CHLORAL AND CHLORAL HYDRATE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chloral

Chem. Abstr. Serv. Reg. No.: 75-87-6

Chem. Abstr. Name: Trichloroacetaldehyde

IUPAC Systematic Name: Chloral

Synonyms: Anhydrous chloral; 2,2,2-trichloroacetaldehyde; trichloroethanal; 2,2,2 trichloroethanal

Chloral hydrate

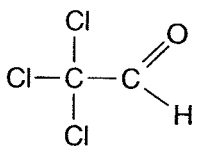
Chem. Abstr. Serv. Reg. No.: 302-17-0

Chem. Abstr. Name: 2,2,2-Trichloro-1,1-ethanediol

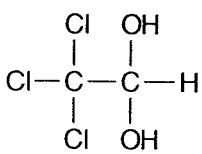
IUPAC Systematic Name: Chloral hydrate

Synonyms: Chloral monohydrate; trichloroacetaldehyde hydrate; trichloroacetaldehyde monohydrate; 1,1,1-trichloro-2,2-dihydroxyethane

1.1.2 Structural and molecular formulae and relative molecular mass



C_2HCl_3O Chloral Relative molecular mass: 147.39



$C_2H_3Cl_3O_2$ Chloral hydrate Relative molecular mass: 165.42

1.1.3 Chemical and physical properties of the pure substance

Chloral

(*a*) *Description*: Colourless, oily hygroscopic liquid with pungent, irritating odour (Budavari, 1989; EniChem America Inc., 1994)

(*b*) *Boiling-point*: 97.8 °C (Lide, 1993)

(*c*) *Melting-point*: –57.5 °C (Lide, 1993)

(*d*) *Density*: 1.51214 at 20 °C/4 °C (Lide, 1993)

(*e*) *Spectroscopy data*: Infrared (prism [4626], grating [36780]), ultraviolet [5-3], nuclear magnetic resonance [8241] and mass [814] spectral data have been reported (Sadtler Research Laboratories, 1980; Weast & Astle, 1985).

(*f*) *Solubility*: Soluble in water, carbon tetrachloride, chloroform, diethyl ether and ethanol (Lide, 1993; EniChem America, Inc., 1994)

(*g*) *Volatility*: Vapour pressure, 35 mm Hg [4.67 kPa] at 20 °C; relative vapour density (air = 1), 5.1 (Verschueren, 1983; EniChem America Inc., 1994)

(*h*) *Stability*: Polymerizes under the influence of light and in the presence of sulfuric acid, forming a white solid trimer called metachloral (Budavari, 1989)

(*i*) *Reactivity*: Forms chloral hydrate when dissolved in water and forms chloral alcoholate with alcohol (Budavari, 1989)

(*j*) *Conversion factor*: $mg/m^3 = 6.03 \times ppm$[1]

Chloral hydrate

(*a*) *Description*: Monoclinic plates from water with aromatic, penetrating and slightly acrid odour and slightly bitter, caustic taste (Budavari, 1989; Lide, 1993)

(*b*) *Boiling-point*: 98 °C (Budavari, 1989)

(*c*) *Melting-point*: 57 °C (Lide, 1993)

(*d*) *Density*: 1.9081 at 20 °C/4 °C (Lide, 1993)

(*e*) *Spectroscopy data*: Infrared (prism [158], grating [41020P]), nuclear magnetic resonance (proton [10362], C-13 [4005]) and mass [1054] spectral data have been reported (Sadtler Research Laboratories, 1980; Weast & Astle, 1985).

(*f*) *Solubility*: Soluble in water, acetone, benzene, chloroform, diethyl ether, ethanol and methyl ethyl ketone (Budavari, 1989; Lide, 1993)

(*g*) *Stability*: Slowly volatilizes on exposure to air (Budavari, 1989)

(*h*) *Octanol/water partition coefficient (P)*: log P, 0.99 (Hansch *et al.*, 1995)

(*i*) *Conversion* factor: $mg/m^3 = 6.76 \times ppm$[1]

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa)

1.1.4 Technical products and impurities

Chloral is available commercially at a purity of 94–99.5% and containing the following typical impurities (max.): water, 0.06%; 2,2-dichloroethanal, 0.3%; 2,2,3-trichlorobutanal, 0.01%; hydrogen chloride, 0.06%; chloroform (see IARC, 1987a), dichloroacetaldehyde and phosgene (Jira *et al.*, 1986; EniChem America Inc., 1994). The *United States Pharmacopeia* specifies that chloral hydrate for pharmaceutical use must contain 99.5–102.5% $C_2H_3Cl_3O_2$ (United States Pharmacopeial Convention, 1989); the *British Pharmacopoiea* specifies values of 98.5–101.0% (Medicines Commission, 1988).

