Methyl bromide / Bromomethane

MAK Value Documentation

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated methyl bromide [74-83-9], considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. Methyl bromide is a neurotoxin and an irritant to the upper respiratory tract. A NOAEC of 3 ml/m³ for effects in the olfactory epithelium was obtained from the 29-month inhalation study in the rat, for irritation, the most sensitive endpoint. Based on this, the maximum concentration at the work place (MAK value) for methyl bromide is set at 1 ml/m³ (3.9 mg/m³). Since the critical effect of methyl bromide is local, Peak Limitation Category I is designated. In rats, signs of irritation are observed only at methyl bromide concentrations of 20 ml/m³ and above, so an excursion factor of 2 can be given.

There is an adequate margin between NOAEC/L for developmental toxicity and the MAK value. However, methyl bromide is a neurotoxin and no data on developmental neurotoxicity exists. Therefore, methyl bromide is assigned to Pregnancy Risk Group D.

Methyl bromide is genotoxic in vitro and an alkylating substance in vivo. No increased tumour incidence was observed in inhalation studies in mice and rats. Together with the possible relationship between methyl bromide exposure and increased occurrence of prostate carcinomas in a historical cohort study in humans, methyl bromide is still suspected of being carcinogenic and remains in Carcinogen Category 3B. No clastogenic or aneugenic effects are found in vivo with prolonged inhalation and therefore the substance is not regarded as a germ cell mutagen. Skin contact is expected to lead to relatively minor contribution to systemic toxicity. There are no data on sensitization.

Because methyl bromide can be absorbed through the skin from the gaseous phase at high concentrations when respiratory protection is used, biomonitoring studies are recommended in addition to personal protective measures. The BLW value of 12 mg bromide/l plasma or serum must be observed.

Keywords

methyl bromide; bromomethane; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Methyl bromide

[74-83-9]

Supplement 2011

MAK value (2010)	**1 ml/m³ (ppm) ≙ 3.9 mg/m³**
Peak limitation (2010)	**Category I, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity (1992)	**Category 3B**
Prenatal toxicity (2010)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BLW (2002)	**12 mg bromide/l plasma or serum**
log K_{OW}[1)]	1.19 (*SRC* 2010)

Since the completion of the last supplement to the documentation for methyl bromide (bromomethane), new data have become available for the epidemiology, genotoxicity, reproductive toxicity and toxicokinetics of the substance which make a re-evaluation necessary. Further new data for the toxicokinetics, metabolism, exposure and effects, and for background exposure are described in the BLW value (Biologischer Leitwert) (documentation "Brommethan" BAT Value Documentation, 2003, only available in German).

Toxicokinetics and Metabolism

Absorption, distribution, elimination

Groups of 10 male Wistar rats were exposed to air saturated with methyl bromide (liquid at the start of exposure) on the shaved dorsal skin (area about 12 cm^2), with the aid of a glass cylinder. The rats were exposed for 30 seconds and 1, 3 or 5 min-

1) octanol/water partition coefficient

utes (see also Section "Local effects on skin and mucous membranes"). Severe skin damage occurred. In all groups, the highest level of bromide ions in plasma was reached after one hour. After 4 to 8 weeks, the plasma level had returned to the range of the control value. The biological half-life of bromide in plasma calculated using a two-compartment model was in the range of 5 to 6.3 days, depending on the duration of exposure (Yamamoto et al. 2000).

Animal experiments demonstrated the absorption of methyl bromide through the skin. They agree with observations made in 6 workers equipped with breathing masks who fumigated an old castle to treat woodworm and beetle infestation. Following two 20-minute exposures to high concentrations of gaseous methyl bromide (estimated concentration in the air 35–40 g/m^3 = 8750–10 000 ml/m^3), skin lesions occurred in all workers after a latency period of several hours in areas that had been covered with clothing during the exposure (axillae, groin area, abdomen). Immediately after exposure, the mean bromide concentration determined in the plasma of the workers was 9.0 mg/l (range: 7.7–11.4 mg/l), which decreased to 6.8 mg/l (range: 4.5–9.4 mg/l) over a period of 12 hours (Hezemans-Boer et al. 1988; Zwaveling et al. 1987).

Additional evidence for the dermal absorption of methyl bromide can be found in a study by Goergens et al. (1994), in which 8 workers with breathing masks were examined after they had fumigated floors in greenhouses and buildings. Compared with in control persons not exposed, the methylcysteine levels in globin samples from the workers were increased by a factor of about 2 and the incidence of SCE (sister chromatid exchange) in lymphocytes was increased by less than 2-fold, which indicates the increased absorption of methyl bromide. The workers examined were exposed to gaseous methyl bromide. As inhalation exposure was excluded by the use of breathing masks, it must be assumed that methyl bromide was absorbed through the skin from the gaseous phase.

Using model calculations for the flux from the aqueous phase and Henry's constant, it is possible to estimate the flux from the gaseous phase for an external concentration of 1 ml/m^3:

Concentration in aqueous phase = $C_{gas} \cdot$ Henry's constant
C_{gas} = 1 $ml/m^3 = 3.9 \cdot 10^{-6}$ g/l; Henry's constant = 3.8 at 24°C;
Concentration in aqueous phase = $1.48 \cdot 10^{-5}$ g/l

The flux in the worst case according to the model of Fiserova-Bergerova et al. (1990) is therefore $5.2 \cdot 10^{-7}$ mg/cm^2 and hour

Assuming 8-hour whole-body exposure (17 000 cm^2) and 70 kg body weight, the uptake of methyl bromide at an external concentration of 1 ml/m^3 is therefore 0.001 mg/kg body weight. Under these conditions, absorption by inhalation is 4 $mg/m^3 \cdot 10$ m^3/70 kg body weight = 0.57 mg/kg body weight. When the MAK value is observed, dermal absorption is therefore very low compared with by inhalation.

Effects in Humans

Single exposures

Methyl bromide is acutely toxic in humans and damages mainly the central nervous system. The symptoms depend on the dose and include headaches, visual disorders, vomiting, acute psychosis, tonic-clonic convulsions and paralysis. As a result of pronounced cerebral oedema, the damage to the CNS can result in death, in particular due to respiratory arrest. The symptoms subside in most cases, although permanent effects have been described (documentation "Brommethan" BAT Value Documentation, 2003, only available in German), Table 1 lists more recent case reports after acute inhalation. Here too, damage to the central nervous system is the main effect, manifest in unsteady gait, balance disorders and a reduced sense of pain. In the described cases of intoxication, the serum bromium concentrations were increased by about 10-fold above the given background exposure. The duration and level of exposure were not reported in the studies.

Table 1 Case reports of acute intoxication after inhalation of methyl bromide

Exposed persons (age)	Bromium concentration	Effects	References
man (24 years)	81.8 mg/l serum	functional disturbance of the cerebellum: ataxia, unsteady gait, vertigo and paraesthesia, vibrations and a decreased sense of pain in both feet for two weeks, hyperactive reflexes in all extremities	Suwanlaong and Phanthumchinda 2008
man (30 years)	43.7 mg/l serum (normal: 3.7–8.6 mg/l)	bilateral symmetrical lesions of high signal intensity in the posterior region of the putamen (core region of the cerebrum), in the subthalamic nuclei and the dorsal medulla oblongata, unsteady gait, paraesthesia in both feet, blepharoptosis	Ichikawa et al. 2001
man (39 years) woman (34 years) child (5 years)	72.9 mg/l, 67.8 mg/l 91.5 mg/l blood plasma (normal < 5 mg/l blood plasma)	vomiting, tonic convulsions and clouding of consciousness	Yamano et al. 2001
woman	2.7 mg/l blood post mortem: 0.29 mg/l blood, 0.17 mg/l gallbladder, 24 µg/g liver, 28 µg/g adipose tissue	fever, seizures, organ failure, death 19 days after exposure	Horowitz et al. 1998

Table 1 (continued)

Exposed persons (age)	Bromium concentration	Effects	References
man, woman newborn baby (single exposure)	child: 170, mother: 130, father: 110 mg/l blood (after 39 hours)	death of the child 12–13 hours after exposure: death caused by acute pneumonia through aspiration after vomiting; mother and father: 5–6 days after exposure dizziness, nausea, sore throat, dry cough, difficulties in walking straight ahead, no unusual findings in neurological examinations, blood, liver and creatinine values normal; father: thorax X-ray normal; mother: after 7 days additional fever, pneumonia, inflammatory parameters in the blood increased, recovery within 14 days after treatment with penicillin	Langård et al. 1996
2 men (39 years)	10–20 g/m³ 48 hours after exposure: 46.6 mg/l blood	1-hour exposure with inadequate breathing mask: dizziness, exhaustion, nausea, vomiting, pains in the chest, shortness of breath, recovery 3 days later	Deschamps and Turpin 1996
(44 years)	10–20 g/m³ 40 hours after exposure: 156 mg/l blood	additionally: confusion, lack of orientation, ataxia, anuria, speech disorders still 5 months later, trembling, aid required in getting up	
9 gardeners (on 2 consecutive days)	200 ml methyl bromide/m³	2 patients: myoclonus, tonic-clonic convulsions	Hustinx et al. 1993

