Chloroform

MAK value (1999)	**0.5 ml/m³ (ppm) ≙ 2.5 mg/m³**
Peak limitation (2001)	**Category II, excursion factor 2**
Absorption through the skin (1999)	**H**
Sensitization	**–**
Carcinogenicity (1999)	**Category 4**
Prenatal toxicity (1999)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	trichloromethane
Chemical name (CAS)	trichloromethane
CAS number	67-66-3
Structural formula	$CHCl_3$
Molecular weight	119.38
Melting point	–63.5°C
Boiling point	61.7°C
Density at 20°C	1.48 g/cm³
Vapour pressure at 20°C	211 hPa
1 ml/m³ (ppm) ≙ 4.962 mg/m³	**1 mg/m³ ≙ 0.202 ml/m³ (ppm)**

In 1999 chloroform was classified in Carcinogen Category 4. According to the criteria valid at the time, classification of the substance in a category for germ cell mutagenicity was not necessary. As, in the meantime, new criteria for the classification of substances in categories for germ cell mutagens have been drawn up (see "Germ cell mutagens", Volume 17, present series), this supplement to Volume 14 of the present series evaluates the data for the genotoxicity of chloroform in the light of the newly defined classification criteria.

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Genotoxicity

On the basis of the documentation from 1999 ("Chloroform", Volume 14), the data for the genotoxicity of chloroform *in vitro* and *in vivo* are summarized and their relevance for classification in one of the categories for germ cell mutagens evaluated.

In vitro

As a result of the high volatility of chloroform, testing in a closed system is recommended for *in vitro* investigations. The conditions under which incubation was carried out are therefore always given in the description of the studies available.

Gene mutation tests

In various bacterial test systems chloroform was not found to be mutagenic in either the presence or absence of a metabolic activation system, even in a closed system. Most of the studies with yeasts and other fungi yielded negative results (see Volume 14). In a study with *Saccharomyces cerevisiae* D7 for mitotic recombination and gene conversion with concentrations between 21 and 54 mM in a closed system, the results were positive only at clearly cytotoxic concentrations. This strain of yeast contains an endogenous cytochrome P450-dependent monooxygenase system (Callen *et al*. 1980).

Also in mammalian cells mutagenic effects were caused only by high concentrations. In the HPRT (hypoxanthine guanine phosphoribosyl transferase) gene mutation test with V79 cells, three experiments were carried out under identical conditions with chloroform concentrations between 100 and 1500 µg/ml in a closed system. In two of the three experiments there was a slight increase in the number of mutants by a factor of 1.8 to 4.6 after concentrations of 1000 µg/ml and more (about 8.4 mM) in the presence of a metabolic activation system (S9 from Aroclor 1254-induced rat liver). Without the addition of a metabolic activation system the test yielded negative results. Cytotoxic effects were not observed; at the highest tested concentration precipitation was observed in a cytotoxicity test previously carried out (Hoechst AG 1987). Another HPRT gene mutation test with V79 cells, which is of limited validity as a result of the inadequate description of the method and presentation of the results, yielded negative results. The concentrations used were between 1 % and 2.5 % (about 124 and 310 mM). Incubation took place after gassing the cells in closed bottles for one hour; the test was carried out only without a metabolic activation system and no positive control was included (Sturrock 1977). The $TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells in an open system yielded negative results in the absence of a metabolic activation system in the tested concentration range between 0.39 and 1.5 µl/ml (about 4.9–8.5 mM) (Caspary *et al*. 1988; Mitchell *et al*. 1988). In the presence of a metabolic activation system (S9 from Aroclor 1254-induced rat liver), weak positive, statistically significant effects were obtained in the cytotoxic range of 0.025 µl/ml and above (= about 0.31 mM). The concentration range tested was between 0.007 and 0.06 µl/ml. The frequency of mutants was increased three-fold relative to that in the control (Mitchell *et al*. 1988).

