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BG RCI
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# TOXICOLOGICAL EVALUATIONS

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**TOXICOLOGICAL EVALUATION**

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# Chloroformic acid methyl ester

**No. 36**

CAS No. 79-22-1

BG Chemie
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# Chloroformic acid methyl ester

Apart from the evaluation of chloroformic acid methyl ester (No. 36), there are also TOXICOLOGICAL EVALUATIONS of chloroformic acid ethyl ester (No. 77), chloroformic acid propyl ester (No. 159) and chloroformic acid butyl ester (No. 160), which may be consulted for comparison.

## 1 Summary and assessment

Chloroformic acid methyl ester is toxic following oral administration ($LD_{50}$ rat oral 40 to 313 mg/kg body weight). Upon inhalation, chloroformic acid methyl ester is very toxic. $LC_{50}$ values for the rat have been determined as approx. 60, approx. 200 and approx. 208, and 450 mg/m³ following 4-hour exposure, and as approx. 345 to 480 mg/m³ following 1-hour exposure. For the mouse, the $LC_{50}$ has been reported as 185 mg/m³ following 2-hour exposure. The high acute inhalation toxicity of chloroformic acid methyl ester, which is due to the chemical's severe corrosiveness in conjunction with its high volatility, became strikingly apparent in inhalation hazard tests, in which all or the majority of rats died after only 3 minutes' exposure to atmosphere enriched or saturated with vapour at 20 °C. When applied to the skin, chloroformic acid methyl ester is systemically harmful to mildly toxic ($LD_{50}$ rabbit dermal 7120 and > 3038 mg/kg body weight, rat dermal 894 and approx. 1230 mg/kg body weight, and mouse dermal 1750 and 2200 mg/kg body weight), but it causes leathery necrosis at the site of application. Dyspnoea, apathy, reeling, tremor, abnormal position, rough coat, salivation and poor general condition have been reported as signs of toxicity following oral administration. Following inhalation exposure the signs of toxicity include, *inter alia*, mucous membrane irritation, closed eyes, reddish ocular and nasal discharges, encrustations around the eyes and nose, escape behaviour and marked dyspnoea, due to the chemical's corrosive effect. In addition, however, inhalation exposure is also associated with general signs of severe intoxication similar to those seen after oral administration. Necropsy findings have also been reported to include, in particular, changes that were attributable to the chemical's corrosive properties; inhalation exposure was associated with pulmonary haemorrhages, emphysema and oedema, *inter alia*, as well as markedly increased lung

weights and hydrothorax; oral administration resulted in blood in the stomach, haemorrhagic corrosive gastritis with sloughing and diffusely reddened gastrointestinal mucosa; and intraperitoneal injection caused intra-abdominal adhesions. Furthermore, necropsies have frequently revealed acute dilatation and congestive hyperaemia of the heart, and a few studies have demonstrated liver changes. Histological examinations following inhalation intoxication have shown severe degeneration of the nasal turbinate and tracheal mucosal epithelium, alveolar haemorrhages, erosion of bronchial and bronchiolar mucosal epithelium and increased permeability of the alveolar septa. The histopathological changes were mostly resolved by 9 or 10 days after exposure in animals which had been exposed to levels of chloroformic acid methyl ester in the range of the $LC_{50}$.

The toxic effects of chloroformic acid methyl ester following repeated whole-body inhalation exposure to concentration levels of 0 (controls), 0.4, 2, 4 or 8 ppm (approx. 0 (controls), 1.6, 7.8, 15.7 or 31 mg/m³) are reported in detail in a subchronic inhalation study with interim necropsies at 0.5, 2 and 4 weeks (with the respective number of exposures totalling 3, 10, 20 and 63) which was carried out in Wistar rats. Inhalation of chloroformic acid methyl ester causes marked damage throughout the respiratory tract. Damage is caused, in particular, to the ciliated respiratory epithelium and the transitional epithelium of the nose and the ciliated epithelium at the base of the epiglottis and the bronchioles. Less pronounced are the histopathological changes of the squamous epithelium and the cuboidal epithelium of the larynx and the ciliated respiratory epithelium of the trachea. The olfactory epithelium remains normal. The epithelia mainly exhibit thickening due to hyperplasia and alterations in terms of squamoid metaplasia, and with increasing number of exposures they also partly undergo keratinisation. Mucus production is markedly enhanced initially; there is hypertrophy of the mucus-producing goblet cells but with prolonged exposure the cells are displaced by the later predominant squamous epithelium. In the region of the nasal cavity and the larynx purulent inflammation develops, whilst in the alveolar and peribronchial regions prominent granulomatous inflammation arises. In the deep regions of the lung there is loss of Clara cells, which normally fulfil a protective function. Due to inflammation of the lower part of the respiratory system absolute and relative lung weights are increased. The local manifestation of lesions and their severity depend to a greater extent upon the levels of exposure administered rather than the

number of exposures, i.e. the cumulative dose. Concentration levels of 4 and 8 ppm (approx. 15.7 and 31 mg/m³) cause histopathological alterations throughout the entire respiratory tract after as few as 3 exposures, whereas lesions induced by 2 ppm (approx. 7.8 mg/m³) remain confined to the nasal cavity and the larynx even after 63 exposures. This indicates that the test substance is effectively removed from the air stream by the upper respiratory tract up to a concentration of approx. 2 ppm. In addition to the histopathological changes discussed above, 24 or more exposures at 8 ppm cause some lethality, and exposure to levels ≥ 4 ppm depress body weight gain. The 8 ppm level causes clinical signs of toxicity which are indicative of treatment-related irritation of the respiratory tract; signs were reported to include rubbing of snouts, sneezing and nasal crusts, intercurrent deaths being associated with abnormal breathing patterns and reduced general state. Substance-related macroscopic changes were confined to the animals that underwent 8 ppm exposure for the entire duration of the study; they exhibited red foci in the lungs. DNA replication measured in the nasal cavity and larynx essentially reflected the histopathological changes. With respect to the assessment of minimal lesions, however, histological examination was more sensitive than measurement of DNA replication. No treatment-related toxicological changes were observed in organs other than the respiratory tract. Irrespective of the number of exposures, the *no observed adverse effect concentration* (NOAEC) is reported as 0.4 ppm (1.6 mg/m³) for 3, 10, 20 and 65 exposures.

There is large agreement between the outcome of the subchronic inhalation study discussed above, in which Wistar rats were given interim necropsies after 3, 10 and 20 exposures, and the findings of a 28-day inhalation study conducted in the Sprague-Dawley rat, according to which the *no observed effect concentration* (NOEC) of chloroformic acid methyl ester is 1.5 mg/m³ (approx. 0.4 ppm). Upon exposure to the next higher test concentration of 4 mg/m³ (approx. 1 ppm), 1 out of 10 animals exhibited localised minor squamous metaplasia of the laryngeal epithelium, a lesion also noted in 1 out of 10 control animals. Comparison of the mortality data from the two studies reveals a striking increase in mortality with increasing concentration levels. Whereas exposure to 8 ppm for a period ≥ 4 weeks in the subchronic study with satellite groups resulted in the death of 4 out of 40 (10%) animals, as many as 3 out of 10 (30%) animals died following exposure to approx. 9 ppm (35 mg/m³) in the fourth week of the subacute study.

In the rabbit, chloroformic acid methyl ester is corrosive to the skin and eye. Even a 1-minute skin exposure causes severe necrosis.

When conducted as a preincubation or standard plate incorporation assay, the Salmonella/microsome test on *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1538 reveals no mutagenic potential for chloroformic acid methyl ester, either in the absence or presence of metabolic activation. In the chromosome aberration test conducted in V79 cells of the Chinese hamster, chloroformic acid methyl ester induced chromosome damage upon metabolic activation with S-9 mix from Aroclor 1254-induced rat liver. In the absence of metabolic activation, cells treated with chloroformic acid methyl ester showed no increase in aberration rate.

