TWA:	100 ppm [353 mg/m^3]
STEL (15 min):	200 ppm [706 mg/m^3]
BLVs :	4 % COHb (see "*Recommendation*")
	0.3 mg methylene chloride / l urine
	1 mg methylene chloride / l blood
Notation:	'Skin'

SCOEL carcinogen group: C (*genotoxic carcinogen for which a practical threshold is supported and a health –based OEL is proposed*)

SUBSTANCE IDENTIFICATION

Methylene chloride

Synonyms	DCM, dichloromethane, methane dichloride, methylene bichloride, methylene dichloride, methylenum chloratum
EINECS No.	602-004-00-3
CAS No.	75-09-2
Molecular formula	CH_2Cl_2
Structural formula	

```
        Cl
        |
   H -  C  - H
        |
        Cl
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MWt	84.96 g/mol[1]
Conversion factor;	At 20°C and 101.3 kPa 1 ppm = 3.53 mg/m^3; 1 mg/m^3 = 0.28 ppm
EU Classification:	Carc Cat 3: R40 Limited evidence of a carcinogenic effect.

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This document is based on the International Program for Chemical Safety (IPCS) Environmental Health Criteria Monograph (1996) to which the reader is referred to for more detailed information, the references therein and studies published since the IPCS monograph, which were identified using the on-line database PubMed.

PHYSICO-CHEMICAL PROPERTIES

Methylene chloride is a clear, colourless, highly volatile, non-flammable liquid with penetrating ether like odour. The odour threshold is 740 mg/m^3. It has a boiling point of 40°C and a melting point of -95.1°C. It has a solubility in water of 13 g/L[1] at 20°C and a vapour pressure of 47.4 kPa at 20°C. Pure methylene chloride vapour is denser than air.

Pure, dry methylene chloride is relatively stable but in the presence of water and light it slowly decomposes to produce small quantities of hydrogen chloride.

Commercial grades of methylene chloride normally contain between 0.005 and 0.02% of a stabiliser (such as methanol, ethanol, amylene, cyclohexane, phenolic compounds or tertiary butyl amine) to prevent acidification and corrosion.

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1. Occurance/use

Methylene chloride is normally produced by the Stauffer process in which methanol is reacted with hydrogen chloride to form methyl chloride, which is then reacted with chlorine. Chloroform and carbon tetrachloride are by-products of the production process. About 254,000 tonnes were produced in Western Europe in 1991. In 1994, a total of 133,000 tonnes of methylene chloride was used in Western Europe. With the exception of 1988, which saw a slight increase in volumes sold, there had been a continuous reduction in the quantity of methylene chloride used since 1985, due largely to increased recycling by end users (Eurochlor, 1997). Since the early 1990s sales of methylene chloride have remained at a fairly stable level in Europe (Eurochlor http://www.eurochlor.org/consumption).

The main uses of methylene chloride are as a paint and varnish remover, as process solvent, in aerosols, in the electronics industry (as a photoresist stripper in the production of printed circuit boards) and in metal cleaning (vapour degreasing). It is the most commonly used solvent in paint stripping, where it is a quick-acting solvent for both wash-off and scrape-off formulations. In food and pharmaceutical processes, methylene chloride is used as an extraction medium as it offers high solvency and purity, and it can be distilled off relatively easily from the final product. In the mid-1990s, the pharmaceutical industry accounted for about 40% of the methylene chloride used in Europe. Methylene chloride is also used as a process solvent for the production of polycarbonate, cellulose esters, triacetate and triacetate esters, in adhesive formulations, plastic processing and refrigerants, and as a secondary blowing agent in polyurethane foam.

METHODS OF EXPOSURE MONITORING AND ANALYSIS

The UK Health and Safety Executive (1995 and 2000) have published general methods for the sampling of volatile organic compounds in workplace air and their analysis by gas chromatography that are suitable for methylene chloride (Methods for Determination of Hazardous Substances 80 and 96) and a more specific method for chlorinated hydrocarbons (MDHS 28).

The US Occupational Safety and Health Administration (OSHA) have published two fully validated methods (OSHA organic method 59, 1986; method 80, 1990) for the measurement of methylene chloride in air in workplace air. Method 59 involves collection on charcoal tubes and analysis by gas chromatography (GC/FID) where the limit of reliable quantification is 102 µg/m^3 (29 ppb). Method 80 involves collection on Carbosieve S-III and analysis by GC/FID with a limit of quantification of 0.70 mg/m^3 (0.196 ppm).

The US National Institute for Occupational Health and Safety (NIOSH) Analytical Method 1005, issue 3 (NIOSH, 1998) also involves collection on charcoal tubes and analysis by GC/FID. The limit of detection is stated as 0.4µg and the method has been validated for sample masses of 1.4-2600   g corresponding to concentrations of 1.4 - 2600 mgm^{-3} (0.4 - 749 ppm) in a one litre air sample.

INRS has also published analytical methods for the determination of methylene chloride in workplace air. One is Metropol Fiche 039 (updated in 2001), which involves collection

on sobent tube containing Carboxen 564, desorption with CS_2 and analysis by GC/FID (Fiche Metropol C). These methods are available at http://www.inrs.fr.

Biological monitoring methods for urinary methylene chloride by gas chromatography have been described by Sakai *et al* (2002), Seno *et al* (1999) and Ukai *et al* (1998). Urinary concentrations of methylene chloride are reported to be well correlated with exposure concentrations in air. Seno *et al* (1999) also described a method for the determination of methylene chloride in blood (see also DFG 1991). It is also possible to measure methylene chloride in exhaled breath (Hotz et al., 1987). Another possibility is determination of the carboxyhaemoglobin (COHb) levels in blood. Advantages and disadvantages of the different procedures are discussed in the following chapter.

2 Health effects

2.1 Toxicokinetics

Experiments in human volunteers have established that between 70 and 75% of inhaled methylene chloride vapour is absorbed following exposure to concentrations of 50-200 ppm (176-706 mgm^{-3}). Experiments in humans and animals have established that methylene chloride is absorbed through the skin and animal data show that it is also absorbed from the digestive tract. Methylene chloride is distributed to all tissues and crosses the blood brain barrier and the placenta. Neither methylene chloride nor its metabolites accumulate in tissue. Methylene chloride is excreted in breast milk (IPCS, 1996).

Three skin penetration studies were found where pure methylene chloride was applied to human (Ursin et al 1995) or rat (Tsuruta 1997) skin in vitro or to mouse skin in vivo (Tsuruta 1975). The three studies reported similar results with transdermal fluxes ranging from 2.7 to 6.6 $mg/cm^2/h$. ECETOC (1993) has suggested that a skin notation should be assigned when the amount of chemical absorbed upon exposure of both hands and lower arms (2000 cm^2) for one hour is expected to contribute more than 10% to the systemic dose, compared to the amount absorbed via inhalation exposure at the OEL during a full work day (assuming that 10 m^3 air is inhaled during an 8-h workday and that 50% is absorbed). Applying this calculation to methylene chloride, the systemic dose from such skin exposure could be 3- to 7-fold greater than that from inhalation exposure at an OEL of 100 ppm.

The main route of metabolism is by cytochrome P450 mediated oxidation to form carbon monoxide and carbon dioxide that are largely exhaled. Some of the carbon monoxide binds to haemoglobin in blood. In human volunteers exposed to 150 ppm (530 mg/m^3) for 7.5 hours each day for five days, peak levels of carboxyhaemoglobin (COHb) were 5.3%. Exposure to 100 ppm produced peak COHb levels of 3.4%. COHb levels returned to normal within 24 hours of exposure to 200 ppm for 7.5 hours. In non-smoking workers, levels of COHb in blood following methylene chloride exposure are dose and time dependent for same day exposure but are independent of exposures on previous days (IPCS, 1996; Amsel *et al*, 2001; Soden *et al*, 1996). COHb levels in exposed workers who smoke are higher than in non-smokers and are not dose-dependent (Soden *et al*, 1996). Exposure to 496 ppm [1770 mg/m^3] methylene chloride for less than 1 hour is associated with COHb levels of 1 to 4%. Measured levels of COHb following exposure to 980 ppm [3500 mg/m^3] for two hours were 10%. COHb levels of more than 8% were found following exposure to 250 ppm [880 mg/m^3] for 7.5 hours. Workers exposed to average concentrations of methylene chloride of 32 ppm [114 mg/m^3] (8-hour TWA) had COHb

levels between 0.8 and 2.5%. At higher levels of exposure (> 250 ppm) the oxidative metabolic pathway becomes gradually saturated, the proportionate increase in COHb becomes smaller, and an increasing proportion of the received dose is exhaled unchanged.

Figure 1 shows monitoring data of COHb in human volunteers exposed to methylene chloride, as reported by Di Vincenzo and Kaplan (1981). This set of experimental data can be opposed to data in exposed workers by Soden et al. (1996; *Figure 2*). In this data set of group means the baseline COHb level of non-exposed persons (>1.5% COHb) appears relatively high, which is likely caused by an influence of smokers in this group. Considering this, the two data sets appear basically consistent with each other.
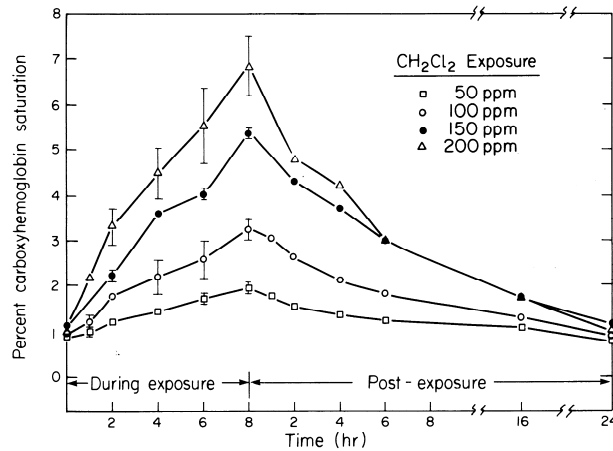

Figure 1: Time-course of the saturation of haemoglobin with carbon monoxide (% COHb) in volunteers exposed to different airborne levels of methylene chloride under resting conditions, according to Di Vincenzo and Kaplan (1981); means and SEM of 4-5 persons. Without exposure, a background COHb level of 1% was reported.
