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# TOXICOLOGICAL EVALUATIONS

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**TOXICOLOGICAL EVALUATION**

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# Chloroformic acid ethyl ester

**No. 77**

CAS No. 541-41-3

BG Chemie
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# Chloroformic acid ethyl ester

Apart from the evaluation of chloroformic acid ethyl ester (No. 77), there are also TOXICOLOGICAL EVALUATIONS of chloroformic acid methyl ester (No. 36), 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 ethyl ester is harmful following acute oral administration ($LD_{50}$ rat oral approx. 205 to 470 mg/kg body weight). Upon inhalation, chloroformic acid ethyl ester is clearly toxic. $LC_{50}$ values for 1-hour exposure have been determined as approx. 625.5 to 890 mg/m³ in the rat. Concentration levels ≥ approx. 900 mg/m³ were lethal to the majority of or all animals that underwent 1-hour exposure. The high acute inhalation toxicity of chloroformic acid ethyl ester, which is due to the chemical's severe corrosiveness in conjunction with its high volatility, became strikingly apparent in the inhalation hazard test, in which 11 out of 12 rats died after only 3 minutes' exposure to atmosphere enriched or saturated with vapour at 20 °C. When chloroformic acid ethyl ester is applied dermally, it is of low systemic toxicity ($LD_{50}$ rabbit dermal > 2280 or 7120 mg/kg body weight, depending on the source of information) but causes severe corrosive damage at the site of application. Dyspnoea, apathy, reeling, lying on the abdomen or side and twitching have been reported as signs of toxicity following oral administration. The signs of toxicity seen upon inhalation exposure include mucous membrane irritation, closed eyes and severe respiratory distress caused by the agent's corrosive effects. Necropsy findings have also, in particular, been found to include 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; oral administration resulted in dark-red discoloration of the gastrointestinal mucosa together with reddish intestinal contents; and intraperitoneal injection caused intra-abdominal adhesions.

In an exploratory subacute inhalation study, rats underwent whole-body exposure to chloroformic acid ethyl ester for 6 hours/day, 5 days/week at 20 ppm (approx. 90 mg/m³) over a period of 2 weeks or at 5 or 1 ppm (ap-

prox. 22.5 and 4.5 mg/m³) over a period of 4 weeks. Ten exposures to 20 ppm chloroformic acid ethyl ester resulted in signs of irritation, respiratory difficulty, poor general condition and body weight loss. At necropsy, the lungs were distended and haemorrhagic. Twenty exposures to 5 ppm only resulted in retarded weight increase, whilst 20 exposures to 1 ppm were tolerated by rats without remarkable findings.

In the rabbit, chloroformic acid ethyl ester is corrosive to the skin and eye. Even a 5-minute skin exposure causes slight necrosis.

Conducted as a preincubation test in accordance with OECD guideline No. 471, the Salmonella/microsome test on *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1538 revealed no mutagenic potential for chloroformic acid ethyl ester either in the absence or presence of metabolic activation.

In an exploratory carcinogenicity study, male Sprague-Dawley rats underwent whole-body exposure to chloroformic acid ethyl ester at levels of 1.5, 3.0 or 6.0 ppm (approx. 6.75, 13.5 and 27 mg/m³ air) 6 hours/day on 5 days/week for a period of 30 days and were subsequently held for life-span observation. One out of 50 animals exposed to 6 ppm and examined at the age of approx. 2 years (700 days) was found to have squamous cell carcinoma of the nasal mucosa. None of the 98 controls exposed to test substance-free air under the same conditions exhibited tumours of that type. Among animals treated with 6 ppm chloroformic acid ethyl ester, 15 out of 50 (30%) exhibited squamous metaplasia of the nasal mucosa and 45 out of 50 (90%) had rhinitis; in the control group, 9 out of 98 (9.1%) displayed squamous metaplasia and 80 out of 98 (approx. 82%) had rhinitis. The investigators did not analyse their findings for statistical significance. Further exploratory carcinogenicity studies in female ICR/Ha Swiss mice (30 to 50 mice/group) receiving lifetime (18 to 22 months) dermal or subcutaneous administrations of chloroformic acid ethyl ester showed no increases in tumour incidences compared with controls. Chloroformic acid ethyl ester was administered to the 30 to 50 animals/group dermally thrice weekly at dose levels of 3, 4.3 or 5.5 mg/administration or subcutaneously once weekly at dose levels of 0.3 or 1.1 mg/administration. According to the investigators, the highest dose levels corresponded to the maximum tolerated dose (MTD) in each case. In parallel, the same investigators carried out an initiation-promotion test with single dermal application of 5.5 mg chloroformic