In humans, poisoning by inhalation of chloroformic acid methyl ester is associated with signs of severe irritation of the eyes and respiratory tract. Depending on the level of exposure and frequently following an asymptomatic period, as is seen in phosgene poisoning, pulmonary oedema may develop and possibly result in death.

In the Federal Republic of Germany, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has established a MAK value (maximum workplace concentration) for chloroacetic acid methyl ester on the suggestion of BG Chemie. It was set in the List of MAK- and BAT Values 2004 at 0,2 ml/m³ (ppm, equivalent to 0,78 mg/m³). Furthermore, chloroacetic acid methyl ester has been assigned to pregnancy risk group C, i.e. substances for which "there is no reason to fear a risk of damage to the embryo or foetus when MAK and BAT values are observed".

## 2 Name of substance

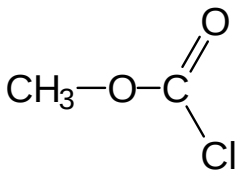
| | | |
|---|---|---|
| 2.1 | Usual name | Chloroformic acid methyl ester |
| 2.2 | IUPAC name | Chloroformic acid methyl ester |
| 2.3 | CAS No. | 79-22-1 |
| 2.4 | EINECS No. | 201-187-3 |

## 3 Synonyms, common and trade names

Carbonochloridic acid, methyl ester
Carbonochloridsäuremethylester
Chlorameisensäuremethylester
Chlorkohlensäuremethylester
Chlorocarbonic acid methyl ester
Formic acid, chloro-, methyl ester
MCF
Methoxycarbonyl chloride
Methyl carbonochloridate
Methylchlorameisensäureester
Methylchlorcarbonat
Methylchlorformiat
Methylchlorkohlensäureester
Methylchlormethanat
Methyl chlorocarbonate
Methyl chloroformate

## 4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_2H_3O_2Cl$

## 5 Physical and chemical properties

| | | | |
|---|---|---|---|
| 5.1 | Molecular mass, g/mol | 94.50 | |
| 5.2 | Melting point, °C | –61 | (BASF, 1997; Bayer, 1998; Hoechst, 1982 a) |
| 5.3 | Boiling point, °C | 70.5 | (Lide and Frederikse, 1997) |
| | | 71 (at 1013 hPa) | (Böhm, 2001; Damle, 1992) |
| | | 71.4 | (Hawley, 1995; Sax, 1995) |
| | | 71.4 (at 1013 hPa) | (Hoechst, 1982 a) |
| | | 71–72 | (BASF, 1997) |

| | | |
|---|---|---|
| 5.4 | Vapour pressure, hPa | 137 (at 20 °C) (BASF, 1997; Bayer, 1998; Hoechst, 1982 a)<br>500 (at 50 °C) (BASF, 1997) |
| 5.5 | Density, g/cm³ | 1.23 (at 15 °C) Hawley, 1995)<br>1.237 (at 15 °C) (BASF, 1997)<br>Ca. 1.22 (at 20 °C) (BASF, 1981 a)<br>1.223 (at 20 °C) (Sax, 1995)<br>1.2231 (at 20 °C) (Lide and Frederikse, 1997)<br>1.228 (at 20 °C) (Hoechst, 1982 a)<br>1.2298 (at 20 °C) (Böhm, 2001)<br>Ca. 1.23 (at 20 °C) (Bayer, 1998)<br>1.250 (at 20 °C) (Damle, 1992) |
| 5.6 | Solubility in water | Insoluble (decomposition by hydrolysis) (Bayer, 1998; Damle, 1992)<br>Low (gradual hydrolysis) (Sax, 1995; BASF, 1988 a)<br>Ca. 5 g/l (at 20 °C); hydrolysis to methanol, hydrochloric acid and carbon dioxide with a $t_{½}$ value of ca. 15 minutes (BASF, 1992)<br>Hydrolysis constant: 14.1 x $10^{-4}$ (at 35 °C); 5.6 x $10^{-4}$ (at 25 °C) (Wardenbach, 1984) |
| 5.7 | Solubility in organic solvents | Miscible with, and soluble in, methanol, ethanol, ethyl ether, benzene and tetrachloromethane (Hawley, 1995; Lide and Frederikse, 1997; Sax, 1995)<br>Miscible with nearly all usual aprotic solvents (BASF, 1981 a) |
| 5.8 | Solubility in fat | Soluble (Gurova et al., 1977) |
| 5.9 | pH value | < 7 (BASF, 1988 a) |
| 5.10 | Conversion factor | 1 ml/m³ (ppm) ≙ 3.92 mg/m³<br>1 mg/m³ ≙ 0.255 ml/m³ (ppm)<br>(at 1013 hPa and 25 °C) |

# 6 Uses

Versatile intermediate used in the manufacture of e.g. dye components, peroxide compounds, herbicides, insecticides as well as pharmaceuticals (Böhm, 2001; Damle, 1992; BASF, 1981 a). Used as a solvent in the photographic industry (Böhm, 2001).

# 7 Experimental results

## 7.1 Toxicokinetics and metabolism

No information available.

## 7.2 Acute and subacute toxicity

### *Acute toxicity*

The acute toxicity data for chloroformic acid methyl ester following oral, inhalation, dermal and intraperitoneal administration are shown in Table 1.

Beginning of Table 1

**Table 1. Acute toxicity studies of chloroformic acid methyl ester**

| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
|---|---|---|---|---|---|---|
| Rat, albino | oral | 40 | n. d. | $LD_{50}$ | n. d. | Industrial Bio-Test, 1977 a |
| Rat | oral | < 50 | n. d. | $LD_{50}$ | n. d. | Eastman Kodak, not dated |
| Rat | oral | 60, given as a solution in oil | n. d. | $LD_{50}$; motor hyperactivity, depression, convulsions, lateral position | n. d. | Gurova et al., 1977 |
| Rat, Sprague-Dawley, female | oral | 110 | n. d. | $LD_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Sprague-Dawley, male | oral | 190 | n. d. | $LD_{50}$ | n. d. | Vernot et al., 1977 |

| Table 1. Acute toxicity studies of chloroformic acid methyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| Rat | oral | ca. 308 (ca. 250 µl) | n. d. | $LD_{50}$; dyspnoea, apathy, prone position, reeling, occasional spastic gait, diarrhoea, poor general condition; necropsy findings: acute cardiac dilatation and congestive hyperaemia, clearly marked gastric vessels, glandular stomach diffusely reddened with eschar formation in several cases, fluid intestinal contents | 14 days | BASF, 1975 |
| Rat, Sprague-Dawley, male, female | oral | 313, given as an emulsion in olive oil | ca. 99% | $LD_{50}$; dyspnoea, rattling respiration, apathy, abnormal position, reeling, tremor, rough coat, salivation, poor general condition; necropsy findings in animals that died intercurrently: heart: acute dilatation and congestive hyperaemia, stomach: haemorrhagic areas, haemorrhagic gastritis with sloughing, intestine: diffuse reddening of the intestinal mucosa, liver: occasionally marks on the periphery of the liver lobules; terminal necropsy findings: thickened forestomach wall, intra-abdominal adhesions | 14 days | BASF, 1981 b |
| Rat, Wistar, male, female | oral | 25–200, given as a solution in distilled water | n. d. | mortality: 0/6 at 25 mg/kg body weight, 2/3 at 200 mg/kg body weight, hence classified as toxic; piloerection, apathy, dyspnoea, squatting position, reeling; necropsy findings in animals that died intercurrently: general congestive hyperaemia, stomach, glandular stomach, severe reddening of the small intestine and appendix, much fluid in the stomach; terminal necropsy was without findings | 14 days | BASF, 1990 |
| Mouse, male | oral | 40 | n. d. | $LD_{50}$ | n. d. | Plzak and Doull, 1969 |
| Mouse | oral | 67, given as a solution in oil | n. d. | $LD_{50}$; motor hyperactivity, depression, convulsions, lateral position | n. d. | Gurova et al., 1977 |
| Guinea pig | oral | 140, given as a solution in oil | n. d. | $LD_{50}$; motor hyperactivity, depression, convulsions, lateral position | n. d. | Gurova et al., 1977 |