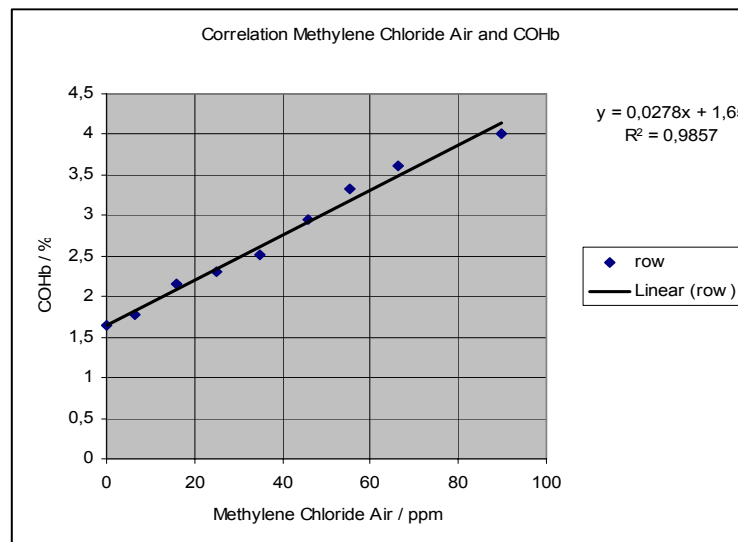

Figure 2: Correlation between airborne methylene chloride concentrations and COHb in workers (reported subgroup means); data of Soden et al. (1996)

A second (minor) metabolic pathway becomes more active at high levels of exposure, especially in mice, in which glutathione-S-transferase (GST) is involved in oxidation of

methylene chloride to formaldehyde and formate, which is further metabolised to form carbon dioxide. *In vitro* experiments with human cells have shown that the formation of formaldehyde from methylene chloride is influenced by the polymorphism of glutathione-S-transferase theta (*GSTT1*), and that this may influence the susceptibility of individuals to adverse effects from methylene chloride exposure (Hallier *et al*, 1994). IPCS (1996) considered this metabolic pathway to be of minor importance in humans, as concentrations of the responsible glutathione transferase in human liver tissue were only a small fraction of those in the mouse (<10%). The metabolic routes of methylene chloride are relevant for the discussions of the mode of action of tumour induction across species. For details of this discussion, see chapter 5.7 (considerations of the mode of action).

Relevant metabolic steps of the two avenues of biological activation, to carbon monoxide and to formaldehyde, are shown in Figure 3.
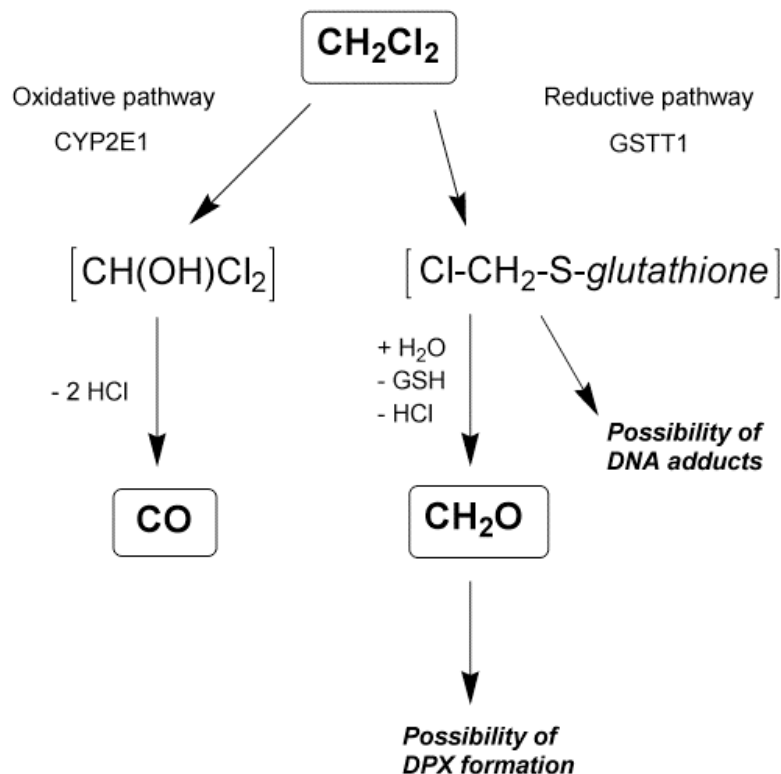

Figure 3: Biological activation of methylene chloride to toxic metabolites. The major oxidative (saturable) pathway leads to carbon monoxide. A minor reductive (glutathione-dependent) pathway leads to potentially reactive metabolites, such as formaldehyde.

Biological monitoring of methylene chloride and metabolites

The parent compound, methylene chloride, and its toxic metabolite carbon monoxide can be determined in biological media for biological monitoring. Different recommendations have been given, and there are advantages and disadvantages of the different procedures, which are compiled in *Table 1*.

Correlations between airborne methylene chloride exposures in workers and resulting methylene chloride concentrations in both blood (Lauwerys / Hoet, see also DFG 2005)

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and urine (Ghittori et al. 1993, Ukai et al. 1998, Sakai et al. 2002) have been established. Taking the available data together, the functions given in *Figure 4* are resulting.

Table 1: Methylene chloride – existing recommendations of biological guidance values and comments (all samples collected at end of exposure). Included are the corresponding OEL proposals

Guidance value recommended by	Analyte, matrix	Value	Advantages	Disadvantages
DFG (2005) (D)	DCM in Blood	0.5mg/l (OEL 50 ppm)	No interference from smoking	Invasive, sample collection time and method critical
Lauwerys / Hoet (B)	DCM in Blood	0.5mg/l (OEL 50 ppm)	No interference from smoking	Invasive, sample collection time and method critical
Lauwerys / Hoet (B)	DCM in breath	15 ppm (OEL 50 ppm)	Non-invasive. No interference from smoking	Sample collection time and method critical , semi-quantitative
ACGIH (USA)	DCM in urine	0.3mg/l (TLV 50 ppm)	Non-invasive. No interference from smoking	Sample collection time and method critical , semi-quantitative
[SCOEL discussions]	COHb	Blood	Endpoint of toxicity	Invasive, interference from smoking
HSE (UK)	CO	Breath (OEL 100ppm)	Non-invasive	Interference from smoking

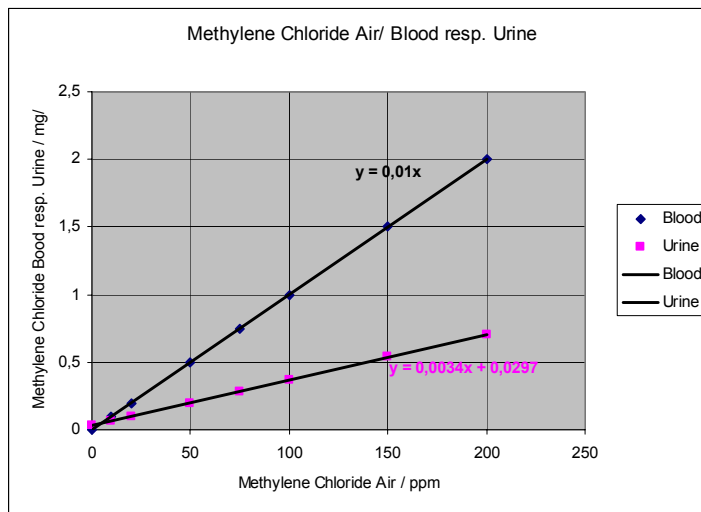

Figure 4: Correlation between airborne methylene chloride exposure and methylene chloride concentrations in blood and urine at end of shift (see text)

Based on these figures, an OEL of 100ppm (as proposed in this document) is equivalent to a urinary excretion of 1 mg methylene chloride per liter urine (end of shift), and to a steady-state blood level (end of the shift) of 0.3 mg per liter.

2.2. Acute toxicity

2.2.1. Human data

A number of industrial accidents involving methylene chloride have been reported (IPCS, 1996). High levels of exposure methylene chloride have led to unconsciousness and, in some cases, death. Other effects include irritation of the eyes and respiratory tract, lung oedema and acute effects on the heart, liver and kidneys. The formation of COHb as a result of methylene chloride metabolism decreases the oxygen-carrying capacity of the blood, giving symptoms of oxygen deprivation such as light-headedness, headache or loss of consciousness. Mahmud and Kales (1999) described a case in a cabinet worker who experienced intermittent headaches, consistent with both methylene chloride intoxication and carbon monoxide poisoning. In another fatal case of methylene chloride poisoning, however, COHb levels were within the normal range, suggesting that the fatal narcosis and respiratory depression were due to the direct effect of methylene chloride on the central nervous system (Zarrabeitia *et al*, 2001).

Experiments in human volunteers have established that the main effect of a single high exposure to methylene chloride is neurotoxicity (IPCS, 1996). Neurobehavioural changes (disturbed vigilance and impaired combined tracking monitoring performance) were observed following exposure to 250 ppm [694 mg/m^3] for 1.5 to 3 hours. In other experiments no effects on visual performance were observed following a short exposure to 700 ppm [2500 mg/m^3] (duration unknown) but effects on visual function were observed after 95 minutes exposure to 290 ppm [1040 mg/m^3]. Light headedness and effects on visual function were observed after 1 hour of exposure to 672 ppm [2400 mg/m^3]. A dose-related increase in serum concentrations of bilirubin was observed in volunteers exposed to 460 ppm [1650 mg/m^3] together with elevated concentrations of COHb.

Chang *et al* (1999) described six cases involving oral ingestion of methylene chloride. Central nervous system depression, tachypnoea, and corrosive gastrointestinal injury were the most common effects. An elevated COHb level was documented in only two patients (35% and 8.4% COHb, respectively). Renal failure, hepatic failure, and acute pancreatitis occurred in the two most severe cases of poisoning.

