The MAK Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Methyl chloride

MAK Value Documentation, addendum – Translation of the German version from 2021

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Keywords

methyl chloride; neurotoxicity; fertility; maximum concentration at the workplace; MAK value; carcinogenicity; developmental toxicity; germ cell mutagenicity; toxicity; skin absorption

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) has re-evaluated the occupational exposure limit value (maximum concentration at the workplace, MAK value) of methyl chloride [74-87-3], considering all toxicological end points. Relevant studies were identified from a literature search and also unpublished study reports were used. Critical effects are neurotoxicity and reduced fertility. The NOAEC for adverse effects on fertility in male rats after 3-month exposure was 150 ml/m^3. A NAEC of 100 ml/m^3 for reduced fertility in male rats was estimated from a 2-year study. The NOAEC for degenerations of the cerebellar granular layer is 150 ml/m^3 in mice. Slight behavioural toxic effects were observed in humans at a concentration of 200 ml/m^3; neurotoxic effects developed at much higher concentrations. On the basis of neurotoxic effects in humans and reduced fertility and severe neurotoxic effects in animals, the maximum concentration at the workplace (MAK value) for methyl chloride has been lowered to 10 ml/m^3. As the critical effect of methyl chloride is systemic, Peak Limitation Category II is retained. The initial half-live in humans is below one hour, so an excursion factor of 1 is determined. Methyl chloride is genotoxic in vitro only at very high concentrations of 8000 ml/m^3 and above. DNA adducts were not observed even with very high concentrations of methyl chloride. From in vivo and metabolism studies it can be concluded that cytotoxic and secondary genotoxic effects are of prime importance. Thus, methyl chloride is not regarded as a germ cell mutagen. The observed kidney tumours occurred only in one species and sex (male mouse) and can be explained with a mechanism, that is of no relevance in humans. In comparison with methyl bromide and methyl iodide, carcinogenic effects of methyl chloride due to alkylating effects are not to be expected. However, a possible formation of DNA adducts cannot be completely excluded. If at all, these are only formed at concentrations far exceeding the MAK value and even then only to a very small extent, so that it is of no significance for the situation at the workplace. Methyl chloride is therefore not classified in a Carcinogen Category. There are no data on developmental neurotoxicity, therefore methyl chloride is assigned to Pregnancy Risk Group D. Skin contact is not expected to contribute significantly to systemic toxicity. There are no data on sensitization.

Citation Note:
Hartwig A, MAK Commission. Methyl chloride. MAK Value Documentation, addendum – Translation of the German version from 2021. MAK Collect Occup Health Saf. 2024 Sep;9(3):Doc064. https://doi.org/10.34865/mb7487e9_3ad

Manuscript completed:
24 Feb 2021

Publication date:
30 Sep 2024

MAK value (2020)	**10 ml/m³ (ppm) ≙ 21 mg/m³**
Peak limitation (2020)	**Category II, excursion factor 1**
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (2020)	**Pregnancy Risk Group D**
Germ cell mutagenicity	–
BAT value	–
Synonyms	monochloromethane
Chemical name (IUPAC)	chloromethane
CAS number	74-87-3
Molecular formula	CH_3Cl
Molar mass	50.49 g/mol
Melting point	–97.4 °C (Greim 1996 a)
Boiling point	–23.7 °C (Greim 1996 a)
Vapour pressure at 25 °C	5730 hPa (NCBI 2020)
1 ml/m³ (ppm) ≙ 2.09 mg/m³	**1 mg/m³ ≙ 0.477 ml/m³ (ppm)**

Documentation for methyl chloride was published in 1992 (Greim 1996 a), followed by a supplement on peak limitation in 2001 (Greim 2001, available in German only).

Cited unpublished toxicological studies from companies have been made available to the Commission.

Methyl chloride is used in the chemical industry as an intermediate in the production of e.g. silicones, methyl cellulose, higher chlorinated methanes and butyl rubber as well as in the production of agricultural chemicals, pharmaceuticals and water treatment chemicals. Methyl chloride has been found also in cigarette smoke. In the past, it has been used as an inhalation anaesthetic or a topical refrigerant anaesthetic (Bause 2019; Westhorpe 1994). It was additionally used as a refrigerant (R 40) before being replaced by incombustible refrigerants (HCFC, CFC) in the 1960s. Most naturally occurring methyl chloride is produced by natural processes, for example, by tropical plants or wood-decaying fungi, in the oceans or during the burning of biomass (Arts et al. 2019; SCOEL 2017). Cattle have been found to emit methyl chloride and trace amounts (2.5–33 ppbv) of methyl chloride were detected in the exhaled air of humans. However, the mechanism of its formation and the source of the determined methyl chloride is unknown (Keppler et al. 2017; Williams et al. 1999).

1 Toxic Effects and Mode of Action

The critical effects of methyl chloride are neurotoxicity and effects on fertility. In humans, the first slight effects of behavioural toxicity are observed at concentrations of 200 ml/m³ and above; neurotoxicity develops only at much higher concentrations. The symptoms are similar to those of alcohol-induced intoxication (headaches, impaired vision,

nausea and vomiting, slurred speech, tremor, memory loss, etc.) and may ultimately lead to seizures and coma. High levels of acute exposure may even be lethal. In mice, the most sensitive end point was the degeneration of the granular layer of the cerebellum; this effect was induced at concentrations of 400 ml/m^3 and above. In male rats, fertility was reduced after 3 months of exposure to concentrations of 475 ml/m^3 and above.

In vitro, methyl chloride was mutagenic in bacteria and mutagenic and clastogenic in mammalian cells at cytotoxic concentrations. In general, however, the primary effects appear to be cytotoxic and are accompanied by secondary genotoxic effects such as inflammation. Even though DNA adducts were not detected in rats and mice in vivo, their development in very small amounts cannot be excluded. Methyl chloride induces kidney tumours in male mice.

At a concentration of 1500 ml/m^3, reduced body weights and delayed ossification were observed in the foetuses of rats. Teratogenicity was not detected. Neither maternal nor developmental toxicity was observed in rabbits after exposure by inhalation to a concentration of 1000 ml/m^3.

There are no data for sensitizing effects.

2 Mechanism of Action

The mechanism of action leading to the induction of toxic effects by methyl chloride is not fully understood. Glutathione conjugation catalysed by GSTT1 (glutathione *S*-transferase T1) is the principal metabolic mechanism (see Section 3.2). The metabolites produced by this process, methanethiol and possibly hydrogen sulfide (Greim 1996 a), induce neurotoxic effects. Methanethiol inhibits cytochrome oxidase in the brain even at low concentrations and may cause the toxic effects on the central nervous system and the degeneration of the granular layer of the cerebellar cortex observed in animal studies. Another metabolite of methyl chloride is formaldehyde. Methyl chloride leads to a marked depletion of glutathione in the kidneys and liver of B6C3F1 mice (Section 5.6.2). This in turn increases formaldehyde concentrations, particularly in the kidneys of mice (Section 3.2). This effect is promoted by formaldehyde dehydrogenase, an enzyme that is likewise dependent on glutathione (BUA 1986). Also the oxidation of methyl chloride by cytochrome P450 (CYP)2E1 leads to the formation of formaldehyde; however, this metabolic pathway plays a subordinate role and is not relevant for humans. The alkylation of proteins may in addition contribute to the development of toxicity (Greim 1996 a). However, no evidence of alkylation activity was found in vivo (Section 5.6.2).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

As described in the documentation published in 1992 (Greim 1996 a), methyl chloride is readily taken up by the lungs and metabolized very rapidly.

In 8 test persons who were exposed by inhalation to methyl chloride in concentrations of 100 ml/m^3 (age of test persons 18–32 years, sex not specified) or 200 ml/m^3 (24 test persons, sex not specified) for 3 hours at rest, methyl chloride reached the steady state in the exhalded breath within 1 hour. At 200 ml/m^3 the mean alveolar exhaled concentration of unchanged methyl chloride was 63 ± 23.6 ml/m^3. The methyl chloride concentration in the exhaled air was above 100 ml/m^3 in 3 of the 24 test persons in the 200 ml/m^3 group, but lower in the remaining test persons. The average methyl chloride concentration in the blood was 11.5 ± 12.3 mg/l. During exposure to 100 ml/m^3, the mean concentrations were 36 ± 12 ml/m^3 in the exhaled air and 7.7 ± 6.3 mg/l in the blood. The concentrations in the exhaled air correlated with the concentrations in the blood ($r = 0.85$, $n = 29$, $p < 0.01$) (Putz-Anderson et al. 1981).

A study investigating a group of test persons exposed to a methyl chloride concentration of 10 ml/m^3 while exercising on a bicycle ergometer at 50 watts found that persons who were GSTT1-positive absorbed 50% to 60% of the methyl chloride by inhalation and persons who were GSTT1-negative absorbed about 16% (see Section 3.2.1; Löf et al. 2000).

Assuming a log K_{OW} of 0.91, water solubility of 5.3 g/l (ECHA 2020 a) and standard conditions (a 2000 cm^2 surface area of skin, exposure for 1 hour), the models developed to estimate the absorption through the skin (Fiserova-Bergerova et al. 1990; Tibaldi et al. 2014) calculated that 404 and 59 mg, respectively, would be absorbed through the skin after exposure to a saturated aqueous solution. However, methyl chloride is unlikely to be used in an aqueous solution. By applying Henry's constant of 0.00882 atm × m^3/mol (NCBI 2020) and assuming a concentration of 10 ml/m^3 in the air, the concentration of methyl chloride in an aqueous film on the skin is calculated to be 0.57×10^{-4} g/l. After whole-body exposure (18 000 cm^2) for 8 hours, according to the 2 models amounts of 313 and 46 µg, respectively, would be absorbed.

The blood:air partition coefficient for exposure to methyl chloride at a concentration of 10 ml/m^3 is in the range of 2.12 to 2.49 in humans and is about 2.47 in rats (US EPA 2001).

3.2 Metabolism

Methyl chloride is primarily metabolized by conjugation with glutathione catalysed by GSTT1. The *S*-methylglutathione formed by this process is further metabolized to *S*-methylcysteine and either excreted with the urine or converted to methanethiol and CO_2. Another metabolic pathway is direct oxidative dehalogenation via CYP2E1 leading to the formation of formaldehyde and formate. This was discussed in detail in the documentation published in 1992 (Greim 1996 a).

3.2.1 Conjugation with glutathione catalysed by GSTT1

In humans, polymorphisms in the GSTT1 gene have been described: the homozygous deletion of the functional gene (*GSTT1*(–/–)) leads to a total loss of GSTT1 activity (non-conjugators). In persons carrying a heterozygous deletion (*GSTT1*(+/–)), the metabolic reaction is catalysed by GSTT1, but to a somewhat lesser extent than in persons with 2 functional alleles. The distribution of the three conjugator types varies widely in different population groups. A study carried out in Sweden, for example, found about 10% non-conjugators (*GSTT1*(–/–)), 50% slow conjugators (*GSTT1*(+/–)) and 40% fast conjugators (*GSTT1*(+/+)). The percentage of non-conjugators is considerably higher in Asian populations (Löf et al. 2000; Warholm et al. 1995).

A study in test persons investigated the metabolism of methyl chloride: groups of 8 persons representing one of the three different *GSTT1* genotypes were exposed to methyl chloride at a concentration of 10 ml/m^3 for 2 hours while exercising on a bicycle ergometer (50 watts). Blood samples were taken before, during and up to 4 hours after the end of exposure. Urine samples were analysed 2, 4, 6, 13 and 22 hours after the end of exposure to determine the concentration of the metabolite *S*-methylcysteine. The test persons in the non-conjugator group were found to have a lower net respiratory uptake of methyl chloride (44 µmol, 95% confidence interval (CI): 29–59) and thus differed with statistical significance from the groups of moderate conjugators (158 µmol, 95% CI: 124–192) and fast conjugators (243 µmol, 95% CI: 174–312). During exposure, exhalation clearance was similar in all three genotypes; however, when the concentration of methyl chloride in the exhaled air was determined after the end of exposure, the levels were much higher in the group of non-conjugators than in the groups of fast and moderate conjugators. The methyl chloride concentrations determined in the exhaled air of non-conjugators 4 hours after exposure were of about the same level as those determined in the fast conjugators after 1 hour. A compartment analysis revealed that metabolic clearance was highest in the group of fast conjugators (mean 4.6 l/min; 95% CI: 3.1–6.1), compared with 2.4 l/min (95% CI: 1.9–2.9) in the group of moderate conjugators, while in the group of non-conjugators no metabolic clearance took place (0 l/min, 95% CI: −0.1 – +0.1). The differences between the 3 groups were statistically significant. However, the methyl chloride levels in the blood did not differ markedly in glutathione conjugators and non-conjugators. The half-life of methyl chloride in the blood was in the minute range (Löf et al. 2000). At the upper confidence limit of 0.1 l/min, non-conjugators metabolize at most only about 2% of methyl chloride via CYP2E1-mediated oxidation.