| Table 1. Acute toxicity studies of chloroformic acid methyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| Rat, Sprague-Dawley, male, female | inhalation | 60 (15 ppm), 4-hour vapour exposure, dynamic inhalation method, whole-body exposure, concentration level determined by analysis | ca. 99% | $LC_{50}$; dyspnoea, gasping, formation of bubbles in front of the nose, closed eyes, reddish ocular and nasal discharges; later occurrence of encrustations around the eyes and nose, unkempt matted fur with partial yellowish discoloration, reeling, distended stomach, poor general condition, escape behaviour, impaired co-ordination, salivation, crouching posture, depressed body weight gain; necropsy findings in animals that died intercurrently: heart: acute dilatation and congestive hyperaemia, lung: infarctoid congestion, in part wet and beefy, oedematous, marginal emphysema, hydrothorax in several cases, liver: occasional, mild, partly only focal peripheral hepatocellular steatosis; terminal necropsy was without findings | 14 days | BASF, 1980 |
| Rat, Wistar/SPF, male | inhalation | ca. 200 (51 ppm), 4-hour whole-body exposure | ≥ 96% | $LC_{50}$; breathing difficulties as well as motor disorders and impaired autonomic reactions, depressed body weight gain; necropsy findings in animals that died intercurrently: dark red to black discoloration of the lungs, pulmonary oedema, haemorrhagic hydrothorax; terminal necropsy findings: occasionally lungs with dark red foci; histological examination: damage to the bronchial epithelium, increased permeability of the alveolar septa | 14 days | Hoechst, 1986 a |
| Test in accordance with OECD guideline No. 403. | | | | | | |
| Rat, Wistar/SPF, female | inhalation | ca. 208 (53 ppm), 4-hour whole-body exposure | ≥ 96% | $LC_{50}$; all other findings identical with those obtained for males tested in parallel (see above) | 14 days | Hoechst, 1986 a |
| Test in accordance with OECD guideline No. 403. | | | | | | |
| Rat | inhalation | 450 (115 ppm), 4 hours | n. d. | $LC_{50}$; excitation, dyspnoea, nasal discharge, convulsions; necropsy findings: lung: haemorrhagic alveolar exudate, alveolar dilatation, focal lymphoid infiltration, liver: slight cytoplasmic swelling and minor cytoplasmic vacuolisation, kidneys: occasional instances of vascular glomerular destruction and luminal dilatation of the collecting tubules | n. d. | Gurova et al., 1977 |

| Table 1. Acute toxicity studies of chloroformic acid methyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| Rat, Sprague-Dawley, male | inhalation | ca. 345 (88 ppm), one hour | n. d. | $LC_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Fischer 344, male | inhalation | 360–480 (92–122 ppm), one hour | n. d. | $LC_{50}$; depressed body weight gain, dyspnoea, lethargy, clear or red tinted ocular and nasal discharges; necropsy findings: lung discoloration, increase in lung weights; histological examination of animals exposed to 430 or 520 mg/m³ (satellite groups): 4 and 24 hours after exposure: severe degeneration of the nasal turbinate and tracheal mucosal epithelium, alveolar haemorrhages, erosion of bronchial and bronchiolar mucosal epithelium, by 9 or 10 days after exposure microscopic respiratory tract lesions were resolved but regeneration of nasal mucosal epithelium was incomplete | 14 days | Battelle, 1981 |
| Rat, Fischer 344, female | inhalation | 390 (99 ppm), one hour | n. d. | $LC_{50}$; all other findings identical with those obtained for males tested in parallel (see above) | 14 days | Battelle, 1981 |
| Rat, Sprague-Dawley, female | inhalation | ca. 404 (103 ppm), one hour | n. d. | $LC_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Wistar, male, female | inhalation | ca. 287 (73 ppm), 4 hours | n. d. | mortality: 9/10, sneezing, intermittent breathing, gasping, whimpering and crackling breathing noises, ruffled fur, narrow or closed palpebral fissures, squatting posture, prone position, drowsiness, reeling movements, uncoordinated gait, weakened reflexes, depressed body weight gain; necropsy findings: dark red foci on the lungs and dark red discoloration of the lungs, appearance of frothy watery liquid upon opening the lung, reddish watery liquid in the abdominal cavity; terminal necropsy of survivors was without findings | 14 days | Hoechst, 1986 b |
| Rat, Sprague-Dawley, male, female | inhalation | atmosphere enriched or saturated with vapour at 20 °C (estimated concentration: 1460 g/m³), 3 minutes | ca. 99% | mortality: 12/12; vigorous escape behaviour, severe mucous membrane irritation, gasping; necropsy findings: lung: acute emphysema with petechial bleeding, heart: acute right ventricular dilatation and acute congestive hyperaemia | 14 days | BASF, 1981 c |

| Table 1. Acute toxicity studies of chloroformic acid methyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| Rat, Wistar/SPF, male, female | inhalation | atmosphere enriched or saturated with vapour at 20 °C, 3 minutes | ≥ 96% | mortality: 10/10; all animals died within 3 minutes, intermittent breathing, excitation, corneal clouding; necropsy findings: brown foci on the outer edges of the lungs; histological examination: haemorrhages of the parenchymal and pleural capillaries | n. d. | Hoechst, 1985 |
| Acute inhalation hazard test (limit test) in accordance with OECD guideline No. 403. | | | | | | |
| Rat | inhalation | atmosphere enriched or saturated with vapour at 20 °C, exposure for 3, 10 or 30 minutes | n. d. | mortality: 11/12, 5/6 and 6/6, respectively; vigorous escape behaviour, extremely severe mucous membrane irritation, corneal clouding, dyspnoea, convulsions; necropsy findings: acute bilateral dilatation of the heart, congestive hyperaemia, lung congestion with severe oedema and moderate acute emphysema | n. d. | BASF, 1975 |
| Mouse | inhalation | 185 (47 ppm), 2 hours | n. d. | $LC_{50}$; excitation, dyspnoea, nasal discharge, convulsions; necropsy findings: lung: haemorrhagic alveolar exudate, alveolar dilatation, focal lymphoid infiltration, liver: slight cytoplasmic swelling and minor cytoplasmic vacuolisation, kidneys: occasional instances of vascular glomerular destruction and luminal dilatation of the collecting tubules | 14 days | Gurova et al., 1977 |
| Cat | inhalation | 1500 (383 ppm), 30 minutes | n. d. | lethal within 3 days | n. d. | Lazarev, 1963 |
| n. d. | inhalation | 450 (115 ppm), one hour | n. d. | $LC_{50}$ | n. d. | Damle, 1992 |
| Rat, Sprague-Dawley, male, female | dermal | 894, undiluted, 24-hour occlusive application to the depilated skin of the trunk | ca. 99% | $LD_{50}$; dyspnoea, apathy, reeling, cyanosis, tonic convulsions, spastic gait, rough coat, application site with marked signs of primary irritation after 24 hours and leathery necrosis after 4 days; necropsy findings in animals that died intercurrently: heart: acute right ventricular dilatation and acute congestive hyperaemia, lung: in part severe extensive haemorrhages (wet and beefy areas); terminal necropsy of survivors was without findings | 14 days | BASF, 1981 d |

