Social Europe

# Recommendation from the Scientific Committee on Occupational Exposure Limits for methyl bromide

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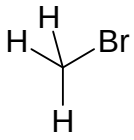
| | |
|---|---|
| 8 hour TWA | : not feasible to derive a health-based limit (see Recommendation) |
| STEL (15 min) | : not feasible to derive a health-based limit (see Recommendation) |
| Notation | : "Skin" |
| SCOEL Carcinogen category | : A (non-threshold genotoxic carcinogen) |

**Substance identification**

Methyl bromide




Synonyms : Bromomethane, monobromomethane, HBr

EINECS No. : 200-813-2

EEC No. : 602-002-00-2

Classification : Mutagenicty, Cat. 3; R68, T R23/25, Xn R48/20, Xi R36/37/38, N R50, N R59

CAS No. : 74-83-9

MWt : 94.95 g/mol

Conversion factor (25 °C): 1 ppm = 3.89 mg/m$^3$; 1 mg/m$^3$ = 0.26 ppm

This document is based on ACGIH (2001), IARC (1999), DFG (1999), IPCS (1995) and the references based therein, as well as on a re-assessment by SCOEL of the recent literature. Further reviewed literature: Calvert et al. (1998), Kaneda et al. (1998), and Wilson et al., (1998), Pletsa et al. (1999).

**Physico-chemical properties**

Methyl bromide is a colourless, non-flammable gas with no taste or odour at low concentrations. At levels well above current limit values of 1 ppm (3.89 mg/m$^3$), a sweetish odour may be observed. Odour thresholds between of 80 and 4000 mg/m$^3$ are reported (Ruth, 1983). The melting point of methyl bromide is -93.66 °C and the boiling point is 3.56°C. Methyl bromide is soluble in water (17.5 g/l at 20°C), in diethyl ether, ethanol, chloroform, carbon disulfide, benzene, and tetrachloromethane. The vapour pressure is 1893 hPa (20°C).

# 1. Occurrence/use

The annual production volume of methyl bromide in the year 1990 was in the EU about 19000 tonnes, and about 28000 tonnes in North America (IPCS, 1995). Methyl bromide is commonly produced by the interaction of methanol and hydrogen bromide (IPCS, 1995). Methyl bromide is used as follows: soil (pre-planting) fumigation (77%), quarantine and commodity fumigation (12%), structural fumigation (5%), and chemical intermediates (6%) (UNEP, 1992). The general use of methyl bromide in fire extinguishers has been abandoned as it was the cause of a number of fatal accidents. However, it is still used for special-purpose fire extinguishers (IPCS, 1995), as also exemplified by a recent case report (Hoizey et al., 2002).

## 1.1. Occupational exposure

Occupational exposure to methyl bromide takes mainly place during manufacture and during fumigation (structural and soil fumigation). The primary route of potential occupational exposure is inhalation, although some intoxications have also been reported after dermal exposure.

*Manufacturing*

In a methyl bromide plant in the USA, workplace air concentrations of 78-116 mg/m$^3$ were recorded using direct measurement (IPCS, 1995). In the worker´s breathing zone (methyl bromide-producing factory in Japan) methyl bromide concentrations were usually below 4 mg/m$^3$, but sometimes exceeded 20 mg/m$^3$ (Kishi et al., 1988).

*Fumigation*

Occupational exposures between < 0.8 and 646 mg methyl bromide/m$^3$ were measured during space, soil or chamber fumigation in a survey of methyl bromide fumigation in Switzerland (Guillemin et al., 1990). During greenhouse fumigation, relatively high methyl bromide concentrations are reported: values range from 320 to 4000 mg/m$^3$ in one investigation (Roosels et al., 1981) and from 117 to 11700 mg/m$^3$ in a further study (van den Oever et al., 1982).

# 2. Health Significance

## 2.1 Toxicokinetics

*Absorption*

*Inhalation*

The uptake of inhaled methyl bromide is about 50 % in F344 rats, beagle dogs and in human volunteers (Andersen et al., 1980, Medinsky et al., 1985, Raabe, 1986, Raabe, 1988). In rat experiments at higher methyl bromide concentrations (above 650 mg/m$^3$) the amount of absorbed material decreased: at 1206 mg/m$^3$ only 27% was absorbed. (Medinsky et al., 1985): Therefore, it appears that the uptake of methyl bromide in experimental animals by inhalation is saturable.

*Dermal*

In fumigation workers who had skin contact with methyl bromide, increased plasma bromide levels provided evidence of the penetration of the substance through the skin

(Iwasaki et al., 1989). This is also supported by experiments on dermal uptake of methyl bromide in rats (Yamamoto et al., 2000). The possibility of absorption by the skin of toxic quantities of methyl bromide has been repeatedly demonstrated (Jordi, 1953, Longley and Jones, 1965, Lifschitz and Gavrilov 2000).

*Distribution*

In rats methyl bromide is rapidly distributed to all tissues after inhalation and rapidly metabolised. A small percentage is cleared slowly and incorporated into metabolic pools. The major organs of [$^{14}$C] distribution are adipose tissue, liver, lung and kidney (Bond et al., 1985, Honma et al., 1985; Jaskot et al., 1988). Methyl bromide concentrations in all tissues described reached a maximum within 1 h after start of exposure and maintained almost the same steady-state level during 8 h of continuous exposure (Honma et al., 1985).

*Metabolism and elimination*

Increased bromide values were found in the blood serum of persons who had immediate skin contact with methyl bromide (Longley and Jones, 1965, Hezemans-Boer et al., 1988), even when they wore adequate respirators (Zwaveling et al., 1987).