acid ethyl ester followed by a 14-day treatment-free period and lifetime thrice-weekly dermal applications of phorbol myristate acetate (PMA) as promoter. According to the investigators, animals treated with chloroformic acid ethyl ester and PMA exhibited marginally increased tumour incidences (6 out of 50 animals developed tumours, 4 papillomas and 2 squamous carcinomas; $p < 0.05$). It is unclear precisely what amount of PMA was used to treat animals upon initiation with chloroformic acid ethyl ester and, consequently, which PMA control group was used for comparison. Therefore, the investigators' evaluation lacks clear plausibility.

Humans accidentally contaminated with chloroformic acid ethyl ester have been observed to develop irritation of the eyes and respiratory tract as well as cyanosis and pulmonary oedema. Chloroformic acid ethyl ester has been assigned to a group of chemicals which in humans causes toxicity-related obstructive respiratory disorders characterised by toxic mucous membrane damage, primarily in the region of the intermediate and lower airways.

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 worked on chloroformic acid ethyl ester on the suggestion of BG Chemie. The MAK-Kommission has assigned chloroformic acid ethyl ester in the List of MAK and BAT Values 2004, to Category 3B of carcinogenic substances (i.e. "Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data. The classification in Category 3 is provisional. Substances for which in vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories. Further studies are required before a final decision can be made. A MAK or BAT value can be established provided no genotoxic effects have been detected.").

## 2 Name of substance

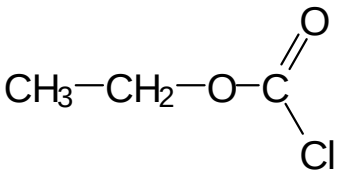
| | | |
|---|---|---|
| 2.1 | Usual name | Chloroformic acid ethyl ester |
| 2.2 | IUPAC name | Chloroformic acid ethyl ester |
| 2.3 | CAS No. | 541-41-3 |
| 2.4 | EINECS No. | 208-778-5 |

## 3 Synonyms, common and trade names

Aethylchlorkohlensäureester
CAEE
Carbonochloridic acid, ethyl ester
Carbonochloridsäureethylester
Cathyl chloride
Chlorameisensäureethylester
Chlorkohlensäureethylester
Chlorocarbonic acid ethyl ester
ECF
Ethoxycarbonyl chloride
Ethyl carbonochloridate
Ethylchlorameisensäureester
Ethylchlorcarbonat
Ethylchlorformiat
Ethylchlorkohlensäureester
Ethylchlormethanat
Ethyl chlorocarbonate
Ethyl chloroformate
Ethyl chloromethanoate
Formic acid, chloro-, ethyl ester

## 4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_3H_5O_2Cl$

## 5 Physical and chemical properties

5.1 Molecular mass, g/mol 108.53

| | | |
|---|---|---|
| 5.2 | Melting point, °C | Ca. –80 (BASF, 1982)<br>–80.6 (Lide and Frederikse, 1996; Sax, 1995)<br>–81 (Bayer, 1992) |
| 5.3 | Boiling point, °C | 93 (at 1013 hPa) (Bayer, 1992)<br>93–95 (Hawley, 1995; BASF, 1982)<br>94 (at 1013 hPa) (Böhm, 2001; Damle, 1992)<br>95 (Lide and Frederikse, 1996) |
| 5.4 | Vapour pressure, hPa | 54 (at 20 °C) (BASF, 1988 a)<br>55 (at 20 °C) (Bayer, 1992)<br>217 (at 50 °C) (BASF, 1988 a) |
| 5.5 | Density, g/cm³ | 1.1442 (at 15 °C) (Sax, 1995)<br>1.13 (at 20 °C) (BASF, 1982)<br>1.1352 (at 20 °C) (Lide and Frederikse, 1996)<br>1.138 (at 20 °C) (Damle, 1992)<br>1.135–1.139 (at 20 °C) (Hawley, 1995)<br>1.14 (at 20 °C) (Bayer, 1992)<br>1.1403 (at 20 °C) (Böhm, 2001) |
| 5.6 | Solubility in water | Insoluble (hydrolysis) (BASF, 1982; Damle, 1992)<br>Hydrolysis (Hawley, 1995; van Duuren et al., 1987)<br>Hydrolysis rate $t_{½}$ (at 30 °C) 19 minutes (Sellakumar et al., 1987) |
| 5.7 | Solubility in organic solvents | Miscible with nearly all usual aprotic solvents (BASF, 1982)<br>Miscible with or highly soluble in alcohol, ether, benzene and chloroform (Hawley, 1995; Lide and Frederikse, 1996; Sax, 1995) |
| 5.8 | Solubility in fat | Dissolves well in fat (Hansen and Andersen, 1988) |
| 5.9 | pH value | No information available |
| 5.10 | Conversion factor | 1 ml/m³ (ppm) ≙ 4.5 mg/m³<br>1 mg/m³ ≙ 0.22 ml/m³ (ppm)<br>(at 1013 hPa and 25 °C) |