2.2.2. Animal data

IPCS (1996) reviewed the acute toxicity of methylene chloride and concluded that it has a low acute toxicity following inhalation or oral administration. In short term inhalation experiments, 6 hour LC_{50} values of 11250 ppm [40200 mg/m^3] to 15650 ppm [55900 mg/m^3] have been reported in rats, mice and guinea pigs. Oral LD_{50} values of 1410-3000 mg/kg bodyweight have been reported. The main effects of exposure are associated with the central nervous system (CNS) and liver with CNS disturbances being reported at 3900 ppm [14000 mg/m^3] or more in mice and slight changes in electroencephalographic (EEG) activity in rats at 496 [1770 mg/m^3]. Slight histological changes of the liver have been reported in mice at concentrations of 15800 ppm [56500 mg/m^3] or more. Exposure to 2000 ppm [7100 mg/m^3] adversely affected Clara cells in the lungs of mice, but not of rats. There is inconsistent evidence for cardiovascular effects at concentrations of 9800 ppm [35000 mg/m^3] in monkeys, dogs and rabbits. Some studies have reported effects on the kidneys with slight histological changes being observed in mice exposed to 15800 ppm [56500 mg/m^3] for 7 hours.

Social Europe

2.3. Irritation

There are no reports linking methylene chloride to respiratory irritation in humans or animals. The IPCS (1996) cite a few case reports in which methylene chloride was caused skin and eye irritation in humans. In animals, two investigations have demonstrated that methylene chloride can cause skin irritation in rabbits including severe erythema and oedema with necrosis and acanthosis (IPCS, 1996). The IPCS (1996) also cite two investigations of the irritancy of methylene chloride to the rabbit eye. Effects were reversible and included moderate to severe changes in the conjunctiva combined with increased corneal thickness and intra-ocular tension.

2.4. Sensitisation

No data are available. The absence of any case reports suggests that methylene chloride is unlikely to cause respiratory or dermal sensitisation in humans.

2.5. Repeated dose toxicity

Human data

Studies of possible morbidity associated with workplace exposure to methylene chloride have all been small scale and not all included control groups (*Table* 2). The data suggest that no adverse effects on health are associated with workplace exposure up to concentrations of about 100 ppm [350 mg/m^3] over several years. Irritation of the eyes and respiratory tract, subtle neurotoxic effects and minor effects on blood have been observed at higher levels of exposure.

With regard to the study of White et al. (1995) it should be mentioned that the persons examined in the printing industry were exposed to a mixture of solvents, including toluene, methyl ethyl ketone, mineral spirits and "beta-ether", in addition to methylene chloride.

Social Europe

Table 2: Studies of morbidity associated with methylene chloride reviewed by IPCS (1996) and more recent studies

Exposed population	Findings	Study
Workers exposed to 114 mg/m^3 (TWA)	No effects on clinical chemistry, haematology or electrocardiograms	Di Vincenzo & Kaplan (1981)
29 men exposed for several years to concentrations "below 100 ppm" [347 mg/m^3]	When compared with age matched controls, no effects on subjective symptoms, neurobehavioural tests, motor nerve conduction velocity, electrocardiograms and clinical examinations	Cherry *et al* (1981)
33 workers exposed to concentrations between 100 and 17000 mg/m^3 over two years [23-4800 ppm]	Irritation of the eyes and respiratory tract and neurasthenic disorders reported in half of the exposed population, digestive disorders reported by one third. No comparison with a control population	Kuzelova & Vlasak (1966)
25 methylene chloride exposed retired airline maintenance workers compared against unexposed retirees (exposures not specified)	Men employed in paint stripping. No significant difference was found between exposed and unexposed workers, although subtle differences in attention and memory were detected.	Becker & Lash (1990), Lash *et al* (1991)
46 subjects exposed to concentrations of 6-24 mg/m^3 for several years (exposures not specified)	Non significant excess of digestive disorders and hypertonia in comparison with controls. Symptoms of gallbladder pathology and swollen liver reported, but no details of drinking or smoking habits provided	Kashin *et al* (1980)
Workers who had worked for at least 10 years exposed to average concentrations of 1677 mg/m^3 [475 ppm]	In comparison with an unexposed group, there were no differences in selected symptoms including irregular heartbeat, dizziness or loss of memory or in SGPT (Serum Glutamic Pyruvic Transaminase), Bilirubin or haemocrit. Only noticeable difference was lower levels of SGOT (Serum Glutamic Oxaloacetic Transaminase) in the exposed group.	Soden (1993)
2-year prospective study of 30 workers in a screen printing business exposed to a mixture of solvents including methylene chloride; exposures typically to about 75-100 ppm for 20 minutes, 6 times/day	Persons with higher acute exposure demonstrated significantly impaired test performance on tasks involving manual dexterity, visual memory, and mood. Those with higher chronic exposure demonstrated significantly poorer performance on visual memory tasks and mood. No obvious clinical disease.	White *et al* (1995)

Social Europe

The outcome of mortality studies provide no evidence to suggest that workplace exposure to methylene chloride at concentrations of up to 1677 mg/m^3 (475 ppm) has an important effect on mortality (*Table 3*). There are no indications that raised COHb levels arising from industrial exposure to methylene chloride are associated with an increased risk of ischaemic heart disease. In the Kodak, Gibbs and Tomenson studies, a ban on workplace smoking may have introduced a negative bias to the study design. The cancer studies are discussed further in section 5.7.

Social Europe

Table 3: Mortality studies reviewed by IPCS (1996), IARC (1999) and more recent studies

Exposed population	Description of study	Findings	Study
Men exposed to methylene chloride at a Kodak photographic film production facility in New York State. Mean (8 hour TWA) exposure concentrations for different groups ranged from 35.3- 402 mg/m^3 (10-114 ppm) with an overall mean of 92 mg/m^3 (26 ppm).	Retrospective cohort mortality – 344 deaths between 1956-76 Proportionate mortality – 754 workers employed during 1964 and followed up to 1976	No increase in risk from ischaemic heart disease, liver or kidney cancer or cancers at other sites	Friedlander *et al* (1978)
	All workers exposed for at least one year between 1964 and 1970 (1013) followed up to 1988	No increase in risk of death from ischaemic heart disease, liver or kidney cancer, possible excess of pancreatic cancer mortality	Hearne & Friedlander (1981), Hearne *et al* (1987, 1990)
Update of Kodak study 1) 1,311 men initially employed between 1946 (when the solvent was first used) and 1970. Average exposure of 138 mg/m^3 (39 ppm; 8-hour time-weighted average) for 17 years 2) 1913 men employed between 1964 to 1970 and followed up to1994. Average exposure of 92 mgm^{-3} (26 ppm) for 24 years;	Median length of follow-up from first exposure was 34 years. Median time from first exposure - 35 years.	Mortality in both cohorts was lower than expected for all causes of death, ischaemic heart disease, and cancer. No statistically significant increases were observed for any cause of death.	Hearne and Pifer (1999)
Workers producing triacetate fibre in a South Carolina plant. Exposure concentrations estimated to range from 494-1677 mgm^{-3} (140-475 ppm) Workers also exposed to methanol and acetone	1271 male and female workers employed for at least 3 months between 1954 and 1977.	No excess of deaths from cardiovascular causes. Significant excess of cancers of the biliary passages and liver. Three of the four liver and biliary cancer deaths in workers with 10+ years of employment and 20+ years since first employment	Ott *et al* (1983), Lanes *et al* (1993)
Workers in a cellulose acetate fibre plant in Maryland. Cohort divided into 3 groups: high exposure to	Retrospective cohort mortality study of 3211 workers employed in or after 1970 and followed	No excess mortality from cancers of the biliary tract, liver, pancreas or lung. Increased mortality from prostate	Gibbs (1992), Gibbs *et al* (1996)

Social Europe

methylene chloride (>1300 mgm^{-3}; 350 ppm), low exposure (176-353 mgm^{-3}; 50-100 ppm) and no exposure.	until 1989.	cancer in men with 20+ years employment and from cervical cancer in women with 20+ years of employment. Possible increased risk of death from cardiovascular causes observed in earlier study not confirmed in 1996 study.	
1785 employees of a factory that produced cellulose triacetate film base Subcohort of 1473 subjects with methylene chloride exposure. The mean duration of exposure was nine years at 67 mgm^{-3} (19 ppm) eight hour time weighted average).	Retrospective cohort study - All male employees with a record of employment at the film factory in 1946-88; 334 deaths identified up to 31 December 1994.	In methylene chloride exposed subcohort, substantially reduced mortality for all causes, all cancers, and cancers of liver, biliary tract, lung and pancreas. Ischaemic heart disease mortality slightly increased compared with local rates but lower in active employees where a direct effect of methylene chloride exposure would be expected. No in service mortality due to ischaemic heart disease in workers with the highest cumulative exposure (2824 mgm^{-3}.years; 800+ ppm-years).	Tomenson *et al* (1997)
Workers in a chlorinated methanes production plant – exposure to a mixture of chlorinated hydrocarbons, no specific information about exposure to methylene chloride	226 men employed for at least one year between 1940 and 1969 and followed for mortality until 1979	No excess of respiratory cancer or circulatory disease. Three cases of pancreatic cancer were observed against 0.9 expected, no information presented about liver cancers	Ott *et al* (1985)

Social Europe

Civilian workers employed at a military aircraft maintenance facility for at least one year between 1952 and 1956; workers exposed to a mixture of solvents; exposures best characterised for trichloroethylene (methylene chloride exposures not specified)	Retrospective cohort study of 14457 workers of whom 1222 were exposed to methylene chloride	No overall excess of cancer deaths or deaths more generally; Non significant excess of non-Hodgkin lymphoma and multiple myeloma in men, increased breast cancer risk in women; no information about other cancers	Blair *et al* (1998)
Lamp manufacturing workers involved in coiling and wire drawing with exposures to methylene chloride (not quantitatively specified), other chlorinated solvents, strong acids and metals including arsenic and chromium	203 women and 46 men employed for 6+ months between 1960 and 1975	Non significant excess of all cancers and of breast cancers in women; significant excess of breast cancers in women with greater than 5 or greater than 15 years employment	Shannon *et al* (1988)

Social Europe

Animal data

The IPCS (1996) reviewed a number of inhalation studies and these are summarised in *Tables 4 and 5*. The results of these studies indicate that repeated or long-term exposure of animals to high concentrations of methylene chloride is associated with reversible CNS effects, slight eye irritation, liver effects and mortality. The lowest exposure concentration reported to cause liver effects was 25 ppm [88 mgm^{-3}] (continuous exposure). No effects were found on the liver following exposure to 250 ppm [880 mgm^{-3}] for 5 hours/day. The lowest exposure concentration associated with reversible CNS effects as 5000 ppm [17700 mgm^{-3}] (6 hours/day) with no effects at 1990 ppm [7,100 mgm^{-3}]. Effects on brain chemistry were observed following exposure to 250 mgm^{-3} (6 hours/day). In a more recent investigation, no effects on immune function or thymus weight were found following exposure of rats to 5000 ppm [17700 mgm^{-3}] of methylene chloride for 6 hours/day, 5 days/week for 28 days, but a significant reduction in relative, but not absolute, spleen weight was observed in female rats (Warbrick *et al*, 2003).