Investigations with rats administered $^{14}$C-labelled methyl bromide showed that about half of the $^{14}$C dose taken up is exhaled as $^{14}CO_2$ (Bond et al., 1985). The rest of the radioactivity appears mainly in urine and a small amount also in the faeces; the distribution between the routes of excretion depends on the mode of administration (inhalation, oral or intraperitoneal; Medinsky et al., 1985). The metabolic pathways of methyl bromide correspond with those of the chemically related substances methyl chloride and methyl iodine. These are represented in Figure 1, according to Kornbrust and Bus (1982) and Bolt and Gansewendt (1993).

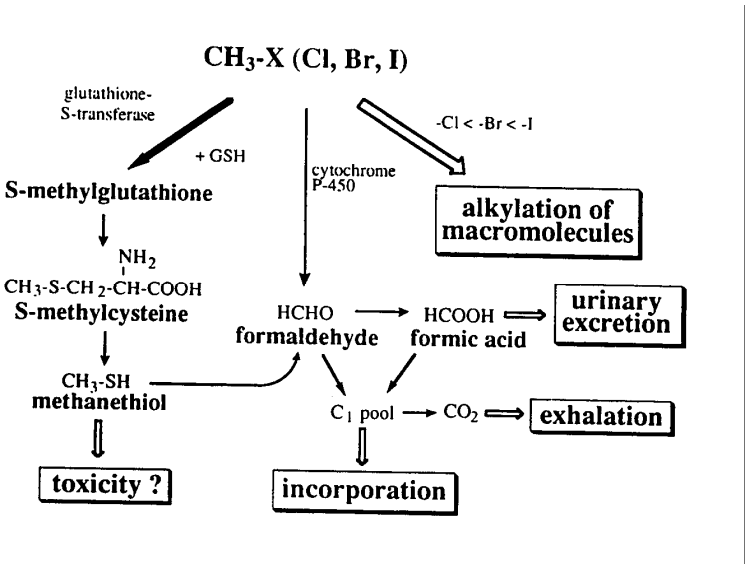



*Figure 1: Metabolic pathways of methyl halides (chloride, bromide, iodide)*

To a small extent the monohalomethanes are oxidised by the cytochrome P-450 system which eliminates the halogen ion (here: bromide) to form formaldehyde and, in consequence, formic acid (Kornbrust and Bus, 1982, 1983).

Methyl halides can be conjugated enzymatically in several tissues including human

erythrocytes, to form *S*-methylglutathione (Redford-Ellis and Govenlock). There are marked species differences; this metabolic pathway could not be detected in erythrocytes of mouse, rat, cattle, sheep, pigs, nor rhesus monkeys (Deutschmann et al., 1990).

When human blood samples from different persons are incubated with methyl bromide, in most cases ("conjugators") this is conjugated with glutathione to form S-methyl-glutathione. Some blood samples (those of "non-conjugators") do not contain this enzyme activity (Hallier et al., 1990a). The responsible enzyme activity is markedly higher towards methyl bromide than towards the other halomethanes. The purification and characterisation of the enzyme has been described (Schröder et al. 1992); it is the glutathione-S-transferase hGSTT1-1 (Pemble et al., 1994). Dependent on ethnicity, the *hGSTT1* gene is deleted in major parts of the population (*Table 1*). This has reflections on inter-individual and inter-ethnic differences of disposition and toxicity of methyl bromide (see 3.2).

Table 1: Percentages of carriers ("conjugators", homozygous and heterozygous) and non-carriers ("non-conjugators") of the hGST1 gene, within different populations/ethnicities (Thier et al. 2003)

| Country/region | % *hGSTT1* carriers | % *hGSTT1* non-carriers | Reference |
|---|---|---|---|
| Germany (1994) | 75% | 25% | Hallier et al. |
| Turkey | 80% | 20% | Oke et al. (1998) |
| Scandinavia (1993) | 85% | 15% | Hallier et al. |
| USA (whites) (1995) | 80% | 20% | Nelson et al. |
| USA ("African American") (1995) | 87% | 22% | Nelson et al. |
| USA ("Mexican American") (1995) | 90% | 10% | Nelson et al. |
| China (Shanghai) | 51% | 49% | Shen et al. (1998) |
| East Asia (Korea, China) (1999) | 38% | 62% | El Masri et al |

## 2.2. Acute toxicity

### 2.2.1. Human data

Cases of severe methyl bromide poisoning in humans, some of them fatal, were frequently reported. Fatal poisoning has resulted from exposures to relatively high concentration (from 33000 mg/m$^3$). Non-fatal poisoning has resulted from exposure to concentrations above
390 mg/m$^3$. The manifestations of methyl bromide poisoning may be delayed. The latent period may vary from 2 to 48 h (Holling and Clarke 1944). Symptoms of acute methyl bromide poisoning are severe pulmonary oedema, headache, visual disturbances, nausea, vomiting, smarting of the eyes, itching of the skin, listlessness, vertigo, and tremor; progressing to convulsions, fever, cyanosis, pallor and death. Several neuropsychiatric signs and symptoms, such as mental confusion, mania, muscular twitches, and slurring of speech, may precede death (Wyers, 1945, Sax et al., 1984, Gosselin et al., 1984, Lifshitz and Gavrilov, 2000, Hoizey et al., 2002).

Garnier et al. (1996) described an intoxication event of two methyl bromide fumigators working together and of which duration and intensity of exposure were considered identical. One person, being of negative GSTT1 phenotype ("non-conjugator") developed only mild neurotoxicity of reversible nature, whereas the other, of GSTT1-positive phenotype ("conjugator"), developed very severe neurotoxicity and persistent infirmity. The GSTT1 negative subject showed higher concentrations, compared to those of the GSTT1 positive subject, of S-methylcysteine adduct in albumin (149 vs. 91 nmol/g protein) and in globin (77 vs, 30 nmol/g globin). This is consistent with the view of glutathione conjugation being a toxifying pathway of methyl bromide (see 3.1). A recent case report of methyl bromide poisoning, also including biomonitoring and GSTT1 phenotyping data, is supportive of this view (Buchwald and Müller, 2001).