Human erythrocytes, unlike those of animals (rats, mice, cattle, pigs, sheep, rhesus monkeys), express GSTT1 and are thus able to metabolize methyl chloride in the blood. The GSTT1 activity in the liver and kidney tissues of fast conjugators was twice that determined in the same tissues of moderate conjugators; the activity was 2 to 7-fold higher in the liver than in the kidneys. By comparing the enzyme activity in the liver and kidneys of humans with that in

rats, mice and hamsters, the following order was established: mouse > fast conjugators > rat > moderate conjugators > hamster > non-conjugators (SCOEL 2017; US EPA 2001).

3.2.2 Oxidation

In rats and mice, methyl chloride is metabolized in the liver via CYP2E1-mediated oxidation, forming formaldehyde. A comparison of males and females did not find any differences in the amount or activity of the enzyme. Methyl chloride was not metabolized by the kidney microsomes of rats. However, CYP2E1 activity was detected in the kidney microsomes of male and female CD-1 mice. The activity and the amount of CYP2E1 present were considerably higher in male CD-1 mice than in female CD-1 mice. It was demonstrated that CYP2E1 activity could be increased in female mice by the administration of testosterone; conversely, the concentration and activity of CYP2E1 in castrated male mice decreased to about the same levels as in females. Unlike CYP2E1 from the mouse and rat liver, the expression of renal CYP2E1 is not induced by ethanol (Dekant et al. 1995).

4 Effects in Humans

After single as well as repeated exposure to high concentrations of methyl chloride, the first effects observed were prenarcotic symptoms, such as headaches, dizziness, impaired consciousness and marked torpor, and gastrointestinal disorders such as nausea and vomiting. Following about 2 days without complaints, neurotoxic effects such as personality changes arising from organic changes in the brain, tremor, tonic-clonic spasms, hiccoughing or transient paralysis were observed. The optical system may likewise be affected. The effects on the target organs were manifest in the form of myocardial damage with characteristic changes in the electrocardiogram (ECG) and, in the liver, as enlargement, icterus and pathological liver function parameters. Focal parenchymal degeneration was detected. In the kidneys, symptoms of nephritis and histopathological changes such as congestion, haemorrhage, focal degeneration and tubules necrosis were observed. Hyperaemia, congestion and haemorrhage were found in the lungs. If the toxic effects of methyl chloride are not lethal, the lesions in the central nervous system and in the other organs may regress fully. Frequently, however, the damage is permanent. Most of the many described cases of occupational poisoning were acute; the concentrations at the workplace were not determined. Chronic toxicity was reported only in isolated cases, again without data for the concentrations in the air (Greim 1996 a).

4.1 Single exposures

The study in test persons described above in Section 3.1 included tests of behavioural toxicity (eye-hand coordination, mental alertness and time discrimination). The test persons were exposed for 3 hours to methyl chloride concentrations of 0, 100 (8 test persons, sex not specified) or 200 ml/m^3 (24 test persons, sex not specified). Each test person was given 70 minutes to practice the tasks. The behavioural tests were carried out before and during exposure. The results determined prior to exposure served as the control data. The results of the behavioural tests obtained from the test persons exposed to 100 ml/m^3 were not included in the statistical evaluation because the number of test persons was too small. The data for the test persons who were exposed to 200 ml/m^3 revealed minimal ($p < 0.053$) effects that were attributed to exposure to methyl chloride. In the 200 ml/m^3 group, test performance was on average 6.7% lower during exposure to methyl chloride than prior to exposure. In the control group without exposure to methyl chloride, performance was on average 2.73% lower than pre-exposure performance. No data for irritation were published (Putz-Anderson et al. 1981).

4.2 Repeated exposure

Two cohort studies investigated the effects on a ship's crew exposed to methyl chloride in 1963 (Rafnsson and Gudmundsson 1997; Rafnsson and Kristbjornsdottir 2014). After methyl chloride was leaking from a defective refrigerator, the appliance was repaired by crew members and then refilled with methyl chloride. The refrigerator was located

directly below the quarters of 17 crew members (deckhands). Eleven other crew members, including 7 officers, were exposed only during the repair of the refrigerator or while taking care of their sick crewmates. The level of exposure on board the ship was not determined; exposure to methyl chloride was estimated to have been roughly in the range from 100 to 1000 ml/m^3 (Rafnsson and Kristbjornsdottir 2014). The first symptoms (no other details) of toxicity were observed 2 days after the discovery of the leak and its ensuing repair. In all, the ship's crew was probably exposed to methyl chloride for 4 days. A follow-up of the cohort began in 1966 and ended in 1997 (Rafnsson and Gudmundsson 1997), another began in 1963 and ended in 2010 (Rafnsson and Kristbjornsdottir 2014). One deckhand died on the second day following the discovery of the leak, 15 others experienced symptoms of methyl chloride poisoning. Several of these crew members were admitted to hospital and treated there for several weeks. Over the course of the 18 months following the accident, 2 crew members developed severe depression and committed suicide, another crew member was lost at sea. One deckhand emigrated to another country 3 years later; no follow-up data could therefore be collected. In all, 24 crew members (18 deckhands, 6 officers) were available during the follow-up period from 1966 to 1997 (Rafnsson and Gudmundsson 1997) and 27 crew members (20 deckhands, 7 officers) were available during the follow-up period from 1963 to 2010 (Rafnsson and Kristbjornsdottir 2014). The reference group consisted of 5 persons for each crew member; these were selected from the register of deckhands and officers to match the crew members in terms of age and occupation (120 and 135 reference persons, respectively). The follow-up data were taken from the national Causes of Death Register of Iceland and the Icelandic Cancer Register. The number of crew members who died of cardiovascular diseases was significantly increased, particularly among the deckhands who had been exposed to higher concentrations (deckhands: 5/18, rate ratio (RR) 3.9; 95% CI: 1.0–14.4; officers: 3/6; RR: 1.7; 95% CI: 0.3–6.4) (Rafnsson and Gudmundsson 1997). This was confirmed by the increase in mortality resulting from cardiovascular diseases reported by the later study (hazard ratio (HR): 2.06; 95% CI: 1.02–4.15). The authors attributed the 2 cases of suicide to methyl chloride exposure (HR: 13.76; 95% CI: 1.18–160.07). Overall, a comparison of the crew members and the reference group demonstrated an increase in mortality (HR: 2.10; 95% CI: 1.28–3.46) (Rafnsson and Kristbjornsdottir 2014). However, the results of the 2 studies are of limited relevance as the exposure levels could only be estimated in retrospect and there are no data for possible confounders.

A total of 9 male test persons were exposed to methyl chloride at rest for 1 (n = 2), 3 (n = 3) or 7.5 (n = 4) hours a day; the group exposed for 7.5 hours also exercised during exposure. The test persons were exposed according to the following scheme: week 1: 2 days 0 ml/m^3; week 2: 5 days 100 ml/m^3; week 3: 4 days 20 ml/m^3; week 4: 2 days 0 ml/m^3; week 5: 5 days 100 ml/m^3 (fluctuating between 50 and 150 ml/m^3); week 6: 2 days 150 ml/m^3 and 1 day 0 ml/m^3. Nine female test persons were exposed for 1 (n = 2), 3 (n = 3) or 7.5 (n = 4) hours a day according to the following scheme: week 1: 1 day 0 ml/m^3; week 2: 5 days 100 ml/m^3; week 3: 1 day 0 ml/m^3. The test persons underwent extensive medical examinations during the study (once a week) and after the last exposure; these included clinical examinations, blood tests, biochemical parameters, ECG and cognitive tests. In the group of male test persons exposed for 7.5 hours, electroencephalograms, visual evoked potentials and electromyograms were taken at several time points. The blood pressure and body temperature of the test persons were checked each day; they were asked about subjective symptoms and urinalysis was performed. The exhaled air was analysed both immediately and 15, 30, 60, 120 and 180 minutes after exposure. The methyl chloride concentrations in the blood and in the exhaled air were increased by a factor of 2 to 6 in 3 male and in 1 female test person. Changes in the results of behavioural/cognitive tests, in the ECG and in ventilation frequency were not observed at any time point after exposure. Slight respiratory acidosis was determined after exposure to a methyl chloride concentration of 100 ml/m^3 (fluctuating) while exercising. Several test persons developed a cold during the study and had a sore throat (Stewart et al. 1980).

A follow-up study investigated mortality in 852 male workers who were employed at a butyl rubber plant for at least 1 month between the years 1943 and 1978. Information about their health status was obtained from employee forms, medical records and death certificates. The age-specific mortality rates of the white, male U.S. population during the same calendar years were used as the control data. The described work activities and the workplaces at which these activities were carried out were assigned to the categories low, moderate or high exposure depending on the possible level of methyl chloride exposure (Section 4.7.2). Mortality was not increased after evaluating all causes of death (standardized mortality ratio (SMR): 82, 95% CI: 68–98) or the deaths caused by cardiovascular diseases (SMR:

97, 95% CI: 76–123) (Holmes et al. 1986). As the SMR for deaths caused by cardiovascular diseases tended toward 100, the EPA (US EPA 2001) concluded that substance-related effects may have been overlaid by a "healthy worker effect". This study is of only limited relevance for the evaluation, as the exposure levels were only estimated and exposure to other chemicals may have occurred.

A study investigating the workers of a chemical production plant who were exposed also to methyl chloride (Olsen et al. 1989) has not been included in the evaluation of the toxicity of methyl chloride because of exposure to other substances and a lack of data for the level and duration of exposure.

4.3 Local effects on skin and mucous membranes

Irritation did not occur in any of 24 test persons exposed for 2 hours to a methyl chloride concentration of 10 ml/m^3 (Löf et al. 2000). Furthermore, the test persons in the study of Stewart et al. (1980) did not report any subjective symptoms after exposure to up to 150 ml/m^3 and the study of Putz-Anderson et al. (1981) did not describe signs of irritation at 200 ml/m^3.

4.4 Allergenic effects

There are no data available.

4.5 Reproductive toxicity

A case study presented the case of a child with sacral agenesis whose mother had been exposed to methyl chloride and ammonia. A second study described an association between the occurrence of sacral agenesis in 5 children and their mothers' exposure to trichloroethylene, methyl chloride and other chemicals during pregnancy. In a study in former female employees of a microelectronics assembly plant in New Mexico that compared 90 mother–child pairs with a group without exposure to chemicals, an increased risk of spontaneous abortion was found among those with exposure to various chemicals. However, methyl chloride was only one substance among a large number of other substances to which the women were exposed such as chlorofluorocarbons, chlorinated hydrocarbons, glycol ethers, isopropyl alcohol, acetone, toluene, xylene and "alcohol" (US EPA 2001). As a result of the small number of cases and the exposure to several substances, the reproductive toxicity of methyl chloride cannot be evaluated on the basis of this study.

4.6 Genotoxicity

There are no data available.

4.7 Carcinogenicity

4.7.1 Case–control studies

A case–control study investigated the relationship between exposure to chemicals at the workplace and pancreatic cancer based on the death certificates of 63 097 persons in 24 US states who had died of pancreatic cancer from 1984 to 1993. The data from the death certificates of 252 386 persons who had not died of cancer served as control. The workplace was determined from the death certificates and potential exposure to certain chemicals, including methyl chloride, was assessed using a job exposure matrix. However, the study is only of limited relevance because it was not possible to obtain detailed information about the workplace, exposure to chemicals and possible confounders from the death certificates. No association was found between the occurrence of pancreatic cancer and the estimated levels of low, medium or high exposure (no other details) to methyl chloride (Kernan et al. 1999).