| Table 1. Acute toxicity studies of chloroformic acid methyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| Rat | dermal | ca. 1230, administered as a 10–50% aqueous emulsion or undiluted | n. d. | $LD_{50}$; dyspnoea, slight apathy; necropsy findings: acute cardiac dilatation, congestive hyperaemia | 14 days | BASF, 1975 |
| Mouse | dermal | 1750, undiluted | n. d. | $LD_{50}$; death occurred within seconds after treatment in the high dose group | n. d. | Gurova et al., 1977 |
| Mouse | dermal | 2200, given as a solution in oil | n. d. | $LD_{50}$; death occurred within seconds after treatment in the high dose group | n. d. | Gurova et al., 1977 |
| Rabbit, albino | dermal | > 3038 | n. d. | $LD_{50}$ | n. d. | Industrial Bio-Test, 1977 a |
| Rabbit, New Zealand | dermal | 7120, 24-hour exposure | n. d. | $LD_{50}$ | n. d. | Vernot et al., 1977 |
| Mouse | intraperitoneal | 40 | n. d. | $LD_{50}$ | n. d. | RTECS, 2001 |
| Mouse | intraperitoneal | ca. 11 (ca. 9 µl), given as a 0.0681–3.16% aqueous solution | n. d. | $LD_{50}$; dyspnoea, apathy, reeling in some cases, twitching in some cases, poor general condition; necropsy findings: occasional intra-abdominal adhesions | 14 days | BASF, 1975 |
| [1] where specified<br>n. d. no data | | | | | | |

End of Table 1

When administered orally, chloroformic acid methyl ester proved to be toxic in rat studies, with $LD_{50}$ values ranging from 40 to 313 mg/kg body weight (BASF, 1975, 1981 b; Gurova et al., 1977; Industrial Bio-Test, 1975; Vernot et al., 1977). Oral $LD_{50}$ values were published as 40 and 67 mg/kg body weight for mice and 140 mg/kg body weight for guinea pigs (Plzak and Doull, 1969; Gurova et al., 1977). Upon inhalation, chloroformic acid methyl ester was very toxic. $LC_{50}$ values for the rat were determined as 60, approx. 200 and approx. 208, and 450 mg/m³ following 4-hour exposure (BASF, 1980; Hoechst, 1986 a; Gurova et al., 1977), and as approx. 345 to 480 mg/m³ following 1-hour exposure (Vernot et al., 1977; Battelle, 1981). The high acute inhalation toxicity of chloroformic acid methyl ester, which is due to the chemical's high volatility, became strikingly apparent in inhalation hazard tests, in which all or the majority of rats died after only 3 minutes' exposure to atmosphere enriched or saturated with vapour at 20 °C (BASF, 1975, 1981 c; Hoechst, 1985). For the mouse, the $LC_{50}$ was reported as 185 mg/m³ following 2-hour exposure (Gurova et al., 1977).

When applied to the skin, chloroformic acid methyl ester was of low systemic toxicity ($LD_{50}$ rabbit dermal 7120 and > 3038 mg/kg body weight, rat dermal 894 and approx. 1230 mg/kg body weight, and mouse dermal 1750 and 2200 mg/kg body weight), but it caused leathery necrosis at the site of application, where reported (BASF, 1975, 1981 d; Industrial Bio-Test, 1975; Gurova et al. 1977; Vernot et al., 1977). Following intraperitoneal administration to mice the $LD_{50}$ was approx. 11 mg/kg body weight. However, the study in question was carried out with chloroformic acid methyl ester in aqueous solution, for which reason it is highly likely that the chemical had undergone hydrolysis (see also Section 5.6). Dyspnoea, apathy, reeling, tremor, abnormal position, rough coat, salivation and poor general condition, *inter alia*, were described as signs of toxicity following oral administration (BASF, 1975, 1981 b, 1990). Following inhalation exposure the signs of toxicity included mucous membrane irritation, closed eyes, reddish ocular and nasal discharges, encrustations around the eyes and nose, escape behaviour and marked dyspnoea, due to the chemical's corrosive effect. In addition, however, inhalation exposure was also associated with general signs of severe intoxication, such as depressed body weight gain, poor general condition, motor disorders and impaired autonomic reactions (BASF, 1980, 1981 c; Hoechst, 1985, 1986 a; Battelle, 1981; Gurova et al., 1977). Necropsy findings also, in particular, included changes that were attributable to the chemical's corrosive properties; inhalation exposure was associated with pulmonary haemorrhages, emphysema and oedema in conjunction with markedly increased lung weights and hydrothorax; oral administration resulted in blood in the stomach, haemorrhagic corrosive gastritis with sloughing and diffusely reddened gastrointestinal mucosa; and intraperitoneal injection caused intra-abdominal adhesions. Furthermore, necropsies frequently revealed acute dilatation and congestive hyperaemia of the heart, and a few studies demonstrated liver changes (BASF, 1975, 1980, 1981 b, c, 1990; Hoechst, 1985, 1986 a; Battelle, 1981; Gurova et al., 1977). Histological examinations following acute inhalation intoxication showed severe degeneration of the nasal turbinate and tracheal mucosal epithelium, alveolar haemorrhages, erosion of bronchial and bronchiolar mucosal epithelium and increased permeability of the alveolar septa (Hoechst, 1986 a; Battelle, 1981). The histopathological changes were mostly resolved by 9 or 10 days after exposure in animals which had been exposed to levels of chloroformic acid methyl ester in the range of the $LC_{50}$ (Battelle, 1981).

The acute toxicity data which are available only from secondary sources ($LC_{50}$ for 1-hour exposure 450 mg/m³ without any indication of the species tested (Damle, 1992), $LD_{50}$ mouse intraperitoneal 40 mg/kg body weight (RTECS, 2001), and mortality in cats given as 3 out of 3 following 30 minutes' exposure to 1500 mg/m³ (Lazarev, 1963)) are suitable only to a limited extent for the assessment of the chemical's acute toxicity, as essential details concerning study conduct, species and data analysis are partly or completely lacking in those sources.

### *Subacute toxicity*

The toxic effects of chloroformic acid methyl ester (99.2% pure) following subacute administration were investigated in male and female Sprague-Dawley rats in an inhalation study conducted in accordance with OECD guideline No. 412. Groups of 5 males and 5 females were exposed to chloroformic acid methyl ester at nominal concentration levels of 0 (controls), 0.5, 1.5, 4.0, 12.0 or 35.0 mg/m³ for 6 hours per day, 5 days per week for 4 weeks (whole-body vapour exposure). The analysed concentration levels were 0 (controls), 0.52, 1.48, 3.94, 12.14 and 34.46 mg/m³. Treatment-related deaths occurred in 2 males and one female from the top concentration group in the final week of the study after they had previously exhibited marked body weight loss. The top concentration group showed clinical signs of toxicity which, in the investigators' interpretation, were attributable to the irritant effect of the chemical; these included hunched posture, blinking of the eyelids, noisy nasal breathing and, during the third week of the study, rapid breathing pattern. Animals exhibited temporary reduction in food consumption in association with reduced body weight gain, with some animals also showing weight loss at later stages of the study. Significant and toxicologically relevant changes in haematological parameters as signs of haemoconcentration were observed in respect of packed cell volume, haemoglobin and red cell numbers, and increased neutrophil counts were associated with inflammatory lesions seen in the respiratory tract. Furthermore, total protein (in males only), globulin and cholesterol levels were increased while albumin and the albumin/globulin ratio were decreased. At necropsy, the lungs of 3 out of 3 males and 4 out of 4 females failed to collapse upon thoracotomy and were congested in 1 out of 4 females and 1 out of 3 males, and enlargement of the tracheobronchial and