### 2.2.2. Animal data

An $LD_{50}$ value after oral administration in rats was 214 mg/kg bw (Danse et al., 1984). The dose-mortality response curve after methyl bromide inhalation is quite steep. The $LC_{50}$ values for rats and mice are shown (details: in *Table 2,* see Appendix)

Clinical signs were decrease in locomotor movement, tremor, convulsion, diarrhoea, bradypnoea, dyspnoea, lacrimation and diarrhoea.

Based on comparative pharmacological studies of methyl bromide and bromide, using hippocampal slices of young rats, it was postulated that the central neurotoxicity of methyl bromide should be due to metabolites or other indirect effects, rather than on methyl bromide itself (Zeise et al. 1999).

## 2.3. Irritation

### 2.3.1. Human data

Liquid methyl bromide and methyl bromide gas has penetrated through all articles of clothing. Liquid methyl bromide caused dermal irritation with superficial burns with much vesication when in contact with skin (ACGIH, 2001, Butler et al., 1945). But also methyl bromide gas is irritating to the skin. Hezemans-Boer et al (1988) reported sharply demarcated erythema with multiple vesicles and large bullae in workers exposed for 40 minutes to about 35000 mg/$m^3$. With the exception of some residual hyperpigmentation, the effects were reversible within 4 weeks.

### 2.3.2. Animal data

Irish et al. (1940) noticed lacrimation in rats after inhalation of methyl bromide levels above 10000 mg/$m^3$. Irritation of the eye membranes in mice at concentrations of 3200 mg methyl bromide/$m^3$ was described by Balander et al. (1962). In rats, local application of methyl bromide to the skin caused morphological changes of epidermal cells, fibroblasts and blood vessels which were attributed to cytotoxicity (Yamamoto et al. 2000).

## 2.4. Sensitisation

No data are available.

## 2.5. Repeated dose toxicity

### 2.5.1. Human data

There are numerous case reports of effects after repeated exposure to high concentrations
of methyl bromide. In most cases there are no data on exposure concentrations given.

Adverse symptoms like lethargy, ataxia, and retrobulbar optic neuritis were reported by workers exposed to a maximum concentration of 58 mg/m$^3$ (Kishi et al., 1988). Nausea, vomiting, headache, and skin lesions were observed in workers exposed for 2 weeks at concentrations generally below 136 mg/m$^3$ (35 ppm) (Watrous, 1942). Mental confusions, speech difficulties, hallucinations, paraesthesia are described following repeated administration to methyl bromide (Johnstone, 1945, Kantarjian and Shaheen, 1963) After chronic intoxication to low methyl bromide concentrations (not detectable by workers) sometimes irreversible CNS lesions with symptoms associated especially with the corpus striatum, cerebellum and pyramidal tract were seen (Dechaume et al., 1948).

Non-fatal poisoning has resulted from exposure to concentrations as low as 390 – 1950 mg/m$^3$ (100-500 ppm). Organs affected by exposure include the nervous system, lung, nasal mucosa, kidney, eye and skin (IPCS, 1995).

### 2.5.2. Animal data

*Inhalation*

Several studies are available investigating the effects after inhalative administration of methyl bromide. The results of the well performed and documented studies, lasting 2 weeks or more are listed (details in *Table 3* see Appendix).

Typical effects after inhalative methyl bromide administration to rats and mice were decreased body weight gain, changes in haematology (Japanese Ministry of Labour, 1992, NTP, 1992), myocardial damage (Kato et al., 1986, Reuzel et al., 1991), degeneration in the brain (Japanese Ministry of Labour, 1992, NTP, 1992) and inflammation and metaplasia of the olfactory epithelium (Japanese Ministry of Labour, 1992, Reuzel et al., 1991). At higher doses also lung congestion, liver and kidney necrosis were seen (Japanese Ministry of Labour, 1992). The LOAEL is 16 mg/m$^3$ based on dose-related inflammation of the nasal cavity.

*Oral*

After oral administration of methyl bromide to rats lesions in the stomach and forestomach were observed (Danse et al., 1984). No adverse effects were observed in beagle dogs (Wilson et al., 1998). The results of these studies are summarized (details in *Table 3,* see Appendix). An NOAEL has been reported to be 2 mg/kg bw.

## 2.6. Genotoxicity

### 2.6.1. Human data

Blood and oropharyngeal cells of 32 methyl-bromide-exposed fumigation workers (4h during the 2 weeks preceding the analysis, exposure concentrations not available) were collected and compared with 28 controls. Micronuclei were measured in lymphocytes and oropharyngeal cells, and hypoxanthine-guanine phosphoribosyl transferase gene

(*hprt*) mutations were measured in lymphocytes. Mean *hprt* variant frequencies and mean oropharyngeal cell micronuclei were elevated in workers compared to reference persons (Calvert et al., 1998).

### 2.6.2. Mutagenicity in vitro

Methyl bromide is clearly genotoxic, both *in vivo* and *in vitro* (Bolt and Gansewendt, 1993, IARC, 1999).

*Bacterial tests*

Ames assays and one modified Ames (SOS-umu) were performed with standard tester strains TA 100, 98, 1535, 1537, 1538 and TA 1535/pSK1002 with and without metabolic activation. Positive results were obtained with TA 100 and TA 1535 (IPCS, 1995). One forward mutations streptomycin resistance assay with *Klebsiella pneumoniae* $ur^-$ $pro^-$ was also positive (Kramers et al., 1985a).