Repeated exposure

Fifty-six male workers at a methyl bromide factory who were exposed to the substance were questioned about acute and chronic symptoms. Symptoms were recorded using a differentiated questionnaire, and the data were checked using a standardized set of questions. The control group consisted of workers not exposed who were employed as ticket inspectors or engineers in a railway company. At the time of questioning, 37 of the workers were still employed at the factory (group 1). In 19 workers (group 2), the exposure had taken place 6 or more months previously. The exposure duration in the individual workers was between less than 1 year and more than 20 years. The concentrations of methyl bromide in the ambient air to which the workers were exposed were determined every 6 months; over the preceding 10 years these had been on average below 5 ml/m³ with peak concentrations of more than 5 ml/m³ (no other details). However, in accidents, concentrations of more than 15 ml/m³ were determined, which led to subacute intoxication (lethargy,

ataxia, retrobulbar neuritis). This was one of the reasons for performing the study. However, the values determined in the air are not reported in the publication. The urinary concentration of bromide in the exposed persons from group 1 was given as 18.9 mg/l ± 10.4 (range: 3.2–54). A significant correlation between the urinary bromide concentrations and the symptoms described (sum value) could not be established. Compared with the workers in group 1, the workers in group 2 reported more frequently tingling and numbness in the hands and legs as acute effects, and an unsteady gait as chronic effects. In all exposed persons, a 'runny nose' with nasal irritation, burning, itching and the formation of bullae on the hands, tingling and numbness in the hands and soles of the feet, nightmares, scaly and dry hands, dizziness and apathy occurred with a significantly greater frequency than in the control group. The duration of the exposure correlated significantly with the total symptom value and the sum value of significantly increased symptoms. No information was given as to whether the workers wore breathing masks or not (Kishi et al. 1991). No details can be found in this study as to what effects occurred at which concentration of methyl bromide and after how long an exposure duration. The authors assume that the exposure levels above 15 ml/m^3 in the past are probably the main reason for the increased reports of chronic symptoms in the exposed persons. No NOAEC (no observed adverse effect concentration) for irritating effects and neurotoxicity can be derived from the study, as the values determined for the concentrations of methyl bromide in the air are not given. The reported symptoms of irritation and neurotoxicity cannot therefore be related to methyl bromide concentrations. In addition, the results of the biomonitoring investigations, which revealed bromide concentrations in a range from 3.2 to 54 µg/ml urine and, in particular, represent the short-term exposure level, did not correlate with the extent of the acute symptoms.

Two other case reports of chronic intoxication with methyl bromide are available. For 4 years, between August and November, a 23-year-old man treated the floors of greenhouses with methyl bromide gas. During this period, he was not exposed to any other chemical. His breathing protection is described as probably inadequate, and the man often wore only shorts because of the high temperatures, so that considerable absorption through the skin must be assumed. Early November he experienced unsteady gait, paraesthesia in the feet, pyramidal tract syndrome, dizziness, and visual disorders. Clinical examination revealed cerebral symptoms, horizontal nystagmus and ataxia. Demyelination was not observed, the cerebrospinal fluid was normal, and the acoustic and sensory evoked potentials were in the normal range. The visually evoked potential was changed. No irritative effects occurred. On day 20 after the symptoms first appeared, the bromide concentration was 24 mg/l in the patient's blood and 19 mg/l in his urine. Within a few months the patient's state of health had improved, although over the following 2 years his vision remained impaired and the hyperreflexia in his lower legs persisted (De Haro et al. 1997). A 33-year-old worker exposed to methyl bromide for 4 years between July and December complained from the beginning of August of muscular weakness in the lower legs, paraesthesia, dizziness, unsteady gait, and impaired colour and night vision. Medical examination revealed hepatic cytolysis. The increased transaminase

values returned to the normal range after 2 weeks without exposure. An electroencephalogram did not yield any unusual findings. After 8 months, no symptoms were found in the patient (De Haro et al. 1997). In both cases, the exposure levels were not reported. The studies cannot therefore be included in the evaluation.

Genotoxicity

The leukocytes of 21 gardeners who used methyl bromide during ground sterilization were examined within 24 hours after its use for the formation of N^7-methylguanine and O^6-methylguanine–DNA adducts in blood. Exposure lasted between 6 and 68 minutes. Personal air sampling revealed methyl bromide concentrations in the range of 11–78 mg/m³ (4.6–20 ml/m³) in 3 gardeners with high exposure, 0.1–22 mg/m³ (0.025–5.6 ml/m³) in 7 gardeners and of up to 2.7 mg/m³ (0.69 ml/m³) in 11 gardeners with low exposure. There were 19 control persons who were not exposed. In one exposed person and one person not exposed, N^7-methylguanine–DNA adducts (4 N^7-methylguanine/10^6 nucleotides) were detected. No O^6-methylguanine–DNA adducts could be found in any of the exposed persons or controls. In the second part of the study, the leukocytes of 6 occupational users of methyl bromide were examined for the formation of N^7-methylguanine and O^6-methylguanine–DNA adducts in blood. Personal monitoring revealed mean exposure concentrations of 23–165 mg methyl bromide/m³ (5.8–41.7 ml/m³). In 2 persons, N^7-methylguanine–DNA adducts (11.5 N^7-methylguanine/10^6 nucleotides; 2.6 N^7-methylguanine/10^6 nucleotides), but no O^6-methylguanine–DNA adducts were detected. The study thus provides evidence that even after high-level methyl bromide exposure only very low quantities of DNA adducts can be detected in leukocytes. The authors also discussed whether the leukocytes represented a suitable target for DNA adducts after methyl bromide exposure. The gardeners and occupational users did not wear breathing masks and did not use any other protective devices (Pletsa et al. 2002).

In 14 workers (4 smokers, 10 non-smokers) who used methyl bromide for fumigation, the incidence of SCE and the increased formation of *S*-methlycysteine were determined in the lymphocytes at the beginning of the season in June and at the end of the season in September. The workers wore breathing masks with filters. In all but one worker, the incidence of SCE in September had increased compared with the June value; the increase was up to twice the initial value. The interindividual variations were explained by the different jobs and the different exposure levels. The levels of *S*-methylcysteine, a metabolite determined in urine as an indicator for internal exposure, increased by a factor of up to 3 in 8 workers compared with the levels in 4 control persons. There was no difference between smokers and non-smokers. No data were provided regarding the concentrations or exposure duration in the individual workers (Goergens et al. 1994).

In 32 workers who used methyl bromide for fumigation, HPRT mutations in lymphocytes, and micronuclei in lymphocytes and in oropharyngeal cells were examined. The median for exposure duration was 3 years (0.3 to 22 years). During the

2-week investigation period, the median exposure duration was 4 hours (0 to 145 hours). The bromide concentration in the urine of the exposed workers was 131 mg/l, that of control persons 110 mg/l. This difference was not statistically significant. In the exposed non-smokers, the geometric mean for HPRT mutations increased in 2 exposure groups (≥ 4 hours; < 4 hours exposure duration) compared with that for the control persons; the increase was not statistically significant. Only in the oropharyngeal cells of the workers exposed for less than 4 hours, was the increase in the incidence of micronuclei (geometric mean value) statistically significant ($p = 0.03$) compared with the incidence in the controls. In the lymphocytes, no increase in micronuclei was observed. According to the authors themselves, the study was limited by the low statistical power, the lack of a validated method to determine exposure to methyl bromide at the workplace and the irregular exposure to methyl bromide within the period determined. Nevertheless, they see a possible association between the observed genotoxic effects and the exposure to methyl bromide. Details regarding respiratory protection were not given (Calvert et al. 1998). In view of the reasons given above, the study is not suitable for demonstrating the genotoxic effects of methyl bromide.

Carcinogenicity

In the prospective cohort study of Alavanja et al. (2003) the relationship between the prostate cancer risk in humans and exposure to 50 different pesticides was investigated. The cohort comprised 55 332 male pesticide users. Details regarding the use and duration of the application of methyl bromide were collected by questionnaire between 1993 and 1997. As a measure of the effects, odds ratios with logistic regressions were calculated (Table 2), adjusted for age and a family history of prostate cancer. For all the exposed persons together, the risk of prostate cancer was not significantly increased compared with persons not exposed (OR = 1.10; 95% CI: 0.77–1.36), however, for the 2 groups with the highest exposure there was a significant increase in the risk (Table 2). This resulted in a significant exposure–effect relationship ($p = 0.004$).