# 6 Uses

Versatile intermediate used in the manufacture of e.g. peroxide compounds, dye components, herbicides, insecticides, flotation agents 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 ethyl ester following oral, inhalation, dermal and intraperitoneal administration are summarised in Table 1.

Beginning of Table 1

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

| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
|---|---|---|---|---|---|---|
| Rat | oral | < 50 | n. d. | $LD_{50}$ | n. d. | Eastman Kodak, not dated |
| Rat | oral | ca. 205 (180 mm³), administered as a 0.1–1% aqueous tragacanth gum emulsion | n. d. | $LD_{50}$; dyspnoea, apathy, reeling, lying on the abdomen and side, twitching in some cases; necropsy: dark-red discoloration of the gastric mucosa, reddish intestinal contents | 14 days | BASF, 1970 |
| Rat, Sprague-Dawley, female | oral | 270 | n. d. | $LD_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Sprague-Dawley, male | oral | 411 | n. d. | $LD_{50}$ | 14 days | Warf Institute, 1972 |

| Table 1. Acute toxicity studies of chloroformic acid ethyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| Rat, Sprague-Dawley, male | oral | 470 | n. d. | $LD_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Sprague-Dawley, male | inhalation | ca. 652.5 (145 ppm), one hour | n. d. | $LC_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Sprague-Dawley, female | inhalation | ca. 765 (170 ppm), one hour | n. d. | $LC_{50}$ | n. d. | Vernot et al., 1977 |
| Rat, Fischer 344, male | inhalation | 840 (185 ppm), one hour | n. d. | $LC_{50}$; reduced body weight gain; necropsy of the animals which died intercurrently and those which survived to the end of the study: greatly increased lung weights, alveolar haemorrhage, red lung coloration; 210 mg/m³ was without findings | 14 days | Battelle, 1981 |
| Rat, Fischer 344, female | inhalation | 890 (196 ppm), one hour | n. d. | $LC_{50}$; reduced body weight gain; necropsy of the animals which died intercurrently and those which survived to the end of the study: greatly increased lung weights, alveolar haemorrhage, red lung coloration; 210 mg/m³ was without findings | 14 days | Battelle, 1981 |
| Rat | inhalation | ca. 900 (198 ppm), one hour | n. d. | mortality: 9/10; mucous membrane irritation, gasping; necropsy findings: congestion and oedema of the lungs, hydrothorax | n. d. | BASF, 1970 |
| Rat, Sprague-Dawley, male | inhalation | 1620 (356 ppm), one hour | n. d. | mortality: 10/10 within one day; closed eyes, severe respiratory distress; necropsy findings: severe haemorrhage in all lung lobes and some areas of the trachea | n. d. | Warf Institute, 1972 |
| Rat, Sprague-Dawley, male | inhalation | 3240 (713 ppm), one hour | n. d. | mortality: 10/10 within 2 hours; closed eyes, severe respiratory distress | n. d. | Warf Institute, 1972 |
| Rat | inhalation | atmosphere enriched or saturated with vapour at 20 °C, 3 minutes | n. d. | mortality: 11/12; vigorous escape behaviour, extremely severe mucous membrane irritation, gasping; necropsy findings: congestion, oedema and emphysema of the lungs | n. d. | BASF, 1970 |
| Mouse | inhalation | 2260, 10 minutes | n. d. | LCLo (no further details) | n. d. | RTECS, 2001 |