*Mammalian tests*

One mouse lymphoma assay was positive (Kramers et al., 1985a). No data are given on metabolic activation. In human lymphocyte cultures the frequency of sister chromatid exchanges was increased with and without S9 (Tucker et al., 1985, 1986). Chromosome aberrations were induced in the presence of S9 in human $G_0$ lymphocytes (Garry et al., 1990). Two UDS assays were negative in concentrations of up to 30 mg/l (McGregor 1981, Kramers et al., 1985a).

### 2.6.3. Mutagenicity in vivo

A sex-linked recessive assay with *Drosophila melanogaster* was negative after a dose level of 750 mg/m$^3$ for 6 h, but positive after exposure to 375 mg/m$^3$ (5 x 6 h) and 200 mg/m$^3$ (15 x 6 h) (Kramers et al., 1985a, b). A sex-linked recessive lethal *Drosophila melanogaster* assay at concentration up to 272 mg/m$^3$ for 5 h was negative (McGregor 1981). The rate of chromosomal aberrations in rat bone marrow cells were not elevated after single or repeated dosage of up to 272 mg/m$^3$ methyl bromide (McGregor, 1981).

Micronucleus assays in mice and rats were clearly positive after administration of 600 – 1712 mg/m$^3$ methyl bromide (6 h/d, 5 d/w, 14 d). Micronuclei were found in the bone marrow of rats and mice and in peripheral blood cells of rats (Ikawa et al., 1986). A further positive micronucleus assay was reported in peripheral erythrocytes of B6C3F1 mice treated with 47-778 mg/m$^3$ for 6 h/d, 5 d/w, 14 d (NTP, 1992). This result could not be reproduced after 13 week treatment. After the same treatment a (not reproducible) positive SCE assay was performed (NTP, 1992)

A dominant lethal assay with male CD rats and a dosage of up to 272 mg/m$^3$ for 5 d (7 h/d) was negative (McGregor 1981).

### 2.6.4. DNA methylation

A DNA binding study of inhaled and orally applied $^{14}$C-methyl bromide in F344 rats and B6C3F1 mice showed, after isolation and hydrolysis of DNA from liver, lung, stomach and forestomach, three methylated bases (3-methyl-adenine, 7-methyl-guanine, $O^6$-methyl-guanine) and the existence of another unidentified DNA adduct. Adducts occurred in all tissues examined. There was a remarkably high level of adducts in stomach and

forestomach after both oral and inhalation exposures (Gansewendt et al., 1991). The latter finding paralleled the finding of Medinsky et al. (1984) of a persistence of $^{14}$C-labeling in the stomach after dosing rats i.p. with $^{14}$C-methyl bromide.

Later, the occurrence of the main DNA adducts $N^7$- and/or $O^6$-methylguanine at comparable levels in various tissues was independently confirmed (among others, in glandular stomach, forestomach, liver) after single (rat: 80, 160 mg/kg bw) or multiple (rat: 30, 60 mg/kg bw, 4 consecutive days, mice: 25 mg/kg bw, 10 consecutive days) oral treatment of rats or □lacZ transgenic mice with methyl bromide. Multiple rat treatment resulted in substantial decreases in the repair enzyme $O^6$-alkylguanine-DNA alkyltransferase (Pletsa et al., 1999).

Thus, a clear, systemic, directly DNA-alkylating potential of methyl bromide is well established which is to be viewed along with its direct mutagenic properties (v.s.).

## 2.7. Carcinogenicity

*Oral*

In subchronic toxicity studies with Wistar rats (0, 0.4, 2, 10, 50 mg/kg bw) severe irritating effects in the forestomach have been found, including inflammation and hyperplasia at doses of 0.5 mg/kg bw and 10 mg/kg bw respectively (Boorman et al., 1986; Danse et al., 1984; Hubbs et al., 1986). In the study of Danse et al. (1984) squamous cell carcinomas of the forestomach were found at 50 mg/kg bw in 13/20 animals. From subsequent examinations of the slides it was concluded that the forestomach lesions represented inflammation and hyperplasia rather than malignant lesions (Pesticide Toxic Chemical News, 1984) (details: *Table 4,* see Appendix). Forestomach hyperplasia and inflammation was also seen after dosage of 50 mg methyl bromide/kg bw to male Wistar rats over 13 weeks (additional 12 weeks of recovery). Evidence of malignancy was seen in one rat (Boorman et al., 1986) (details: *Table 4*, see Appendix).

Sixty male and female F344 rats were fed diets fumigated with methyl bromide (80, 200, 500 mg total bromide/kg diet; equal to 2.7, 6.8 and 17 mg total bromide/kg bw). The only effect was a slightly reduced body weight gain in males at 500 ppm group. No carcinogenic effects were observed (Mitsumori et al., 1990).

*Inhalation*

As shown (details in *table 2*; see Appendix) no increased tumour incidence was seen in the 13 week- and in carcinogenicity studies with rats (Wistar, F344) and mice (B6C3F1, Crj:BDF1) (Japanese Ministry of Labour, 1992, NTP, 1992, Reuzel et al., 1991).

## 2.8. Reproductive toxicity

*Fertility*

Adverse effects on male fertility after inhalative administration of methyl bromide were observed in several studies (details are shown in *table 4*). Male rats and mice showed testis atrophy (Eustis et al., 1988), incomplete spermatogenesis (Kato et al., 1986), decreased or increased testis weights (Morrissey et al., 1988), reduced sperm motility and increased percentages of abnormal sperm (Kato et al., 1986, Morrissey et al., 1988). The LOAEL was 117 mg/m$^3$ for rats and 39 mg/m$^3$ for mice.