IPCS (1996) also reviewed effects following oral administration. A study in rats established a no effects level for the liver of 125 mg/litre in drinking water. No conversion to mg/kg bodyweight per day is provided, but assuming a rat body weight of 0.35 kg and an intake of 0.049 litres water/day, this is approximately equivalent to 17.5 mg/kg/day. Another drinking water study found small effects on weight gain and indications of liver damage at intakes equivalent to 607 to 1469 mg/kg/day. In another drinking water study, mice exposed to between 226 and 1911 mg/kg/day showed subtle centrilobular fatty changes in the liver.

There is limited evidence that exposure to methylene chloride may potentiate the toxicity of other chlorinated hydrocarbons. Kim (1997) reported that methylene chloride significantly potentiated the hepatotoxicity of carbon tetrachloride in rats.

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Table 4: Effects in rats in medium to long term repeated dose studies [conversion factor: 1 mgm^{-3} = 0.28 ppm]

Exposure regime	Concentration mgm^{-3}	Observed effects	Study
2 hours/day for 15 days	3500	Decreased body weight, effects on liver lipids	Ito *et al* (1990)
2 hours/day for 20 days	3500	Effects on the liver: hypertrophic hepatocytes, increased lipid peroxidation and glutathione peroxidase activity	Takashita *et al* (1991)
6 hours/day for 3 days	250, 1100, 3500	Effects on dopamine and noradrenaline turnover in the brain	Fuxe *et al* (1984)
6 hours/day for 19 days	5740, 11500, 22900, 45900, 56500	Intermittent scratching, ataxia and hyperactivity in rats exposed to 22900 mgm^{-3} or more, dyspnoea, anaesthesia and some death in animals exposed to 45900 mgm^{-3} or more	NTP (1986)
6 hours/day for 10 days	7100, 14100	No or limited lung or liver lesions	Hext *et al* (1986)
5 hours/day for 28 days	880	No effects on the liver	Norpoth *et al* (1974)
5 hours/day for 5 days/week for 4 weeks	12800	Inflammatory response and cell damage in the lungs	Sahn and Lowther (1981)
6 hours/day, 5 days/week for 19 exposures	0, 8800, 17700, 28200	Anaesthesia, increased liver weights and reduced body weights in the two highest dose groups	Nitschke *et al* (1981)
6 hours/day, 5 days/week, 13 weeks	177, 710, 7100	No evidence of irreversible neurological damage	Mattsson *et al* (1990)
7 hours/day for 6 months	35000	CNS depression during period of exposure	Heppel *et al* (1944)
6 hours/day for 90 days	35000	Slight redness of conjunctiva lasting for 1 to 10 hours after each exposure, no other signs of toxicity	Leuschner *et al* (1984)
6 hours/day, 5 days/week for 13 weeks	1850, 3700, 7400, 14800, 29700	One rat exposed to 29700 died before the end of the study; foreign body pneumonia was observed in some rats exposed to 7400 mgm^{-3} or more; mean body weights reduced in high dose group, reduced liver lipid to liver weight ratios in high dose males and females and in females at 14800 mgm^{-3}	NTP (1986)
Continuous exposure for 100 days	88350	Slight cytoplasmatic vacuolisation of the liver and tubular degeneration in the kidney	Haun *et al* (1972)
6 hours/day, 5 days/ week for 2 years	0, 3500, 7100, 14100	No effects on body weight gain	NTP (1986)

Table 5: Effects in other species in repeated dose studies [conversion factor: 1 mgm^{-3} = 0.28 ppm]

Species	Exposure regime	Concentration mgm^{-3}	Observed effects	Study
Mice	6 hours/day, 3 weeks	14100	Increased liver weight and increased mitotic activity in hepacytes, a few animals showed necrosis of some epithelial cells of the bronchi and bronchioles together with reactive hyperplasia of adjacent lymphoid tissue	Eisenbrandt and Reitz (1986)
Mice, hamsters	6 hours/day, 5 days/week - 18 (mice) or 19 (hamster) exposures	0, 8800, 17700, 28200	Anaesthesia and reduced body weights in highest dose group, effects on aspartate aminotransferase in female mice at 17,700 mgm^{-3}	Nitschke *et al* (1981)
Mice	Continuous exposure for 7 days	17700	Reduced body weight, increased relative liver weights, liver damage	Weinstein *et al* (1972)
Mice	Continuous exposure, 30 days Intermittent exposure for 1-12 hours/day, 30 days Continuous exposure for 4, 8, 15, 90 days	130-1059 2118-25416 1059	Liver weight increased in dose-related manner, fatty accumulation and increased blood levels of butyryl cholinesterase found at 265 mgm^{-3}, all effects reversible	Kjellstrand *et al*, 1986
Mice	6 hours/day, 5 days/week for 13 weeks	1850, 3700, 7400, 14800, 29700	Some deaths and reduced body weights at 297800 mgm^{-3}, hepatic centrilobular hydropic degeneration in males and females at 29700 mgm^{-3} and in females only at 14800 mgm^{-3}.	NTP (1986)
Mice	Continuous exposure for 100 days	88 350	Slight cytoplasmatic vacuolisation at both levels, decrease in microsomal P-450 content at the higher dose	Haun *et al* (1972)
Mice	Continuous exposure for 10 weeks	350	Fatty infiltration, vacuolisation and enlarged hepatocyte nuclei, reversible increase in plasma triglycerides	Weinstein and Diamond (1972)

Social Europe

Dogs, monkeys, rabbits, guinea pigs	7 hours/day, 5 days/week for 6 months	1,7700	Fatty degeneration of the liver and pneumonia in 3/14 guinea pigs	Heppel *et al* (1944)
		35,000	Reduced activity in all species, fatty degeneration of the liver in dogs	
Dogs	Continuous exposure for up to 100 days	3,500	Abnormal haematology and increased activity of serum enzymes after 4 weeks	Haun *et al* (1972)
		17,350	Oedema of the brain	
Dogs	6 hours/day for 90 days	17,700	Slight sedation and erythema lasting for up to 10 hours after exposure	Leuschner *et al* (1984)
Gerbils	Continuous exposure for 3 months	340	Decreased levels of neurotransmitter amino acids in the brain	Briving *et al* (1986)
Gerbils	Continuous inhalation followed by 4 months recovery	1240	Increased brain concentrations of two astroglial proteins and decreased levels of DNA in the hippocampus and cerebellum	Rosengren *et al* (1986)
		740	Decreased hippocampal DNA levels, possibly as a result of the loss of nerve cells	
Monkeys	Continuous exposure for 28 days	88.25	Raised COHb levels	Haun *et al*, 1972)

Social Europe

2.6. Mutagenicity

The mutagenicity of methylene chloride has been reviewed by IARC (1999) and also IPCS (1996). Methylene chloride is consistently mutagenic in microorganisms. Weaker and less consistent responses are seen in mammalian systems. Methylene chloride induced sister chromatid exchanges, chromosome breakage and chromosome loss *in vitro* in human cells. *In-vitro* results in rodent cells were inconclusive or negative. Methylene chloride induced DNA single-strand breaks in mammalian cell cultures, but inconclusive or negative effects were reported for induction of gene mutations. It did not induce unscheduled DNA synthesis either *in vivo* in rodents or in human fibroblast cultures. It was genotoxic in fungi but not in *Drosophila* in the sex-linked recessive lethal assay.

2.7. Carcinogenicity

Human data

No consistent relationships between methylene chloride and cancer at any particular site have been reported in studies of workers exposed to methylene chloride (*Table 3*). Two studies observed an excess of pancreatic cancer and OjaJärvi *et al* (2001) found a small excess risk of pancreatic cancer, which was not statistically significant (RR = 1.42-0.80; 2.53). The authors could not find a relation to the level of exposure. Other individual studies reported an excess of liver and biliary tract cancers among longer-term employees, an excess of prostate cancer in men and excess of cervical cancer in women that appeared to increase with level of exposure and a possible exposure-related increase in breast cancer risk. In most studies, workers were exposed to a number of other chlorinated solvents and some were also exposed to other potential carcinogens including metals. This limited the power of published studies to detect an effect that is specific to methylene chloride. In case control studies (*Table 6*) possible associations have been reported between exposure to methylene chloride and cancers of the bladder, breast (in women), rectum and possibly the lung. Dell et al. (1999) concluded that there was no strong or consistent finding for any site of cancer, despite several studies of large occupational cohorts of workers potentially exposed to high concentrations of methylene chloride.

IARC (1999) concluded that there was not a sufficiently consistent elevation of risk across studies of any cancer type to make a causal interpretation credible. The results of more recent studies do not support a revision of this conclusion.

Animal data

A number of long term inhalation studies have been performed (Table). The NTP (1986) reported clear evidence of carcinogenicity in male and female B6C3F1 mice, as shown by increased incidences of alveolar/bronchiolar neoplasms and of hepatocellular neoplasms. Although, a high incidence of leukaemia in both exposed and unexposed rats in the NTP study is potentially a cause for concern, the outcome of the NTP studies are broadly consistent with other studies (Table 6). Maronpot *et al* (1995) found evidence that early life exposure to methylene chloride gave rise to an increased lung cancer risk in mice even in the absence of continuing exposure whereas the development of liver cancer was more closely associated with continuing exposure.