| Table 1. Acute toxicity studies of chloroformic acid ethyl ester | | | | | | |
|---|---|---|---|---|---|---|
| Species, strain, sex[1] | Route | Dose (mg/kg body weight or mg/m³) | Purity | Effect | Observation period | Reference |
| n. d. | inhalation | 1090, one hour | n. d. | $LC_{50}$ | n. d. | Damle, 1992 |
| Rabbit, albino, male | dermal | ca. 2280 (2 ml), 24-hour occlusive exposure | n. d. | mortality: 0/2; mortality was also 0/2 for the other two test dose levels of 0.5 and 1.0 ml/kg body weight; no effect on body weight gain, application areas exhibited skin corrosion | 14 days | Warf Institute, 1972 |
| Rabbit, New Zealand | dermal | 7120, 24-hour exposure | n. d. | $LD_{50}$ | n. d. | Vernot et al., 1977 |
| Mouse | intraperitoneal | > ca. 57 (50 mm³), administered as a 0.1% aqueous tragacanth gum emulsion | n. d. | $LD_{50}$; dyspnoea, lying on the abdomen; necropsy findings: adhesions in the abdominal cavity | 14 days | BASF, 1970 |
| Mouse | intraperitoneal | 15 | n. d. | LDLo (no further details) | n. d. | RTECS, 2001 |
| Mouse | intraperitoneal | < 14.25 (12.5 mm³), administered as a 0.5% aqueous tragacanth gum emulsion | n. d. | $LD_{50}$; dyspnoea, lying on the abdomen; necropsy findings: adhesions in the abdominal cavity | 14 days | BASF, 1970 |

[1] where specified
n. d. no data

End of Table 1

When administered orally, chloroformic acid ethyl ester proved to be harmful in rat studies, with $LD_{50}$ values ranging from 270 to 470 mg/kg body weight (Vernot et al., 1977; Warf Institute, 1972). Upon inhalation, chloroformic acid ethyl ester was clearly toxic. $LC_{50}$ values for 1-hour exposure were determined as approx. 625.5 to 890 mg/m³ in the rat (Vernot et al., 1977; Battelle, 1981). Concentration levels ≥ approx. 900 mg/m³ were lethal to the majority of or all animals that underwent 1-hour exposure (BASF, 1970; Warf Institute, 1972). The high acute inhalation toxicity of chloroformic acid ethyl ester, which is due to the chemical's high volatility, became strikingly apparent in the inhalation hazard test, in which 11 out of 12 rats died after only 3 minutes' exposure to atmosphere enriched or saturated with vapour at 20 °C. When applied dermally, chloroformic acid ethyl ester was of low systemic toxicity ($LD_{50}$ rabbit dermal > 2280 or 7120 mg/kg body weight) but caused severe corrosive damage at the site of application (Vernot et al., 1977; Warf Institute, 1972). Dyspnoea, apathy, reeling, lying on the abdomen or side and twitching were reported as signs of toxicity following oral administration. The signs of toxicity seen upon inhalation ex-

posure included mucous membrane irritation, closed eyes and severe respiratory distress caused by the agent's corrosive effects (BASF, 1970; Warf Institute, 1972). 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; oral administration resulted in dark-red discoloration of the gastrointestinal mucosa together with reddish intestinal contents; and intraperitoneal injection caused intra-abdominal adhesions (BASF, 1970; Battelle, 1981; Warf Institute, 1972).

Chloroformic acid ethyl ester undergoes hydrolysis in aqueous solution (see also Section 5.6). Therefore, the oral $LD_{50}$ values determined for aqueous tragacanth gum emulsions of chloroformic acid ethyl ester (rat oral approx. 205 mg/kg body weight, mouse intraperitoneal > approx. 16 mg/kg body weight and < approx. 14.25 mg/kg body weight; BASF, 1970) are suitable only to a limited extent for the assessment of the chemical's acute toxicity, as the possibility can not be precluded that the test substance contained in those formulations was partially hydrolysed. The acute toxicity data which are available only from secondary sources ($LD_{50}$ rat oral < 50 mg/kg body weight (Eastman Kodak, year not given), $LC_{50}$ (one hour) 1090 mg/m³ (Damle, 1992) and LCLo mouse inhalation (10 minutes) 2260 mg/m³ and LDLo mouse intraperitoneal 15 mg/kg body weight (RTECS, 2001)) are also 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*