In one dominant lethal assay (details: *Table 5*, see Appendix) no effects on frequency of pregnancy, number of corpora lutea per pregnancy and the frequency of early deaths were observed (McGregor, 1981).

*Two-generation toxicity study*
In a two-generation toxicity study with Sprague-Dawley rats (American Biogenics Corporation, 1986) maternal toxicity (reduced body weight gains, increased relative liver weights and decreased mean brain weights) was seen at 350 mg/m$^3$. The body weights of the pups were reduced in the $F_{1a}$, $F_{2a}$ and $F_{2b}$ generations at ≥ 117 mg/m$^3$. In the $F_{1a}$ generation a reduced pup survival was recorded at 350 mg/m$^3$. The female fertility index was slightly reduced in the $F_{2a}$ litters at 350 mg/m$^3$. The NOAEL for maternal toxicity was 117 mg/m$^3$ (based on brain weights and body weight gain) and the NOAEL for effects on the offspring is 12 mg/m$^3$ (based on pup body weight).

*Developmental toxicity*
The design and the results of developmental toxicity studies are presented in *Table 6* (see Appendix).

Developmental toxicity was found only at maternally toxic levels in studies with rats and rabbits. In the study of Peters et al. (1982) with rats at 50 mg/kg bw total resorptions of all embryos was found, but no effects were observed at 25 mg/kg bw which was maternal toxic. Increased incidences of fused sternebrae, reduced foetal weights and malformations (missing gallbladder of missing caudal lobe of the lung) were observed in one study with New Zealand rabbits at 311 mg/m$^3$ (Breslin et al., 1982). Signs of maternal toxicity at this dose level were reduced body weights and brain lesions. The NOAEL (maternal toxicity and teratogenicity) derived for this study was 156 mg/m$^3$.

*Biological monitoring*
Determination of the bromide concentrations in blood or urine was recommended (Tanaka et al., 1991) although the concentrations only correlate badly with the external exposure to methyl bromide (Rathus and Landy, 1961, van den Oever, 1978,) as there are no practicable alternatives. With high exposures, this value together with clinical parameters can provide a reason to remove a fumigator from the workplace (van den Oever et al., 1984). The fact that the bromide concentration does not correlate with the severity of the neurological symptoms of intoxication makes evaluation of the concentration more difficult (Verberk et al., 1979). Death has been observed with levels of bromide in serum of 30 mg/l while concentrations of over 200 mg/l were not reported to be lethal (Hustinx et al. (1993). The bromide concentration in the urine of professional fumigators investigated by Hallier (1995) was within the background of the general population, of about 5 mg/l.

The determination of reaction products (adducts) with macromolecules in blood, in particular serum albumin and globin, proved to be a suitable parameter for the biological monitoring of exposure to methyl bromide during fumigation. S-Methyl-cysteine in globin has been suggested as a parameter (Iwasaki, 1988); however, there was a lack of reproducibility of results, also in animal experiments (Iwasaki, 1988a). With methyl bromide fumigators, also considerable interindividual variability of the results was noticed (Iwasaki and Kagawa, 1989). An advantage of the determination of S-methyl-cysteine in serum albumin and in globin is that this parameter is not influenced by smoking habits (Iwasaki and Kagawa, 1989, Hallier, 1995).

Measuring S-methyl-cysteine in serum albumin and in globin (Müller et al., 1995) provides a basis for biological monitoring of persons exposed during fumigation with methyl bromide. Although a health-based Biological Exposure Limit cannot however be established (DFG,

1999) an orientation for the biological monitoring of methyl bromide can be based on the existing occupational experience, i.e. on the adduct values found during fumigation under various conditions. Base values in the general population, according to Müller *et al.* (1995) are located at 15 nmol S-methyl-cystein per gram albumin. According to Hallier (1995) a guide value of 50 to 60 nmol S-methyl-cysteine per gram protein in practice seems to provide a reasonable safety margin to toxic dose ranges. If this value is exceeded it is usually the result of inadequate occupational safety measures.

## Recommendations

The use of methyl bromide as a fumigant is based on its reactivity as a methylating agent. In a variety of biological systems, in vitro and in vivo, it methylates macromolecules (proteins and DNA) and displays genotoxic properties. In long-term experiments (rats, gavage), methyl bromide has induced neoplastic prestages in the forestomach and stomach (hyperplasia, inflammation), and in one experiment and at the highest dose (50 mg/kg per day) it has induced squameous cell carcinomas. No such tumours were observed in mice. Upon inhalation, there are local effects of inflammation and metaplasia of the rat olfactory epithelium. An LOAEL of inflammation in the rat nasal cavity was 16 mg/m$^3$ [4 ppm].

The preponderant systemic effect of methyl bromide is neurotoxicity which is evidently related to metabolites. The metabolic process leading to such toxicity in humans is triggered by the glutathione S-transferase hGSTT1-1. As this enzyme is genetically polymorphic (in about 20% of the European population the *hGST1* gene is deleted) there is a wide variation in individual susceptibility to the neurotoxic effects of methyl bromide.

Systemic uptake of methyl bromide via the skin has been clearly demonstrated, in humans and in experimental animals, which calls for a use of biological monitoring. Determinations of bromide concentrations in blood and/or urine and of methylated cystein in blood proteins (albumin, haemoglobin) may be used. However, there are no sufficient data to establish a health-based Biological Exposure Limit.