It was not possible to establish a significant relationship with the prostate cancer risk in any of the other pesticides and pesticide groups investigated. The number of cases in the two high exposure categories for methyl bromide are, however, very low. The high internal consistency of the results (Iowa and North Carolina subcohorts, private and commercial pesticide sprayers, stability of the results using other exposure parameters such as “days/year” or “total days” as well as after adjustment for other pesticides) speaks for a causal interpretation of the increased risks following methyl bromide exposure as described by Alavanja et al. (2003). In this study, however, no a priori hypothesis about the relationship between methyl bromide and prostate carcinomas was formulated. Therefore, the observed results may also be a chance finding due to the large number of tests. All in all, this study is therefore not sufficient to justify the conclusion that there is a causal relationship between methyl bromide exposure and an increased prostate cancer risk.

Table 2 Odds ratios for the prostate cancer risk after exposure to methyl bromide (Alavanja et al. 2003)

Exposure categories for methyl bromide	Cases	Odds ratio [95% CI]
I (lowest)	23	1.01 (0.66–1.56)
II	22	0.76 (0.47–1.25)
III	11	0.7 (0.38–1.28)
IV	6	**2.73 (1.18–6.33)**
V (highest)	5	**3.47 (1.37–8.76)**

In addition, for the evaluation of the prostate cancer risk in humans, cohort studies, case–control studies and meta-analyses are available for the occupational group of farmers (see Tables 3, 4 and 5).

Table 3 Cohort studies of the relative risk in farmers of contracting prostate cancer

Cases	Exposure	Relative risk [95% CI]	References
1148	exposure to herbicides (exposure recorded according to the field surface sprayed; no data for methyl bromide exposure)	**2.23** (1.3–3.84)	Morrison et al. 1993
401	exposure to herbicides (exposure not recorded, no data for methyl bromide exposure)	**1.13** (1.02–1.24)	Dich and Wiklund 1998
81	exposure unclear	**1.7** (1.0–2.7)	Parker et al. 1999
167	exposure unclear	0.93 (0.76–1.13)	Mills and Kwong 2001
733	exposure unclear	0.74 (0.41–1.33)	Zeegers et al. 2004

Table 4 Case–control studies of the relative risk in farmers of contracting prostate cancer

Cases/ controls	Exposure	Relative risk [95% CI]	References
19/2532	exposure to pesticides (no data for methyl bromide exposure)	**1.09** (0.57–2.08)	Aronson et al. 1996
192/not specified	exposure to pesticides (no data for methyl bromide exposure)	0.7 (0.6–0.9)	Krstev et al. 1998 a
30/2296	exposure to pesticides (no data for methyl bromide exposure, not dependent on the time of exposure)	**2.17** (1.18–3.98)	Krstev et al. 1998 b
222/1330	methyl bromide exposure (no other details)	**1.16** (0.77–1.75)	Mills and Yang 2003

Table 5 Meta-analyses of the relative risk of contracting or dying from prostate cancer

Study	Number of studies	Comments	Relative risk [95% CI]
Blair et al. 1992	22	7753 cases	**1.08** (1.06–1.11)
Keller-Byrne et al. 1997	24 (all studies)	methyl bromide exposure unclear	**1.12** (1.01–1.24)
	13 (retrospective studies)		**1.29** (1.1–1.51)
	6 (mortality studies)		0.93 (0.77–1.11)
Acquavella et al. 1998	11	methyl bromide exposure unclear	0.95 (0.93–0.98)
Van Maele-Fabry and Willems 2004	22	no exposure recorded	**1.24** (1.06–1.45)
Van Maele-Fabry et al. 2006	16	no data for exposure to methyl bromide in any of the studies listed	**1.28** (1.05–1.58)

The available epidemiological studies indicate a statistically significant increase in the prostate cancer risk for farmers. However, the studies do not contain, with the exception of the study of Alavanja et al. (2003), any data regarding possible exposure to methyl bromide. The studies are therefore not suitable for evaluating the carcinogenicity of methyl bromide in humans.

Animal Experiments and in vitro Studies

Acute toxicity

Inhalation

Groups of 6 male F344 rats were exposed once for 6 hours to methyl bromide concentrations of 0 or 175 ml/m^3. The olfactory epithelium in the dorsal medial meatus was disrupted, fragmented, and exfoliated down to the basal cell layer. Four other groups, also consisting of 6 rats, were exposed once for 6 hours to methyl bromide concentrations of 0 or 175 ml/m^3 and then again after an exposure-free interval of 28 days. The rats were killed 24 hours after the second exposure. During the 4-week recovery period, the olfactory epithelium regenerated (Bolon et al. 1991).

Male and female Sprague Dawley rats (number of animals not specified) were exposed to methyl bromide concentrations of 0, 30 or 350 ml/m^3 for 6 hours. Clinical effects and body weights were examined. All the animals survived up to the end of the study. The body and brain weights were unaffected. Behavioural effects were investigated 3 hours, and 7 and 14 days after the exposure. After three hours of exposure, reduced body temperatures and the following effects on behaviour were

observed in the male and female rats of the high concentration group: decreased motor activity, an increased incidence of eyelid closure, piloerection and decreased rearing onto the hind legs. The tail pinch response was decreased in the males. The females' urination was increased and they could no longer rear themselves normally. No histological changes were found in the nervous system and in the nasal tissue (OECD 2002).

Subacute, subchronic and chronic toxicity

Inhalation

Studies are available in rats and mice for the chronic toxicity of methyl bromide (NTP 1992; Reuzel et al. 1991); these were already described in the documentation from 1992 (documentation "Methyl bromide" 1996) and are presented here once again in context. A re-evaluation is available for the effects in the nose of rats described in the study of Reuzel et al. (1991) which makes a re-assessment of the irritative effects necessary (US EPA 1997). This re-evaluation was carried out in order to evaluate the individual animal data so far not sufficiently accounted for in the original study and to check the accuracy and consistency of the data. In addition, new studies with rats, mice (Gotoh et al. 1994; Yang et al. 1995) and dogs (Schaefer et al. 2003) have been published.

Table 6 shows the inhalation studies with methyl bromide in rats, mice and dogs.

Table 6 Effects of methyl bromide after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 5 ♂, 5 ♀	**14 days**, 0, 12, 25, 50, 100, 200 ml/m³ 6 hours/day, 5 days/week	**12 ml/m³**: trembling, nervousness, paralysis (no other details, unclear whether also in control animals) **50 ml/m³ and above**: pronounced toxic effects on behaviour (trembling, nervousness, paralysis) **200 ml/m³**: deaths, hyperaemia in lungs, liver and kidneys, blood in urine	NTP 1992
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks,** 0, 10, 20, 40, 80, 120 ml/m³ 6 hours/day, 5 days/week	**20 ml/m³**: **NOAEC (♂)** **40 ml/m³ and above**:♂: mean haemoglobin level and mean cell volume reduced, erythrocyte count increased **80ml/m³**: **NOAEC (♀)** **120 ml/m³**: mortality ↑ (4/24 ♂, additional 14 animals from other studies), body weight gains ↓ (♂), clinical symptoms (changed posture of fore and hind limbs)	NTP 1992