A case–control study investigated the occurrence of cancer of the respiratory tract (trachea, bronchi, lungs or pleura) in persons who had worked at a silicone production plant between 1943 and 1980. An earlier retrospective mortality

study had found an increase in the number of respiratory cancers among the 1942 persons who were employed at the plant during this time period; however, the increase was not statistically significant (observed: 30; expected: 24.9; SMR: 120.5; 95% CI: 81.3–172.0). There were 18 cases of cancer among the workers who had been employed there for more than 20 years (expected: 8.5, SMR: 211.9; 95% CI: 131.9–351.6). A case–control study was carried out to investigate these findings in greater detail. For each person with cancer, four controls were chosen from a roster of all employees who had ever worked at the plant. These were matched with the cases within 5 years of birth year and calendar date at hire. The primary sources for identifying the cancer cases were death certificate information and health insurance files. Information about smoking habits was obtained either from medical questionnaires completed during occupational health surveillance examinations carried out by the company or by interviewing co-workers. A possible association between the occurrence of respiratory cancer and the workplace within the plant, the job title and exposure to chemicals was investigated. A historical survey was carried out to determine which substances were present at the work areas in question: (1) substances that are known to cause respiratory cancer (including asbestos, chromium, cadmium), (2) substances that are suspected of causing respiratory cancer (including dimethyl sulfate, formaldehyde, vinyl chloride) and (3) substances that could potentially be inhaled (mainly during silicone production) or for which there are too few data available to confirm their safety (including chlorosilanes, methyl chloride, amorphous silica). To estimate exposure levels, each plant job was classified as having no contact with a specific substance, occasional contact (the substance was used in the general work area, but not directly handled) and routine contact. The following odds ratios were determined for exposure to methyl chloride for all persons irrespective of duration of employment: occasional contact: 0.39 (95% CI: 0.17–0.92), routine contact: 0.91 (95% CI: 0.41–2.02) and no contact: 0.56 (95% CI: 0.26–1.20); for persons who were employed for more than 5 years: occasional contact: 0.28 (95% CI: 0.08–0.96), routine contact: 0.53 (95% CI: 0.17–1.59) and no contact: 0.43 (95% CI: 0.18–1.00); and for persons employed for more than 10 years: occasional contact: 0.29 (95% CI: 0.06–01.27), routine contact: 0.87 (95% CI: 0.27–2.79) and no contact: 0.48 (95% CI: 0.18–1.22). It was thus not possible to establish a significant association between the occurrence of cancer of the respiratory tract and exposure to methyl chloride (Dow Corning Corporation 1994). The study has considerable shortcomings (including the relatively small number of cancer cases, no determination of exposure concentrations, an inaccurate description of the cases, controls and methods, an unusually large fraction of smokers, both in the group of persons with cancer and in the group of controls). The validity of this study is therefore severely limited and the study cannot be used for the evaluation.

4.7.2 Cohort studies

The cohort studies described above in Section 4.2 that were carried out to investigate an incident involving accidental exposure to methyl chloride on a ship (Rafnsson and Gudmundsson 1997; Rafnsson and Kristbjornsdottir 2014) did not find evidence that methyl chloride had an effect on mortality from cancer in general (deckhands: RR: 0.6; 95% CI: 0.0–4.4; officers: RR: 5.0; 95% CI: 0.4–43.8) or from lung cancer (deckhands: RR: 2.7; 95% CI: 0.1–53.6; officers: no lung tumours) (Rafnsson and Gudmundsson 1997). However, the cohorts and number of cases were very small, the 95% CIs are very wide and include the 1. It is therefore not possible to draw any clear conclusions about cancer risk. This is true also for the follow-up study carried out by Rafnsson and Kristbjornsdottir (2014). In this study, the hazard ratio for all types of cancer was 2.07 (95% CI: 0.85–5.04) and for renal cancer 9.35 (95% CI: 1.28–68.24). This study does not include data for possible confounders (Rafnsson and Kristbjornsdottir 2014).

In the follow-up study described above in Section 4.2 that was carried out in workers of a butyl rubber manufacturing plant (Holmes et al. 1986), no statistically significant increase in mortality from malignant neoplastic lesions was found with respect to the time of initial hire (SMR: 1943–1950: 58; 1951–1960: 107; 1961–1978: –; 95% CI not specified), the duration of exposure to methyl chloride (SMR: less than 12 months: 92; 12 to 60 months: 25, more than 60 months: 64; 95% CI not specified) or the level of exposure (SMR: low exposure: 42; medium: 45, high: 65; 95% CI not specified). Overall, the workers (white workers: SMR: 66, 95% CI: 40–103; non-white workers: SMR: 63, 95% CI: 32–113) were not found to have increased mortality from malignant neoplasms (ICD (International Statistical Classification of Diseases) 140–209: digestive organs/peritoneum (ICD 150-159), respiratory system (ICD 160-163), lymphatic tissue (ICD 200-209), other tissue (ICD 190-199)) (Holmes et al. 1986). As the level of exposure was only estimated, confounders were not

taken into consideration and there may have been exposure to other chemicals, this study cannot be used for the evaluation of methyl chloride.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

Only one study of acute toxicity has become available since the publication of the supplement in 1992. This is an unpublished study that is described in the REACH dossier (ECHA 2020 a) and was carried out according to OECD Test Guideline 403. Groups of 5 male and 5 female HsdRccHan (TM):WIST rats were exposed nose-only by inhalation to 99.99% methyl chloride at concentrations of 4020, 8420 or 21 800 mg/m^3 (1918, 4016, 10 399 ml/m^3) for 4 hours, followed by an observation period of 14 days. The breathing frequency was increased in all animals during exposure, at the time point of removal from the exposure chamber and 1 hour after exposure (the animals of the low concentration group did not exhibit any noticeable changes after this time point, no further data are available for the other 2 concentration groups). In the high concentration group, slightly reduced body weights and reduced body weight gains were observed in 2 females during the first week after exposure; these effects were observed in 1 of the 2 animals also in the second week. In 2 females and 1 male of the medium concentration group, slightly reduced body weights or reduced body weight gains were likewise observed during the first week after exposure. The body weight gains of all the other animals remained in the normal range over the course of the study. Dark spots on the lungs and kidneys with a mottled appearance were detected in several animals (no other details) by gross-pathological examination. The 4-hour LC_{50} was above 21 800 mg/m^3.

5.2 Subacute, subchronic and chronic toxicity

The inhalation studies that investigated the lowest concentrations and a 2-year study are described in detail below. The studies are shown in Table 1.

The functional (performance in the accelerated rotarod test) and morphological effects of continuous and intermittent exposure to methyl chloride were investigated in an 11-day study in female C57BL/6 mice. Groups of 12 mice were exposed to methyl chloride (purity 99.5%) continuously for 22 to 22.5 hours or intermittently for 5.5 hours a day. Two different exposure series were used: in the first series, the groups were exposed continuously to concentrations of 0, 100, 200 or 400 ml/m^3 and intermittently to 0, 400, 800 or 1600 ml/m^3. In the second series, the groups were exposed continuously to concentrations of 0, 15, 50 or 150 ml/m^3 and intermittently to 0, 150 or 2400 ml/m^3. The mice were trained for the neurofunctional rotarod test 4 times a week for a total of 2 weeks. Only mice that were able to stay for 1 minute on a rod rotating at a speed of 20 revolutions per minute (rpm) were chosen to participate in the neurofunctional test. Additional training trials were held on days 6, 8 and 10 of exposure, routinely at a rotating speed of 20 rpm. The animals were tested on days 4, 8 and 11 of exposure on a rod with accelerating speed (from 10 to a maximum of 70 rpm) and timed for the length of time they were able to remain on the rod. In the first exposure series, 6 randomly selected animals were examined morphologically; in the second series, all 12 animals were examined. As specified in the protocol, animals were sacrificed and examined on days 4 and 8 and after the end of exposure. Additionally, several animals of the control group and of the group continuously exposed to 150 ml/m^3 underwent necropsy on days 1, 2 and 6. The cerebellum, cerebrum, brain stem, peripheral nerves, vertebrae, spinal cord and a routine spectrum of other tissues (including liver, kidneys, thymus) were examined. The animals that were **continuously** exposed to a concentration of 400 ml/m^3 had to be sacrificed prematurely after 4 days because of their moribund state. After 3 days of continuous exposure to 200 ml/m^3, ataxia and reduced feed consumption were observed; after 5 days, the animals had died or were moribund. After 10.5 days of continuous exposure to 150 ml/m^3, the animals were emaciated and had to be sacrificed because of their moribund condition. After 5 days of **intermittent** exposure to 2400 ml/m^3, the animals were moving more slowly, had ruffled fur and were emaciated. They had to be sacrificed after 8 or 9 days because of their moribund state. Less severe effects were observed in the animals of the 1600 ml/m^3 group: the hind legs were

stiff after exposure for 11 days. Several mice of this group exhibited increased excitability and 2 mice developed a tendency to rear up on their hind legs. However, these effects usually disappeared overnight during the recovery period.

After **continuous** exposure to concentrations of 100 ml/m^3 and above, degenerative lesions (pyknosis and karyorrhexis) were detected in the cerebellum of all animals by histopathological examination; these effects primarily affected the granular cell layers (100 ml/m^3: slight degeneration, 150 ml/m^3: moderate degeneration, 200 and 400 ml/m^3: severe degeneration). At concentrations of 100 ml/m^3 and above, effects that are probably attributable to glycogen depletion were observed in the liver. The severity of the effects increased with the concentration. No substance-related changes were determined in the groups continuously exposed to 15 or 50 ml/m^3. In the rotarod test used to evaluate neurofunctional performance, a statistically significant decrease in performance was observed in the animals exposed to 150 ml/m^3 on days 4 and 8 (no tests were carried out on day 11 because it had been necessary to sacrifice the animals earlier due to their moribund condition). The animals of the 200 and 400 ml/m^3 groups could not be tested because of their moribund condition. The NOAEC (no observed adverse effect concentration) for continuous exposure was 50 ml/m^3, the LOAEC (lowest observed adverse effect concentration) was 100 ml/m^3.

After **intermittent** exposure to concentrations of 400 ml/m^3 and above, slight degeneration of the granular cell layer of the cerebellum was observed in several animals (400 ml/m^3: 33% of the animals affected, 800 ml/m^3: 67%, 1600 ml/m^3: 65%). In the group with intermittent exposure to 2400 ml/m^3, slight to moderate degeneration was determined in all animals. Effects on the liver (no other details) developed after intermittent exposure to concentrations of 400 ml/m^3 and above; these were attributed to glycogen depletion. However, neither degeneration nor necrosis was observed. Signs of renal toxicity (very slight multifocal degeneration and regeneration of the tubules, eosinophilic staining in the tubules) were found only in the group exposed to 2400 ml/m^3. In the groups exposed to 800 and 1600 ml/m^3, the performance in the rotarod test was decreased on day 4 of exposure, but not on days 8 and 11. The animals exposed to 2400 ml/m^3 performed less well on the rotarod on days 4 and 8. It was not possible to test the animals on day 11 because of their moribund state. Overall, on the basis of the effects that were observed after intermittent exposure, a NOAEC of 150 ml/m^3 and a LOAEC of 400 ml/m^3 were determined for the effects on the cerebellum and a NOAEC of 400 ml/m^3 and a LOAEC of 800 ml/m^3 for performance in the rotarod test.

The NOAEC and LOAEC for continuous and intermittent exposure are about proportional to the product of exposure concentration and duration and thus follow Haber's rule (concentration × time = effect). However, a steeper concentration–effect relationship was determined for the performance in the rotarod test after continuous exposure (Landry et al. 1985).

In a 2-year inhalation study (Battelle Columbus Laboratories 1981) with F344 rats and B6C3F1 mice, groups of 120 animals per sex were exposed to methyl chloride concentrations of 0, 50, 225 or 1000 ml/m^3 (purity 99.97%) (6 hours/day, 5 days/week, except on holidays). The animals were examined for signs of toxic effects and their body weights were monitored over the entire course of the study (only the average of the total weight of the mice that were in each cage (4 or fewer animals) was determined). Ten animals per sex and concentration group were sacrificed after 6, 12, 18 and 24 months. A histopathological examination was carried out and the blood and urine were analysed. The remaining animals were examined histopathologically after 24 months. In the **rats**, corneal opacity was observed after 6 months, in some cases in combination with conjunctivitis. This effect was observed in animals from all groups including the control groups. After 18 months, however, the frequency of this effect was increased with statistical significance only in the females in comparison with the frequency determined in the controls. After 24 months, no statistically significant differences were found between the exposed groups and the controls. According to the summary, the effects after 18 months may have been substance-related. In addition, slight corneal opacity was observed only after 12 months; this effect occurred more frequently in the exposed animals than in the control animals, but concurrently with a viral infection. The viral infection may have led to reduced lacrimal secretion, which left the eyes more vulnerable to the irritant effects of the substance. Bilateral diffuse degeneration and atrophy of the seminiferous tubules were increased with statistical significance 12 and 18 months after exposure to a methyl chloride concentration of 1000 ml/m^3 (4/10 after 12 months, controls 1/10; 10/20 after 18 months). After 24 months, interstitial cell hyperplasia and adenomas were found in all male rats; these occur as a natural part of the aging process. As a result, however, it was no longer possible to detect any further substance-related degeneration and atrophy of the seminiferous tubules. A number of

histopathological findings suggest that methyl chloride promotes cellular hyperplasia; however, with a concurrent reduction in the size of the interstitial tumours (Battelle Columbus Laboratories 1981; US EPA 2001).