Because of the clear systemic mutagenic effects of methyl bromide, a health-based Occupational Exposure Limit cannot be derived. Based on the LOAEL for local inflammation in the upper airways (v.s.), the exposure, in any case, should be kept well below 1 ppm, and appropriate protective measures should minimise both dermal and inhalational contact.

The data base is presently not sufficient to derive a Biological Limit Value. However, in order to provide a provisional guidance, it should be noted that human fatalities have occurred at plasma or serum bromide levels above 30 mg/l, and that EEG changes have been reported at bromide levels above 12 mg/l. As the background bromide level in serum or plasma is about 5 mg/l, a provisional tolerable range for occupationally exposed persons could be between 5 and 12 mg $Br^-$ per liter plasma or serum (DFG, 2003).

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# Appendix: Details of experimental toxicity tests with methyl bromide

Table 2: Acute inhalation toxicity

| Species | Concentration ($mg/m^3$) | Exposure time | Reference |
|---|---|---|---|
| Mouse | 6600 | 30 min | Bakhishev, 1973 |
| Mouse | 4680 | 1 h | Alexeeff et al., 1985 |
| Mouse | 1540 | 2 h | Balander et al 1962 |
| Mouse | 1575 | 4 h | Yamano 1991 |
| Rat | 11000 | 30 min | Bakhishev 1973 |
| Rat | 7300 | 1 h | Zwart 1988, Zwart et al., 1992 |
| Rat | 3034 | 4 h | Kato et al., 1986 |
| Rat | 1175 | 8 h | Honma et al., 1985 |

Table 3: Repeated dose toxicity after inhalation

| Species | Exposure time | Dose (mg/m³) | Effects | Reference |
|---|---|---|---|---|
| Rat<br>SPF Wistar<br>6 m | 6 h/d, 5 d/w<br>2 w | 0, 150, 375, 750 | ≥ 150 mg/m³: body weight gain ↓, liver weight ↓,<br>750 mg/m³: hyperaemic lung<br>**NOAEL: < 150 mg/m³** | NTP 1992 |
| Rat<br>F344/DuCrj<br>10 m, 10 f | 6 h/d, 5 d/w<br>2 w | 0, 599, 778, 1011, 1315, 1712 | 599 mg/m³ f: body weight gain ↓,<br>≥ 599 mg/m³: metaplasia of olfactory epithelium<br>≥ 778 mg/m³: body weight gain ↓, vacuolisation in adrenal glands, myocardial damage,<br>≥ 1315 mg/kg bw: mortality ↑, 1712 mg/kg bw: lung: congestion and haemorrhage, liver: necrosis, fatty changes, kidney: necrosis<br>**NOAEL: < 599 mg/m³** | Japanese Ministry of Labour, 1992 |
| Rat<br>SPF Wistar<br>6 m, 6 f | 6 h/d, 5 d/w (w 1, 2, 3)<br>6 h/d, 7 d/w (w 4)<br>4 w | 0, 70, 200, 600 | ≥ 200 mg/m³: body weight gain ↓,<br>600 mg/m³: mortality ↑, histopathological changes in heart and lungs<br>**NOAEL: 70 mg/m³** | NTP 1992 (Dutch study) |
| Rat<br>Sprague-Dawley<br>10-12 m | 4 h/d,<br>6 w | 0, 584, 778, 1167, 1556 | ≥ 584 mg/m³: adrenal glands weight ↓, heart changes, ≥ 778 mg/m³: body weight gain ↓, organ weights (heart – not dose dependant, liver) ↓, ≥ 1167 mg/m³: testis weights ↓, changes in testes; 1556 mg/m³: brain, kidney changes, spleen weight ↓<br>**NOAEL: < 584 mg/m³** | Kato et al., 1986 |
| Rat<br>Wistar<br>10 m, 10 f | 6 h/d, 5 d/w<br>13 w | 0, 4, 25, 166 | 166 mg/m³: liver: minimal changes<br>**NOAEL: 25 mg/m³** | Wilmer et al., 1983 |
| Rat<br>F344/N<br>18 m, 18 f | 6 h/d, 5 d/w<br>13 w | 0,117, 234, 467 | ≥ 234 mg/m³ f: body weight gain ↓, 467 mg/m³: body weight gain ↓, Hct ↓, Hb ↓, RBC ↓, olfactory epithelial: dysplasia, cysts<br>**NOAEL: 117 mg/m³** | Haber et al., 1985 (abstract), NTP 1990 |
| Rat<br>10 m, 10 f | 6 h/d, 5 d/w<br>13 w | 0, 29, 73, 183, 455, 1140 | ≥ 73 mg/m³: biochemical changes in blood, ≥ 455 mg/m³: body weight gain ↓, Hct ↑, MCV ↑, platelet (m) ↑, 1140 mg/m³: brain: necrosis, degeneration of granular layer of cerebellum, thymus: haemorrhage, atrophy, kidney: necrosis, testis: atrophy, respiratory tract: interstitial pneumonia, metaplasia of olfactory epithelium, adrenal gland: vacuolisation, myocardial damage<br>**NOAEL: 29 mg/m³** | Japanese Ministry of Labour, 1992 |