Chloroformic acid ethyl ester was investigated together with 108 other chemicals in an exploratory subacute inhalation study. Groups of 4 male and 4 female Alderley Park rats weighing approx. 200 g underwent whole-body exposure to chloroformic acid ethyl ester for 6 hours/day, 5 days/week at 20 ppm (approx. 90 mg/m³) over a period of 2 weeks or at 5 or 1 ppm (approx. 22.5 and 4.5 mg/m³) over a period of 4 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 ethyl ester. Ten exposures to 20 ppm chloroformic acid ethyl ester caused signs of irritation, respiratory difficulty, poor general condition and body weight loss. At necropsy, the lungs were distended and haemorrhagic. Twenty exposures to 5 ppm only resulted in retarded weight increase, whilst 20 exposures to 1 ppm were 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 ethyl 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, the treated skin exhibited severe irritation and corrosive effects which were dependent upon the duration of exposure to chloroformic acid ethyl ester. Necrosis occurred when the duration of exposure was ≥ 5 minutes (BASF, 1970).

**Table 2. Irritant effects of chloroformic acid ethyl ester on the rabbit skin and their dependence on the duration of exposure (based on BASF, 1970)**

| Application site | Duration of exposure | Findings after 24 hours | Findings after 8 days |
|---|---|---|---|
| Back | 1 minute | slight reddening | severe reddening |
| Back | 5 minutes | severe reddening | slight necrosis with severe reddening at the edges, mild oedema |
| Back | 15 minutes | slight reddening, partly anaemic | severe necrosis with severe reddening at the edges |
| Back | 20 hours | slight necrosis, anaemic, severe reddening at the edges | severe necrosis with severe reddening at the edges |
| Ear | 20 hours | slight reddening, severe oedema, slight necrosis | severe necrosis |

In order to validate a test method said to allow more differentiated classification of a chemical as corrosive or irritating to the skin, a screening study was conducted which also included chloroformic acid ethyl ester. Groups of 6 New Zealand white rabbits (weighing 2 to 4 kg) underwent a single 1-hour or 4-hour exposure of the depilated uninjured skin of the flank to patches impregnated with 0.5 ml undiluted chloroformic acid ethyl ester (purity not specified). The study was carried out under both occlusive and semi-occlusive conditions. Each animal underwent 4 tests in parallel. The

effects were assessed after 1, 24, 48 and 72 hours and after 7 days in accordance with OECD guideline No. 404. Without specifying their individual findings, the investigators reported that chloroformic acid ethyl ester caused skin irritation following 1-hour semi-occlusive or occlusive and 4-hour semi-occlusive exposure, while 4-hour occlusive exposure had a corrosive effect on the skin (Potokar et al., 1985).

Without specifying individual findings, they reported that 24-hour semi-occlusive application of 0.5 ml chloroformic acid ethyl ester to the intact or scarified depilated dorsal or flank skin of albino rabbits resulted in irreversible damage to the skin. Readings were carried out 24 and 72 hours after the beginning of exposure. Chloroformic acid ethyl ester was evaluated by the investigators as corrosive to the skin (Warf Institute, 1972).

Chloroformic acid ethyl ester exhibited severely corrosive effects to the eye in a rabbit study. One and 24 hours following instillation of 50 µl chloroformic acid ethyl ester (purity not specified) into the rabbit eye, severe reddening, severe oedema and severe clouding were observed, and 24 hours after instillation haemorrhages were noted in addition. At 8 days, the severely corrosive effect was even more manifest, with mild oedema, severe clouding and severe reddening, staphyloma and purulent discharge being observed. Negative controls treated with sodium chloride were without abnormal findings at all scheduled examinations (BASF, 1970).

A further study was conducted in which 6 New Zealand rabbits each had 100 mg chloroformic acid ethyl ester instilled into one eye before being placed under observation for 72 hours. The chemical was not diluted, nor were the eyes rinsed. The investigators reported irreversible damage to the mucous membranes. They evaluated chloroformic acid ethyl ester as corrosive to the eye (Warf Institute, 1972).

## 7.4 Sensitisation

No information available.

## 7.5 Subchronic and chronic toxicity

No information available.

## 7.6 Genotoxicity

### 7.6.1 In vitro

In the Salmonella/microsome test carried out in accordance with OECD guideline No. 471, chloroformic acid ethyl ester (98% 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 ethyl ester at concentrations ranging from 0.0005 to 0.4 µl/plate, strain TA 1535 being exposed to chloroformic acid ethyl ester levels of 0.0005 to 5.0 µl/plate. Ethanol served as the solvent. In the presence of S-9 mix concentration levels ≥ 0.2 µl/plate exhibited bacteriotoxicity while in the absence of metabolic activation even lower concentrations ≥ 0.015 µl/plate were toxic. Incubation of *Salmonella typhimurium* strains with chloroformic acid ethyl ester did not result in any significant increase in revertant counts. Tests with 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 c).