Social Europe

Relatively few investigations have been made of the carcinogenicity of methylene chloride following oral administration. Studies in rats and mice reviewed by IPCS (1996) did not find an excess risk of cancer at doses of up to 250 mg/kg bodyweight/day for two years or 500 mg/kg/day for 64 weeks.

IARC (1999) has concluded that there was *inadequate evidence* in humans for the carcinogenicity of methylene chloride, but *sufficient evidence* in experimental animals. The overall evaluation was that methylene chloride is *possibly carcinogenic to humans (Group 2B)*.

Considerations of the mode of action

Long-term animal experiments have demonstrated that methylene chloride is associated with the development of cancers in mice whereas similar effects were not observed in rats and hamsters. Based on the formation of reactive metabolites, such as formaldehyde, through the reductive pathway of metabolism (see *Figure 3*) the paradigm has bee advanced that this pathway may be connected with carcinogenesis, and that species differences between the balance of the oxidative and reductive pathway should explain species differences in carcinogenicity (Green et al. 1988; Green 1997). Mechanistic studies have demonstrated that metabolism of methylene chloride by the glutathione-S-transferase-dependent reductive pathway is highest in the mouse. This pathway is mediated by the isoenzyme GSTT1-1 and produces reactive intermediates that are held responsible for the liver and lung tumour formation (Thier et al. 1993, 1996, 1998, Wheeler et al. 2001, Marsch et al. 2004). Upon incubation of isolated hepatocytes from mice, rats, Syrian golden hamsters and humans with methylene chloride, only mouse hepatocytes formed detectable amounts of DNA-protein crosslinks (DPX; Casanova et al. 1997). When V79 cells were transfected with the mouse enzyme (mGSTT1-1) and incubated with high concentrations (2.5-10 mM) methylene chloride, DPX were formed that were typical for formaldehyde (Hu et al. 2006). For the development of lung tumours in the mouse, damage to mouse lung Clara cells and increased cell division were seen as decisive components. The susceptibility of the mouse was seen as a consequence of very high activity and specific cellular and nuclear localisation of the theta class GST, unique to the mouse (Green 1997).

The markedly lower levels of the methylene chloride metabolising GST in rats and hamsters are consistent with the lack of liver and lung tumours in these species. The levels of this enzyme in humans are even lower than found in rats and hamsters. Hence, the mouse model may not be informative about cancer risks in humans because of the differences in the metabolism of methylene chloride (IARC, 1999).

Recently, this concept has received further experimental support. Sherratt et al. (2002) directly compared the relative capacity and locality of methylene chloride activation in mouse and human tissues. The results showed that the mouse mGSTT1-1 was more efficient in catalysing the conjugation of methylene chloride with GSH than the orthologous human enzyme (hGST1-1). In addition, the mouse expressed higher levels of the enzyme than humans in the liver. Histochemical analysis confirmed the presence of GSTT1-1 in the nucleus of mouse liver cells. In human liver lower levels of GSTT1-1 were detected in bile duct epithelial cells and hepatocyte nuclei, but the enzyme was also present in the cytoplasm. The species differences (mouse/humans) of a higher metabolic efficiency of mGSTT1 *vs*. hGSTT1-1 are also explained by differences in the primary structure. The amino acid residue 234 is the determinant of these differences. The replacement of TRP[234] in hGST1-1 by arginine, as found in the rodent enzyme, enhanced

Social Europe

the enzymatic activity by an order of magnitude (Shokeer et al. 2005). The enzymatic differences are paralleled by differences in the crystal structures of the proteins (Tars et al. 2005).

Some peculiarities of human GSTT1-1 are also relevant. The *hGST1* gene is polymorphic; about 20% of the European population shows a complete deletion (*GSTT1*0*). Furthermore, GSTT1-1 metabolising methylene chloride is present in human erythrocytes, which is not the case in other species including rodents (review: Bolt and Thier 2006). Hence, part of the human population (*GSTT1*0*) does not possess the activating pathway leading to reactive metabolites, and for the rest of the population (*GSTT1*1*) the blood compartment may act as an additional "sink" to eliminate methylene chloride (Sherratt et al. 2002).

Published assessments of carcinogenic risks

Since the 1980s a number of articles have presented quantitative comparisons of methylene chloride metabolism, in order to derive calculations and argumentations regarding possible carcinogenic risks in humans (Andersen et al. 1987, Green et al. 1988, Liteplo et al. 1998, El Masri et al. 1999, Jonsson et al. 2001, Jonsson and Johanson 2001, Preston and Williams 2005). An improved procedure has been adopted recently (Starr et al. 2006).

Against the background of the previous developments, David et al. (2006) recently presented an updated and refined physiologically-based pharmacokinetic (PBPK) model for methylene chloride in humans. This model was based on the PBPK model developed by Andersen et al. (1987) and later developments, and it was supplemented by a Bayesian modelling using the Markov Chain Monte Carlo (MCMC) technique (Marino et al. 2006). The modelling incorporated the advances in understanding the metabolism and in statistical approaches, in order to better reflect the variability in humans. By this way, the new information on human *GSTT1* polymorphism was incorporated using the probabilistic design (El Masri et al. 1999, Jonsson and Johansson (2001). Physiological parameters for input into the MCMC analysis were selected according to current scientific evidence for each parameter. Metabolic data for individual subjects from five human studies were combined into a single data set and population values were derived. These were used for calibration of the human model. The PBPK model, using the calibrated metabolic parameters was then taken to perform a cancer risk assessment for methylene chloride, relying on the experimental tumour incidence in mice and on human exposure data of current IRIS entries. Unit risks, i.e., the risk of cancer from exposure to 1 microg/m^3 over a lifetime, were estimated using the calibrated human model. The results indicated skewed distributions for liver and lung tumour risks, alone or in combination, with a mean unit risk (per microg/m^3) of 1.05×10^{-9}, considering both liver and lung tumours. Adding the distribution of genetic *GSTT1* polymorphisms for metabolism to the presumed ultimate carcinogen, the unit risks ranged from 0 (which is expected given that approximately 20% of the population is estimated to be *GSTT1*0* "non-conjugators") up to a unit risk of 2.70×10^{-9} at the 95th percentile. The median, or 50th percentile, was 9.33×10^{-10}.

If one takes such a unit risk of 10^{-9}, the risk of exposure to 1 microg/m^3 methylene chloride for human working lifetime (assumed as14% of lifetime) would be 1.4×10^{-10}. Accordingly, a lifetime risk for exposure at 100 ppm [353 mg/m^3] would be 4.9×10^{-5}. This is a risk level that will never be detected in epidemiological studies. Even this estimate represents an over-estimation of the human risk, as the "sink function" of the human erythrocytes (*v.s.*)

Social Europe

in persons possessing the active *GSTT1* gene and GSTT1-1 enzyme has not been considered.

Conclusion

Taking together the current knowledge on the potential of human metabolic activation of dichloromethane, it appears unlikely that this compound poses a practical carcinogenic risk to humans, under conditions of current occupational exposures. In consequence, SCOEL has decided to assign dichloromethane to the SCOEL carcinogen group C with a "practical" threshold (Bolt and Huici-Montagud 2008) and to derive an OEL based on non-cancer endpoints.

Social Europe

Table 6: Epidemiological investigations of the carcinogenicity of methylene chloride in humans

Exposed population	Description of study	Findings	Study
119 cases and 108 controls potentially exposed to methylene chloride	Case control study of astrocytic brain cancer	Risks increased with increased potential intensity of exposure and duration of employment; exposure assessment severely criticised by Norman (1996)	Heineman *et al* (1994)
Mortality records from 24 states, gathered from 1984 to 1989 coded for occupation and industry. After excluding homemakers, 33509 cases and 117794 controls remained.	Case-control study of breast cancer with separate analyses for blacks and whites.	After adjusting for socioeconomic status, suggestive associations for styrene, several organic solvents (methylene chloride, carbon tetrachloride, formaldehyde), and several metals/metal oxides and acid mists.	Cantor *et al* (1995)
3630 histologically confirmed cancer cases, of whom 257 had rectal cancer, 533 population controls, 1295 subjects with cancers at sites other than the rectum, lung, colon, rectosigmoid junction, small intestine, and peritoneum	Case-control study investigating the relationship between solvent exposure and rectal cancer (building on earlier study by Siemiatycki, 1991)	Possible association between methylene chloride and rectal cancer, not possible to separate the relative importance of different potential risk factors	(Dumas *et al*, 2000)
12980 female cases of CNS cancer in 24 US states in 1984-1992 and 51920 female controls who died from diseases other than malignancies and neurological disorders	Death certificate-based case-control study	20-30% CNS cancer increase in women exposed to electromagnetic fields (EMF), methylene chloride, insecticides, fungicides, and contact with the public. Risk unrelated to probability and intensity of exposure.	Cocco *et al* (1999)

Social Europe

Table 6: Investigations of carcinogenicity in long term animal experiments [conversion factor: 1 mgm^{-3} = 0.28 ppm]

Species	Exposure regime	Concentrations mgm^{-3}	Observed effects	Study
Rats	for 6 hours per day, 5 days per week for 2 years	0, 3530, 7060 and 14120	Reduced survival of exposed and unexposed male rats due to leukaemia. Reduced survival of high dose females. Increased incidences of benign mammary gland adenomas and fibroadenomas in exposed males and females. Liver damage in exposed animals with a dose-related increase in the incidence of hepatocellular neoplastic nodules or hepatocellular carcinomas (combined) in females. Increased incidence of squamous metaplasia of the nasal cavity in high dose females. In males, increased incidence of mesotheliomas (arising primarily from the tunica vaginalis)	NTP (1986)
Rats	6 hours/day, 5 days/ week for 2 years	0, 1770, 5300, 12400	Increase in sarcomas in the mid-cervical area in the region of the salivary gland in mid and high-dose males. Slight dose-related increase in benign mammary tumours in males, dose related increase in benign mammary tumours in females	Burek *et al* (1984)
Rats	6 hours/day, 5 days/ week for 20 months (males), 24 months (females)	0, 177, 710, 1770	Increase in benign mammary tumours in females at 1770 mgm^{-3}. NOEL 710 mgm^{-3}	Nitschke *et al* (1988a)
Rats	4 hours/day, 5 days/week for 7 weeks, then 7 hours/day, 5 days/week for 97 weeks	350	No effect on incidence of benign or malignant tumours	Maltoni *et al* (1988)
Rats	As above for 15 and 104 weeks	350	No effect on incidence of benign or malignant tumours	Maltoni *et al*, 1988)

Mice	6 hours/day, 5 days/week for various periods up to 104 weeks	7100	An excess of lung and liver tumours observed in mice exposed for over 1 year	Kari *et al* (1992)
Mice	6 hours/day, 5 days/week for 26, 52, 78 or 104 weeks	7100	All exposed groups showed excesses of lung and liver tumours	Kari *et al* (1992)
Mice	6 hours per day, 5 days per week for 2 years	0, 7060, 14120	The survival of exposed males and high dose females was reduced. Increased incidences of lung tumours were observed in exposed mice. Cytologic degeneration of the liver and increased Incidences of hepatocellular adenomas or hepatocellular carcinomas (combined) were observed in high dose male and exposed female mice. Dose-related increases in the numbers of mice bearing multiple lung or liver neoplasms and in the incidences of testicular atrophy in male mice and uterine and ovarian atrophy in female mice.	NTP (1986)
Mice	6 h per day, for varying durations up to 104 weeks.	7060	Exposure caused an increase in liver and lung neoplasia in the absence of overt cytotoxicity. Measurement of replicative DNA synthesis after 13, 26, 52 and 78 weeks of exposure showed a significant decrease in the hepatocyte labelling index at 13 weeks. Replicative DNA synthesis in pulmonary airways after 1, 2, 3, 4, 13 and 26 weeks of exposure was significantly lower than in controls.	Maronpot *et al* (1995)
Hamster	6 hours/day, 5 days/week for 2 years	0, 1770, 5300, 12400	No significant increase in tumours	Burek *et al* (1984)

Social Europe

2.8. Reproductive toxicity

Humans

Methylene chloride crosses the placenta and is also excreted in breast milk.