Both in the female and in the male **mice**, mortality was very high in all groups (number of animals that died during the study (female mice/male mice): controls: 33/75; 50 ml/m^3: 34/62; 225 ml/m^3: 25/62; 1000 ml/m^3: 73/93). This was attributed to the circumstance that several animals were housed together, leading to dominance fights, particularly during the first 6 months. Due to the high mortality in the high concentration group, the study was terminated in male mice after 21 months and in female mice after 22 months. Signs of neurofunctional impairment (clutch response) and central nervous system (CNS) toxicity such as tremor and paralysis were observed in almost all male and female mice exposed to 1000 ml/m^3 (SCOEL 2017; US EPA 2001). Other effects determined histopathologically only in the high concentration group were degeneration and atrophy of the granular layer of the cerebellum in 3 of 7 males and 6 of 8 females after exposure for 18 months (Battelle Columbus Laboratories 1981; US EPA 2001) and in 17 of 18 females after 22 months. Cerebellar lesions were found in the animals that died spontaneously during the first 7 months (female mice: 9/20, male mice: 15/24), and also in the animals that died spontaneously between 18 and 21 or after 22 months (female mice: 35/37, male mice: 45/47). In 7 male mice (of a total of 43 examined animals) that died prematurely or were sacrificed between 18 and 21 months, germinal cell degeneration and giant cell formation in the testes coupled with atrophy of the seminiferous tubules were observed (controls: 1/20 after 24 months). The hepatocellular lesions found in the male mice of the group exposed to 1000 ml/m^3 (vacuolization, karyomegaly, cytomegaly, multinucleated hepatocytes, degeneration) were observed also in the female mice of the high concentration group, but were not as severe. Hyperplasia and karyomegaly of the renal tubules and renal adenomas and carcinomas were observed in the male mice of the high concentration group from 12 months of exposure onwards (Section 5.7). In the 1000 ml/m^3 group, lesions in the spleen (ranging from lymphoid depletion to atrophy) developed in mice of both sexes from 6 months of exposure onwards. The US EPA (2001) concluded that the occurrence of cysts in the renal cortex of mice of the 1000 ml/m^3 group (7 males, 1 female), in 1 male and 1 female of the 225 ml/m^3 group and in 6 males of the 50 ml/m^3 group (controls: 1 male) cannot be attributed to exposure to the substance, as the incidences were within the range of that for the historical controls for this mouse strain. Axonal swelling and degeneration of the spinal nerves were observed in all groups of mice including the control group (multifocal axonal swelling and degeneration of the lumbar spinal nerves after exposure for 24 months: controls: ♀: 36/39, ♂: 11/12; 50 ml/m^3: ♀: 33/39, ♂: 16/16; 225 ml/m^3: ♀: 48/49, ♂: 21/21; 1000 ml/m^3: no data because of termination after 21 or 22 months); however, no concentration–effect relationship and no underlying functional anomalies were found (Battelle Columbus Laboratories 1981; US EPA 2001). Overall, a NOAEC for rats and mice of 225 ml/m^3 was determined from the findings of the 2-year study. A LOAEC of 1000 ml/m^3 was established for rats on the basis of the effects on the testes; in mice, mortality and neurotoxicity were observed at 1000 ml/m^3.

Considerable errors were made while carrying out the study (including the incorrect determination of the sex of the mice, resulting in pregnancies, and switching of the concentrations given to the mice of the 50 and 1000 ml/m^3 groups on 3 consecutive days). However, the authors concluded that the validity of the findings was not affected by these errors (Battelle Columbus Laboratories 1981; US EPA 2001).

Other studies of toxicity after repeated exposure are found in Table 1.

Tab. 1 Toxicity of methyl chloride after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 40 ♂, 40 ♀	2 or 3 days, 24 hours/day, 0, 200, 500, 1000, 2000 ml/m³ observation for 12 days	2 days: **200 ml/m³ and above**: ♂ and ♀: liver: AP ↓ (not statistically significant); **500 ml/m³ and above**: ♂ and ♀: body weights ↓; liver: slight discoloration (only ♂), AP ↓ (only ♂ statistically significant); epididymis: inflammation, degeneration, interstitial oedema, sperm granulomas, sperm ↓, (effects more pronounced during observation period, also testicular atrophy); **1000 ml/m³ and above**: all animals lethargic; adipose tissue ↓; liver: accumulation of lipids (only ♂); kidneys: necrosis, degeneration, cytoplasmic heterogeneity, regeneration, accumulation of lipids (only ♀), changes in urine parameters; blood: red blood cells, haematocrit, haemoglobin ↑ (resulting from dehydration and haemoconcentration in moribund animals); observation period: 1 ♀ died; **2000 ml/m³**: all animals lethargic, moribund or dead (14/20 ♂ and 10/20 ♀); blood: BUN, AST, ALT, total bilirubin ↑; AP ↓; observation period: all animals died; 3 days: **200 ml/m³ and above**: LOAEC; ♂: body weight gains ↓; ♂ and ♀: liver: effects increasing with the concentration (at 500 ml/m³ and above (only ♂) also during the observation period): dark discoloration, necrosis, inflammation, degenerative lesions, lipid levels ↑, AP ↓ (only ♀ with statistical significance); **500 ml/m³ and above**: ♂ and ♀: body weights ↓; liver: AP ↓ (♂ statistically significant); epididymis: inflammation, degeneration, interstitial oedema, sperm granulomas, sperm ↓ (effects found also during the observation period); **1000 ml/m³ and above**: ♂ and ♀: all animals ill, moribund; kidneys: necrosis, degeneration, cytoplasmic heterogeneity, regeneration, changes in urine parameters; testes: absolute and relative weights ↓; blood: red blood cells, haematocrit, haemoglobin ↑ (resulting from dehydration and haemoconcentration in moribund animals), BUN, AST, ALT, total bilirubin ↑, slight neutrophilia; observation period: 6/10 ♂ and 8/10 ♀ died; **2000 ml/m³**: mortality 100%	Burek et al. 1981; US EPA 2001
rat, F344, 10 ♂, 10 ♀	11 days (5 days – 2 days break – 4 days), 6 hours/day, 0, 2000, 3500, 5000 ml/m³	controls: no lesions observed; **2000 ml/m³**: LOAEC; liver: hepatocellular degeneration (♂ 0/10; ♀ 8/10); kidneys: degeneration and necrosis of renal tubules (♂ 8/10; ♀ 0/10); testes: degeneration (♂ 10/10); epididymis: sperm count ↓, exfoliated spermatocytes, giant cells, cellular debris, eosinophilic hyaline droplets; **3500 ml/m³**: 2/10 ♀ sacrificed in extremis on day 5; diarrhoea (♂ 1/10); discoloration of the urine (♀ 3/10); liver: hepatocellular degeneration (♂ 9/10; ♀ 9/10); kidneys: degeneration and necrosis of renal tubules (♂ 10/10; ♀ 5/10); testes: degeneration (♂ 10/10); epididymis: sperm cells ↓, exfoliated spermatocytes, giant cells, cellular debris, eosinophilic hyaline droplets; adrenal glands: fatty degeneration (♂ 4/10; ♀ 10/10); **5000 ml/m³**: 6/10 ♂ and 5/10 ♀ sacrificed in extremis on day 5; diarrhoea (♂ 10/10 and ♀ 1/10 on day 3); lack of coordination of the front legs, paralysis of the hind legs (day 5, ♂ 2/10 and ♀ 1/10); discoloration of the urine (♂ 3/10; ♀ 2/10); liver: hepatocellular degeneration (♂ 10/10; ♀ 9/10); kidneys: degeneration and necrosis of renal tubules (♂ 10/10; ♀ 10/10); cerebellum: degeneration of the granular layer (♂ 3/10; ♀ 2/9); testes: degeneration (♂ 10/10); epididymis: sperm cells ↓, exfoliated spermatocytes, giant cells, cellular debris, eosinophilic hyaline droplets; adrenal glands: fatty degeneration (♂ 10/10; ♀ 10/10)	Morgan et al. 1982

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, F344, 10 ♂, 10 ♀	90 days, 6 hours/day, 5 days/week, 0, 375, 750, 1500 ml/m³	**375 ml/m³**: NOAEC; **750 ml/m³**: ♂ and ♀: body weights ↓ (weeks 6–12); liver: vacuolar changes in the cytoplasm of the hepatocytes (7/18, controls: 7/19; 5 times more frequent in ♀ (no other details)); **1500 ml/m³**: ♂ and ♀: body weights ↓ (weeks 3–13); liver: vacuolar changes in the cytoplasm of the hepatocytes (9/14, 5 times more frequent in ♀ (no other details)); liver infarction in 1 ♀	US EPA 2001
rat, Sprague Dawley, 10 ♂, 10 ♀	93–95 days, 6 hours/day, 5 days/week (64–66 exposures in total), 0, 50, 150, 400 ml/m³	**150 ml/m³**: ♀: specific urine weights ↓ (not at higher concentrations); **400 ml/m³**: NOAEC; ♂: liver: relative weights ↑, several hepatocytes revealed slight, reversible changes in appearance (5/10); specific weight of urine ↓	McKenna et al. 1981; US EPA 2001
rat, F344, 120 ♂, 120 ♀	24 months, 6 hours/day, 5 days/week, 0, 50, 225, 1000 ml/m³ interim examination after 6, 12, 18 months	**225 ml/m³**: NOAEC; **1000 ml/m³**: ♀ and ♂: body weights ↓, body weight gains ↓; brain: absolute weights ↓ (♀ only 12 and 18 months); ♂: liver: relative weights ↑; kidneys: relative weights ↑; testes: absolute weights ↓, bilateral diffuse degeneration and atrophy of the seminiferous tubules; ♀: liver: absolute weights ↓; kidneys: absolute weights ↓; lungs: relative weights ↓	Battelle Columbus Laboratories 1981
mouse, C3H, 5 ♂, 5 ♀	12 days, consecutive, 6 hours/day, 0, 500, 1000, 2000 ml/m³	controls: no lesions observed; **500 ml/m³**: LOAEC; liver: hepatocellular degeneration (♂ 2/5); **1000 ml/m³**: ♂ 1/5 dead on day 11; kidneys: basophilia of renal tubules (♂ 2/5; ♀ 5/5), degeneration and necrosis of renal tubules (♂ 1/5); haematuria (all ♀, day 8); **2000 ml/m³**: all animals dead or moribund on day 5; liver: hepatocellular degeneration (♂ 4/5); kidneys: degeneration and necrosis of renal tubules (♂ 5/5; ♀ 5/5), haematuria (5/5 ♀, day 4; 5/5 ♂, days 4 and 5)	Morgan et al. 1982
mouse, C57BL/6, 5 ♂, 5 ♀	12 days, consecutive, 6 hours/day, 0, 500, 1000, 2000 ml/m³	controls: no lesions observed; **500 ml/m³**: LOAEC; liver: hepatocellular degeneration (♂ 3/5; ♀ 2/5); **1000 ml/m³**: liver: hepatocellular degeneration (♂ 3/5; ♀ 3/5); kidneys: basophilia of renal tubules (♂ 2/5), haematuria (all ♀, day 8); cerebellum: degeneration of the granular layer (♂ 3/5; ♀ 2/5); **2000 ml/m³**: all animals dead or moribund on day 5 (1 ♂ on day 2); liver: hepatocellular degeneration (♂ 5/5); kidneys: degeneration and necrosis of renal tubules (♂ 3/5; ♀ 5/5), haematuria (all ♀, day 4); cerebellum: degeneration of the granular layer (♂ 0/5; ♀ 4/5)	Morgan et al. 1982
mouse, B6C3F1, 5 ♂, 5 ♀	12 days, consecutive, 6 hours/day, 0, 500, 1000, 2000 ml/m³	controls: no lesions observed; **500 ml/m³**: LOAEC; kidneys: basophilia of renal tubules (♂ 1/5); **1000 ml/m³**: kidneys: basophilia of renal tubules (♂ 3/5; ♀ 2/5), haematuria (all ♀, day 8); **2000 ml/m³**: all animals dead or moribund (♂ on day 2, ♀ on day 5); liver: hepatocellular degeneration (♂ 5/5; ♀ 4/5); kidneys: degeneration and necrosis of renal tubules (♂ 1/5; ♀ 5/5), haematuria (all ♀, day 4); cerebellum: degeneration of the granular layer (♂ 0/5; ♀ 2/5)	Morgan et al. 1982