### 7.6.2 In vivo

No information available.

## 7.7 Carcinogenicity

Groups of male Sprague-Dawley rats (aged 9 to 10 weeks, weighing 325 ± 16.8 g) underwent whole-body exposure to chloroformic acid ethyl ester at levels of 1.5, 3.0 or 6.0 ppm (approx. 6.75, 13.5 and 27 mg/m³ air) 6 hours/day on 5 days/week for a period of 30 days and were subsequently held for life-span observation. Controls (98 animals) were exposed to test substance-free air under the same conditions. The chloroformic acid ethyl ester used in the study was > 95% pure and administered at analytically determined concentration levels of 1.5 ± 0.07, 2.9 ± 0.1 and 6.0 ± 0.2 ppm. The exposure regimen was selected on the basis of the hydrolysis rate (chloroformic acid ethyl ester $t_{½}$ at 30 °C = 19 minutes) as the investigators worked under the hypothesis that tumour response to electrophilic com-

pounds was correlated with the hydrolysis rate in an inversely proportional manner. At necropsy, the nasal passages were first flushed with 10-percent formalin solution and subsequently the entire head and other organs were also fixed in 10-percent formalin solution. The head was decalcified and processed into stepwise perpendicular cross-sections, starting just posterior to the nostrils and extending as far as the orbit. Further histological sections were taken from each lobe of the lung and from the trachea, larynx, liver, kidneys, testes and any other organs exhibiting macroscopic gross pathology. The findings are given in Table 3 below.

**Table 3. The carcinogenicity of chloroformic acid ethyl ester in rats after 30 days' exposure and subsequent life-span observation (based on Sellakumar et al., 1987)**

| Concentration (ppm) | No. of animals | Median life-span (days) | Number of animals with nasal mucosal pathology | | |
|---|---|---|---|---|---|
| | | | Rhinitis | Squamous metaplasia | Squamous cell carcinoma |
| 0 (controls) | 98 | 613 | 80 | 9 | 0 |
| 1.5 | 50 | 576 | 44 | 13 | 0 |
| 3.0 | 50 | 573 | 43 | 12 | 0 |
| 6.0 | 50 | 617 | 45 | 15 | 1 |

The outcome of the study was that 1 out of 50 animals (2%) from the top dose group exhibited squamous cell carcinoma of the nasal mucosa at the age of approx. 2 years (700 days). The remainder of the tumour spectrum was comparable with that seen in the controls and animals of the lower dose groups. The publication provides no information on non-tumourigenic findings in the other organs studied. In the investigators' opinion, the lack of a brisk tumour response may have been due to the concentration range studied being too low although the hydrolysis rate seemed to be in the correct range for a tumourigenic response (Sellakumar et al., 1987).

Chloroformic acid ethyl ester (> 99% pure) was investigated together with other chemicals for tumourigenic effects in three different carcinogenicity studies in the mouse by van Duuren et al. (1987); bioassays consisted in an initiation-promotion test with dermal application and two chronic studies with dermal application or subcutaneous administration. Doses used in these investigations were selected on the basis of not further specified preliminary 6 to 8-week studies in groups of 5 mice/dose level. The doses selected as maximum tolerated doses (MTD) for chronic bioassays were

those which resulted in little or no inflammation at the site of administration, no clinically observable toxic effects, and normal weight gain (no further details). During all three studies body weights were recorded, and tumour observations were carried out daily. Animals that became moribund or died intercurrently were necropsied and examined macroscopically. Routine sections for histopathology were taken from the area of administration and the lung, liver, kidney, spleen, colon and urinary bladder and from all other tissues and organs that appeared clinically abnormal. All three substudies employed 6 to 8-week-old female ICR/Ha Swiss mice, also referred to as Hsd:ICR(BR) mice according to the investigators, which received lifetime treatment (for 18 to 22 months) as follows.