A case control study of 44 women within a cohort of workers employed in Finnish Pharmaceutical factories reported that methylene chloride was associated with an increased risk of spontaneous abortion (Odds Ratio of 2.3 with a 95% confidence interval of 1.0-5.7). There was weak evidence that risk increased with increasing frequency with which methylene chloride was handled but the odds ratios for other solvents were also raised, so the significance of the findings for methylene chloride is uncertain (Taskinen *et al*, 1986).

A study of 50 men working on aircraft maintenance at an US Air Force installation exposed to jet fuel and solvent concentrations below 6 ppm (i.e. 21 mgm^{-3} for methylene chloride) for individual solvents found some effects on sperm generation but no clear association was found between biological exposure measurements and spermatogenic changes (Lemasters *et al* 1999).

Animals

The IPCS (1996) reviewed several studies. Slight skeletal malformations but no significant teratogenic effects or evidence of foetal toxicity was observed in rats and mice exposed to concentrations of methylene chloride of 1230 ppm [4400 mgm^{-3}] for 7 hours/day on days 6 to 15 of pregnancy (Schwetz *et al*, 1975). Maternal COHb levels of 12% were measured during exposure. No teratogenic effects were observed in rats exposed to 4550 ppm [16260 mgm^{-3}] for 6 hours/day during 17 days of pregnancy but foetal body weights were reduced (Hardin and Manson, 1980). No effects were seen in behavioural assays but there were changes in the general activity level of pups from about 10 days that were still present at 150 days. It was unclear whether this was a result of exposure. Maternal COHb levels during exposure ranged from 7.1-10.1 %.

In a two generation study, rats were exposed to 0, 100, 500, or 1500 ppm [0, 350, 1770 or 5300 mgm^{-3}] for 6 hours per day for 5 days each week. No evidence was found of any adverse effect on any reproductive parameter, neonatal survival or neonatal growth in either the F_0 or F_1 generation (Nitschke *et al*, 1988a and b).

Recommendation

High levels of exposure methylene chloride have led to unconsciousness and, in some cases, death.

The formation of COHb as a result of methylene chloride metabolism decreases the oxygen-carrying capacity of the blood, giving symptoms of oxygen deprivation such as light-headedness, headache or loss of consciousness. In non-smoking workers, COHb levels in blood of more than 8% were found following exposure to about 250 ppm [880mg/m^3] for 7.5 hours.

Other effects include irritation of the eyes and respiratory tract, lung oedema and acute effects on the heart, liver and kidneys. Experiments in human volunteers have found neurobehavioral changes following exposure to 250 ppm [694 mg/m^3] for 1.5 to 3 hours and effects on liver function at 460 ppm [1650 mg/m^3].

Social Europe

At the workplace, no evidence of adverse effects on health has been found following exposure concentrations of about 100 ppm [350 mg/m³] over several years.

In animals, repeated or long-term exposure to high concentrations of methylene chloride is associated with reversible CNS effects, slight eye irritation, liver effects and mortality. The lowest exposure concentration reported to cause liver effects is 25 ppm [88 mg/m³] (for continuous exposure). No effects were found on the liver following exposure to 250 ppm [880 mg/m³] for 5 hours per day. The lowest exposure concentration associated with reversible CNS effects is 2000 ppm [17700 mg/m³] (6 hours/day) with no effects at 500 ppm [7100 mg/m³]. Effects on brain chemistry were reported following exposure to 70 ppm [250 mg/m³] (6 hours/day).

Despite a large number of epidemiological investigations, no clear association between methylene chloride and cancer in humans has been demonstrated. The studies with the largest cohorts and longest follow up times are those published by Hearne and Pifer (1999), Lanes et al (1993) and Tomenson et al (1997).

Inhalation exposure to methylene chloride is associated with the development of cancers in mice but not in rats and hamsters. The mouse model may not be informative about cancer risks in humans because of large quantitative differences in the metabolism of methylene chloride. IARC (1999) concluded that that methylene chloride is *possibly carcinogenic to humans (Group 2B)*.

Human reproductive data are limited and do not provide clear evidence of an effect. No teratogenic effects were observed in rats exposed to 4560 ppm [16300 mg/m³] but foetal body weights were reduced. Slight skeletal malformations have been reported at 1230 ppm [4400 mg/m³]. No effects were found in a two generation study at concentrations of up to 1480 ppm [5300 mg/m³].

As mentioned above, no evidence of adverse effects on health has been found at the workplace following occupational exposure concentrations of about 100 ppm [350 mg/m³] over several years. Experimental studies appear consistent with this figure.

A relevant toxic metabolite of methylene chloride is carbon monoxide. For carbon monoxide SCOEL has recommended an OEL of 20 ppm, compatible with a biological limit (BLV) of 4% COHb (SCOEL/SUM/57D).

Experimental exposures of human volunteers to 100 ppm methylene chloride have resulted in 3% COHb at the end of an 8 h exposure period (*Figure 1*). This is basically supported by field studies in exposed workers (Soden 1993, Soden et al. 1996; *Figure 2*)

Taking these informations together, an OEL (8 h TWA) of 100 ppm is recommended for methylene chloride. Based on possible short-term prenarcotic effects, a STEL (15 min) is set to 200 ppm.

The biological limit (BLV) recommended for carbon monoxide (4%COHb) also to methylene chloride. It should be observed that this limit may be exceeded in cases of heavy smokers, due to the COHb increment by tobacco smoking. Therefore, for practical purposes the determination of methylene chloride in blood or urine (both at end of the shift) may be advantageous. According to the explanations given in chapter 5.1, BLVs are proposed for methylene chloride in blood of 1 mg/liter, and for methylenhe chloride in urine of 0.3 mg/liter.

Methylene chloride is carcinogenic to mice, producing lung and liver tumours. This carcinogenicity is conferred by a reductive, GST-dependent, metabolic pathway.

Social Europe

Quantitative comparisons of the toxicokinetics in several experimental animal species and in humans indicate that this toxifying pathway is not relevant in humans (see chapter 5.7 for details). This conclusion is also compatible with epidemiological data, which did not point to clear carcinogenic effects in humans, although there have been significant occupational exposures to methylene chloride since many decades. The compound is assigned to the SCOEL carcinogen group C, implying the existence of a "practical threshold (see chapter 5.7).

As specified in chapter 5.1, there may be a significant contribution of skin to the total uptake of methylene chloride under workplace conditions. Consequently, a skin notation is applied.

References

Amsel J, Soden KJ, Sielken RL Jr, Valdez-Flora C. (2001). Observed versus predicted carboxyhemoglobin levels in cellulose triacetate workers exposed to methylene chloride. American Journal of Industrial Medicine.;40:180-11.

Andersen ME, Clewell HJ, Gargas ML, Smith FA, Reitz RH (1987) Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Toxicol 87:185-205

Becker CE & Lash A. (1990)/ Study of neurological effects of chronic methylene chloride exposure in airline maintenance mechanics [abstract 7]. Veterinary and Human Toxicology;32: 342.

Blair A, Hartge P, Stewart PA, McAdams M, Lubin J. (1998).Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow up. Occupational and Environmental Medicine; 55:161-171

Bolt HM, Huici-Motagud A (2008) Strategy of the Scientific Committee on Occupational Exposure Limits (SCOEL) in the derivation of occupational exposure limits for carcinogens and mutagens. Arch Toxicol 82: 61-64.

Bolt HM, Thier R. (2006) Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Curr Drug Metab.; 7:613-628.

Briving C, Hamberger A, Kjellstrand P, Rosengren L, Karlsson JE, & Haglid KG. (1986). Chronic effects of dichloromethane on amino acids, glutathione and phosphoethanolamine in gerbil brain. Scandinavian Journal of Work, Environment & Health;12: 216-220.

Burek JD, Nitschke KD, Bell TJ, Wackerle DL, Childs RC, Beyer JE, Dittenber DA, Rampy LW, & McKenna MJ. (1984.) Methylene chloride: A two- year inhalation toxicity and oncogenicity study in rats and hamsters. Fundamental and Applied Toxicology; 4: 30-47.

Casanova M, Bell DA, Heck HD. (1997) Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and Ma genes. Fundam Appl Toxicol.; 37: 168-180.