mediastinal lymph nodes was noted in 3 out of 3 males and 3 out of 4 females. Lung weights were increased in both sexes. Histological examination revealed changes of the nasal mucosa (acute exudative sinusitis) in 4 out of 5 males and 5 out of 5 females; minimal squamous metaplasia with minor epithelial erosion and focal inflammation of the larynx in 3 out of 5 males and 4 out of 4 females; inflammatory changes and minimal epithelial hyperplasia of the trachea in 1 out of 5 males; pneumonia together with intra-alveolar exudation and alveolar macrophage aggregation in all males and females, and additionally oedema, congestion, bronchiolitis, squamous metaplasia of terminal bronchiolar epithelium with focal necrosis in some animals and granulomatous lesions in 1 out of 5 females. In the group exposed to the second-highest concentration, 12.14 mg/m³, only 1 out of 5 males exhibited noisy nasal breathing during the second week of the study. All other animals from this concentration group showed no clinical signs of toxicity. Body weight gain, food consumption, haematological and biochemical parameters and necropsy findings were normal in the animals from the group exposed to the second-highest concentration. Histopathological changes in the form of exudative sinusitis were noted in 1 out of 5 females and in the form of localised minor squamous metaplasia of the laryngeal epithelium in 2 out of 5 males and 1 out of 5 females. Similar changes of the laryngeal epithelium were also observed in one male from the intermediate concentration group and one female from the control group. Nominal concentrations of 0.5 and 1.5 mg/m³ (analytical concentrations of 0.52 and 1.48 mg/m³) produced no effects (HRC, 1992).

The concentration levels used in the 28-day inhalation study described above (HRC, 1992) were identified on the basis of the results of a 5-day range-finding study in which groups of 5 male and 5 female Sprague-Dawley rats were exposed to chloroformic acid methyl ester levels of 0 (control), 7.5, 24 or 75 mg/m³ (analytical concentrations of 0 (control), 7.5, 24 and 74 mg/m³) and 7.5 mg/m³ was tolerated by the animals without abnormal findings. The clinical signs of toxicity observed during exposures included blinking of the eyelids, licking the inside of the mouth, ruffled fur, sneezing in the top and intermediate dose groups, and irregular breathing, which was confined to the top group. In between exposures, the high-concentration males sneezed and exhibited noisy nasal breathing. Reduced food and water consumption was associated with body weight loss in the animals from the top concentration group. At necropsy 3 days after

the end of exposure, the lungs failed to collapse in 1 out of 5 intermediate-dose males and in 3 out of 5 males and 5 out of 5 females from the top concentration group. The lungs of 1 out of 5 intermediate-dose males and 5 out of 5 males and 1 out of 5 females from the top concentration group exhibited petechial bleeding. All females from the top concentration group had increased lung weights. Organ weights could not be evaluated for males due to an error occurring during necropsy. Histological examination revealed inflammatory and erosive mucous membrane lesions in the nose, larynx, trachea and lungs (bronchiolitis, pneumonia) in animals from the top concentration groups as well as focal epithelial hyperplasia of the nasal mucosa in animals from the intermediate and top concentration groups. Comparison of the histopathological findings for a satellite group examined immediately after the end of exposure to those obtained for animals which were examined 3 days after the end of exposure demonstrated clear signs of regeneration and repair of epithelial lesions. There were no findings at the lowest concentration level of 7.5 mg/m³ (HRC, 1992).

Chloroformic acid methyl ester was investigated together with 108 other chemicals in an exploratory subacute inhalation study. Groups of 4 male and 4 female Alderly Park SPF rats weighing approx. 200 g underwent whole-body exposure to chloroformic acid methyl ester at concentration levels of 20, 5 or 1 ppm (approx. 78.4, 19.6 and 3.92 mg/m³) for 6 hours/day, 5 days/week over a period of 3 weeks. There were no concurrent controls in the study. Assessments included clinical signs of toxicity, body weight gain, necropsy and histological examination of at least the lungs, liver, kidneys, spleen and adrenal glands. Clinical chemistry and haematology tests were probably not carried out in animals treated with chloroformic acid methyl ester. Exposure to 20 ppm chloroformic acid methyl ester caused nose irritation, respiratory difficulty, lethargy, poor general condition and weight loss. At necropsy, the lungs were distended and haemorrhagic. Histological examination revealed areas of consolidation and collapse, oedema and haemorrhage in the lungs, and congestion of the kidneys. Exposure to 5 ppm only produced signs of irritation and lethargy, whilst exposure to 1 ppm was tolerated by rats without remarkable findings (no further details; Gage, 1970).

## 7.3 Skin and mucous membrane effects

The acute skin irritancy of undiluted chloroformic acid methyl ester (purity not specified) was tested in rabbits. The chemical was applied to the dorsal skin for 1, 5 or 15 minutes or 20 hours, or to the skin of the ear for 20 hours. The observation period was 8 days. As shown in Table 2 below, even a 1-minute exposure to chloroformic acid methyl ester caused severe reddening of the area of exposure and beyond, severe oedema and, subsequently, severe leathery necrosis which extended beyond the area of exposure. Chloroformic acid methyl ester was evaluated as corrosive by the investigators (BASF, 1975).

**Table 2. Irritant effects of chloroformic acid methyl ester on the rabbit skin and their dependence on the duration of exposure (based on BASF, 1975)**

| Application site | Duration of exposure | Findings after 24 hours | Findings after 8 days |
|---|---|---|---|
| Back | 1 minute | severe reddening of the area of exposure and beyond, very severe oedema | severe necrosis, leathery and extending beyond the area of exposure |
| Back | 5 minutes | severe reddening of the area of exposure and beyond, partly livid, very severe oedema | severe necrosis, leathery and extending beyond the area of exposure |
| Back | 15 minutes | slight necrosis, surrounded by slight reddening and very severe oedema | severe necrosis, leathery and extending beyond the area of exposure |
| Back | 20 hours | slight necrosis, surrounded by severe reddening and severe oedema | severe necrosis, leathery and extending beyond the area of exposure |
| Ear | 20 hours | partly slight necrosis, partly severe reddening and severe oedema | partly slight necrosis, anaemic, partly superficial scabs |

In order to validate a test method allowing more differentiated classification of a chemical as corrosive or irritating to the skin, a screening study was conducted which also included chloroformic acid methyl ester. Groups of 6 New Zealand white rabbits underwent a single 1-hour or 4-hour exposure of the depilated uninjured skin of the flank to gauze pads impregnated with 0.5 ml undiluted chloroformic acid methyl ester (purity not specified) under both occlusive and semi-occlusive conditions. The 4 exposure variants were tested in parallel in each animal. The effects were assessed after 1, 24, 48 and 72 hours and after 7 days in accordance with OECD guideline No. 404. All 4 types of exposure produced extensive areas of discoloured skin (grey-white, yellowish brown) and necrosis as well as encrustation and

induration at the sites of application. After occlusive application, in particular, there was also reddening beyond the sites of application. Chloroformic acid methyl ester was evaluated as corrosive to the skin in all 4 substudies, i.e. after 1-hour and 4-hour exposure under both semi-occlusive and occlusive conditions (Hoechst, 1982 b; Potokar et al., 1985).

Another skin irritation test with chloroformic acid methyl ester was conducted in 3 New Zealand white rabbits which received 3-minute applications of the test substance to the depilated intact skin of the flank under occlusive as well as semi-occlusive conditions. Examinations were carried out in accordance with OECD guideline No. 404. Damage reached deep into the skin of the rabbits. The sites of application displayed white-grey and/or brown discoloration, accompanied by necrosis and/or very marked or severe oedema as well as erythema together with eschar formation and encrustation. The treated flanks were reddened beyond the area of application in animals which underwent occlusive exposure. In general, findings were somewhat less marked in animals receiving semi-occlusive treatment than in those with occlusive treatment. Chloroformic acid methyl ester was evaluated as severely irritating to corrosive (Hoechst, 1982 b).