Study 1: An initiation-promotion test was carried out in 50 female ICR/Ha Swiss mice. They received a single application of chloroformic acid ethyl ester, 5.5 mg/mouse in 0.1 ml acetone, to the shaved skin of the inter-scapular region. Following a 14-day treatment-free period, the animals were given thrice-weekly lifetime dermal applications of phorbol myristate acetate (PMA) as promoter, again dissolved in 0.1 ml acetone, to the same site. It is unclear from the publication whether the animals were treated with 0.0025 or 0.005 mg PMA/animal and administration. Two groups of 50 and 30 animals were treated as described above but with the promoter PMA alone at respective dose levels of 0.0025 and 0.005 mg/animal. Three groups of vehicle controls comprising a total of 110 animals (two groups of 30 and one of 50 animals) were treated with 0.1 ml acetone/animal and administration. The results are shown in Table 4 below.

**Table 4. Results of an initiation-promotion study in female ICR/Ha Swiss mice given a single dermal application of chloroformic acid ethyl ester (CAEE) followed by a 14-day treatment-free period and thrice-weekly lifetime dermal applications of phorbol myristate acetate (PMA) (based on van Duuren et al., 1987)**

| | 5.5 mg CAEE/animal once, followed by thrice-weekly lifetime PMA treatment[1] | 0.0025 mg PMA/animal as thrice-weekly lifetime treatment | 0.005 mg PMA/animal as thrice-weekly lifetime treatment | 0.1 ml acetone/animal as thrice-weekly lifetime treatment (vehicle controls) |
|---|---|---|---|---|
| Median survival time (days)[2] | 545/640 | 455/635 | > 490/490 | 515/665<br>> 575/575<br>> 610/610 |
| Days to first tumour | 135 | – | 420 | – |
| Number of animals with tumours at the site of administration/number of animals tested | 6/50 | 0/50 | 3/30 | 0/110 |
| Number of animals with tumours at the site of administration | 4 papillomas<br>2 squamous carcinomas<br>($p < 0.05$)[3] | 0 | 2 papillomas<br>1 sarcoma | 0 |

[1] It is unclear from the publication whether the animals received 0.0025 or 0.005 mg PMA/animal and administration. Presumably, however, 0.0025 mg/animal was administered, because if 0.005 mg/animals had been administered, the respective controls would definitely have been sacrificed too early.

[2] The significance of the second figure is not specified in the publication. It probably refers to maximum lifetime, i.e. the day on which the group was terminated.

[3] Significance level (p value) compared with the PMA control.

Compared with the two PMA control groups, in which 0 out of 50 and 3 out of 30 animals developed tumours (2 papillomas, one sarcoma), a single initiation with chloroformic acid ethyl ester followed by lifetime administration of the promoter PMA resulted in tumours (4 papillomas, 2 squamous carcinomas) in 6 out of 50 animals. Tumour incidences displayed marginal significant increases ($p < 0.05$) according to the authors (no further details). No tumours occurred in the vehicle control groups. Note: It is unclear precisely what amount of PMA was used to treat animals upon initiation with chloroformic acid ethyl ester and, consequently, which PMA control group was used for comparison. Therefore, the investigators' evaluation lacks clear plausibility.

Study 2: In the second study, female ICR/Ha Swiss mice received lifetime treatment with chloroformic acid ethyl ester which was applied thrice weekly in 0.1 ml acetone to the shaved skin of the interscapular region at dose levels of 3 (50 mice), 4.3 (30 mice) or 5.5 mg/mouse (50 mice). The

vehicle controls were comparable to those in the initiation-promotion study. The results are shown in Table 5 below.

**Table 5. Results of a long-term carcinogenicity study in female ICR/Ha Swiss mice given thrice-weekly lifetime dermal applications of chloroformic acid ethyl ester (CAEE) (based on van Duuren et al., 1987)**

| | 3.0 mg CAEE/animal | 4.3 mg CAEE/animal | 5.5 mg CAEE/animal | 0.1 ml acetone/animal (vehicle control) |
|---|---|---|---|---|
| Median survival time (days)[1] | 480/660 | > 565/565 | 490/660 | 515/665<br>> 575/575<br>> 610/610 |
| Number of animals with tumours at the site of administration/number of animals tested | 0/50 | 1/30 | 0/50 | 0/110 |
| Number of animals with tumours at the site of administration | 0 | 1 sarcoma | 0 | 0 |

[1] The significance of the second figure is not specified in the publication. It probably refers to maximum lifetime, i.e. the day on which the group was terminated.

Following lifetime dermal application of chloroformic acid ethyl ester, no significant increases in tumour incidences were found in female ICR/Ha Swiss mice when compared with control groups (no further details).