Chang YL, Yang CC, Deng JF, Ger J, Tsai WJ, Wu ML, Liaw HC, Liaw SJ. (1999). Diverse manifestations of oral methylene chloride poisoning: report of 6 cases. Journal of Toxicology. Clinical Toxicology; 37:497-504.

Cherry N, Venables H, Waldron HA, Wells GG. (1981). Some observations on workers exposed to methylene chloride. British Journal of Industrial Medicine; 38: 351-355.

Cocco P, Heineman EF, Dosemeci M. (1999). Occupational risk factors for cancer of the central nervous system (CNS) among US women. American Journal of Industrial Medicine.;36:70-74

Social Europe

David RM, Clewell HJ, Gentry PR, Covington TR, Morgott DA, Marino DJ (2006) Revised assessment of cancer risk to dichloromethane. II. Application of probabilistic models to cancer risk determinations. Regulatory Toxicol Pharmacol.; 45:55-65.

Dell LD, Mundt KA, McDonald M, Tritschler JP, Mundt DJ.: (1999) Int Arch Occup Environ Health 72:429-442.

DFG [Deutsche Forschungsgemeinschaft] (1991) Anilin. In: Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe. Bd. 2. Analysen in biologischen Material. 10. Lieferung. VCH, Weinheim.

DFG [Deutsche Forschungsgemeinschaft] (2005) Anilin. In: The MAK Collection, BAT Value Documentations, Vol. 4, Wiley-VCH, Weinheim

Di Vincenzo GD, Kaplan CJ (1981) Uptake, metabolism and elimination of methylene chloride vapour by humans. Toxicology and Applied Pharmacology; 59: 130-140

Dumas S, Parent ME, Siemiatycki J, Brisson J. (2000). Rectal cancer and occupational risk factors: a hypothesis-generating, exposure-based case-control study. International Journal of Cancer.;87:874-879.

ECETOC (1993) Strategy for assigning a skin notation. Document No. 31. ECETOC, Brussels.

Eisenbrandt DL,Reitz RH. (1986). Acute toxicity of methylene chloride: Tumorigenic implications for B6C3F1 mice. Toxicologist, 6: 662.

El Masri HA, Bell DA, Portier CJ (1999) Effects of glutathione transferase theta polymorphism on the risk estimates of dichlormethane to humans. Toxicol Appl Pharmacol 158:221-230.

EUROCLOR (1997). Methylene chloride: an update on human and environmental effects. Solvents digest. Brussels: European Chlorinated Solvent Association. Belgium. (originally published 1995). www.eurochlor.org.

Friedlander BR, Hearne T, & Hall S (1978) Epidemiologic investigation of employees chronically exposed to methylene chloride. Journal of Occupational Medicine; 20: 657-666.

Fuxe K, Andersson K, Hansson T, Agnati LF, Eneroth P, & Gustafsson JA. (1984). Central catecholamine neurons and exposure to dichloromethane. Selective changes in amine levels and turnover in tel- and diencephalic DA and NE nerve terminal systems and in secretion of anterior pituitary hormones in the male rat. Toxicology, 29: 293-305.

Ghittori S, Marraccini P, Franco G, Imbriani M (1993) Methylene chloride exposure in industrial workers. Am Ind Hyg Ass J 54: 27-31.

Gibbs GW. (1992). The mortality of workers employed at a cellulose acetate and triacetate fibers plant in Cumberland Maryland, a "1970" cohort followed 1970-1989. Final report. Winterburn: Safety Health Environmental International Consultants.

Gibbs GW, Amsel J, Soden K .(1996). A cohort mortality study of cellulose triacetate-fiber workers exposed to methylene chloride. Journal of Occupational and Environmental Medicine;38:693-697.

Social Europe

Goulle JP, Lacroix C, Vaz E, Rouvier P, Proust B. (1999). Fatal case of dichloromethane poisoning. Journal of Analytical Toxicology;23:380-383.

Green T., (1997) Methylene chloride induced mouse liver and lung tumours: an overview of the role of mechanistic studies in human safety assessment. Hum Exp Toxicol.; 16:3-13.

Green T, Provan WM, Dugard PH, Cook SK.; (1988) Methylene chloride (dichloromethane): human risk assessment using experimental animal data. ECETOC Technical Report no. 32. European Chemical Industry Ecology & Toxicology Centre, Brussels. ISSN 07773-8072-32.

Hallier E, Schröder KR, Asmuth K, Dommermuth A, Aust B, Goergens HW. (1994) Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: influence of polymorphism of glutathione transferase theta (GST T1-1). Arch Toxicol. 1994;68(7):423-7.

Hardin BD & Manson JM. (1980). Absence of dichloromethane teratogenicity with inhalation exposure in rats. Toxicology and Applied Pharmacology; 52: 22-28.

Haun CC, Vernot EH, Darmer KI, & Diamond SS. (1972). Continuous animal exposure to low levels of dichloromethane. In: Proceedings of the 3rd Annual Conference on Environmental Toxicology. Dayton, Ohio: Wright-Patterson Air Force Base, Aerospace Medical Research Laboratory. (Paper No. 12; AMRL-TR-130): 199-208.

Hearne FT, Grose F, Pifer WJ, Friedlander BR, Raleigh RL. (1987.) Methylene chloride mortality study: Dose-response characterization and animal model comparison. Journal of Occupational Medicine; 29: 217-228.

Hearne FT & Friedlander BR.(1981). Follow-up of methylene chloride study. Journal of Occupational Medicine; 23: 660.

Hearne FT, Pifer JW, & Grose F. (1990). Absence of adverse mortality effects in workers exposed to methylene chloride. An update. Journal of Occupational Medicine; 32: 234-240.

Hearne FT, Pifer JW. (1999). Mortality study of two overlapping cohorts of photographic film base manufacturing employees exposed to methylene chloride. Journal of Occupational and Environmental Medicine.;41:1154-1169.

Heppel LA, Neal PA, Perrin TL, Orr ML, & Porterfield VT. (1944). Toxicology of dichloromethane (methylene chloride). I. Studies on effects of daily inhalation. The Journal of Industrial Hygiene and Toxicology; 26: 8-16.

Hext PM, Foster J, & Millward SW. (1986). Methylene chloride (Dichloromethane): 10-day inhalation toxicity study to investigate the effects on rat and mouse liver and lungs. ICI Report No. CTL/P/1432.

Hotz P, Hoet P, Lauwerys R, Buchet JP. (1987) of a method to monitor low molecular mass hydrocarbons in exhaled breath of man: preliminary evaluation of its interest for detecting a lipoperoxidation process in vivo. Clin Chim Acta. Feb 15;162(3):303-10.

Hu YP, Kabler SL, Tennant AH, Townsend AJ, Kligerman AD.; (2006) Induction of DNA-protein crosslinks by dichloromethane in a V79 cell line transfected with the murine glutathione-S-transferase theta 1 gene. Mutat Res.; 607:231-239.

Social Europe

IARC (1999) Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide (Part One). Lyon: WHO. (International Agency for Research on Cancer, Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Volume 71): 251-315

IPCS. (1986). Methylene chloride (2nd ed). (International Programme on Chemical Safety Environmental Health Criteria 164). (Second edition). Geneva: WHO.

Jonsson F, Johanson G.; (2001) A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. Toxicol Appl Pharmacol.; 174:99-112.

Jonsson F, Bois F, Johanson G.; (2001) Physiologically based pharmacokinetic modelling of inhalation exposure of humans to dichloromethane during moderate to heavy e4xercise. Toxicol Sci.; 59:209-218.

Ito A, Kawata F, Takeshita T, Ito M. (1990). [Experimental studies of effects of methylene chloride on living body (1)]. Hochudoku; 8: 64-65 (in Japanese).

Kari FW, Maronpot RR, & Anderson MW. (1992). Testimony for the OSHA hearing on the proposed occupational standard for methylene chloride. North Carolina:National Institute of Environmental Health Sciences.

Kashin LM, Makotchenko VM, Malinina-Putsenko VP, Mikhailovskaja LF, & Shmuter LM. (1980). [Experimental and clinico-hygienic investigations of methylene chloride toxicity.] Vrachebnoe Delo;1: 100-103 (in Russian).

Kim YC. (1997). Dichloromethane potentiation of carbon tetrachloride hepatotoxicity in rats. Fundamental and Applied Toxicology;35:138-41.

Kjellstrand P, Bjerkemp M, Adler-Maihofer M, & Holmquist B. (1986). Effects of methylene chloride on body and organ weight and plasma butyrylcholinesterase activity in mice. Acta Pharmacologica Toxicologica; 59: 73-79.

Kuzelova M & Vlasak R. (1966). [The effect of methylene chloride on the health of workers in production of film-foils and investigation of formic acid as a methylene-dichloride metabolite]. Pracovni Lekarstvi; 18: 167-170 (in Czech).

Lanes SF, Rothman KG, Dreyer NA, & Soden KJ. (1993). Mortality update of cellulose fiber production workers. Scandinavian Journal of Work, Environment & Health;19(6): 426-428.

Lash AA, Becker CE, So Y, & Shore M. (1991). Neurotoxic effects of methylene chloride: Are they long lasting in humans? British Journal of Industrial Medicine; 48: 418-426.

Lemasters GK, Olsen DM, Yiin JH, Lockey JE, Shukla R, Selevan SG, Schrader SM, Toth GP, Evenson DP, Huszar GB. (1999). Male reproductive effects of solvent and fuel exposure during aircraft maintenance. Reproductive Toxicology;13:155-166.

Leuschner F, Neumann BW, & Hubscher F. (1984). Report on subacute toxicological studies with dichloromethane in rats and dogs by inhalation. Arzneimittel-Forschung; 34: 1772-1774.

Social Europe

Liteplo RG, Long GW, Meek ME. (1998) Relevance of carcinogenicity bioassays in assessing potential health risks associated with exposure to methylene chloride. Hum Exp Toxicol.; 17: 84-87.

Mahmud M, Kales SN. (1999). Methylene chloride poisoning in a cabinet worker. Environmental Health Perspectives.;107:769-772.