In a further skin test in albino rabbits, a skin irritation index was determined as 110 out of a maximum score of 110, and hence chloroformic acid methyl ester was evaluated as extremely irritating, and corrosive (no further details; Industrial Bio-Test, 1975).

In contradiction with the above-mentioned findings (BASF, 1975; Industrial Bio-Test, 1975; Potokar et al., 1985) according to which chloroformic acid methyl ester is corrosive to the skin, another report claimed, without giving details, that single application of undiluted chloroformic acid methyl ester or a solution of the chemical in oil to the skin of mice, rats and rabbits produced no signs of irritation whatsoever. Reportedly, the only signs seen after 3 or 4 applications were mild hyperaemia and subsequent mild pigmentation (Gurova et al., 1977). As essential details concerning experimental design and assessment of effects (e.g. duration of exposure, mode of application, scope and time points of examination) are lacking, the study is unsuitable for evaluating the skin irritancy of chloroformic acid methyl ester.

Chloroformic acid methyl ester exhibited severely corrosive effects to the eye in a rabbit study. One hour following instillation of 50 µl chloroformic acid methyl ester (purity not specified) into the rabbit eye, findings included mild reddening, severe oedema, severe clouding of the eye, white nictitating and mucous membranes, and 24 hours upon instillation vascular injection and greasy films were observed in addition. At 8 days, corrosive effects were even more manifest, with slight reddening, severe oedema and severe clouding, corrosion, iritis, staphyloma, purulent discharge, scars and encrustations of the eyelids being observed. Negative controls treated with sodium chloride were without abnormal findings at all scheduled examinations (BASF, 1975).

In a further test for mucous membrane effects in albino rabbits, chloroformic acid methyl ester was evaluated as extremely irritating, and corrosive. An irritation index of 8.0 out of a maximum score of 8.0 was determined (no further details; Industrial Bio-Test, 1975).

Without providing further details, one source reports that a single instillation of an oily formulation of chloroformic acid methyl ester into the eyes of rabbits produced severe inflammation, purulent discharge and general intoxication (Gurova et al., 1977). As essential details concerning experimental design and assessment of effects (e.g. concentration of chloroformic acid methyl ester in the formulation tested, duration of exposure, scope and time points of examination) are lacking, the study is unsuitable for evaluating the irritating effects of chloroformic acid methyl ester on mucous membranes.

## 7.4 Sensitisation

No information available.

## 7.5 Subchronic and chronic toxicity

The toxic effects of chloroformic acid methyl ester following subchronic exposure were investigated in an inhalation study in Wistar rats which was conducted in accordance with OECD guideline No. 413, EPA TSCA guideline 40 CFR § 798.2450 and EC Directive 87/302/EEC. Groups of 10 males and 10 females per concentration level underwent whole-body exposure to chloroformic acid methyl ester at nominal levels of 0 (control), 0.4, 2, 4 or 8

ppm (approx. 0 (control), 1.6, 7.8, 15.7 or 31 mg/m³). Test concentrations were selected taking into consideration the findings from the subacute inhalation study in the Sprague-Dawley rat discussed above (HRC, 1992; see Section 7.2). Mean concentration levels as determined by analysis were 0 (control), 0.40, 2.15, 3.98 and 7.83 ppm (approx. 0 (control), 1.6, 8.4, 15.6 and 30.7 mg/m³). The chloroformic acid methyl ester used for exposure was > 99.4% pure at the beginning of the study; upon reanalysis at the end of the study purity was found to be 98.5%. In addition to the groups of animals which underwent a total of 65 exposures lasting 6 hours/working day over the entire study duration of 92 days, satellite groups of 10 males and 10 females for interim necropsies after 3, 10 or 20 exposures were included for each test concentration and scheduled time point of examination and treated under identical conditions. Per group, the nasal cavity and larynx of 4 females pretreated with 5-bromo-2'-deoxyuridine were studied by determining DNA replication as a measure of cell proliferation. No haematology or clinical chemistry studies were carried out. Four male rats exposed to 8 ppm died, the deaths being recorded after 24, 32, 36 and 41 exposures. Clinical signs of toxicity were confined to the top concentration group and indicated treatment-related irritation of the respiratory tract; signs included rubbing of snouts, sneezing, nasal crusts and in the animals which subsequently died, abnormal breathing patterns and reduced general state. Body weight change exhibited slight reduction in males from the intermediate and top concentration groups. Slight yet statistically significant decreases in absolute body weights were seen, but only in males of top concentration group which were sacrificed after 3 exposures or at the end of the study. Substance-related macroscopic changes were confined to the animals that underwent 8 ppm exposure for the entire duration of the study; they exhibited red foci in the lungs. Animals from this concentration group which died intercurrently exhibited emphysema and/or multifocal discoloration of the lungs. Animals from the top concentration group, except for those sacrificed for the first interim necropsy after 3 exposures, displayed increases in relative and absolute lung weights. All other organ weights showed no treatment-related or toxicologically relevant changes when compared with the controls. Histopathological changes were restricted to the respiratory tract. An overview of the frequency and severity of the lesions observed in various parts of the respiratory tract is provided in Table 3 below.

**Table 3. Histopathological findings and increased cell proliferation rates[1] in the Wistar rat following 3, 10, 20 or 65 whole-body exposures to chloroformic acid methyl ester (subchronic inhalation study with interim necropsies at 0.5, 2 and 4 weeks), based on BASF (1999)**

| Concentration | Exposures | Examination | Nose | Larynx | Trachea | Lung |
|---|---|---|---|---|---|---|
| 0.4 ppm | 3 | Histopathology | no findings | no findings | no findings | no findings |
| | | Cell proliferation | no findings | no findings | -- | -- |
| | 10 | Histopathology | no findings | no findings | no findings | no findings |
| | | Cell proliferation | no findings | no findings | -- | -- |
| | 20 | Histopathology | no findings | no findings | no findings | no findings |
| | | Cell proliferation | no findings | no findings | -- | -- |
| | 63 | Histopathology | no findings | no findings | no findings | no findings |
| | | Cell proliferation | no findings | no findings | -- | -- |
| 2 ppm | 3 | Histopathology | + | + | no findings | no findings |
| | | Cell proliferation | no findings | no findings | -- | -- |
| | 10 | Histopathology | + | + | no findings | no findings |
| | | Cell proliferation | no findings | no findings | -- | -- |
| | 20 | Histopathology | + | ++ | no findings | no findings |
| | | Cell proliferation | + | + | -- | -- |
| | 65 | Histopathology | ++ | + | no findings | no findings |
| | | Cell proliferation | ++ | + | -- | -- |
| 4 ppm | 3 | Histopathology | + | + | + | no findings |
| | | Cell proliferation | no findings | + | -- | -- |
| | 10 | Histopathology | ++ | ++ | ++ | no findings |
| | | Cell proliferation | + | + | -- | -- |
| | 20 | Histopathology | ++ | ++ | ++ | + |
| | | Cell proliferation | + | + | -- | -- |
| | 63 | Histopathology | ++ | ++ | + | + |
| | | Cell proliferation | +++ | ++ | -- | -- |
| 8 ppm | 3 | Histopathology | ++ | +++ | ++ | + |
| | | Cell proliferation | no findings | ++ | -- | -- |
| | 10 | Histopathology | ++ | +++ | +++ | ++ |
| | | Cell proliferation | ++ | ++ | -- | -- |
| | 20 | Histopathology | +++ | +++ | +++ | ++ |
| | | Cell proliferation | ++ | ++ | -- | -- |
| | 65 | Histopathology | +++ | +++ | +++ | ++ |
| | | Cell proliferation | +++ | +++ | -- | -- |

[1] Determination of DNA replication following 5-bromo-2'-deoxyuridine pretreatment
\+ slight
++ moderate
+++ severe
-- not studied