Table 6 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 5–8 ♂, 7–8 ♀	**13 weeks, study of behavioural toxicity,** 0, 20, 40, 80 ml/m³ 6 hours/day, 5 days/week	**20 ml/m³: NOAEC** **40 ml/m³**: ♀ **week 12**: toxic effects on behaviour: fear response increased, changed exploratory behaviour; ♂ **week 6**: novel side crossing significantly ↓ **80 ml/m³**: ♂ **week 6**: changed exploratory behaviour, pain latency ↑	NTP 1992
mouse, B6C3F1, 50 ♂, 50 ♀	**103 weeks,** 0, 10, 33, 100 ml/m³ 6 hours/day, 5 days/week	**33 ml/m³: NOAEC** **100 ml/m³**: activity ↓, sensitivity of fear response ↑, pain latency ↑, hind limb grip strength ↑, mortality ♂ ↑, cerebral degeneration, sternal dysplasia, necrosis and metaplasia of the olfactory epithelium	NTP 1992
mouse, Crj:BDF1, 50 ♂, 50 ♀	**103 weeks**, 0, 4, 16, 64 ml/m³ 6 hours/day, 5 days/week	no effect on mortality **16 ml/m³: NOAEC** **64 ml/m³**: body weight gains ↓, slight atrophy of the granular layer of the brain	Gotoh et al. 1994
rat, Wistar, 6 ♂	**2 weeks,** 0, 39, 96, 193 ml/m³ week 1: 6 hours/day, 5 days/week week 2: 6 hours/day, 3 days/week	**39 ml/m³ and above**: concentration-dependent decrease in brain weights **193 ml/m³**: body weight gains and liver weights ↓, trembling, impaired motor coordination, lungs hyperaemic, haemorrhage	Yang et al. 1995 (review) study Dutch Government Technical Report
rat, Wistar, 6 ♂, 6 ♀	**4 weeks,** 0, 18, 51, 154 ml/m³ weeks 1–3: 6 hours/day, 5 days/week week 4: 6 hours/day, 7 days/week	**18 ml/m³: NOAEC** **51 ml/m³ and above**: neurotoxic effects, (tremor, unsteady gait) **154 ml/m³**: mortality ↑ ♂ 5/6, ♀ 3/6, body weight gains ↓, food consumption ↓, degeneration in heart and lungs, pneumonia	Yang et al. 1995 (review) study Dutch Government Technical Report
rat, Wistar, 10 ♂, 10 ♀	**13 weeks,** 0, 1, 7, 49 ml/m³ (0, 1, 6.5, 42.6 ml/m³) 6 hours/day, 5 days/week	**6.5 ml/m³: NOAEC** **42.6 ml/m³**: ♂/♀: alkaline phosphatase ↓, relative liver weights ↓, reduced hepatocyte size, ♂: leukocyte count ↑, ♀: albumin concentration ↑, no neurotoxic effects	Yang et al. 1995 (review) study Dutch Government Technical Report
rat, F344, 10 ♂, 10 ♀	**13 weeks,** 0, 30, 60, 120 ml/m³ 6 hours/day, 5 days/week	**30 ml/m³: NOAEC** **60 ml/m³**: ♀: body weight gains ↓, olfactory epithelial dysplasia **120 ml/m³**: ♂/♀: body weight gains ↓, olfactory epithelial dysplasia and cysts ♀: haematocrit, haemoglobin and erythrocyte count ↓	NTP 1992

Table 6 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, F344, 8 ♂, 8 ♀	**13 weeks, study of behavioural toxicity** 0, 30, 60, 120 ml/m³ 6 hours/day, 5 days/week	**60 ml/m³**: NOAEC for behavioural toxicity **120 ml/m³**: minor behavioural changes	NTP 1992
rat, Sprague Dawley, ♂, ♀ no other details	**13 weeks study of neurotoxicity**, 0, 30, 70, 140 ml/m³ 6 hours/day, 5 days/week	**30 ml/m³ and above**: ♀: absolute brain weights ↓ (5% ↓ as controls) **70 ml/m³ and above**: ♀: body weights ↓, motor activity ↓, ♂: grip strength of forelimbs ↓ **140 ml/m³**: body weights ↓, absolute brain weights ↓, minimal regenerative dysplasia of the olfactory epithelium, minimal degeneration of peripheral nerves ♂: mortality ↑, convulsions, tremor, hyperactivity, rapid respiration, salivation, hind limb splay ↑, abnormal righting reflex, moderate to severe brain haemorrhage in 2 animals, lesions in the brain, neuronal oedema in the hippocampus ♀: motor activity ↓, rearing ↓	OECD 2002
rat, F344, 50 ♂, 50 ♀	**103 weeks**, 0, 4, 20, 100 ml/m³ 6 hours/day, 5 days/week	No effect on mortality, **0 ml/m³**: inflammation and respiratory metaplasia in the olfactory epithelium as a sequel of a reaction to foreign bodies **4 ml/m³ and above**: ♂: increased incidence of inflammation in the olfactory epithelium ♀: increased incidence of respiratory metaplasia in the olfactory epithelium only at the lowest concentration **20 ml/m³ and above**: ♂ significantly increased incidence of inflammation in the olfactory epithelium **100 ml/m³**: ♂, ♀: body weight gains ↓, increased incidence of necrosis in the olfactory epithelium	Gotoh et al. 1994
rat, Wistar, 90 ♂, 80 ♀	**29 months**, 0, 3, 30, 90 ml/m³ 6 hours/day, 5 days/week	**3 ml/m³**: **NOAEC** (hyperplasia in the olfactory epithelium not confirmed in re-evaluation) **30 ml/m³ and above**: evaluation-relevant degenerative and hyperplastic changes of the nasal olfactory epithelium **90 ml/m³**: mortality ↑, body weight gains ↓, changes in the heart (thrombi, myocardial degeneration), in the forestomach and oesophagus (hyperkeratosis)	Reuzel et al. 1991; US EPA 1997

Table 6 (continued)

Species, strain, number per group	Exposure	Findings	References
dog, beagle, 4 ♂, 4 ♀	**4 weeks,** 0, 5, 10, 25, 50, 100 ml/m³ 7 hours/day, 5 days/week	**100 ml/m³: NOAEC**	OECD 2002
	for 2 further weeks, all dogs of the 5 ml/m³ group and dogs of the 10 ml/m³ group exposed to 150 ml/m³	**5 ml/m³**: no effects **150 ml/m³**: body weights ↓ after 5–6 days: ataxia, tremor, nystagmus, depression, base-wide stance, inability to stand, animals sacrificed because of severity of findings, vacuoles in the granular layer of the cerebellum	
dog, beagle, 4 ♂, 4 ♀	**6 weeks,** 0, 5, 10, 20 ml/m³ 7 hours/day, 5 days/week	**20 ml/m³: NOEC**	Schaefer et al. 2003

Irritation

From the 2-year inhalation study in mice, a NOAEC of 33 ml/m³ for effects on the olfactory epithelium can be derived. At the next-higher concentration of 100 ml/m³, metaplasia or necrosis occurred. In rats, hyperplasia of the olfactory epithelium was found after 29 months even at concentrations of 3 ml/m³ (Reuzel et al. 1991). This was, however, classified as very slight, and these changes occurred also in the animals of the control group (Table 7). No clear concentration-dependence was found for low-grade hyperplasia; this was established only for moderate to severe hyperplasia. In the re-evaluation (US EPA 1997), the distribution was slightly changed as a result of the re-assessment of the findings, and the threshold for significance was raised from 3 to 30 ml/m³. The pathologist of the original study agreed with this evaluation. Slight hyperplasia is frequently reversible, and 3 ml/m³ is therefore considered to be the NOAEC for changes in the olfactory epithelium. The study results also show that this effect is a consequence of long-term exposures and that after high, but short-term exposures no changes occur (see Table 7).

In a 2-year inhalation study with rats and mice, increased incidences of inflammation in the olfactory epithelium of the nose occurred in the male rats and respiratory metaplasia in the female rats at the low concentration of 4 ml/m³ and above. The concentration-dependent increase in the number of male animals with inflammation was significant at concentrations of 20 ml/m³ and above. No concentration-dependence can be derived from the data for respiratory metaplasia (Gotoh et al. 1994). The results of the study are not suitable for the evaluation of methyl bromide as the spontaneous incidence of inflammation in the control ani-

Table 7 Hyperplasia of the nasal olfactory epithelium of rats after inhalation exposure to methyl bromide (Reuzel et al. 1991; US EPA 1997)

	12 months				24 months				29 months			
	Concentration [ml/m³]				**Concentration [ml/m³]**				**Concentration [ml/m³]**			
	0	**3**	**30**	**90**	**0**	**3**	**30**	**90**	**0**	**3**	**30**	**90**
number of ♂ animals examined	10	8	9	10	10	7	9	9	46	48	49	48
hyperplasia												
very slight	3/1[1)]	0	1	0	1	0	3/4	0	2/3	9/6	7/5	8/10
slight	0	0/1	0	2	2/0	0	1	4/5	2/1	3/2	12/11	14**
moderate	0	0	0	3	0	0	0	2/1	0	1/1	4	9**
total	3/1	0/1	1	5	3/1	0	4/5	6	4	13*/9	23**/20**	31**/33**
number of ♀ animals examined	10	8	8	10	9	10	10	9	58	58	59	59
hyperplasia												
very slight	0	0	1	0	1/2	1	0/1	2	7/6	17/9	13/12	10/13
slight	0	0	0	2	2/1	1	4/3	2	2/0	2	9/7	23**/18
moderate	0	0	0	0	1/0	0	1	3	0	1/0	3	9**/8
total	0	0	1	2	4/3	2	5	7	9/6	19*/11	25*/22*	42**/39**

*$p < 0.05$; **$p < 0.01$; [1)] after re-evaluation

mals was very high as a result of a reaction to foreign bodies and no increase in the incidence was found between the concentrations of 20 and 100 ml/m³. In addition, the quality of the study is questionable because of the very short description of the effects.