Maltoni C, Cotti G, & Perino G (1988) Long-term carcinogenicity bioassays administered by ingestion to Sprague-Dawley rats and Swiss mice and by inhalation to Sprague-Dawley rats. Annals of the NY Academy of Sciences; 534: 352-366.

Marino DJ, Clewell HJ, Gentry PR, Covington TR, Hack CE, David RM, Morgott DA (2006) Revised assessment of cancer risk to dichloromethane. Part II: Bayesion PBPK and dose-response modelling in mice. Regul Toxicol Pharmacol. 45:44-54.

Maronpot RR, Devereux TR, Hegi M, Foley JF, Kanno J, Wiseman R, Anderson MW. (1995). Hepatic and pulmonary carcinogenicity of methylene chloride in mice: a search for mechanisms. Toxicology.;102: 73-81.

Marsch GA, Botta S, Martin MV, McCormick WA, Guengerich FP. (2004) Formation and mass spectrometric analysis of DNA and nucleoside adducts by S-(1-acetoxymethyl)glutathione and by glutathione S-transferase-mediated activation of dihalomethanes. Chem Res Toxicol.; 17:45-54.

Mattsson JL, Albee RR, & Eisenbrandt DL. (1990). Neurotoxicologic evaluation of rats after 13 weeks of inhalation exposure to dichloromethane or carbon monoxide. Pharmacololgy, Blochemistry, and Behaviour; 36: 671-681.

Nitschke KD, Stevens GA, Kociba RJ, Keyes DG, & Rampy LW. (1981). Methylene chloride: a four week inhalation toxicity study in rats, hamsters and mice. (Internal report). Midland, Michigan: Dow Chemical Company.

Nitschke KD, Burek JD, Bell TJ, Kociba RJ, Rampy LW, & McKenna MJ. (1988a). Methylene chloride: A 2-year inhalation toxicity and oncogenicity study in rats. Fundamental and Applied Toxicology; 11: 48-59.

Nitschke KD, Eisenbrandt DL, Lomax LG, Rao KS (1988b) Methylene chloride: Two-generation inhalation reproductive study in rats. Fundamental and Applied Toxicology; 11: 60-67.

Norpoth K, Witting U, & Springorum M.(1974)/ Induction of microsomal enzymes in the rat liver by inhalation of hydrocarbon solvents. Int Arch Arbeitsmed.; 33: 315-321.

NTP.(1986). Toxicology and carcinogenesis studies of dichloromethane (methylene chloride) (CAS No. 75-09-2) in F344/N rats and B6C3F1 mice (inhalation studies). RTP, North Carolina: NTP. (Technical Report No. 306; NIH Publication No. 86-2562).

Ojajärvi A, Partanen T, Ahlbom A, Boffetta P, Hakulinen T, Jourenkova N, Kauppinen T, Kogevinas M, Vainio H, Weiderpass E, Wesseling C. (2001). Risk of pancreatic cancer in workers exposed to chlorinated hydrocarbon solvents and related compounds: a meta-analysis. American Journal of Epidemiology;153:841-850.

Social Europe

35

Ott MG, Skory LK, Holder BB, Bronson JM, & Williams PR. (1983). Health evaluation of employees occupationally exposed to methylene chloride. Scandinavian Journal of Work Environment and Health; 9 (Suppl 1): 1-38.

Preston RJ, Williams GM. (2005) DNA-reactive carcinogens: mode of action and human cancer hazard. Crit Rev Toxicol.; 35:673-683.

Rosengren LE, Kjellstrand P, Aurell A, & Haglid KG. (1986). Irreversible effects of dichloromethane on the brain after long term exposure: A quantitative study of DNA and the glial cell marker proteins S-100 and GFA. British Journal of Industrial Medicine; 43: 291-299.

Sahn SC & Lowther DK. (1981). Pulmonary reactions to inhalation of methylene chloride: Effects on lipid peroxidation in rats. Toxicology Letters; 8(4-5): 253-256.

Sakai T, Morita Y, Wakui C. (2002). Biological monitoring of workers exposed to dichloromethane, using head-space gas chromatography. Journal of Chromatography. B. Analytical Technologies in the Biomedical and Life Sciences;778:245-250.

Schwetz BA, Leong BKJ, Gehring PJ. (1975). The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicology and Applied Pharmacology; 32: 84-96.

Sherratt PJ, Williams S, Foster J, Kernohan N, Green T, Hayes JD. (2002) Direct comparison of the nature of mouse and human GSTT1-1 and the implications on dichloromethane carcinogenicity. Toxicol Appl Pharmacol.; 179:89-97.

Shokeer A, Larsson AK, Mannervik B. (2005) Residue 234 in glutathione transferase T1-1 plays a pivotal role in the catalytic activity and the selectivity against alternative substrates. Biochem J.; 388:387-392.

Seno H, Ishii A, Watanabe K, Suzuki O, Kumazawa T. (1999). Extraction of chloroform and methylene chloride in human whole blood and urine by headspace solid phase microextraction (SPME). Medical Science and the Law.;39:332-6.

Shannon HS, Haines T, Bernholz C, Julian JA, Verma DK, Jamieson E, Walsh C. (1988). Cancer morbidity in lamp manufacturing workers. American Journal of Industrial Medicine; 14:281-90.

Soden KJ (1993) An evaluation of chronic methylene chloride exposure. Journal of Occupational Medicine; 35: 282-286.

Soden KJ, Marras G, Amsel J (1996) Carboxyhemoglobin levels in methylene chloride-exposed employees. Journal of Occupational and Environmental Medicine;38:367-371.

Starr TB, Matanoski G, Anders MW, Anderson ME (2006) Workshop overview: Reassessment of the cancer risk of dichloromethane in humans. Toxicol Sci 91: 20-28.

Takashita T, Ito A, Kawata F, Ito M, Ito K.(1991). [Experimental studies of effects of methylene chloride on living bodies (2)]. Hochudoku, 9: 100-101 (in Japanese).

Tars K, Larsson AK, Shokeer A, Olin B, Mannervik B, Kleywegt GJ (2006) Structural basis of the suppressed catalytic activity of wild-type human glutathione transferase T1-1 compared to its W234R mutant. J Mol Biol. 355:96-105.

Taskinen H, Lindbohm ML, Hemminki K .(1986). Spontaneous abortions among women working in the pharmaceutical industry. British Journal of Industrial Medicine; 43: 199-205.

Thier R, Taylor JB, Pemble SE, Humphreys WG, Persmark M, Ketterer B, Guengerich FP. (1993) Expression of mammalian glutathione S-transferase 5-5 in Salmonella typhimurium TA1535 leads to base-pair mutations upon exposure to dihalomethanes. Proc Natl Acad Sci USA. 90(18):8576-80.

Thier R, Pemble SE, Kramer H, Taylor JB, Guengerich FP, Ketterer B. (1996) Human glutathione S-transferase T1-1 enhances mutagenicity of 1,2-dibromoethane, dibromomethane and 1,2,3,4-diepoxybutane in Salmonella typhimurium. Carcinogenesis 17:163-166.

Thier R, Wiebel FA, Hinkel A, Burger A, Brüning T, Morgenroth K, Senge T, Wilhelm M, Schulz TG. (1998) Species differences in the glutathione transferase GSTT1-1 activity towards the model substrates methyl chloride and dichloromethane in liver and kidney.
Arch Toxicol. 72(10):622-9.

Tomenson JA, Bonner SM, Heijne CG, Farrar DG, Cummings TF (1997) Mortality of workers exposed to methylene chloride employed at a plant producing cellulose triacetate film base. Occupational and Environmental Medicine.;54: 470-476.

Tsuruta H (1975) Percutaneous absorption of organic solvents. (I) Comparative study of the in vivo percutaneous absorption of chlorinated solvents in mice. Industrial Health, 13:227-236.

Tsuruta H (1977) Percutaneous absorption of organic solvents. (II) A method for measuring the penetration rate of chlorinated solvents through excised rat skin. Industrial Health, 15:131-139.

Ukai H, Okamoto S, Takada S, Inui S, Kawai T, Higashikawa K, Ikeda M. (1998) Monitoring of occupational exposure to dichloromethane by diffuse vapor sampling and urinalysis.Int Arch Occup Environ Health. 71(6):397-404.

Ursin C, Hansen CM, Van Dyk JW, Jensen PO, Christensen IJ & Ebbehoej J (1995) Permeability of commercial solvents through living human skin. American Industrial Hygiene Association Journal, 56(7):651-660.

OSHA [U.S. Occupational Safety and Health Administration]. (1991). OSHA Method (available from www.osha.gov)

Warbrick EV, Kilgour JD, Dearman RJ, Kimber I, Dugard PH. (2003). Inhalation exposure to methylene chloride does not induce systemic immunotoxicity in rats. Journal of Toxicology and Environmental Health. Part A. 66:1207-19

Weinstein RS, Boyd D, Back KC. (1972). Effects of continuous inhalation of dichloromethane in the mouse: morphologic and functional observations. Toxicology and Applied Pharmacology; 23: 660-679.

Social Europe

Weinstein RS, Diamond SS. (1972). Hepatotoxicity of dichloromethane (methylene chloride) with continuous exposure at a low dose level. In: Proceedings of the 3rd Annual Conference on Environmental Toxicology. Dayton, Ohio, Wright-Patterson Air Force Base, Aerospace Medical Research Laboratory (AMRL-TR-72-130, 209-220).

Wheeler JB, Stourman NV, Thier R, Dommermuth A, Vuilleumier S, Rose JA, Armstrong RN, Guengerich FP. (2001) Conjugation of haloalkanes by bacterial and mammalian glutathione transferases: mono- and dihalomethanes. Chem Res Toxicol. (2001) 14:1118-1127.

White RF, Proctor SP, Echeverria D, Schweikert J, Feldman RG. (1995). Neurobehavioral effects of acute and chronic mixed-solvent exposure in the screen printing industry. American Journal of Industrial Medicine; 28:221-31.

Zarrabeitia MT, Ortega C, Altuzarra E, Martinez MA, Mazarrasa O, Calvet R. (2001). Accidental dichloromethane fatality: a case report. Journal of Forensic Sciences. 46:726-727.

Social Europe