Histopathological changes of the respiratory tract were noted after only three exposures to levels of chloroformic acid methyl ester ≥ 2 ppm. **Alterations of the nasal cavity** consisted in squamous epithelial metaplasia and focal or diffuse hyperplasia of the ciliated respiratory epithelium and the transitional epithelium. Associated with enhanced mucus production was hypertrophy of the mucus-producing goblet cells but with prolonged

exposure the cells were displaced by the later predominant squamous epithelium. By the end of the study the squamoid, greatly thickened transitional epithelium additionally exhibited marked surface keratinisation. Beginning at the transitional epithelium but by the end of the study also affecting the respiratory epithelium, there was purulent inflammation which was associated with necrotic cells sloughing off. DNA replication in the respiratory epithelium of animals treated at 8 ppm depended upon the duration of exposure and was found to be slightly increased following 3 exposures, relative to controls, whilst respective increases were 2.5 and 4.5-fold after 10 and 20 exposures and 33-fold at the end of the study. Animals exposed to 2 or 4 ppm were noted to have significant increases in DNA replication at the third interim necropsy and thereafter, attaining 7.9-fold and 17.6-fold values relative to controls by the end of the study. The enormous increase in DNA replication was explained by the investigators in the context of markedly enhanced cell renewal rates in squamoid epithelium. In the transitional epithelium, DNA replication was found to be increased after 3 or more exposures in the concentration groups treated at 8 or 4 ppm and after 20 or more exposures in the concentration group treated at 2 ppm. Strikingly, there was a drop in proliferation rate of the transitional epithelium in the front portion of the nasal cavity at the end of the study, a finding which the investigators explained in terms of increasing keratinisation of the tissue. Squamous and olfactory epithelia of the nasal cavity were without significant findings. **Alterations of the larynx** noted in the region of the ciliated epithelium at the base of the epiglottis consisted in squamous metaplasia with minimal to moderate hyperplasia and partly purulent inflammation of the submucosa and were found to be concentration-dependent at the first and later interim necropsies in animals from the upper three concentration groups. Squamous epithelial metaplasia was multifocal after 3 treatment days and exhibited diffuse extension after 10 treatment days. As the study progressed, the cells of the top layer of the ciliated epithelium became increasingly flatter and showed signs of keratinisation. Necrotic cells were found after 10 and 20 exposures but they disappeared by terminal necropsy after 63 exposures, a finding which the investigators considered to be due to protective squamous cell layers. Normally not keratinised, the squamous epithelium of the larynx was inflamed, hyperplastic and keratinised and exhibited single necrotic cells in the upper three concentration groups at the end of the study. The same alterations were also seen in the top concentration group after 3 exposures. The top

concentration group exhibited minimal to moderate hyperplasia of the cuboidal epithelium of the larynx at all time points of investigation. Increases in DNA replication observed in the ciliated epithelium in the three upper concentration groups were 2.3 to 31.1-fold relative to controls up to the third interim necropsy, while in the squamous epithelium they were 5 and 8.3-fold in the two upper concentration groups up to terminal necropsy. A slight but significant increase in DNA replication was observed in the cuboidal epithelium of the 8 ppm animals after 3 treatment days and at the end of the study. **Alterations of the trachea** were confined to the two upper concentration groups. They occurred in a concentration-dependent manner after 3 or more exposures and consisted in squamous metaplasia and hypertrophy of the goblet cells, which was associated with increased production of mucus. Additionally, the top concentration group exhibited focal epithelial hyperplasia. **Alterations of the lungs** were confined to the two upper concentration groups, similarly to the histopathological alterations of the trachea. After 3 exposures, hyperplastic and metaplastic changes in the pulmonary epithelium of animals exposed to 8 ppm were observed in the terminal bronchioles, particularly at the junctions to the alveolar ducts, the alterations expanding to all bronchioles including the segmental bronchioles by the end of the study. Clara cells, which fulfil a protective function, had disappeared. The respiratory epithelium was either extremely flattened or hyperplastic. The alveoles and the peribronchial tissue exhibited prominent granulomatous inflammation, which in part also extended focally into the parenchyma. Some animals had large accumulations of mucus in the alveolar ducts and bronchioles. Animals treated at 4 ppm showed marked histopathological changes of the lung only at terminal necropsy after 65 exposures. **In summary,** inhalation of chloroformic acid methyl ester by rats caused prominent morphological alterations in the ciliated respiratory epithelium and the transitional epithelium of the nose as well as in the ciliated epithelium at the base of the epiglottis and in the bronchioles. Less pronounced were the histopathological changes of the squamous epithelium and the cuboidal epithelium of the larynx and the ciliated respiratory epithelium of the trachea. Alterations of the epithelia mainly consisted in hyperplasia and squamous metaplasia. DNA replication measured in the nasal cavity and larynx essentially reflected the histopathological changes. With respect to the assessment of minimal lesions, however, histological examination appeared to be more sensitive than measurement of DNA replication. Concentrations of 8 and 4 ppm caused

lesions throughout the entire respiratory tract, whereas with 2 ppm lesions were restricted to the nasal cavity and the larynx, irrespective of the number of exposures. This indicates that the test substance is effectively removed from the air stream by the upper respiratory tract up to a concentration of approx. 2 ppm. The concentration-response relationship was driven mainly by the concentrations used for exposure rather than the cumulative dose. Irrespective of the number of exposures, the *no observed adverse effect concentration* (NOAEC) was 0.4 ppm (1.6 mg/m³) for 3, 10, 20 and 65 exposures (BASF, 1999).

In a study with exposure for up to 4 months, male mice were exposed to chloroformic acid methyl ester at levels of 0 (controls), 0.185, 0.64 or 2.06 mg/m³, and male rats were exposed to chloroformic acid methyl ester at 0 (controls), 0.197, 0.72 or 2.15 mg/m³. Essential details concerning experimental design and scope of examination, e.g. strains of animals used, numbers of animals per group, determination of concentrations, mode and duration of exposure, are lacking. Histological examination of the animals which died intercurrently and those sacrificed at the end of the study was without findings in the low concentration group. The intermediate study groups displayed swelling of the bronchial epithelium; the liver and kidneys were normal. Histological findings in the high exposure groups included thickening of the alveolar septa, lymphoid infiltration of the bronchi, vacuolisation of liver cells and lesions in the proximal tubular epithelium of the kidney. There are no indications as to whether examinations were not carried out in both species, nor are any data given regarding the incidence or severity of the findings. Without giving any further information regarding the nature, severity or assignment of findings to exposure levels, additional findings were reported as changes in nerve-muscle conduction, body temperature, respiratory rate, red blood cell count and osmotic stability of erythrocytes, reticulocyte count, bromosulphophthalein test, organ weights of the lungs, heart, liver, kidneys, spleen and adrenal glands as well as the vitamin C levels in individual organs (Gurova et al., 1977). As essential details concerning experimental design and assessment of effects are lacking and documentation as a whole is inadequate, the study is unsuitable for evaluating the chronic toxicity of chloroformic acid methyl ester.

## 7.6 Genotoxicity

### 7.6.1 In vitro

In the Salmonella/microsome test carried out in accordance with OECD guideline No. 471, chloroformic acid methyl ester (99.2% pure) was devoid of mutagenicity both in the absence and presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The study was conducted as a preincubation test in which *Salmonella typhimurium* strains TA 98, TA 100 and TA 1537 were incubated with chloroformic acid methyl ester at concentrations ranging from 0.0005 to 1.0 µl/plate, *Salmonella typhimurium* strain TA 1535 being exposed to chloroformic acid methyl ester levels from 0.0005 to 5.0 µl/plate. Ethanol served as the solvent. In the presence of S-9 mix, concentration levels ≥ 0.5 µl/plate exhibited bacteriotoxicity while in the absence of metabolic activation even lower concentrations ≥ 0.01 µl/plate were toxic. Incubation of *Salmonella typhimurium* strains with chloroformic acid methyl ester did not result in any significant increase in revertant counts. Tests employing the positive controls 2-aminoanthracene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylenediamine, 9-aminoacridine chloride monohydrate and dimethylcarbamyl chloride gave the expected results (BASF, 1988 b).