Study 3: In the third substudy, female ICR/Ha Swiss mice received thrice-weekly lifetime subcutaneous injections of chloroformic acid ethyl ester in 0.05 ml trioctanoin (also referred to as tricaprylin) into the left flank at dose levels of 0.3 or 1.1 mg/mouse. Three groups of vehicle controls comprising a total of 130 animals (two groups of 50 and one of 30 animals) were given lifetime subcutaneous treatment with 0.05 ml tricaprylin/animal and administration once weekly. The results are shown in Table 6.

**Table 6. Results of a long-term carcinogenicity study in female ICR/Ha Swiss mice given once-weekly lifetime subcutaneous administrations of chloroformic acid ethyl ester (CAEE) (based on van Duuren et al., 1987)**

| | 0.3 mg CAEE/animal | 1.1 mg CAEE/animal | 0.05 ml tricaprylin/animal (vehicle controls) |
|---|---|---|---|
| Median survival time (days)[1] | 480/660 | > 590/590 | > 595/595<br>490/660<br>> 525/560 |
| Number of animals with tumours at the site of administration/number of animals tested | 1/50 | 0/30 | 0/30<br>2/50<br>0/50 |
| Number of animals with tumours at the site of administration | 1 squamous carcinoma | 0 | 2 haemangiomas[2] |

[1] The significance of the second figure is not specified in the publication. It probably refers to maximum lifetime, i.e. the day on which the group was terminated.
[2] Tumours to which the investigators referred as subcutaneous sarcomas in the text.

Following lifetime subcutaneous administration of chloroformic acid ethyl ester, no significant increases in tumour incidences were found in female Swiss mice when compared with controls (no further details).

The above-described long-term studies conducted by van Duuren et al. (1987) in the female ICR/Ha Swiss mouse yielded no significantly increased tumour incidences following lifetime dermal or subcutaneous administration of chloroformic acid ethyl ester. According to the investigators, tumour incidences showed marginally significant increases when chloroformic acid ethyl ester was given as a single dermal application (initiation) followed by lifetime dermal treatment with phorbol myristate acetate (promotion). No data were given in respect of any additional parameters (e.g. body weight development, clinical signs, necropsy findings, histopathological changes, other causes of death, etc.) which are likely to have been investigated (van Duuren et al., 1987).

## 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

Rats were exposed to 3 different concentration levels of chloroformic acid ethyl ester 7 hours/day on 5 days/week for 6 weeks. The measure used to assess direct carcinogenic potential was the proliferation rate of the nasal epithelium as determined by means of the “3H-TdR pulse labelling index”. The proliferation index showed a dose-dependent increase within 18 hours, remained unchanged at the same elevated level throughout the 6-week exposure period and declined after the exposures were ended. The investigators did not mention any criteria for the evaluation of their data (no further details; Burns et al., 1989).

## 8 Experience in humans

A worker who came into contact with chloroformic acid ethyl ester due to an industrial accident initially developed irritation of the eyes and the mucous membranes of the respiratory tract. About 3½ hours after exposure he developed severe difficulty in breathing accompanied by cyanosis. Clinical and x-ray exams revealed pulmonary oedema, which cleared without complications after appropriate treatment. The worker, who was wearing an overall, apron, safety footwear, long gloves and a full-face fresh air mask, became contaminated with chloroformic acid ethyl ester when the chemical splashed on his leg while he was dispensing it. Still wearing his air-fed hood, he immediately washed down the contaminated clothing and also the underlying skin with running water. He then had a shower, the contaminated clothing being with him in the shower room. The author presumed that it was only in the shower room that chloroformic acid ethyl ester, aided by the warmth there, evaporated from the contaminated clothing and skin, producing the fumes which were then inhaled. The skin under the clothing that had been contaminated with chloroformic acid ethyl ester was only slightly reddened (Bowra, 1981).

Chloroformic acid ethyl ester was assigned to a group of chemicals which causes toxicity-related obstructive respiratory disorders characterised by

toxic mucous membrane damage, primarily in the region of the intermediate and lower airways (Reichel, 1984).

# 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 worked on chloroformic acid ethyl ester on the suggestion of BG Chemie. The MAK-Kommission has assigned chloroformic acid ethyl ester in the List of MAK and BAT Values 2004, to Category 3B of carcinogenic substances (i.e. "Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data. The classification in Category 3 is provisional. Substances for which in vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories. Further studies are required before a final decision can be made. A MAK or BAT value can be established provided no genotoxic effects have been detected."; DFG, 2004; Greim, 2004).

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