Trade names for chloral have included: Grasex and Sporotal 100. Trade names for chloral hydrate have included: Aquachloral, Bi 3411, Dormal, EPA Pesticide Chemical Code 268100, Felsules, Hydral, Kessodrate, Lorinal, Noctec, Nycoton, Nycton, Phaldrone, Rectules, Somnos, Sontec, Tosyl and Trawotox.

1.1.5 Analysis

Gas chromatography (GC) can be used for quantitative analysis of chloral and its hydrate, which releases chloral on vaporization (Jira *et al.*, 1986). High-performance liquid chromatography has been used for the determination of nanogram amounts of aldehydes, including chloral, in air, water and other environmental samples. Chloral was separated as the 2,4-dinitrophenylhydrazone derivative using isocratic solvent elution and ultraviolet detection (Fung & Grosjean, 1981).

Determination of trichloroethylene metabolites, including chloral hydrate, in rat liver homogenate has been reported on the basis of selective thermal conversion of chloral hydrate into chloroform, which is determined by headspace GC and electron capture detection (Køppen *et al.*, 1988).

A multi-channel, microwave-induced plasma atomic spectroscopic GC detector has been used to characterize the profiles of chlorinated humic acid on capillary columns and the content of carbon, chlorine and bromine in drinking-water. This technique makes it possible to estimate the empirical formulae of separated compounds with sufficient accuracy for useful peak identification. Chloral was among the compounds characterized by this method (Italia & Uden, 1988).

Headspace analysis and GC–mass spectrometry were used to identify volatile organic substances in the presence of aggressive oxidants, including chloral in drinking-, natural, demineralized and wastewater (Pilipenko *et al.*, 1988).

A spectrophotometric method for the determination of chloral hydrate in drugs is based on the reaction of quinaldine ethyl iodide with chloral hydrate to produce a stable blue cyanine dye, with an absorption maximum at about 605 nm (Helrich, 1990).

Chloral hydrate has been determined by GC in biological materials using four columns with different packings. Elution of the compound was monitored with two flame ionization detectors. The limit of detection was about 0.01 mg per sample (Mishchikhin & Felitsyn, 1988).

The iodide ion produced by oxidation of chloral hydrate with iodine in chloroform solution was measured using an iodide ion-selective electrode, by either direct measurement, addition of a standard or potentiometric titration with silver nitrate solution (Zaki, 1985).

1.2 Production and use

1.2.1 Production

Chloral was synthesized by J. von Liebig in 1832 and introduced (as the hydrate) as the first hypnotic drug in 1869. It is made by chlorination of ethanol (Jira *et al.*, 1986) but has also been prepared by chlorination of a mixture of ethanol and acetaldehyde (see IARC, 1987b) (French patent 612 396, 1929), from chloral hydrate by azeotropic distillation (United States patent 2584 036, 1952) or from hypochlorous acid and trichloroethylene (see monograph, this volume) (United States patent 2 759 978, 1956) (Budavari, 1989). Chloral is also formed as a by-product of the oxychlorination of ethylene (IARC, 1994a) to produce vinyl chloride (see IARC, 1987c; Cowfer & Magistro, 1983).

Chloral is an intermediate in the production of the insecticide DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; see IARC, 1991a). After the discovery of DDT in 1939, the demand for chloral increased, and production reached a peak around 1963, when 40 000 tonnes were produced in the United States. When use of DDT was banned in the United States in 1972, and subsequently in many other countries, the demand rapidly declined. DDT is still produced in the United States for use in tropical countries. Use of chloral in the production of other pesticides was 1400 tonnes in 1972 (Jira *et al.*, 1986).