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, C57BL/6, 12 ♀	11 days, consecutive, 22–22.5 hours/day, series 1: 0, 100, 200, 400 ml/m³, series 2: 0, 15, 50, 150 ml/m³	**50 ml/m³**: NOAEC; **100 ml/m³ and above**: liver: hepatocytes smaller in size; cerebellum: degeneration of the granular layer; **150 ml/m³ and above**: all animals moribund (sacrificed after 10.5 days), emaciation, body weights ↓; kidneys: relative weights ↑; liver: small and pale livers, absolute weights ↓, necrosis; thymus and spleen: smaller in size; performance in the rotarod test ↓; **200 ml/m³ and above**: ataxia; feed consumption ↓; all animals moribund or dead (after 5 days); **400 ml/m³**: all animals moribund (sacrificed after 4 days)	Landry et al. 1985
mouse, C57BL/6, 12 ♀	11 days, consecutive, 5.5 hours/day, series 1: 0, 400, 800, 1600 ml/m³, series 2: 0, 150, 2400 ml/m³	**150 ml/m³**: NOAEC; **400 ml/m³ and above**: liver: effects caused by glycogen depletion (without degeneration or necrosis); cerebellum: lesions; **800 ml/m³ and above**: performance in the rotarod test ↓ (only on day 4, not on days 8 and 11); **1600 ml/m³ and above**: feed consumption ↓; stiffness in the hind legs (after 11 days); liver: absolute and relative weights ↑ (not at 2400 ml/m³!); thymus: smaller in size, weights ↓; performance in the rotarod test ↓ (only on day 4, not on days 8 and 11); **2400 ml/m³**: all animals moribund (sacrificed after 8–9 days), movements slow, ruffled fur, emaciation, body weights ↓; spleen: enlarged; thymus: weights ↓; anaemia; red urine; kidneys: slight multifocal degeneration and regeneration of the tubules, eosinophilic staining in the tubules; performance in the rotarod test ↓	Landry et al. 1985
mouse, C57BL/6, 10 ♀	2 weeks, 6 hours/day, 5 days/week, 0, 1500 ml/m³	**1500 ml/m³**: 2/10 animals dead in week 2; ataxia; kidneys: slight degeneration of proximal tubules (2/10); no clinical signs of CNS impairment; inner granular layer of the cerebellum: 1) coagulative necrosis (also in the controls, but to a lesser extent and involving fewer cells) 2) focal malacia (nuclear condensation, karyorrhexis, necrosis, separation of myelinated axons, microvacuolation)	US EPA 2001
mouse, B6C3F1, 10 ♂, 10 ♀	90 days, 6 hours/day, 5 days/week, 0, 375, 750, 1500 ml/m³	**375 ml/m³**: NOAEC; **750 ml/m³**: ♂ and ♀: liver: relative weights ↑; **1500 ml/m³**: ♂ and ♀: liver: relative weights ↑, liver infarction in 1 ♂	US EPA 2001
mouse, CD-1, 10 ♂, 10 ♀	93–95 days, 6 hours/day, 5 days/week (64–66 exposures in total), 0, 50, 150, 400 ml/m³	**400 ml/m³**: NOAEC; ♀: liver: relative weights ↑	McKenna et al. 1981; US EPA 2001
mouse, B6C3F1, 120 ♂, 120 ♀	24 months, 6 hours/day, 5 days/week, 0, 50, 225, 1000 ml/m³ interim examination after 6, 12, 18 months	**225 ml/m³**: NOAEC; ♀: heart: relative weights ↑; **1000 ml/m³**: ♀ and ♂: mortality ↑; body weights ↓, body weight gains ↓; CNS toxicity, neurofunctional disorders (“clutch response”); cerebellum: degeneration and atrophy of the granular layer; absolute brain weights ↓; spleen: atrophy, lymphoid depletion; liver: relative and absolute weights ↑ (♂ only relative); ♂: liver: ALT ↑, hepatocellular degeneration and necrosis (vacuole formation, karyomegaly, cytomegaly, multinuclear hepatocytes, degeneration, less pronounced in ♀); kidneys: absolute weights ↓ (6 and 12 months), hyperplasia, karyomegaly; lungs: relative weights ↑; testes: atrophy and degeneration of the seminiferous tubules (7/43; controls: 1/20); ♀: liver: absolute and relative weights ↑; kidneys: relative weights ↑; heart: absolute and relative weights ↑	Battelle Columbus Laboratories 1981

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
dog, beagle, 3 ♂	3 days, 23.5 hours/day, 0, 200, 500 ml/m³	**200 ml/m³**: NOAEC; **500 ml/m³**: animals calmer, weak but alert, stiff extremities, incoordination, occasional falls, incapable of sitting or standing, tremor, salivation, body weights ↓; neutrophils ↑, lymphocytes ↓ (symptoms had improved in all animals by the end of the study on day 27 after the last exposure); slight lesions in the brain and spinal cord (possibly caused by a viral infection)	US EPA 2001
dog, beagle, 4 ♂	93–95 days, 6 hours/day, 5 days/week (64–66 exposures in total), 0, 50, 150, 400 ml/m³	**50 ml/m³**: liver: slight swelling of the hepatocytes (2/4); **150 ml/m³**: liver: slight swelling of the hepatocytes (1/4); **400 ml/m³**: NOAEC; liver: slight swelling of the hepatocytes (2/4)	McKenna et al. 1981; US EPA 2001
cat, 3 ♂	3 days, 23.5 hours/day, 0, 200, 500 ml/m³	**200 ml/m³ and above**: NOAEC; lesions in the brain and spinal cord in 1/3 animals (controls 1/3); **500 ml/m³**: body weights ↓ (not statistically significant); lesions in brain and spinal cord in 3/3 animals (controls 1/3)	US EPA 2001

ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; BUN: blood urea nitrogen

5.3 Local effects on skin and mucous membranes

Exposure to methyl chloride concentrations of 250 to 465 ml/m³ on 5 days for 90 seconds caused conjunctival hyperaemia in the eyes of 2 rabbits. Corneal lesions did not occur; the light and pupillary reflexes were normal (Greim 1996 a; SCOEL 2017). A recent study of acute inhalation toxicity (Section 5.1) did not find any adverse effects on the respiratory tract or signs of irritation in the airways after nose-only exposure for 4 hours up to 21 800 mg/m³ (10 399 ml/m³) (ECHA 2020 a; SCOEL 2017).

5.4 Allergenic effects

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

All studies were previously described in the 1992 documentation (Greim 1996 a).

Numerous studies in rats reported effects in male animals such as inflammation and granulomas in the epididymis, testicular atrophy and reduced fertility at concentrations of about 500 ml/m³ and above. Sterility was induced at even higher concentrations of 1000 and 3000 ml/m³ (ATSDR 1998; Battelle Columbus Laboratories 1981; Chapin et al. 1984; Greim 1996 a; Hamm et al. 1985; Working et al. 1985 a, b).

In a 2-generation study with exposure of F344 rats to concentrations of 0, 150, 475 and 1500 ml/m³, fertility was reduced in the males at 475 ml/m³ and above. This was manifest in the form of a reduced number of litters. In addition, body weight gains were decreased in the animals of the F0 generation. At 1500 ml/m³, all male F0 animals were sterile with degenerated testes and atrophied seminiferous tubules (Greim 1996 a; Hamm et al. 1985). The NOAEC for fertility was 150 ml/m³ and the NOAEC for effects on the male reproductive organs was 475 ml/m³.

In a 2-year inhalation study in F344 rats, bilateral diffuse degeneration and atrophy of the seminiferous tubules were found in 1 animal of the 1000 ml/m³ group at the interim examination after 6 months of exposure. The incidence for

this effect was increased after 18 months (Battelle Columbus Laboratories 1981; Greim 1996 a). The NOAEC for effects on the male reproductive organs was 225 ml/m^3.

In the 2-year studies, mice were less susceptible to effects on the testes than rats. Thus, the incidences for these types of effects were lower and the lesions developed later in mice than in rats (Battelle Columbus Laboratories 1981).

The results of a dominant lethal test in rats showed that implantation losses were induced by exposure to a methyl chloride concentration of 3000 ml/m^3 (40.9%; 31.4% pre-implantation losses and 9.5% post-implantation losses) (Working et al. 1985 a). However, the pre-implantation losses were primarily caused by toxic effects on the quality and mobility of the spermatocytes, leading to the infertility of the male animals (Working et al. 1985 b). The post-implantation losses may have been secondary effects caused by the epididymal inflammation induced by methyl chloride. The post-implantation losses did not occur if epididymal inflammation was prevented by the administration of the anti-inflammatory agent BW 755C (Chellman et al. 1986; see Section 5.6.2).

5.5.2 Developmental toxicity

With the exception of a new study in rabbits, all studies were already described in detail in the 1992 documentation (Greim 1996 a).

These studies are briefly reviewed below.

In a prenatal developmental toxicity study in F344 rats, delayed body weight gains were observed in the dams and reduced body weights and delayed ossification were observed in the foetuses at the highest methyl chloride concentration tested of 1500 ml/m^3. Teratogenicity was not detected (CIIT 1981 b; Greim 1996 a; Wolkowski-Tyl et al. 1983 b). A NOAEC of 500 ml/m^3 was determined for developmental and maternal toxicity induced by methyl chloride.

In a prenatal developmental toxicity study carried out in C57BL/6 mice, marked maternal toxicity was observed at a methyl chloride concentration of 1500 ml/m^3, resulting in the early sacrifice of the animals. At a concentration of 500 ml/m^3, 16.7% of the foetuses were found to have heart anomalies (a reduction or absence of the atrioventricular valve, chordae tendineae and papillary muscles on the left side (bicuspid valve) in 3 foetuses and on the right side (tricuspid value) in 6 foetuses, in total 9/56 foetuses, 6/17 litters) (CIIT 1981 b; Greim 1996 a; Wolkowski-Tyl et al. 1983 b). The NOAEC for developmental toxicity induced by methyl chloride was 100 ml/m^3 and the NOAEC for maternal toxicity was 500 ml/m^3. According to a comprehensive database that categorizes toxic effects on development (BfR 2020), the absence, but not the reduction in the size, of the heart valve is regarded as a malformation. The publication does not differentiate between reduction in the size and absence of the valve.

In a follow-up study using the same treatment scheme, ataxia, tremor, convulsions, increased mortality and delayed body weight gains were observed in the dams treated with a methyl chloride concentration of 750 ml/m^3. At concentrations of 500 ml/m^3 and above, the following heart anomalies were found in the foetuses: absent or abnormal tricuspid valve, a reduced number of papillary muscles or chordae tendineae on the right side, small right ventricle, globular heart and white spots in the left ventricular wall (CIIT 1981 a; Greim 1996 a; Wolkowski-Tyl et al. 1983 a). The heart anomalies observed in this follow-up study are similar, but do not correspond either qualitatively or quantitatively with the severity of the findings of the first study. The 2 studies used different fixation techniques. In the first study, Bouin's solution was used as a fixative and the trunk was examined by applying a modified Staples' technique (Wolkowski-Tyl et al. 1983 b). In the follow-up study, the visceral examination of the chest was carried out according to Staples' fresh tissue dissection method. All abnormal hearts and representative control samples were subsequently preserved in Carnoy's solution (Wolkowski-Tyl et al. 1983 a). The dissection and analysis of structures as tiny as those found in the mouse heart are technically intricate processes. There are no historical control data. On the basis of the findings of the follow-up study, the NOAEC for the developmental toxicity induced by methyl chloride was 250 ml/m^3 and the NOAEC for maternal toxicity was 500 ml/m^3.

In a letter to the editor, the point was made that no heart defects were found in the foetuses of C57BL mouse dams exposed to methyl chloride concentrations of 250 or 300 ml/m^3 (24 hours) from gestation days 11.5 to 12.5 or to a concentration of 1000 ml/m^3 (12 hours) from gestation days 11.5 to 12. According to the authors, the treatment was

timed to occur during the critical window for cardiac development. In addition, the authors described a large degree of variability in the papillary muscles of the control animals. Furthermore, they suggested that the heart anomalies described were an artefact of the dissection technique (John-Greene et al. 1985). In response, reference was made to the different exposure protocols. Moreover, the critical window for cardiac development in mice is around gestation day 14 (Tyl 1985). In mice, the atrioventricular valves begin to form on gestation day 11; this process is completed sometime between gestation days 14 to 16 (Arts et al. 2019).

In a prenatal developmental toxicity study in groups of 22 New Zealand White rabbits that was carried out according to OECD Test Guideline 414, the animals were exposed to methyl chloride concentrations of 0, 250, 500 or 1000 ml/m^3 from gestation days 6 to 28 (whole body, 6 hours a day). The animals were examined on gestation day 29. No effects of maternal or developmental toxicity were determined up to the highest concentration tested. The NOAEC for developmental toxicity induced by methyl chloride was 1000 ml/m^3, the highest concentration tested (Triskelion 2016).