In the study with dogs, no data are given for irritation.

Neurotoxicity

In the 14-day study with mice (NTP 1992), symptoms such as trembling, nervousness and paralysis were reported at the low concentration of 12 ml/m³ and above.

These symptoms were most pronounced at concentrations of 50 ml/m³ and above. The description of these symptoms in the text is, however, very imprecise, and it is unclear whether the control group was also affected. Because of the inadequate presentation of the results and methods, no decision can be made as to whether the effects observed at 12 ml/m³ are to be considered as adverse. Pathophysiological changes in the peripheral and central nervous systems were not found in any concentration group.

In the 13-week studies with mice and rats (NTP 1992), standardized procedures were used to identify toxic effects on behaviour. In the mice, significant effects were observed at concentrations of 40 ml/m³ in both the females and the males. However, these were not concentration-dependent as, in the 80 ml/m³ group, they were either not observed at all or found only at later times in the experiment. Significant effects occurred in two parameters only in the male animals at the high concentration of 80 ml/m³. A NOAEC of 20 ml/m³ for behavioural toxicity can thus be derived from the 13-week inhalation study with mice. In the 2-year inhalation study in mice, the test results were very inconsistent. In the behavioural tests, sporadic effects occurred, some of which were slight or transient and were seen only at one specific observation time. After 24 months of exposure to 33 ml/m³, however, no significant effects were found, which means that a NOAEC of 33 ml/m³ can be derived from this study

In the 13-week inhalation study with rats, in addition to a marked decrease in body weights, occasional significant changes in behavioural tests (for example decreased fright reaction, increased or changed exploration behaviour, reduced grip strength of limbs) were found in male and female animals at concentrations of 120 ml/m³. However, these effects were significant only at one time during the experiment. Therefore, a NOAEC of 60 ml/m³ can be derived from this study for behavioural toxicity in the rat.

There are, however, two other inhalation studies available in the rat with 4 and 13-week exposure, which are presented in a review (Yang et al. 1995). After 4-week exposure, pronounced neurotoxic effects, such as unsteady gait and tremor, were observed even in the animals of the middle concentration group at 51 ml/m³. At the low concentration of 18 ml/m³ and in the 13-week study with concentrations of up to 42.6 ml/m³, these effects were not found in the rats investigated. From these studies, therefore, a NOAEC of 42.6 ml/m³ can be derived for neurotoxic effects.

Groups of 4 male and 4 female beagle dogs were exposed to methyl bromide concentrations of 0, 5, 10 and 20 ml/m³ (whole body exposure) for 7 hours a day, on 5 days a week, for 6 weeks. Behavioural effects (functional observational battery), body weights, food consumption and clinical effects were investigated and microscopic examination of tissues from the brain, spinal cord and peripheral nerves was carried out. No gross pathological, microscopic or neurotoxic effects were observed. Therefore, from this study, a NOAEC of 20 ml methyl bromide/m³ can be derived for neurotoxicity in the dog (Schaefer et al. 2003). In another study with groups of 4 male and 4 female beagle dogs, no effects were observed after 4-week exposure to the highest concentration tested of 100 ml methyl bromide/m³. How-

ever, after another 2-week exposure to 150 ml/m³, severe neurotoxicity occurred, as a result of which the dogs were killed. The study has not been published and is not available in the original. These results were taken from the OECD Report of 2002 (OECD 2002).

Local effects on skin and mucous membranes

Skin

Groups of 10 male Wistar rats were exposed to air saturated with methyl bromide (liquid at the start of exposure) on the shaved dorsal skin (area about 12 cm²) with the aid of a glass cylinder. The rats were exposed for 30 seconds, and 1, 3 or 5 minutes. In the group exposed for 5 minutes, 3 animals died within 24 hours. After 30 seconds exposure, only mild oedema and minor haemorrhage in the skin were observed. After 1 hour, oedematous changes occurred in the groups exposed for 1, 3 and 5 minutes, haemorrhage in the skin after 6 hours and necrosis after 12 hours. The necrosis increased in size after 24 to 48 hours and formed scabs. The skin started to regenerate after 48 hours, and the scabs became detached after one week. One to three hours after exposure, histological changes were found: in the upper layer of the epidermis, degenerative keratinocytes with pyknotic cell nuclei were observed. The eosinophilic degeneration progressed within 6 to 12 hours, and necrosis was observed (Yamamoto et al. 2000).

Reproductive and developmental toxicity

Fertility

In a 2-generation study in Sprague Dawley rats with daily inhalation exposure to methyl bromide for 6 hours, which is described in the documentation from 1992 (documentation "Methyl bromide" 1996), only the high concentration of 90 ml/m³ led to a marginal reduction in body weight gains in both the parental and the filial generation. No effects were found at 3 or 30 ml/m³. The NOAEC for systemic toxicity can therefore be regarded as 30 ml/m³, the NOAEC for fertility as 90 ml/m³.

In a 2-generation study, groups of 23 to 24 male and female CD(SD) rats were fed a diet fumigated with methyl bromide, so that total bromide concentrations in the diet of 0, 80, 200 or 500 mg/kg were obtained. The intake of total bromide was given as 1.7–2.1, 7.8–10, 17.3–22.3 and 41.6–50.7 mg/kg body weight and day, respectively. Each generation was exposed for 18 weeks. The exposure started 10 weeks prior to the mating of the F0 generation. Histopathological examinations of the pituitary gland and the reproductive organs were carried out in all animals of the control group and the high dose group, and in the animals of the low and middle dose groups that did not mate or had no offspring. No clinical effects and no increase in mortality was found in the F0 and the F1 generations. The body weights of the animals of the F0 and F1 generations were unaffected. In the high dose group, the

body weights of the female offspring of the F2 generation were significantly reduced during lactation. Food intake was significantly reduced in the female animals of the F1 generation of the high dose group during weeks 9 and 10 and during lactation. The following end points remained unaffected in the F0, F1 and F2 generations compared with the findings in the control group: oestrus cycle, mating index, fertility index, gestation index, duration of gestation, number of implantations, sperm count, sperm morphology, litter size, sex ratio, survival index and litter weights. Toxic effects on behaviour were not investigated (Kaneda et al. 1993). This study is suitable for evaluating the effects of bromide, but not the direct effects of methyl bromide.

Developmental toxicity

Rats

In the documentation from 1992 (documentation "Methyl bromide" 1996), a study is presented in which groups of 40 Wistar rats were exposed to methyl bromide concentrations of 20 or 70 ml/m^3 three weeks prior to mating up to gestation day 19, for 7 hours a day, on 5 days a week. Neither maternal nor developmental toxicity was observed.

Groups of 23 to 24 male and female CD(SD) rats were given gavage doses of methyl bromide of 0, 3, 10, or 30 mg/kg body weight and day in corn oil from days 6 to 15 of gestation. On day 20, the animals were killed and the foetuses were examined for organ and skeletal malformations. In the dams of the high dose group, the body weight gains and food intake were significantly reduced. Pathological changes such as erosion and thickened walls in the stomach were found. There were no clinical effects. The number of corpora lutea, implantations and live foetuses, the sex ratio, the incidence of resorptions, the number of dead foetuses, and the foetal and placental weights were unaffected. In the high dose group, the total number of foetuses and litters with variations was slightly, but not significantly, increased (16 compared with 7 foetuses in the controls and 9 compared with 6 litters in the controls, respectively). The authors considered the significantly increased incidence of foetuses with presacral (free) vertebrae not to be substance-related. No other developmental effects were found. In the rat, a NOAEL (no observed adverse effect level) of 30 mg/kg body weight can be given for embryotoxicity and a NOAEL of 10 mg/kg body weight for maternal toxicity (Kaneda et al. 1998).

Rabbits

In the documentation from 1992 (documentation "Methyl bromide" 1996) a developmental toxicity study is presented in which the high concentration of 70 ml/m^3 led to convulsions, paresis in the hind limbs and death in New Zealand White rabbits after daily inhalation exposure for 7 hours a day between gestation days 1 and 15. Neither maternal nor developmental toxicity was observed at methyl bromide concentrations of 20 ml/m^3.