| Species | Exposure time | Dose (mg/m³) | Effects | Reference |
|---|---|---|---|---|
| rat<br>Wistar<br>90 m, 80 f | 6 h/d, 5 d/w,<br>29 m | 0, 12, 117, 350 | ≥ 12 mg/m³: changes in nasal olfactory epithelium (was not considered as relevant for NOAEL by ACGIH)<br>350 mg/m³: mortality ↑, body weight gain ↓, brain: weight ↓, heart: myocardial degeneration, thrombi, oesophagus, forestomach: hyperkeratosis<br>**NOAEL: 117 mg/m³** | Dreef-van der Meulen et al., 1989, Reuzel et al., 1991 |
| rat<br>F344/DuCrj<br>50 m, 50 f | 6 h/d, 5 d/w,<br>2 y | 0, 16, 78, 389 | ≥ 16 mg/m³ m: nasal cavity: incidence, severity of inflammation dose related<br>≥ 78 mg/m³ m: protein in urine ,<br>389 mg/m³: body weight gain ↓, changes in haematology, blood biochemistry, urinalysis, olfactory epithelium: necrosis, metaplasia<br>**NOAEL m: < 16 mg/m³** | Japanese Ministry of Labour, 1992 |
| Mouse<br>B6C3F1<br>10 m 10 f | 6 h/d, 5 d/w<br>2 w | 0, 47, 97, 195, 389, 778 | 778 mg/m³: mortality ↑<br>**NOAEL: 389 mg/m³** | NTP, 1992 |
| Mouse<br>Crj:BDF1<br>10 m, 10 f | 6 h/d, 5 d/w<br>2 w | 0, 467, 599, 778, 1011, 1315, 1712 | ≥ 467 mg/m³: mortality ↑, body weight gain ↓, histological findings in brain, thymus, kidney heart adrenal glands, F: MCV ↑, protein in urinalysis ↑<br>**NOAEL: < 467 mg/m³** | Japanese Ministry of Labour, 1992 |
| Mouse<br>B6C3F1<br>15 m, 15 f | 6 h/d, 5 d/w<br>6 w | 0, 622 | Lethargy, tremors, body weight gain ↓, organ weights: lung, heart, thymus, brain, liver ↓, neuronal necrosis, nephrosis, atrophy in adrenal cortex testicular degeneration, RBC ↓, f: WBC ↑ | Eustis et al., 1988 |
| mouse<br>B6C3F1<br>18-30 m, 18-30 f | 6 h/d, 5 d/w<br>13 w | 0, 39, 78, 156, 311, 467 | 156 mg/m³ m: Hb ↓, MCV ↓, RBC ↑<br>467 mg/m³: mortality ↑, body weight ↓, curling and crossing of hindlimbs, twitching of forelimbs<br>**NOAEL: 78 mg/m³** | NTP, 1992 |
| mouse<br>Crj:BDF1<br>10 m, 10 f | 6 h/d, 5 d/w<br>13 w | 0, 29, 58, 117, 234 | 234 mg/m³: body weight gain↓, F: MCV ↑, protein in urinalysis ↑<br>**NOAEL: 117 mg/m³** | Japanese Ministry of Labour, 1992 |
| mouse<br>B6C3F1<br>86 m, 86 f | 6 h/d, 5 d/w,<br>2 y (interim sacrifice at 6 and 15 m) | 0, 39, 128. 389 | 389 mg/m³: mortality ↑, body weight gain ↓, thymus weight ↓, nonneoplastic lesions in brain, bone, heart, and nose, behavioural effects<br>**NOAEL: 128 mg/m³** | NTP, 1992 |
| mouse<br>Crj:BDF1<br>50 m, 50 f | 6 h/d, 5 d/w,<br>2 y | 0, 16, 62, 250 | 250 mg/m³: body weight gain ↓, changes in blood biochemistry, brain: atrophy of granular layer of the cerebellum<br>**NOAEL: 62 mg/m³** | Japanese Ministry of Labour, 1992 |

Table 4: Repeated dose toxicity after oral administration

| Species | Exposure time | Dose | Effects | Reference |
|---|---|---|---|---|
| Rat<br>Wistar<br>10 m, 10 f<br>Gavage | 5 d/w<br>13 w | 0, 0.4, 2, 10, 50 mg/kg bw | ≥ 10 mg/kg bw: forestomach mucosa: proliferative changes<br>50 mg/kg bw: stomach: squamous cell carcinomas (13/20) (represented inflammation and hyperplasia), haematological changes<br>**NOAEL: 2 mg/kg bw** | Danse et al., 1984, Pesticide Toxic Chemical News, 1984 |
| Rat<br>15 not specified<br>not specified | 13-25 w<br>12 w recovery | 0, 50 mg/kg bw | Forestomach: acanthosis, fibrosis, pseudoeptheliomatous hyperplasia, hyperplastic lesions (stomach lesions regressed, but adhesions, fibrosis, and mild acanthosis remained after recovery) | Boorman et al., 1986 |
| Rat<br>not specified<br>gavage | 5 d/w<br>up to 17 w (4-8 w recovery) | 0, 25, 50 mg/kg bw | ≥ 25 mg/kg bw: forestomach: ulceration, pseudoepitheliomatous hyperplasia (inclomplete regression after recovery); evidence of malignancy in one rat<br>**NOAEL: < 25 mg/kg bw** | Hubbs et al., 1986 |
| Rat<br>F344<br>60 m, 60 f | Diet<br>2 y | 0, 3, 7 mg/kg bw | 7 mg/kg bw m: body weight ↓<br>**NOAEL: 3 mg/kg bw** | Mitsumori et al., 1990 |
| Beagle Dog<br>3 m, 1 f<br>Diet | 1 y | 0, 0.06/0.07, 0.13/0.12, 0.28/0.27 mg/kg bw (m/f) | **NOAEL: 0.28 mg/kg bw** | Wilson et al., 1998 |