Following an extensive analysis of the developmental toxicity induced by methyl chloride, the following conclusions were drawn: 1. The "normal" anatomy of the tiny papillary muscles found in the foetal mouse heart and the variability of their appearance (John-Greene et al. 1985) complicate any evaluation. A degree of variability in the number, length and shape of these muscles is considered "normal" also in humans. 2. Pregnant mice are more susceptible than pregnant rats or rabbits to systemic toxicity (mostly CNS), which is most probably due to a species difference in metabolism at higher concentrations. 3. Methyl chloride is extensively metabolized in various tissues and has species-specific metabolic characteristics. This makes it difficult to define a suitable animal model for humans. For this reason, in 2010 the REACH consortium classified methyl chloride in Category 2 for reproductive toxicity according to GHS/CLP (suspected human reproductive toxicant) as a precautionary measure without having any further data for additional species. This classification was based on the conflicting data found in rats and mice, in spite of the doubts expressed by various experts and regulatory bodies regarding the relevance and reproducibility of the foetal mouse data. 4. The mouse strain investigated by the study that developed foetal heart defects is not commonly used for developmental toxicity testing, which complicates the interpretation of the relevance of the collected data. This means that in addition to the low incidences and the lack of reproducibility, there is also a lack of historical controls for these defects. A number of experts have suggested that the kind, number and degree of the heart defects, if not an artefact due to the large inter-animal variability, may also be related to the technical difficulty in performing these examinations in such small objects. In agreement with the conclusions of the working group "Classification and labelling of dangerous substances" as well as with REACH and CLP, the authors concluded that methyl chloride no longer meets the classification criteria for developmental toxicity. This conclusion is supported by the high quality of the overall database and an additional negative dataset from a non-rodent species. The evaluation of the existing scientific evidence does not support the classification of methyl chloride as a developmental toxicant (Arts et al. 2019).

In the 2-generation study in F344 rats mentioned above, no differences in litter size, survival until postnatal day 4 and body weights on postnatal days 0 and 4 were found at methyl chloride concentrations of 150 and 475 ml/m^3 in comparison with the values determined in the control group. All male F0 animals in the 1500 ml/m^3 group were sterile. The number of litters was reduced at 475 ml/m^3 (Greim 1996 a; Hamm et al. 1985). This study did not investigate neurotoxic or behavioural end points in the offspring. None of the available studies investigated these end points in the offspring of dams exposed in utero.

On the basis of 2 dominant lethal tests in rats, the pre-implantation losses were attributed to the infertility of the males that was caused by toxic effects on the quality and mobility of the spermatocytes (see Section 5.6; Chellman et al. 1986; Greim 1996 a; Working et al. 1985 b). Therefore, the NOAEC for perinatal toxicity induced by methyl chloride was 475 ml/m^3.

5.6 Genotoxicity

A number of studies were evaluated in the 1992 documentation (Greim 1996 a). As this supplement includes an evaluation of the end point germ cell mutagenicity, the studies are discussed again in detail in this section.

5.6.1 In vitro

Methyl chloride induced an adaptive response to DNA alkylation in Escherichia coli. This response is regulated by the Ada protein (O^6 methylguanine DNA methyltransferase I). During the repair of methylphosphotriesters in methylated DNA, self-methylation of the Ada protein occurs. This in turn activates transcription and thus the formation of more Ada proteins. A direct methylation of the Ada protein by methyl chloride seems unlikely as an unadapted cell contains only about 2 to 4 Ada molecules. However, Ada incubated with the DNA of Micrococcus luteus pre-treated with methyl chloride did not initiate transcription and thus did not lead to the formation of additional Ada proteins. This suggests that methyl chloride is not able to methylate the DNA sufficiently at the phosphate group to induce Ada as a transcriptional activator. On the basis of these findings, the authors concluded that the response induced by methyl chloride is caused by a very small number of methylphosphotriesters in the DNA. The efficient repair of this damage leads to the self-methylation of Ada and thus to the generation of additional Ada proteins. Higher levels of Ada protein in the cells increase the likelihood that direct methylation will occur and thus the further induction of transcription (Vaughan et al. 1993).

At concentrations of 0.8% and above, gaseous methyl chloride was directly mutagenic in the Salmonella typhimurium strains TA1535 and TA100 (used to detect base-pair substitutions) both with and without the addition of metabolic activation (Du Pont 1977; Greim 1996 a; Longstaff et al. 1984; Simmon et al. 1977). Methyl chloride concentrations in the range from 1% to 7% were not mutagenic in the Salmonella typhimurium strains TA1537 and TA98 (used to detect frame-shift mutations) (Du Pont 1977). In the 8-azaguanine resistance test, methyl chloride concentrations of 10% and above were mutagenic in the Salmonella typhimurium strain TM677 without metabolic activation (Fostel et al. 1985; Greim 1996 a).

In human lymphoblasts (TK6 cells), methyl chloride caused sister chromatid exchange at concentrations of 1% and above with a concurrent reduction in the mitotic index and a delayed cell cycle (Fostel et al. 1985). No induction of DNA strand breaks, as measured by alkaline unwinding, was observed (Fostel et al. 1985). A UDS test (DNA repair synthesis) in primary rat hepatocytes and spermatocytes produced positive results at concentrations of 1% and above without metabolic activation; however, in the tracheal epithelial cells of rats, the test yielded negative results up to the highest concentration tested of 10% (Greim 1996 a; Working et al. 1986).

In a gas exposure system using rotating vessels, methyl chloride caused chromosomal aberrations in CHL/IU cells (Chinese hamster lung cells). Exposure to 4% methyl chloride for 6 hours without metabolic activation induced structural aberrations in 60% of the metaphase cells; with metabolic activation, structural aberrations were found in only about 20% of the cells. In addition, a dose–response relationship was established that was dependent on the duration of exposure (6, 24 and 48 hours) (Asakura et al. 2008).

In a $TK^{+/-}$ test with human lymphoblasts (TK6 cells), methyl chloride induced mutations at concentrations of 2% and above without the addition of metabolic activation. Delays in growth were observed even at the lowest concentrations tested (Fostel et al. 1985). The data for genotoxicity in vitro are shown in Table 2.

Tab. 2 Genotoxicity of methyl chloride in vitro

End point	Test system	Concentration in the gas phase; duration	Effective concentration	Cytotoxicity	Results		References
					–m. A.	+m. A.	
gene mutation	Salmonella typhimurium TA100	0, 2.5%–20%; 8 hours	≥ 2.5%	no data	+	+	Simmon et al. 1977
gene mutation	Salmonella typhimurium TA1535	0, 0.5%–20.7%; 72 hours	≥ 0.8%	at 23%	+	+	Andrews et al. 1976
gene mutation	Salmonella typhimurium TA1535	0%, 1%, 4%, 7%; 48 hours	≥ 4%	1%–7% no effect on cell growth	+	+	Du Pont 1977
	Salmonella typhimurium TA100		≥ 1%		+	+	
	Salmonella typhimurium TA1537				–	–	
	Salmonella typhimurium TA98				–	–	

Tab. 2 *(continued)*

End point	Test system	Concentration in the gas phase; duration	Effective concentration	Cytotoxicity	Results		References
					–m. A.	+m. A.	
gene mutation	Salmonella typhimurium TA1535	0%, 1%, 2%, 5%, 10%; 48 hours	≥ 5%	no cytotoxicity up to 10%	+	+	ECHA 2020 a
	Salmonella typhimurium TA100		≥ 1%		+	+	
	Salmonella typhimurium TA1537				–	–	
	Salmonella typhimurium TA98				–	–	
gene mutation	Salmonella typhimurium TA100	only concentration with the maximum effect given; 72 hours	maximum effect at 10%	no data	+	+	Longstaff et al. 1984
	Salmonella typhimurium TA1535		maximum effect at 5%		+	+	
gene mutation	Salmonella typhimurium TA98	0, 0.001, 0.002, 0.003, 0.007, 0.013, 0.027 µg/plate (sealed desiccator), no other data		≥ 0.027 µg/plate	–	–	ECHA 2020 a
	Salmonella typhimurium TA100		0.001 µg/plate	≥ 0.013 µg/plate	+	+	
gene mutation	Salmonella typhimurium TM677 8-azaguanine resistance	0%, 5%–30%; 3 hours	≥ 10%	50% survival at 20%	+	n. t.	Fostel et al. 1985
SCE	human lymphoblasts TK6	0%, 0.3%–3%; 3 hours	1%	50% survival at 3%, ≥ 1% (reduction in the mitotic index and delayed cell cycle)	+	n. t.	Fostel et al. 1985
DNA strand breaks	alkaline elution, human lymphoblasts TK6	0%, 1%, 3%, 5%			–	n. t.	Fostel et al. 1985
UDS	rat hepatocytes	0%, 0.1%–3%; 18 hours and 0%, 1%–10%; 3 hours	≥ 1%	≥ 3% (18 hours); or 10% (3 hours)	+	n. t.	Working et al. 1986
	rat spermatocytes		≥ 1%	≥ 3% (18 hours); no cytotoxicity (3 hours)	+	n. t.	
	tracheal epithelial cells rat	0%, 1%–10%; 3 hours		≥ 5%	–	n. t.	
CA	CHL/IU	0%, 2%, 4%, 6% –m. A.: 6, 24, 48 hours +m. A.: 6 hours	–m. A: 24 and 48 hours: ≥ 2%; 6 hours: ≥ 4% +m. A: 6 hours: ≥ 4%	6 hours: 50% cell growth at 6% 24 and 48 hours: 50% cell growth at ≥ 2% and ≥ 3%	+	+	Asakura et al. 2008
gene mutation	$TK^{+/-}$ test, human lymphoblasts TK6	0%, 1%–5%; 3 hours	≥ 2%	50% survival at 3%, delays in growth at ≥ 0.3%	+	n. t.	Fostel et al. 1985

CA: chromosomal aberration; m. A.: metabolic activation; n. t.: not tested; SCE: sister chromatid exchange; UDS: test for the induction of DNA repair synthesis

5.6.2 In vivo

An SLRL (sex-linked recessive lethal) test in adult male Drosophila melanogaster (males: Canton-S, females: Basc) found that treatment with 20% methyl chloride for 1.75 hours or 50 minutes induced recessive lethal mutations in the germ cells of all post-meiotic phases (University of Wisconsin 1982). In the alkaline unwinding assay, no DNA–DNA or DNA–protein crosslinks were found in the kidneys of male B6C3F1 mice (5 animals per group), but a small number

of DNA single strand breaks were determined after inhalation exposure to methyl chloride at 1000 ml/m^3 for 6 hours a day on 4 days. The animals were sacrificed 6 hours after the end of exposure (Jäger et al. 1988; Ristau et al. 1989). Male and female B6C3F1 mice were exposed once by inhalation to a methyl chloride concentration of 1000 ml/m^3 for 8 hours. Exposure was followed by the dissection of the liver and kidneys of the animals. DNA–protein crosslinks were found, but only in the kidneys of the males and only if the animals were sacrificed directly after exposure. If the animals were examined 5 hours after exposure, single strand breaks were detected, but no DNA–protein crosslinks. After 48 hours, neither DNA–protein crosslinks nor single strand breaks were found (Greim 1996 a; Ristau et al. 1989, 1990). After repeated exposure of males of the same mouse strain to methyl chloride at 1000 ml/m^3 for 6 hours a day on 4 days, DNA–protein crosslinks were likewise found in the kidneys if the animals were examined directly after exposure. Single strand breaks were detected 5 hours after the end of exposure (ATSDR 1998; Greim 1996 a; Ristau et al. 1990). As discussed in detail in the 1992 documentation, exposure at these high concentrations leads to a marked depletion of glutathione in the kidneys of male mice, which impairs the metabolic pathway that is dependent on glutathione, but facilitates a secondary pathway, oxidative metabolism, and thus the formation of formaldehyde. Glutathione depletion also inhibits formaldehyde dehydrogenase activity, which is likewise dependent on glutathione and responsible for the detoxification of formaldehyde. This leads to an accumulation of the metabolite that is responsible for the formation of DNA–protein crosslinks. In addition to these characteristic lesions, which can rapidly be repaired, the depletion of glutathione leads to an increase in DNA single strand breaks through the formation of reactive oxygen species. These are repaired more slowly (Greim 1996 a). Groups of 3 or 6 male F344 rats (CDF(344)/CrlBr) were exposed by inhalation to ^{14}C-methyl chloride for 6 hours at concentrations of 500 or 1500 ml/m^3. Radioactivity was detected in the nucleobases of the RNA and DNA. However, an analysis of the DNA of the liver, kidneys, lungs and testes found that the radioactively labelled bases were unmethylated (Greim 1996 a; Kornbrust et al. 1982).