Groups of 26 New Zealand White rabbits were exposed to methyl bromide concentrations of 0, 20, 40 or 80 ml/m³ for 6 hours a day from gestation days 7 to 19. At the high concentration, food intake and body weights were reduced in the dams, and lethargy, head tilting, ataxia and lateral position were observed. No effects were found at the two lower concentrations. There were no effects on the incidence of pregnancy or resorptions, the litter size, foetal weights, sex ratio and uterus weights. After exposure to methyl bromide concentrations of 80 ml/m³, the incidences of absent gallbladders and a missing lobe of the lungs in the foetuses were increased. As both malformations occurred also in the control group with an unusually high incidence (absent gallbladder 2/26 controls; 0/3597 laboratory controls, no other details), the authors assumed that these effects were not substance-related, but could have been produced by a genetic defect in the male animals used. A second study was carried out with rabbits with the highest concentration of 80 ml/m³, in which the offspring were again found to have an increased incidence of absent gallbladders and a missing pulmonary lobe. The authors attributed this to maternal toxicity and excluded a genetic defect. For maternal and embryotoxic effects, they give a NOAEC of 40 ml methyl bromide/m³ (Methyl Bromide Industry Panel 1990).

Groups of 18 Kbl:JW rabbits were given gavage doses of methyl bromide of 0, 1, 3 or 10 mg/kg body weight and day in corn oil from days 6 to 18 of gestation. The animals were killed on day 27 and the foetuses were examined for organ and skeletal malformations. At the high dose, the body weight gains and food intake of the dams were significantly reduced. Embryotoxic effects were not observed. Therefore, in this study, the NOAEL for developmental toxicity in rabbits was 10 mg/kg body weight and the NOAEL for maternal toxicity 3 mg/kg body weight (Kaneda et al. 1998).

Genotoxicity

In vitro

The data for the genotoxicity of methyl bromide in vitro are given in Table 8. These data were already described in the documentation from 1992 (documentation "Methyl bromide" 1996) and in the 2002 documentation of the BLW (Biologischer Leitwert) (documentation "Brommethan" BAT Value Documentation, 2003, only available in German). and show that methyl bromide is genotoxic in vitro.

In vivo

The data for the genotoxicity of methyl bromide in vivo are given in Table 9.

The formation of DNA adducts in the rat was investigated after the inhalation (40.7 µmol/kg body weight corresponding to 3.8 mg/kg body weight) or oral administration (8.3 µmol/animal corresponding to 0.8 mg/kg body weight) of ^{14}C-methyl bromide in F344 rats. After isolation and hydrolysis of the DNA from the stomach, forestomach, liver and lungs, three identifiable DNA methylation products

Table 8 The genotoxicity of methyl bromide in vitro

End point	Test system	Concentration range	Effective concentration*	Cytotoxicity*	Result		References
					–m. a.	+m. a.	
bacteria							
gene mutation	Salmonella typhimurium TA100 (in exsiccator)	0.02–0.2%	0.02%	no data	+	not determined	Simmon et al. 1977
gene mutation	Salmonella typhimurium TA100 (suspension test)	10–10 000 mg/l	285 mg/l and above	no data	+	+	Kramers et al. 1985
	TA98				–	–	
gene mutation	Salmonella typhimurium TA100 (plate incorporation test)	400–50 000 mg/m^3	19 000 mg/m^3 and above	no data	+	+	Kramers et al. 1985
	T98				–	–	
gene mutation	TA100, 1535	500–5000 mg/m^3	500 mg/m^3 and above	–	+	+	Moriya et al. 1983
gene mutation	TA98, 1537, 1538	500–5000 mg/m^3	–	–	–	–	Moriya et al. 1983
gene mutation	TA100	0.004–2.4 M (380–227 856 mg/l)	0.004 M and above	0.9 M and above	+	+	NTP 1992
	TA98				–	–	
gene mutation	Escherichia coliWP2	500–5000 mg/m^3	no data	–	+	+	Moriya et al. 1983
mammalian cells							
UDS	rat hepatocytes	10–30 mg/l	–	no data	–	not determined	Kramers et al. 1985
SCE	human lymphocytes	4.3% (100 seconds)	4.3%	no data	+	not determined	Tucker et al. 1985
SCE	whole blood/ G_0 lymphocytes	2–24 mg/l (0.5 hours)	2 mg/l and above	no data	+	+	Garry et al. 1990
SCE	whole blood of conjugators	5000 ml/m^3 (1 hour)	–	no data	–	not determined	Schröder et al. 1991

Table 8 (continued)

End point	Test system	Concentration range	Effective concentration*	Cytotoxicity*	Result		References
					–m. a.	+m. a.	
SCE	whole blood of non-conjugators	5000 ml/m³ (1 hour)	5000 ml/m³	no data	+	not determined	Schröder et al. 1991
CA	total blood/G_0 lymphocytes	12, 18, 95 mg/l (0.5 hours)	18 mg/ml and above	95 mg/ml	–	+	Garry et al. 1990
gene mutation	L5178Y mouse lymphoma cells on TK and HPRT locus	0.03–30 mg/l	0.03 mg/l and above	0.3 mg/l and above	+	not determined	Kramers et al. 1985

(3-methyladenine, 7-methylguanine, O^6-methylguanine) and one unidentified DNA adduct were detected. The radioactivity of the DNA adducts from the stomach and forestomach was 8 to 10 (oral) and 5 to 10 (inhalation) times higher than that of the DNA adducts from the liver and lungs. From the dose of 40.7 µmol/kg body weight (male rat) a theoretical inhalation concentration of 4.2 ml/m³ can be calculated (conversion based on 40.7 µmol/kg body weight = 3.8 mg/kg body weight = 0.95 mg/250 g rat; 117 ml/min respiratory minute volume for the rat, 6-hour exposure = total respiratory volume of 42 litres; 0.95 mg/42 l = 23 mg/m³ ⇒ 17 mg/m³/8 hours = 4.2 ml/m³) (Gansewendt et al. 1991).

In a 14-day study, groups of 4 mice of both sexes were exposed to 0, 12, 25, 50, 100 or 200 ml/m³ (6 hours a day, 5 days a week). The high concentration was lethal for 4 males and 1 female. Methyl bromide increased the SCE values in the bone marrow, which were not significant in the pairwise comparison of the individual values with the corresponding controls. The result of the trend test for linear dose-dependence was significant. In a subsequent 13-week study, 4 mice of both sexes were exposed to 0, 10, 20, 40, 80 or 120 ml/m³ (6 hours a day, 5 days a week). Four males exposed to the high concentration died. At this concentration, the body weights were reduced by 12%. After 12 weeks, no increase in the incidence of SCE was found, and there were no changes in the time phases of the cell cycle (NTP 1992).

In the 14-day study, in the same mice, the formation of micronuclei in the peripheral erythrocytes was investigated. In the females, the micronucleus incidence was significantly increased at concentrations of 12 ml/m³ and above. The trend test for linear dose-dependence was significant. In the male mice, the result of the micronucleus test was negative. The high concentration was lethal for 4 males and 1 female. There was no cytotoxicity. In the subsequent 13-week study, no increase in the incidence of micronuclei in the treated groups was found after exposure for 4, 8 or 12 weeks. No cytotoxicity occurred. After 4 weeks, concentrations of 80 ml/m³

and above caused a significant reduction in the micronucleus incidence compared with that in controls. The authors suggested that the decrease in responses with increasing exposure duration could be due to metabolic alterations or changes in bone marrow sensitivity (NTP 1992).

The micronucleus test (14-day study, 5 mice of both sexes per group, 6 hours/day; 5 days/week, inhalation) was repeated at concentrations of 0, 12, 25, 50 or 100 ml/m^3. In the male animals, the incidence of micronuclei was significantly increased only at the concentrations of 50 and 100 ml/m^3; at these two concentrations neurotoxic symptoms had already been observed in the mice (NTP 1992). The trend test yielded a significant result. In the female mice, the incidence of micronuclei was significantly increased at 12, 25 and 50 ml/m^3. The trend test result for a linear increase with the dose was, however, not statistically significant, as the value for the lowest concentration was highest, and the other values formed a plateau with a negative tendency. No cytotoxicity occurred (Witt et al. 2000).

Groups of 10 male and 10 female BDF_1 mice and F344 rats were exposed to methyl bromide concentrations of 0, 154, 200, 260, 338 and 440 ml/m^3 for 6 hours a day, for 2 weeks. All animals were killed on day 15. Of the mice, one male died at the concentration of 154 ml/m^3, 5 males and 8 females died at 200 ml/m^3 and all female mice and all except one male mouse died at 260 338 and 440 and above ml/m^3. In the mice, necrosis of the kidneys, stomach ulcers, atrophy of the testes, spleen and lymph nodes, and necrosis of the heart were found even at the low concentration. The incidence of induced micronuclei in polychromatic and normochromatic erythrocytes was significantly increased in the bone marrow of male and female mice after exposure to methyl bromide concentrations of 154 and 200 ml/m^3. Micronuclei were induced in the peripheral blood of female mice at 154 ml/m^3 and above, and in male mice at 200 ml/m^3 and above although, here too, cytotoxicity was found. In the rats, deaths occurred only at 338 ml/m^3 and above. An increased incidence of micronuclei in the bone marrow was found only in the male rats at 338 ml/m^3 (Araki et al. 1995). As the induction of micronuclei occurred only with simultaneous mortality, toxicity and cytotoxicity, and no further investigations were conducted below this concentration, it is not possible to decide whether micronuclei would be induced also without the observed toxicity.