Indicator tests

In human lymphocytes, induction of sister chromatid exchange (SCE) was not detected after treatment of the cells with chloroform concentrations of up to 400 µg/ml without a metabolic activation system (about 3.4 mM) (no details of the incubation conditions; Kirkland *et al.* 1981). After chloroform concentrations of 10 mM and more, an increase in SCE count per cell by a factor of 1.2 to 1.8 was observed (no details of the incubation conditions; Morimoto and Koizumi 1983). In K_3D cells (permanent leukaemia cell line, Long-Evans rats) the SCE frequency increased significantly at 1 mM in the presence of a metabolic activation system (S9 from PCB-induced rat liver) to 10.0 SCEs per cell; in the absence of a metabolic activation system the results (7.4 SCEs) were, according to the authors, negative (no details of the incubation conditions; Fujie *et al.* 1993). The results of the negative control with a metabolic activation system are missing in the publication. Chloroform concentrations of 0.1 to 10 mM caused a statistically significant increase in the frequency of SCE in SHE cells (a cell line derived from Syrian hamster embryo) by a factor of 1.1 to 1.6 relative to that in the control (Suzuki 1987). The gassing of CHO cells (a cell line derived from Chinese hamster ovary) with chloroform concentrations of 7000 ml/m^3 and S9 from Aroclor 1254-induced rat liver for one hour did not cause an increase in the frequency of SCE (White *et al.* 1979). The data available indicate that SCE is induced only at high concentrations in the presence of a metabolic activation system. Numerous UDS tests with different cell types (rat and mouse hepatocytes, human lymphocytes, SHE) yielded negative results even in the presence of an activation system (see "Chloroform", Volume 14); these tests were either carried out in an open system or details of incubation are missing. A test for the induction of DNA strand breaks using the alkaline elution method yielded negative results with rat hepatocytes in the tested concentration range of 0.03 to 3 mM. The highest concentration of 3 mM was not yet cytotoxic; the test was carried out in a closed system (Sina *et al.* 1983). In freshly isolated hepatocytes from $B6C3F_1$ mice and F344 rats, chloroform concentrations of 0.1 to 5 mM led to the time and concentration-dependent induction of DNA strand breaks, which were detected by means of pulsed field gel electrophoresis. The concentrations used did not produce any cytotoxic effects (no other details; Ammann and Kedderis 1997). The incubation of calf thymus DNA with 1 mM ^{14}C-chloroform and liver microsomes from rats treated with phenobarbital led to covalent DNA binding (0.46 nmol bound/mg DNA/h) (no details of incubation conditions; DiRenzo *et al.* 1982).

Cytogenetic tests

A chromosomal aberration test with human lymphocytes yielded negative results in the presence of a metabolic activation system (S9 Aroclor 1254-induced rat liver) up to the maximum chloroform concentration tested of 400 µg/ml (about 3.4 mM) (Kirkland *et al.* 1981). No details were given about the cytotoxicity of the substance or how incubation was carried out.

Overall, the *in vitro* genotoxicity tests with chloroform presented an inconsistent picture. Genotoxic effects were observed, even in those studies in which incubation was carried out in a closed system, as a rule only at high, cytotoxic concentrations.

In vivo

Somatic cells

Gene mutation tests

Chloroform did not induce mutations or mitotic recombination in *Drosophila* larvae. The eye mosaic test yielded negative results after inhalation exposure to concentrations between 2000 and 16000 ml/m^3. The highest concentration was lethal, concentrations as low as 8000 ml/m^3 caused a reduced spot frequency (mosaic light spots) relative to that in the controls (Vogel and Nivard 1993).

Groups of 10 female LacI transgenic B6C3F$_1$ mice were exposed by inhalation to chloroform concentrations of 0, 10, 30 or 90 ml/m^3, for 6 hours a day, on 7 days a week for 10, 30, 90 or 180 days. A gene mutation test with the DNA isolated from the liver of these animals yielded negative results. After concentrations of 30 ml/m^3 and more, histopathological changes and the induction of regenerative cell proliferation in the liver were observed in transgenic and non-transgenic animals tested in parallel. In the DNA isolated from the livers of these animals, no gene mutations were detected in the LacI target gene. The authors draw attention, however, to the two weaknesses of the test system: its insensitivity, i.e. weak mutagens cannot be detected, and its suitability only for detecting gene mutations (Butterworth *et al.* 1998).

A host-mediated assay with mice with the *Salmonella* strains TA1535 and TA1537 is described as yielding weak positive results (San Agustin and Lim-Sylianco 1978). There are no data for the route of administration or the doses used.

Indicator tests

In the bone marrow cells of mice given oral doses of chloroform of 25 to 200 mg/kg body weight for four days, doses of 50 mg/kg body weight and above caused an increase in SCE count per cell, which at the highest dose was of the factor 1.5 (Morimoto and Koizumi 1983). There are no data for toxicity available. Also after inhalation exposure of mice to chloroform concentrations of 300 ml/m^3 for 3 and 6 hours, the number of SCEs per cell was increased (no other details; Iijima *et al.* 1982).

UDS tests in hepatocytes from rats (after chloroform doses of 0, 40, 400 mg/kg body weight) and mice (after doses of 0, 238, 477 mg/kg body weight) yielded negative results even with hepatotoxic doses (Larson *et al.* 1994; Mirsalis *et al.* 1982). In the hepatocytes of male B6C3F$_1$ mice, chloroform doses of 200 and 500 mg/kg body weight induced an increase in DNA synthesis in the S-phase, an indirect measure of hepatocellular proliferation (Mirsalis *et al.* 1989).

Alkaline elution revealed that DNA strand breaks were not induced in the kidneys of mice after oral chloroform doses of 1.5 mmol/kg body weight (about 180 mg/kg body weight). The body and kidney weights of the treated animals were unchanged relative to those of the controls (Potter *et al.* 1996).

After oral administration of the substance, tests for covalent binding to DNA yielded weak positive results in the kidneys and liver of the rat, and negative results in the mouse

(Pereira *et al.* 1982). The doses given were 48 mg/kg body weight in the rat and 119 mg/kg body weight in the mouse. In a second investigation, no covalent binding was detected in the liver of the mouse after intraperitoneal doses of 15 mg/kg body weight (Diaz-Gomez and Castro 1980). In addition, it was reported in a third publication that no DNA binding in the liver and kidneys of mice was detected (Reitz *et al.* 1982). The radioactivity was determined in all investigations of covalent binding. DNA adducts were not identified.