Another DNA binding study with ^{14}C-methyl chloride (initial concentration in the chamber 700 ml/m^3, average concentration over 6 hours 21–25 ml/m^3) in F344 rats and B6C3F1 mice likewise did not find evidence of methylation at the O^6 and N^7 positions of guanine in the DNA of the liver and kidneys of the animals. Among the examined organs, the radioactivity was most strongly associated with the DNA of the kidneys of B6C3F1 mice (Greim 1996 a; Peter et al. 1985). ^{14}C-Methyl chloride may not be taken up directly by the DNA but by metabolic incorporation via the C1 pool (Kornbrust et al. 1982). This is one difference between methyl chloride and the structurally related methyl halides methyl bromide and methyl iodide. DNA binding studies carried out with these substances in rats detected 3 different DNA adducts after oral exposure (amount tested in males and females: methyl bromide: 8.3 µmol, methyl iodide: 7.2 µmol) and after inhalation exposure (average exposure levels in 6 hours: methyl bromide: females: 9.2 ml/m^3, males: 3.4 ml/m^3; methyl iodide: females: 3.6 ml/m^3, males: 2.3 ml/m^3). However, these 2 studies likewise found a high level of macromolecular incorporation of ^{14}C into the nucleobases (Gansewendt et al. 1991 a, b).

Groups of 3 male CDF (F344)/CrlBR rats were exposed by inhalation to a methyl chloride concentration of 3500 ml/m^3 for 6 hours a day for 1, 3 or 5 days. UDS was not induced in the tracheal epithelial cells or in the hepatocytes or spermatocytes. The test concentration corresponded to the maximum tolerable exposure level at which mortality in F344 rats does not reach statistical significance. Another group of 3 animals was exposed to a methyl chloride concentration of 15 000 ml/m^3 (equivalent to the maximum tolerable exposure level) for 3 hours. At this concentration, UDS was not observed in the tracheal epithelial cells and in the spermatocytes but was induced to a small extent in the hepatocytes. According to the authors, the fact that the activity of the repair mechanisms was increased slightly only at the very high concentration and only in the hepatocytes demonstrated that methyl chloride was systemically available, but not able to damage the germ cells in the testes. This interpretation is supported by the results of the dominant lethal tests described below, which found that dominant lethal mutations were induced in the sperm present in the vas deferens and epididymis at the time of exposure, but not in the spermatogonia, spermatocytes or spermatids in the testes. The negative results obtained in the tracheal epithelial cells, also at the highest concentration tested, correspond with the negative results obtained in vitro at similar or higher concentrations (see Section 5.6.1; Working et al. 1986).

In a dominant lethal test, groups of 40 male F344 rats were exposed by inhalation to methyl chloride concentrations of 0, 1000 or 3000 ml/m^3 for 6 hours a day on 5 consecutive days. Each of the exposed animals was mated weekly with a single female over a period of 8 weeks. In the group exposed to 1000 ml/m^3, no changes in comparison with the

control group were found other than a slight, but significant increase in pre-implantation losses in the third week following the last exposure. Exposure to methyl chloride at 3000 ml/m^3 led to a decrease in the number of living and dead implants and increased the percentage of pre-implantation and post-implantation losses to 40.9% (pre-implantation losses: 31.4%; post-implantation losses: 9.5%). Pre-implantation losses were significantly increased 2, 3, 4, 6 and 8 weeks after exposure, which suggests that the germ cells in the epididymis and early spermatogenesis in the testes (spermatids and primary spermatocytes) were affected. A statistically significant increase in post-implantation losses was observed only in the first week after exposure; the sperm present in the vas deferens and the epididymis would have been exposed during this time. In the 3000 ml/m^3 group, 30% of the males had sperm granulomas in one or both of the epididymides 17 weeks after the end of exposure (Greim 1996 a; Working et al. 1985 a). In a parallel study, another group of 40 male F344 rats was exposed according to the method described above. Spermatogenesis and the testes were then examined in the rats. Five males per group were sacrificed weekly over a period of 8 weeks and another 5 animals per group were sacrificed 16 weeks after the end of exposure. There were no differences in the effects observed in the animals exposed to a methyl chloride concentration of 1000 ml/m^3 and those found in the controls. More than 50% of the animals in the 3000 ml/m^3 group had unilateral and bilateral sperm granulomas in the epididymides. From week 3 of exposure onwards, the testis weights of these animals were decreased with statistical significance and signs typical for cytotoxicity were observed in the testes: a delay in spermatogenesis and a reduction in the number of spermatogonial stem cells by 60% to 70%. This led to infertility in the treated male animals, which was reversible after 16 weeks (Working et al. 1985 b). In another fertility study, female rats were sacrificed 12 hours after ovulation, which was approximately the time they were mated with male animals that had been exposed to methyl chloride concentrations of 1000 or 3000 ml/m^3. The egg cells in the fallopian tubes were examined. In the 1000 ml/m^3 group, almost 90% of the egg cells were fertilized. In the 3000 ml/m^3 group, only 3.4% of the egg cells were fertilized in week 2; the fraction of fertilized egg cells increased to 72% in week 8. The percentage of unfertilized egg cells over the 8-week period thus corresponds to the percentage of pre-implantation losses determined in the dominant lethal test (Working and Bus 1986). The reduced sperm motility and the increased number of sperm head anomalies are assumed to be caused by chronic inflammation of the epididymis and the formation of sperm granulomas. The authors suggest that the inflammation of the epididymides and the reduced number and quality of the sperm may be the cause of the infertility in males in the dominant lethal tests. On the basis of these findings, the pre-implantation losses are not caused by a genotoxic, but by a cytotoxic mechanism (Greim 1996 a; Working et al. 1985 b).

Another dominant lethal test was used to investigate the role of epididymal inflammation in the formation of dominant lethal mutations: groups of 40 male F344 rats were exposed by inhalation to a methyl chloride concentration of 3000 ml/m^3 for 6 hours a day on 5 days. One group was concurrently given the anti-inflammatory agent BW 755C (4,5-dihydro-1-(3-(trifluoromethyl)phenyl)-1H-pyrazol-3-amine) in daily injections of 10 mg/kg body weight 1 hour before and 1 hour after exposure. Beginning 2 days after exposure, the animals were mated with 1 female weekly over a period of 3 weeks. Methyl chloride induced mutations only in the sperm present in the epididymides at the time of exposure; this was evident because the post-implantation losses occurred in the first week after the end of exposure (number per pregnant female: 0.84; controls: 0.29). This conclusion was further supported by the ratio of dead implantations to the total number of implantations in the first (0.10; controls: 0.04) and second week (0.24; controls: 0.06). Mutations in the sperm that were moving towards the epididymis at the time of exposure would have been observable in the third week after exposure. However, no differences were found between the test and control animals at this point in time. Concurrent treatment with BW 755C reduced both the number of post-implantation losses (number per pregnant female: 0.35) and the ratio of dead implantations to the total number of implantations in the first (0.04) and second week (0.18). If epididymal inflammation was inhibited by treatment with BW 755C, this simultaneously prevented the occurrence of post-implantation losses. This suggests that the effects are caused by inflammatory and not genotoxic mechanisms (Chellman et al. 1986; Greim 1996 a).

5.6.3 Summary

Overall, the findings of the present studies suggest that methyl chloride causes mutagenic and clastogenic effects in vitro at high concentrations. DNA alkylation has not been observed in vivo to date. The positive results obtained in the dominant lethal tests could be explained by cytotoxicity.

5.7 Carcinogenicity

No new data have become available. A 2-year inhalation study in F344 rats and B6C3F1 mice was described and discussed in detail in the 1992 documentation (Greim 1996 a). Groups of 110 animals per sex and concentration were exposed to methyl chloride concentrations of 0, 50, 225 or 1000 ml/m^3 for 12 to 24 months (6 hours/day; 5 days/week). The tumour incidence was not increased in rats of both sexes and in female mice. Kidney tumours (cyst adenomas, adenomas of the renal cortex and papillary cyst adenocarcinomas) were found in the male mice of the high concentration group (18 of 86 animals that could be examined) and in 2 animals of the 225 ml/m^3 group. However, the study had considerable methodological shortcomings and the mortality among the mice was very high, particularly in the high concentration group. Therefore, the study was terminated in this concentration group after only 21 (female mice) and 22 months (male mice). The mechanisms and the conditions necessary for the development of renal tumours, their occurrence only in male mice and the relevance of these findings for humans were presented in detail in the 1992 documentation (Greim 1996 a). For this reason, they are only briefly summarized here: the highest concentration tested is in the range of the concentration (1500 ml/m^3) that was found to lead to increased cell proliferation in the renal tissue of mice. In addition, at 1000 ml/m^3, glutathione levels were depleted to below 5% of the initial levels in mice. This leads to an increase in lipid peroxidation and in the degradation of methyl chloride via CYP2E1 (oxidative metabolic pathway). Glutathione depletion leads to a decrease in cytosolic formaldehyde dehydrogenase activity. Under these special conditions, formaldehyde accumulates as a reactive metabolite. CYP2E1 levels are higher in the renal tissue of male mice than in that of female mice (see Section 3.2).

5.7.1 Comparison with other methyl halides

Carcinogenicity studies were carried out with the methyl halides **methyl bromide** and **methyl iodide**; these were described in detail in the documentation for methyl bromide (Hartwig and MAK Commission 2017 a) and that for methyl iodide (Greim 1996 b). In carcinogenicity studies with inhalation exposure, **methyl bromide** did not lead to an increase in the tumour incidences in B6C3F1 mice (2 years, methyl bromide concentrations: 0, 10, 33 or 100 ml/m^3) or in Wistar rats (29 months, methyl bromide concentrations: 0, 3, 30 or 90 ml/m^3). In an oral study, male and female Wistar rats were given gavage doses of methyl bromide in arachis oil of 0, 0.4, 2, 10 or 50 mg/kg body weight and day (5 days/week, 90 days). At 2 mg/kg body weight and above, local irritation induced by methyl bromide led to a dose-dependent increase in the incidence of hyperplasia of the forestomach in all rats and squamous cell carcinomas in the forestomach of rats of the high dose group (Greim 1996 a).

Several studies that investigated the carcinogenic effects of **methyl iodide** are available. In the studies included in the documentation for methyl iodide that was published in 1981 (Greim 1996 b), rats (BD) were given methyl iodide by subcutaneous injection either as a single dose of 50 mg/kg body weight or once a week for a year at doses of 10 or 20 mg/kg body weight. Local tumours at the injection site were the primary effect; the tumours were attributed to the alkylating properties of the substance. A mouse strain (A/He) that is susceptible to lung tumours was given methyl iodide by intraperitoneal injection 3 times a week (24 injections in total) at doses of 0, 8.5, 21.3 or 44.0 mg/kg body weight. The animals were examined 24 weeks after the first injection. The average number of lung adenomas per mouse was increased slightly, but with statistical significance. However, a clear dose–response relationship could not be established because of the high level of mortality in the high dose group (Poirier et al. 1975). Two new carcinogenicity studies have become available since the last documentation for methyl iodide was published in 1992. In a 2-year inhalation study carried out according to OECD Test Guideline 453, male and female Sprague Dawley rats were exposed whole-body to **methyl iodide** at concentrations of 0, 5, 20 or 60 ml/m^3. The total number of adenomas and carcinomas in the thyroid gland was increased with statistical significance only in the males of the high concentration group (15/70 animals;

controls: 4/60). The increase in the incidence of follicular adenomas (13/70, controls: 2/60) and carcinomas (4/70, controls: 2/60) was not statistically significant in this concentration group. The incidence of astrocytomas in both sexes was not regarded as substance-related. The effects observed in the thyroid gland may be attributed to a perturbation of homeostasis of the pituitary-thyroid axis caused by iodide (ECHA 2013, 2020 b).

In an oral study, male and female CD-1 mice were given microencapsulated methyl iodide with the feed (0, 60, 200 or 600 mg/kg feed, equivalent to methyl iodide doses of 0, 8, 28 or 84 mg/kg body weight in the males and 0, 10, 35 or 100 mg/kg body weight in the females) for 78 weeks. In the male mice of the high dose group, a slight but statistically significant, dose-dependent increase in follicular adenomas and carcinomas of the thyroid gland was determined by trend test (3/49, controls: 0/50). A pairwise comparison with the control group did not yield any statistically significant results. The tumour in the thyroid gland (not specified whether adenoma or carcinoma) of 1 male of the 28 mg/kg group was not regarded as substance-induced. The findings of benign fibromas in the uterus and cervix were likewise not regarded as substance-induced. In this study as well, the effects on the thyroid gland were attributed to a perturbation of homeostasis of the pituitary–thyroid axis caused by iodide (ECHA 2013, 2020 b).