In a chromosomal aberration test, no change in the incidence of aberrations was found in the bone marrow of male and female CD rats after the inhalation of 0, 78 or 272 mg/m^3 (0, 20, 70 ml/m^3) for 7 hours a day, for 5 days, compared with that in the controls (McGregor 1981).

Germ cells

Groups of 10 male F344 rats were exposed to methyl bromide concentrations of 0, 75, 150 and 250 ml/m^3 (whole animal exposure) for 6 hours a day, for 5 days. Two animals died at the high concentration. In addition, ataxia, convulsions, lethargy and exhaustion were observed in the surviving animals of this concentration group. In 3 to 5 animals, the homogenate of the testes was examined for single strand breaks between one and 24 hours after the 5-day exposure. After one and 24

hours, there was a significant increase in DNA single strand breaks after exposure to 250 ml/m³. At the two lower concentrations, no toxic effects occurred and there was no increase in single strand breaks (Bentley et al. 1995). The study does not provide evidence for effects on the germ cells, but it shows that the testes and thus also the germ cells are affected by the substance.

In a dominant lethal test, groups of 10 male CD rats inhaled methyl bromide concentrations of 0, 78 or 272 mg/m³ (corresponding to 0, 20, 70 ml/m³) for 7 hours a day, for 5 days. The animals were subsequently mated with untreated female rats (one male to two females) for 10 weeks. Compared with in the controls, no sperm abnormalities were found and no changes in fertility, the number of corpora lutea or the number of pre or post-implantation losses (McGregor 1981).

Table 9 The genotoxicity of methyl bromide in vivo

Test system		Exposure	Result	Comments	References
somatic cells					
DNA adducts, liver, lungs, stomach, forestomach (bound radioactivity)	rat, F344, groups of 5 ♂, 5 ♀	8.3 µmol/animal (0.8 mg/kg body weight), single, gavage	+	3-methyladenine, 7-methylguanine, O^6-methylguanine (radioactivity in the DNA of the stomach and forestomach 8–10 times higher than in the DNA of the liver and lungs)	Gansewendt et al. 1991
DNA adducts, liver, lungs, stomach, forestomach (bound radioactivity)	rat, F344, groups of 5 ♂, 5 ♀	♂: 40.7 µmol/kg body weight (3.9 mg/kg body weight) ♀: 113.3 µmol/kg body weight (10.8 mg/kg body weight) single, 6 hours inhalation	+	3-methyladenine, 7-methylguanine, O^6-methylguanine (radioactivity in the DNA of the stomach and forestomach 5–10 times higher than in the DNA of the liver and lungs)	Gansewendt et al. 1991
SCE, bone marrow	mouse, B6C3F1, groups of 4 ♂, 4 ♀	0, 12, 25, 50, 100, 200 ml/m³, inhalation, 6 hours/day, 5 days/week, 2 weeks	+	SCE ↑, significant dose-dependent trend, but not significant on pairwise comparison with controls; 200 ml/m³: lethal for 4 ♂ and 1 ♀; no cytotoxicity	NTP 1992
SCE, bone marrow	mouse, B6C3F1, groups of 4 ♂, 4 ♀	0, 10, 20, 40, 80, 120 ml/m³, inhalation, 6 hours/day, 5 days/week, 12 weeks	–	120 ml/m³: lethal for 4 ♂; body weights ↓ by 12%; no cytotoxicity	NTP 1992

Table 9 (continued)

Test system		Exposure	Result	Comments	References
MN, bone marrow	mouse, BDF1, groups of 10 ♂, 10 ♀	0, 154, 200, 260, 338, 440 ml/m³, inhalation, 6 hours/day, 5 days/week, 2 weeks	+	154 ml/m³ and above: MN ↑ toxicity: body weights ↓; mortality: ♂: 1/10, 5/10, 9/10, 10/10, 10/10 ♀: 0/10, 8/10, 10/10, 10/10, 10/10	Araki et al. 1995
MN, bone marrow	rat, F344, groups of 10 ♂, 10 ♀	0, 154, 200, 260, 338, 440 ml/m³, inhalation, 6 hours/day, 5 days/week, 2 weeks	+	♂ 338 ml/m³: MN ↑, toxicity: ♂: at 200 ml/m³ and above and ♀ at 154 ml/m³ and above: body weights ↓; mortality ♂: 0/10, 0/10, 0/10, 7/10, 10/10 ♀: 0/10, 0/10, 0/10, 1/10, 10/10	Araki et al. 1995
MN, peripheral erythrocytes	mouse, BDF1, groups of 10 ♂, 10 ♀	0, 154, 200, 260, 338, 440 ml/m³, inhalation, 6 hours/day, 5 days/week, 2 weeks	+	154 ml/m³ and above ♀: MN ↑, 200 ml/m³ and above ♂: MN ↑, toxicity: body weights ↓; mortality 1/10, 5/10, 9/10, 10/10, 10/10	Araki et al. 1995
MN, peripheral erythrocytes	mouse, B6C3F1, groups of 5 ♂, 5 ♀	0, 12, 25, 50, 100, 200 ml/m³, inhalation, 6 hours/day, 5 days/week, 2 weeks	–		NTP 1992
			+	12 ml/m³ and above: ♀: MN ↑; significant in the trend test; 200 ml/m³: lethal for 4 ♂ and 1 ♂; no cytotoxicity	
MN, peripheral erythrocytes	mouse, B6C3F1, groups of 5 ♂, 5 ♀	0, 12, 25, 50, 100 ml/m³, inhalation, 6 hours/day, 5 days/week, 2 weeks	+	50 ml/m³ and above: ♂: MN ↑; significant in the trend test, 100 ml/m³ data from 3 ♂ only	Witt et al. 2000
			+	12 ml/m³ and above: ♀: MN ↑; but no significant trend; no cytotoxicity	

Table 9 (continued)

Test system		Exposure	Result	Comments	References
MN, peripheral erythrocytes	mouse, B6C3F1, groups of 6–8 ♂, 6–8 ♀	0, 10, 20, 40, 80, 120 ml/m³, inhalation, 6 hours/day, 5 days/week, 4, 8, 12 weeks	–	80 and 120 ml/m³ and 4 weeks: MN ↓ significantly; no cytotoxicity	NTP 1992
CA, bone marrow	rat, CD, groups of 9 ♂, 9 ♀	0, 78, 272 mg/m³ (0, 20, 70 ml/m³), inhalation, 7 hours/day, 5 days, examination 6 hours after the end of treatment	–		McGregor 1981
germ cells					
DNA single strand breaks, testis, alkaline elution	rat, F344 groups of 3–5 ♂	0, 75, 150, 250 ml/m³, inhalation, 6 hours/day, 5 days; examination 1 and 24 hours after the end of treatment in testis homogenate	+	250 ml/m³ (after 1 and 24 hours): toxicity, mortality 2/10	Bentley et al. 1995
DLT	rat, CD, groups of 10 ♂	0, 78, 272 mg/m³ (0, 20, 70 ml/m³), inhalation, 7 hours/day, 5 days, mating 1 week each in the ratio 1♂:2♀ for 10 weeks	–		McGregor 1981

SCE: sister chromatid exchange; MN: micronuclei; CA: chromosomal aberrations; DLT: dominant lethal test

Summary

In vitro, methyl bromide was found to be genotoxic in Salmonella mutagenicity tests with TA100 in the presence and absence of metabolic activation, and in SCE tests and in gene mutation tests with mouse lymphoma cells. In vivo, DNA adducts (7-methylguanine and O^6-methylguanine) were detected in the rat in the liver, lungs and particularly in the stomach and forestomach after oral administration and inhalation. These data do not allow the quantitative evaluation of a dose–response relationship.