A further Salmonella/microsome assay using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver) also did not demonstrate mutagenic potential of chloroformic acid methyl ester at levels from 1 to 100 µg/plate. The test was carried out as a standard plate incorporation test with 3 plates per concentration. There were no increases in revertant counts up to the highest test concentration of 100 µg/plate. A preliminary study had demonstrated bacteriotoxicity at 333 µg/plate both with and without metabolic activation. The positive controls sodium azide, 4-nitro-o-phenylenediamine and 2-aminoanthracene and the negative control methanol (solvent) yielded the expected results (LMP, 1985).

These findings confirmed the results of an earlier Salmonella/microsome assay carried out in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 both in the absence and presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). Two plates per concentration level were incubated with chloroformic acid methyl ester at 0 (nega-

tive control ethanol), 0.25, 0.1, 0.02 or 0.004 µl/plate. No details were reported regarding the purity of the test substance or the bacteriotoxicity seen at the concentrations tested. Chloroformic acid methyl ester did not increase revertant counts. As expected, the positive controls 2-acetylaminofluorene, benzo(a)pyrene, β-naphthylamine, neutral red, 4-nitro-o-phenylenediamine and streptozotocin induced marked increases in revertant counts (Hoechst, 1977).

In the chromosome aberration test conducted in V79 cells of the Chinese hamster, chloroformic acid methyl ester induced chromosome damage upon metabolic activation with S-9 mix from Aroclor 1254-induced rat liver. Tests were carried out both with and without metabolic activation at chloroformic acid methyl ester levels of 0.1, 1.0 and 2.5 µl/ml. Cells were prepared at 7 (top concentration only), 18 or 28 (top concentration only) hours. For each scheduled time point of assessment, 400 metaphases were scored per concentration. In the absence of metabolic activation, cells treated with chloroformic acid methyl ester showed no increase in aberration rate. In the presence of metabolic activation, cells prepared after 7 or 28 hours were found to have markedly increased aberration rates relative to the negative control (solvent), regardless of whether gaps were included or not. Cells prepared at 18 hours showed no increase in aberration rate. The respective mitotic indices for the top concentration observed at the three scheduled time points of preparation were 38, 80.6 and 80.5%. The investigators explained the different results obtained for the individual time points of assessment as temporary changes in cell cycle phases in the damaged cells. Tests with the positive controls ethylmethane sulphonate and cyclophosphamide yielded the expected results. The investigators concluded that in this in-vitro cytogenetics study in V79 cells chloroformic acid methyl ester was mutagenic in the presence of metabolic activation (LMP, 1986).

### 7.6.2 In vivo

No information available.

## 7.7 Carcinogenicity

No information available.

### 7.8 Reproductive toxicity

No information available.

### 7.9 Effects on the immune system

No information available.

### 7.10 Neurotoxicity

No information available.

### 7.11 Other effects

No information available.

## 8 Experience in humans

Thiess and Hey (1968) reported a total of 14 cases of human poisoning by inhalation of chloroformic acid methyl ester, including one with fatal outcome. The fatal poisoning (following exposure at a level of approx. 40000 ppm (approx. 156800 mg/m³), as estimated from the air flow, temperature and physical constants) was the result of grossly negligent disregard of safety regulations when cleaning a container for chloroformic acid methyl ester. As an immediate response to inhalation of chloroformic acid methyl ester, the exposed worker developed a strong urge to cough, shallow respiration, retrosternal pain, dyspnoea, tachycardia, irritation of the conjunctivae and severe restlessness. Upon admission to hospital after first-aid treatment at the company's medical department, the following findings were noted: good general condition, marked conjunctivitis, urge to cough, no cyanosis, no marked dyspnoea at rest, slightly diminished vesicular breathing and occasional bronchial rales, normal findings for the heart, circulation, abdominal organs and CNS and normal temperature. The thoracic x-ray exhibited diffuse, confluent foci over both mid and lower lung fields and the beginning of pleural effusion on the right side. Despite intensive therapy (cortisol, antibiotics, circulatory support, sedatives, oxygen tent) the patient died of pulmonary oedema four days after inhalation exposure

to chloroformic acid methyl ester. In the remaining 13 cases, which the authors considered trivial poisonings, the signs included lacrimation together with irritation of the conjunctivae and in some cases laryngitis symptoms with dry cough. The reported signs were completely reversible within 1 to 2 hours. The general findings – including thoracic x-rays – were unremarkable (Thiess and Hey, 1968; Hey and Thiess, 1968).

In one case of accidental exposure to chloroformic acid methyl ester a worker inhaled no more than two or three breaths of the gas. Approximately one hour later, he presented at the company's medical department complaining of a slight burning of the eyes and a mild urge to cough. Pulmonary auscultation was largely unremarkable except for the presence of a very occasional rhonchus over the upper fields on both sides. The worker received symptomatic treatment and was completely free of symptoms by 6 hours after exposure. The next morning, coughing and slight dyspnoea as well as rhonchus and dry rale over the entire lung developed. Examination by x-ray two days after exposure revealed diffuse increase in markings over the right lower lung field. Following therapy with antibiotics, corticoids, cough medicines and bed rest, lung auscultation was normal one week after exposure. In the subsequent period of time, however, a severe urge to cough repeatedly occurred, particularly in the mornings, after periods completely free of symptoms. At a later point in time (no precise details given), comprehensive examination at a lung hospital revealed no pathological findings. The author particularly emphasised the long latency period between exposure and the manifestation of severe clinical signs and symptoms following intoxication with chloroformic acid methyl ester (Schuckmann, 1972).

A worker in a Russian production plant was exposed to chloroformic acid methyl ester when the skin of his back came into contact with the chemical during repair work. The man was wearing a respirator at the time of the accident. Immediately after the accident he only experienced itching and burning of the exposed skin areas. While in the shower, he noticed short-lasting irritation of the eyes and throat (for 3 to 5 minutes) and the urge to cough. Only after a period of 4 to 5 hours during which the man was free of signs and symptoms, did he present with dyspnoea, a cough and throatache at the plant's treatment-and-prevention facility, where he was treated with calcium chloride and dimedrol (diphenhydramine) and sent home. After short improvement, his condition deteriorated to the point that he was

admitted to hospital 22 hours after the accident occurred. Clinical and x-ray exams revealed pulmonary oedema, which cleared without complications after appropriate treatment (Penknovich and Anikin, 1988).

The concentrations of chloroformic acid methyl ester determined in volunteers as the odour threshold and the threshold of irritation were 1 mg/m³ (equivalent to 0.255 ppm) and 2 mg/m³ (equivalent to 0.51 ppm), respectively. A concentration level of 5 mg/m³ (equivalent to 1.3 ppm) was reported as severely irritating to the mucous membranes of the eye and to the respiratory tract in humans (no further details; Gurova et al., 1977).

# 9 Classifications and threshold limit values

In the Federal Republic of Germany, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has established a MAK value (maximum workplace concentration) for chloroacetic acid methyl ester on the suggestion of BG Chemie. It was set in the List of MAK- and BAT Values 2004 at 0,2 ml/m³ (ppm, equivalent to 0,78 mg/m³). Furthermore, chloroacetic acid methyl ester has been assigned to pregnancy risk group C, i.e. substances for which "there is no reason to fear a risk of damage to the embryo or foetus when MAK and BAT values are observed" (DFG, 2004; Greim, 2003).

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