Cytogenetic tests

In a chromosomal aberration test in bone marrow cells of the Chinese hamster, the effects of chloroform doses of 0, 40, 120 or 400 mg/kg body weight were investigated. The substance was administered by gavage and preparation was carried out 6, 24 and 48 hours later. In the highest dose group, in which the animals exhibited reduced spontaneous activity and eyelid closure and did not consume any food, a slight but statistically significant increase in the incidence of aberrations was determined (higher than in the controls by a factor of 1.9 and 4.5) 6 and 24 hours after administration; according to the authors, this was, however, within the range of the historical controls of the laboratory. The authors reported the occasional occurrence of cells with severe chromosomal damage: one cell with an unspecified exchange aberration in three of the four treated groups of animals and one cell with an undefined multiple aberration in two of the four treated groups (1000 cells evaluated in each case). The authors did not regard these results as positive, but do not exclude the possibility that chloroform has clastogenic potential (Hoechst AG 1988). In bone marrow cells of the rat, chromosomal aberrations were induced even after single, intraperitoneal doses of 1.2 mg/kg body weight. Also five oral doses of 119.4 mg/kg body weight led to the induction of chromosomal aberrations (Fujie *et al.* 1990). There are no data for toxicity available. Chromosomal aberration tests in bone marrow cells of the mouse produced inconsistent results. Positive results were obtained after subcutaneous doses of 100 or 200 mg/kg body weight (Sharma and Anand 1984), negative results after intraperitoneal administration of up to 1000 mg/kg body weight (Shelby and Witt 1995). Seen in this light, the positive results for chromosomal aberrations obtained in the rat after intraperitoneal injection of the low dose are not explicable and are therefore not included in the present evaluation. There are five publications available about micronucleus tests in the bone marrow of mice. In most cases high doses were used. Data is lacking, however, for the occurrence of systemic-toxic effects in the treated animals. Also the ratio of normochromatic to polychromatic erythrocytes, which would provide information about cytotoxic effects and entry of the substance into the bone marrow, is not given in any of the publications available. Intraperitoneal injection of chloroform doses up to 800 mg/kg body weight carried out on three consecutive days did not cause the induction of micronuclei. In a second experiment with the same dose pattern, in which doses of 400 and 600 mg/kg body weight were tested, but not 800 mg/kg body weight, weak positive effects were obtained (Shelby and Witt 1995). In the micronucleus tests that yielded negative results, two intraperitoneal injections of chloroform in doses of up to 90 mg/kg body weight (Tsuchimoto and Matter 1981), of up to 952 mg/kg body weight (Gocke *et al.* 1981) and of around 80 % of the LD_{50} (Salamone *et al.* 1981) were

tested. In a publication, which cannot be included in the evaluation as a result of inadequate description of the method, a positive result was obtained with 700 mg/kg body weight. Doses of 100 to 600 mg/kg body weight and 800 and 900 mg/kg body weight yielded negative results (San Agustin and Lim-Sylianco 1978). Data are lacking for the route of administration, and the sex and number of animals, and there was no positive control.

Male rats, from which a kidney had been removed and which were given folic acid to stimulate cell proliferation after nephrectomy, were treated with oral chloroform doses of 4 mmol/kg body weight (about 480 mg/kg body weight). According to the authors, this dose is about 50 % of the LD_{50}. In the kidney cells, the frequency of cells containing micronuclei increased from a control value of 0.13 % to 0.44 % after administration of chloroform (Robbiano *et al.* 1998). The method used in this investigation is very unusual, and in addition only one concentration was tested. This test can therefore only be included in the evaluation with reservations.

Germ cells

In two studies, the test for sex-linked recessive lethal mutations with *Drosophila melanogaster* yielded negative results after administration of chloroform with the diet (Gocke *et al.* 1981; Vogel *et al.* 1981).

A test for morphologically abnormal sperms in mice treated via inhalation with chloroform revealed an increase in the number of morphologically abnormal sperms; a test after intraperitoneal treatment yielded negative results (Land *et al.* 1981; Topham 1980). Changes in sperm morphology are not, however, a reliable indicator of mutations; the relevance of the effects for germ cell mutagenicity is questionable (ICPEMC 1983; Salamone 1988; Wild 1984).

The available *in vivo* genotoxicity tests with mammals indicate a weak clastogenic potential, but only at high and usually toxic doses.

Manifesto (MAK value/classification)

Chloroform is not mutagenic in bacteria. In eukaryotes, the substance was found to have a weak mutagenic and clastogenic potential *in vitro* only in cytotoxic concentrations. There was no evidence of mutagenic effects in somatic and germ cells of *Drosophila melanogaster*. A gene mutation test with transgenic LacI mice yielded negative results. There are no investigations available of the mutagenic effects in the germ cells of mammals. The available *in vivo* genotoxicity tests with mammals indicate a weak clastogenic potential, but only at high and usually toxic doses. On the basis of this data, chloroform has therefore not been classified in a category for germ cell mutagens.

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