In the bone marrow, the frequency of micronuclei was increased in the mouse at the low concentration of 154 ml/m³ and above, and in the rat at 338 ml/m³ and above after exposure for 2 weeks; simultaneous toxicity was observed. In a micronucleus test, inhalation of 12 to 200 ml/m³ for 2 weeks increased the incidence of micronuclei in the peripheral erythrocytes at 12 ml/m³ and above only in female mice. In a repeat study with concentrations in the range of 12 to 100 ml/m³, micronuclei were induced in female mice at concentrations of 12 ml/m³ and above and in male mice at 50 ml/m³ and above, in the female animals, however, without a

linear dose–response relationship. In contrast, in the 13-week inhalation study (10 to 120 ml/m³) the frequency of micronuclei was in the range of the control values after 4, 8 and 12 weeks. As a steady-state situation is reached for micronuclei in the peripheral erythrocytes after prolonged exposure, an increased frequency would be expected also after 4, 8 or 12 weeks. One possible explanation for the discrepancy in the results after 2-week and 4 to 12-week inhalation exposure could be that methyl bromide is initially (2 weeks) in a position to induce micronuclei, but no longer able to do so later on (4, 8, 12 weeks) as a result of the counter measures (enzyme induction) initiated by the cells. The authors (NTP 1992) suggested that the decrease in the effects with increasing exposure duration could be the result of metabolic changes or diminished bone marrow sensitivity.

In the rat, no chromosomal aberrations were induced after 5-day inhalation of 20 or 70 ml/m³.

The detection of DNA single strand breaks in the homogenate of the testes shows that the germ cells are reached; however, a dominant lethal test in rats yielded negative results after 5-day inhalation exposure. Overall, clastogenic or aneugenic effects were found only after 2-week inhalation exposure in mice in the non-cytotoxic range. After prolonged inhalation (≥ 4 weeks) of concentrations in the range of 10 to 120 ml/m³, these clastogenic or aneugenic effects no longer occurred. Although methyl bromide is systemically available, it does not induce dominant lethal mutations in the rat.

Carcinogenicity

The studies of the carcinogenicity of the substance were already described in the documentation from 1992 (documentation "Methyl bromide" 1996). Further long-term studies are not available. In a 2-year inhalation study in B6C3F1 mice, no increase in tumour incidences occurred after exposure to methyl bromide concentrations of 0, 10, 33 or 100 ml/m³. Also in the Wistar rat, no increased tumour incidences were observed after 29-month inhalation exposure to methyl bromide concentrations of 3, 30 or 90 ml/m³.

Groups of 10 male and 10 female Wistar rats were given gavage doses of methyl bromide of 0, 0.4, 2, 10 or 50 mg/kg body weight and day in arachis oil on 5 days a week, for 90 days. After doses of 2 mg/kg body weight and above, the incidences of forestomach hyperplasia were increased in all rats in a dose-dependent manner. In the high dose group, 13 of 20 animals were found to have squamous cell carcinomas in the forestomach. No effects occurred in the low dose group at 0.4 mg/kg body weight and day. The hyperplasia and squamous cell carcinomas were interpreted as sequelae of the local irritation in the forestomach caused by methyl bromide (see documentation "Methyl bromide" 1996).

Manifesto (MAK value/classification)

The most sensitive end points for the evaluation of methyl bromide are its neurotoxic and irritative effects.

MAK value. From the available data in humans, no NOAEC can be given for the irritative and neurotoxic effects. To derive a MAK value, therefore, animal studies are used. For irritation, a NOAEC of 3 ml/m³ for effects in the olfactory epithelium was obtained from the 29-month inhalation study in the rat. In a 2-year inhalation study in mice, irritation occurred only at the much higher concentration of 100 ml/m³, the NOAEC was 33 ml/m³. The NOAEC for systemic effects, including neurotoxic and behavioural effects was 30 ml/m³ for mice and rats. Irritation therefore represents the most sensitive end point. On the basis of a NOAEC of 3 ml/m³ after long-term inhalation exposure, a MAK value of 1 ml/m³ has been established. In the rat, the LOAEC (lowest observed adverse effect concentration) is markedly higher than the NOAEC, which means that the actual NAEC (no adverse effect concentration) may be higher than 3 ml/m³; in mice the NOAEC is 30 ml/m³.

Because methyl bromide can be absorbed through the skin from the gaseous phase, biomonitoring studies are recommended in addition to personal protective measures. The BLW of 12 mg bromide/l serum (documentation "Brommethan" BAT Value Documentation, 2003, only available in German) must be observed.

Peak limitation. As the main effects are local effects, methyl bromide is classified in Peak Limitation Category I. As, in rats, signs of irritation are observed only at methyl bromide concentrations of 20 ml/m³ and above, an excursion factor of 2 can be given.

Absorption through the skin. There are no quantitative data available for the dermal penetration of methyl bromide. Observations in exposed workers and animal studies confirm, however, that methyl bromide can be absorbed dermally and be absorbed through the skin from the gaseous phase. However, in 6 workers exposed exclusively via the skin to methyl bromide concentrations of 8750 to 10 000 ml/m³ for 40 minutes, the BLW of 12 mg bromide/l plasma, which is based on the neurotoxicity of the substance, was not exceeded in any of the persons, and there was also no systemic toxicity. However, severe skin lesions occurred in the exposed persons. As the short-term exposure to these high methyl bromide concentrations, which were more than 17 000 times higher than the MAK value, produced no signs of systemic toxicity and the BLW was not exceeded, dermal absorption is not to be expected to make a toxicologically relevant contribution when the MAK value is observed. Model calculations confirm the relatively minor contribution of dermal absorption compared with inhalation. Methyl bromide is therefore not designated with an "H" (for substances which can be absorbed through the skin).

If the MAK value is exceeded, the whole body must be protected as methyl bromide can be absorbed through the skin from the gaseous phase; the wearing of breathing masks alone is not sufficient.

Sensitization. There are no animal data available for sensitization, and cases of sensitization in humans are unknown. The substance is, therefore, not designated with either "Sa" or "Sh" (for substances which cause sensitization of the airways and skin).

Carcinogenicity. In inhalation studies in mice and rats, no increased tumour incidences were observed. In 1992, methyl bromide was, however, classified in Section IIIB for carcinogenic substances (corresponding to Carcinogen category 3B), as it has systemic genotoxic potential and a section of the human population is particularly sensitive to its genotoxic effects as a result of a genetic polymorphism in glutathione-dependent conjugation ("conjugators" and "non-conjugators") (see also documentation "Methyl bromide" 1996). There are no other animal studies available for the carcinogenicity of the substance. More recent studies confirm the in vitro genotoxicity and in vivo alkylating effects of methyl bromide. Whether methyl bromide is carcinogenic in humans cannot be decided at present. The available historical cohort study (Alavanja et al. 2003) shows a possible relationship between methyl bromide exposure and the increased occurrence of prostate carcinomas in humans. It must, however, be borne in mind that the authors had no a priori hypothesis concerning methyl bromide and that the only statistically significant exposure–response relationship among the 50 pesticides analysed could be a chance finding. It must still be confirmed by at least one further study. Overall, methyl bromide is still suspected of being carcinogenic so that the substance remains in Carcinogen category 3B. In vivo, clastogenic or aneugenic effects are found in mice only after 2-week inhalation exposure. After prolonged inhalation (≥ 4 weeks) of concentrations in the range of 10 to 120 ml/m^3, these clastogenic or aneugenic effects are no longer observed. Although methyl bromide is systemically available, it does not induce dominant lethal mutations in the rat. Therefore, after long-term exposure in the low concentration range, genotoxic effects cannot be detected. As increased tumour incidences were not found in the long-term studies with rats and mice after inhalation, the derivation of a MAK value is justified.

Germ cell mutagenicity. In vitro, methyl bromide is genotoxic, and in vivo it has alkylating properties. Clastogenic or aneugenic effects were found in mice in the non-cytotoxic range only after 2-week inhalation exposure. After prolonged inhalation (≥ 4 weeks) of concentrations in the range of 10 to 120 ml/m^3, these clastogenic or aneugenic effects were no longer found. Although methyl bromide is systemically available, it does not induce dominant lethal mutations in the rat. The substance is therefore not classified in one of the categories for germ cell mutagens.

Prenatal toxicity. In studies of the toxic effects on prenatal development with inhalation or oral exposure, developmental toxicity was found only at concentrations or doses which caused maternal toxicity. The NOAEC for developmental toxicity is 70 ml/m^3 in rats and 40 ml/m^3 in rabbits. In a 2-generation study in the rat, a NOAEC of 30 ml/m^3 was obtained for developmental toxicity. When the MAK value of 1 ml/m^3 is observed, therefore, no prenatal toxicity is to be expected. However, methyl bromide is neurotoxic, and studies of the developmental neurotoxicity are

not available. Behavioural effects were observed in adult mice at concentrations of 40 ml/m³ and above, and in adult rats at 50 ml/m³ and above. As it cannot be excluded that young animals are more sensitive to the neurotoxic effects than adult animals, and as it is not known at which concentrations neurotoxic effects occur in humans, methyl bromide is classified in Pregnancy Risk Group D.

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