Table 5: Effects on fertility

| Study/animals<br>Authors | Type of study<br>Treatment | Specific investigations | Toxicological findings<br>NOAEL |
|---|---|---|---|
| Rat<br>10 m<br>Kato et al., 1986 | Subacute study<br>0, 584, 778, 1167, 1556 mg/m³<br>4 h/d, 5 d/w, 6 w | Histopathology of reproductive organs | ≥ 584 mg/m³: histopathological changes in kidney, heart, spleen<br>≥ 1167 mg/m³: incomplete spermatogenesis, giant cells in seminal tubules, accumulation of necrotic spermatocytes, testis weights ↓<br>**NOAEL: 778 mg/m³** |
| F344 rat<br>m<br>Hurtt et al., 1988 | Subacute study<br>778 mg/m³<br>6 h/d, 5 d | Histopathology of testes, testis weight | 778 mg/m³: plasma testosterone, testicular nonprotein sulfhydryl concentrations |
| F344 rats<br>m, f<br>Morrissey et al., 1988 | Subchronic study<br>0, 117, 233, 467 mg/m³<br>13 w | Histopathology of testes, sperm morphology, vaginal cytology, reproductive organ weights | ≥ 117 mg/m³: cauda epididymis weight ↓, testis weights ↑, sperm motility ↓<br>**NOAEL: < 117 mg/m³** |
| CD rats<br>10 m<br>McGregor, 1981 | Dominant lethal assay<br>0, 78, 272 mg/m³, 7 h/d, 5 d<br>mated with untreated females (1 m/2 f) | Frequency of pregnancy, number of corpora lutea per pregnancy, frequency of early deaths | No effects<br>**NOAEL: 272 mg/m³** |
| Rats, mice<br>m<br>Eustis et al., 1988 | 622 mg/m³<br>6 h/d, 5 d/w, 6 w | Histopathology of testes | 622 mg/m³: testicular degeneration and atrophy<br>rats>mice |
| B6C3F1 mice<br>10 m<br>McGregor, 1981 | 0, 78, 272 mg/m³<br>7 h/d, 5 d | Sperm investigations | No findings<br>**NOAEL: 272 mg/m³** |
| B6C3F1 mice<br>m, f<br>Morrissey et al., 1988 | Subchronic study<br>0, 39, 156, 467 mg/m³<br>13 w | Histopathology of testes, sperm morphology, vaginal cytology, reproductive organ weight | ≥ 39 mg/m³ m: epididymides, testis weight ↑, sperm density ↓, % of abnormal sperms ↑<br>**NOAEL: < 39 mg/m³** |

| | | | |
|---|---|---|---|
| CD Sprague-Dawley rats<br>m, f<br>American Biogenics Corporation, 1986 | Two-generation-study<br>0, 12, 117, 350 mg/m³ 6 h/d, 5 d/w, 8 m | Effects on growth, reproduction, offspring | 350 mg/m³: relative liver weight ↑ ($F_0$)<br>350 mg/m³ m: body weight ↓ ($F_0$, $F_1$f), mean brain weight ↓ ($F_0$, $F_1$m+f)<br>≥ 117 mg/m³: pups body weights ↓ ($F_{1a}$, $F_{2a}$, $F_{2b}$)<br>350 mg/m³: pup survival ↓ ($F_{1a}$)<br>350 mg/m³ f: fertility index ↓ ($F_{2a}$)<br>**NOAEL: 12 mg/m³** |

Table 6: Developmental Toxicity

| **Animals**<br>**Sex** | **Treatment** | **Maternal effects**<br>**NOAEL** | **Developmental effects**<br>**NOAEL** | **References** |
|---|---|---|---|---|
| rats<br>Wistar<br>f | 0, 78, 272 mg/m³<br>7 h/d, 5 d/w, 3 w before mating<br>+ from day 1-19 of gestation | **NOAEL: 272 mg/m³** | **NOAEL: 272 mg/m³** | Sikov et al., 1981 |
| Rats<br>f (pregnant) | 0, 0.5, 5, 25, 50 mg/kg bw gavage<br>day 5-20 of gestation | ≥ 25 mg/kg bw:<br>maternal toxicity (no details available)<br>**NOAEL: 5 mg/kg bw** | 50 mg/kg bw: total resorption of embryos<br>**NOAEL: 25 mg/kg bw** | Peters et al., 1982 |
| Rats<br>Crj:CD<br>24 copulated f | 0, 3, 10, 30 mg/kg bw gavage<br>day 6-15 of gestation<br>deaths at day 20 | 30 mg/kg bw: body weight ↓, erosive lesions in stomach<br>**NOAEL: 10 mg/kg bw** | **NOAEL: 30 mg/kg bw** | Kaneda et al., 1998 |
| rabbits<br>New Zealand<br>24 f | 0, 78, 272 mg/m³<br>7 h/d, 5 d/w<br>day 1 (artificial insemination) - 24 of gestation (272 mg/m³: exposure stop at d 15); deaths at day 30 | 272 mg/m³: mortality ↑<br>**NOAEL: 78 mg/m³** | 78 mg/m³: no effect<br>272 mg/m³: no evaluation | Sikov et al., 1981 |
| rabbits<br>New Zealand<br>f (inseminated) | 0, 78, 156, 311 mg/m³<br>d 7-19 of gestation<br>necropsy at day 28 of gestation | 311 mg/m³: body weight ↓, brain lesions<br>**NOAEL: 156 mg/m³** | 311 mg/m³: foetal weights ↓, fused sternebrae and malformations (missing gallbladder, missing caudal lobe of the lung) ↑<br>**NOAEL: 156 mg/m³** | Breslin et al., 1990 |
| Rabbits<br>Kbl:JW<br>18 inseminated f | 0, 1, 3, 10 mg/kg bw gavage<br>day 6-18 of gestation<br>deaths at day 27 | 10 mg/kg bw: body weight ↓, erosive lesions in stomach<br>**NOAEL: 3 mg/kg bw** | **NOAEL: 10 mg/kg bw** | Kaneda et al., 1998 |