5.7.2 Summary

Renal tumours were found only in male mice and only at the highest concentration tested as a result of a sex-specific increase in the expression of CYP2E1. The structurally related methyl halides methyl iodide and methyl bromide did not cause carcinogenic effects by alkylation in long-term animal studies.

6 Manifesto (MAK value/classification)

The critical end points are fertility and neurotoxicity. Studies with test persons did not report findings of irritation up to a methyl chloride concentration of 200 ml/m^3.

MAK value. There are no data for humans that are suitable for the derivation of a MAK value.

In a 2-generation study in male rats, the number of offspring was reduced after exposure to a methyl chloride concentration of 475 ml/m^3 for 3 months. The NOAEC was 150 ml/m^3. Atrophy of the seminiferous tubules was determined histopathologically at 1500 ml/m^3 (Hamm et al. 1985). A 2-year study in mice and rats detected atrophy of the seminiferous tubules at a methyl chloride concentration of 1000 ml/m^3 (Battelle Columbus Laboratories 1981). As fertility impairment is a more sensitive effect than testicular tubule atrophy, which was not investigated in this study, it is assumed that the NAEC (no adverse effect concentration) is one tenth of the LOAEC for testicular tubule atrophy, as in the 3-month study. Thus, based on the findings of the 2-year study, the NAEC for effects on fertility induced by methyl chloride in rats is assumed as 100 ml/m^3. A workplace concentration of 38 ml/m^3 has been extrapolated from this value (NAEC: 100 ml/m^3, extrapolation from an animal study to humans: 1:2, adjustment of the duration of exposure from 6 hours a day to 8 hours a day at the workplace. As the blood:air partition coefficient is < 5, it is not necessary to consider the higher respiratory volume at the workplace in comparison with that of animals exposed at rest). According to the preferred value approach, a MAK value of 20 ml/m^3 would result.

Slight behavioural toxicity was observed in humans at methyl chloride concentrations of 200 ml/m^3 and above (Putz-Anderson et al. 1981). Severe neurotoxic effects were induced after accidental exposure to much higher concentrations.

After 2-year exposure of male and female B6C3F1 mice to a methyl chloride concentration of 1000 ml/m^3, neurotoxic effects were observed in the form of degeneration and atrophy of the granular layers of the cerebellum. The NOAEC was 225 ml/m^3 (Battelle Columbus Laboratories 1981). These kinds of effects were not observed in rats. The findings of the 2-year study were used to extrapolate a methyl chloride concentration of 84 ml/m^3 for the workplace (NOAEC: 225 ml/m^3, extrapolation from an animal study to humans: 1:2, adjustment of the duration of exposure from 6 hours a day to 8 hours a day at the workplace). With the preferred value approach, a MAK value of 50 ml/m^3 would result for methyl chloride.

An 11-day study was carried out in female C57BL/6 mice. The mouse is the most sensitive species and this particular strain is the most susceptible of the tested mouse strains. Slight degeneration of the granular layer of the cerebellum was observed in this strain after exposure to a methyl chloride concentration of 400 ml/m^3 for 5.5 hours a day. At this concentration, the performance in the rotarod test was not yet impaired. The NOAEC for methyl chloride was 150 ml/m^3 (Landry et al. 1985). On the basis of the NOAEC of 150 ml/m^3 and taking into consideration the extrapolation from animals to humans (1:2), the adjustment of the duration of exposure from 5.5 hours a day to 8 hours a day at the workplace (5.5 hours/day to 8 hours/day) and from 7 days of exposure to 5 days (7:5) as well as a time extrapolation for chronic exposure (1:6), this results in a concentration in the workplace air of 12 ml/m^3. According to the preferred value approach, a MAK value of 10 ml/m^3 would result.

As the 2-year inhalation study investigated only histopathological effects and not behavioural toxicity, neurotoxic effects were induced in humans and severe neurotoxic effects were observed in animal studies (degeneration of the granular layer of the cerebellum), a steep concentration–effect curve was determined after continuous exposure and there are no data for a mechanism of action, a MAK value of 10 ml/m^3 has been established.

Peak limitation. As the MAK value has been derived from a systemic effect, the substance remains classified in Peak Limitation Category II. The initial half-life of methyl chloride is much shorter than 1 hour (Löf et al. 2000; Nolan et al. 1985). For this reason, an excursion factor of 1 has been set (Hartwig and MAK Commission 2017 b).

Carcinogenicity. There are no new data for carcinogenicity. The findings of epidemiological studies do not allow conclusions to be made regarding carcinogenicity in humans. In a 2-year inhalation study with rats and mice, renal tumours were observed in the high concentration group only in 1 species and in 1 sex (male mouse) (Battelle Columbus Laboratories 1981). It is quite probable that the tumours were caused by the sex-specific expression of CYP2E1 in the kidneys of mice (Section 3.2). A metabolism study with test persons (Löf et al. 2000) demonstrated that the metabolic pathway via CYP2E1 plays a subordinate role in humans. Additionally, the CYP2E1 enzyme is not expressed in the kidneys of humans. However, the renal tumours are not considered relevant for humans because neither renal tumours nor other types of tumours developed in rats and female mice up to a concentration of 1000 ml/m^3 and the renal tumours that were observed in the male mouse at the highest methyl chloride concentration tested of 1000 ml/m^3 can be explained by a sex-specific increase in the expression of CYP2E1. DNA binding did not occur in mice and rats (Greim 1996 a). In comparison, after exposure to the methyl halides methyl bromide and methyl iodide, DNA adducts were found in the liver, lungs, stomach and forestomach. However, the long-term studies with inhalation exposure did not reveal carcinogenic effects that can be attributed to alkylation (Section 5.7). Methyl chloride did not induce DNA adducts even at concentrations 150-fold higher (500–1500 ml/m^3) than the MAK value (Kornbrust et al. 1982; Peter et al. 1985). For this reason, genotoxic or carcinogenic effects are not expected to occur in vivo. Overall, the findings of the genotoxicity studies that were evaluated demonstrate that the genotoxic potential only becomes relevant at very high concentrations. However, the formation of DNA adducts cannot be ruled out completely. If these form, then only at levels of exposure far above the MAK value and only in very small amounts that are not significant for the workplace.

As renal tumours are sex-specific effects that occur only in male mice and as a result of the slight genotoxic potential, the substance has not been classified in a category for carcinogenicity.

Germ cell mutagenicity. In vitro, methyl chloride is mutagenic in bacteria and mutagenic and clastogenic in mammalian cells at very high concentrations (0.8% methyl chloride and above).

In vivo, DNA protein crosslinks were found only in male mice and only if the tests were carried out directly after exposure. In male rats, UDS was detected only in liver cells and at very high concentrations of methyl chloride, but not in tracheal epithelial cells or spermatocytes. In a dominant lethal test, methyl chloride induced both post-implantation and pre-implantation losses in rats at a concentration of 3000 ml/m^3. It was possible to attribute the pre-implantation losses to the infertility of the male animals caused by toxic effects on spermatogenesis. The post-implantation losses were secondary effects caused by epididymal inflammation induced by methyl chloride (Section 5.6.2). Additional

evidence of a non-primarily genotoxic effect of chloromethane in vivo is provided by two DNA binding studies which show that chloromethane does not alkylate DNA (Kornbrust et al. 1982; Peter et al. 1985).

The data for metabolism suggest that only glutathione conjugation is relevant for humans (Löf et al. 2000). The oxidative metabolic pathway appears to lead to the formation of formaldehyde and thus to the observed DNA protein crosslinks only in male mice. This sex-specific and species-specific formation of formaldehyde is probably due to CYP2E1, which is more strongly expressed in the kidneys of male mice than in those of female mice or humans.

Overall, the studies that investigated genotoxicity in vivo and metabolism demonstrated that cytotoxic and secondary genotoxic effects are of primary relevance for methyl chloride. For this reason, the substance has not been classified in a category for germ cell mutagens.

Prenatal toxicity. At the previous MAK value of 50 ml/m^3 (100 mg/m^3), methyl chloride was classified in Pregnancy Risk Group B.

Developmental toxicity:

Two prenatal developmental toxicity studies in C57BL/6 mice found heart anomalies (reduction or absence of atrioventricular valve, chordae tendineae and papillary muscles, small right ventricle, globular heart and white spots in the left ventricular wall) at methyl chloride concentrations of 500 ml/m^3 and above. The second study was not able to reproduce the changes found in the first study (CIIT 1981 b; Greim 1996 a; Wolkowski-Tyl et al. 1983 b) either qualitatively or quantitatively (CIIT 1981 a; Greim 1996 a; Wolkowski-Tyl et al. 1983 b). Additionally, it needs to be taken into consideration that the type, number and severity of heart defects may be artefacts caused by the high degree of variability between the animals or result from the dissection and fixation techniques used. The interpretation of the data is made more difficult by the lack of historical controls for this mouse strain, which is not the standard strain used for this type of study. A NOAEC of 250 ml/m^3 for developmental toxicity induced by methyl chloride was established on the basis of the studies in mice; this includes also teratogenic effects. In a prenatal developmental toxicity study in F344 rats, developmental delays were observed in the foetuses concurrently with slight maternal toxicity (reduced body weight gains) at the highest methyl chloride concentration tested of 1500 ml/m^3. The NOAEC for developmental toxicity was 500 ml/m^3 (CIIT 1981 b; Greim 1996 a; Wolkowski-Tyl et al. 1983 a). In a study carried out in New Zealand White rabbits according to OECD Test Guideline 414, no maternal or developmental toxicity was observed up to the highest methyl chloride concentration tested of 1000 ml/m^3. The NOAEC for developmental toxicity was therefore 1000 ml/m^3 (Arts et al. 2019). Teratogenic effects were not observed in rats or in rabbits. A 2-generation study in F344 rats determined a NOAEC for perinatal toxicity of 475 ml/m^3; fertility was decreased at this concentration as a result of toxic effects on the spermatocytes (Greim 1996 a; Hamm et al. 1985).

As the blood:air partition coefficient is < 5, the evaluation does not need to take the increased respiratory volume at the workplace into consideration. There are 25, 50, 100 and 48-fold margins, respectively, between the NOAECs for developmental toxicity of 250 (mouse), 500 (rat) and 1000 (rabbit) ml/m^3 and the NOAEC for perinatal toxicity of 475 (rat) ml/m^3 and the MAK value of 10 ml/m^3.

The margins between the extrapolated NOAECs for developmental toxicity/perinatal toxicity and the MAK value are sufficiently large. This value affords protection against the heart anomalies that were found in one species, the mouse, but that were difficult to interpret. On the basis of the developmental toxic effects, methyl chloride could justifiably be re-classified from Pregnancy Risk Group B into Group C.

Developmental neurotoxicity:

Since 2016, a statement on developmental neurotoxicity in the foetus has been required for substances with MAK values derived from neurotoxic effects.

The 2-generation study in F344 rats did not investigate neurotoxic or behavioural end points in the offspring (Greim 1996 a; Hamm et al. 1985). There are also no studies available that investigated these types of end points in the offspring of dams that were exposed in utero. There are no data available for toxicokinetics, metabolism or the mechanism

of action that would make it possible to evaluate whether foetuses are less susceptible than adult animals. On the basis of the findings of neurotoxic changes in adult animals, the occurrence of developmental neurotoxicity in the offspring cannot be ruled out with certainty. Therefore, it is not possible to draw conclusions about the induction of developmental neurotoxicity in foetuses by methyl chloride. For this reason, methyl chloride has been re-classified from Pregnancy Risk Group B into Group D.

Absorption through the skin. Methyl chloride is a gas; there are no studies available that investigated absorption through the skin or dermal toxicity. Dermal exposure to an aqueous solution is unlikely to occur.

As calculated in Section 3.1, 313 or 46 µg of methyl chloride are taken up from the gas phase after whole-body exposure to a methyl chloride concentration of 10 ml/m^3 for 8 hours. After exposure at the level of the MAK value, maximally 60% of the substance is absorbed by inhalation (Section 3.1), which is equivalent to an absorbed amount of 126 mg at a respiratory volume of 10 m^3. As model calculations determined that a negligible amount of substance is absorbed from the gas phase and exposure to an aqueous solution is unlikely, methyl chloride is no longer designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are no findings in humans relating to sensitizing effects and no findings from animal studies or in vitro studies. Methyl chloride is therefore not designated with "Sh" or with "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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