Chloral hydrate has been produced for use as a hypnotic drug in relatively low volume for many years. United States production for this purpose was about 135 tonnes in 1978 (Jira *et al.*, 1986).

Anhydrous chloral is produced by 11 companies in China and by one company each in Brazil, France, Germany, Italy, Japan, Mexico and the Russian Federation. Chloral hydrate is produced by two companies each in Brazil, Japan and Germany and by one company in Spain (Chemical Information Services, Inc., 1994).

Estimated production and use of chloral in the Member States of the European Union in 1984 was 2500 tonnes (Environmental Chemicals Data and Information Network, 1993).

1.2.2 Use

The principal use of chloral is in production of the insecticide DDT (Sax & Lewis, 1987). Much smaller amounts are used to make other insecticides, including methoxychlor (see IARC, 1987d), naled, trichlorfon (see IARC, 1987e) and dichlorvos (see IARC, 1991b). Chloral is also used as an intermediate in the production of the herbicide trichloroacetic acid (see monograph, this volume) and the hypnotic drugs chloral hydrate, chloral betaine, α-chloralose and triclofos sodium (Jira *et al.*, 1986). Chloroform was first prepared by treating chloral with alkali (DeShon, 1979). Chloral has also been used in the production of rigid polyurethane foam (see IARC, 1987f; Boitsov *et al.*, 1970) and to induce swelling of starch granules at room temperature (Whistler & Zysk, 1978).

Estimated use of chloral in the United States in 1975 was about 40% in the manufacture of DDT, about 10% in the manufacture of methoxychlor, dichlorvos and naled and about 50% in other applications (SRI International, 1975). Chloral hydrate is used as a sedative and hypnotic drug (Medicines Commission, 1988; Goodman Gilman *et al.*, 1991).

1.3 Occurrence

1.3.1 Natural occurrence

Chloral is not known to occur as a natural product.

1.3.2 Occupational exposures

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 2757 employees in the United States of America were potentially exposed to chloral (United States National Institute for Occupational Safety and Health, 1994). The estimate is based on a survey of companies and did not involve measurements of actual exposures.

Chloral has been detected in the work environment during spraying and casting of polyurethane foam (Boitsov *et al.*, 1970). It has also been identified as an autoxidation product of trichloroethylene during extraction of vegetable oil (McKinney *et al.*, 1955). It has been identified at the output of etching chambers in semiconductor processing (Ohlson, 1986).

1.3.3 Air

No data were available to the Working Group.

1.3.4 Water

Chloral is formed during aqueous chlorination of humic substances and amino acids (Miller & Uden, 1983; Sato *et al.*, 1985; Trehy *et al.*, 1986; Italia & Uden, 1988). It may therefore occur in drinking-water as a result of chlorine disinfection of raw waters containing natural organic substances (see IARC, 1991c). The concentrations of chloral measured in drinking-water in the United States are summarized in Table 1.

Table 1. Concentrations of chloral (as chloral hydrate) in drinking-water in the United States

Water type (location)	Concentration (μg/L)	Reference
Tap water (reservoir)	7.2–18.2	Uden & Miller (1983)
Surface, reservoir, lake and groundwater	1.7–3.0	Krasner *et al.* (1989)
Tap water	0.01–5.0	US Environmental Protection Agency (1988)
Distribution system	0.14–6.7	Koch & Krasner (1989)
Surface water	6.3–28	Jacangelo *et al.* (1989)

Chloral has also been detected in the spent chlorination liquor from bleaching of sulfite pulp after oxygen treatment, at concentrations of < 0.1–0.5 g/tonne of pulp (Carlberg *et al.*,

1986). It has been found in trace amounts from photocatalytic degradation of trichloroethylene in water (Glaze *et al.*, 1993).

1.3.5 Other

Chloral is a reactive intermediate metabolite of trichloroethylene (Cole *et al.*, 1975; Davidson & Beliles, 1991).

1.4 Regulations and guidelines

In most countries, no exposure limits have been recommended. A guideline limit of 5 mg/m^3 for short-term occupational exposure (ILO, 1991) and a tentative safe exposure limit of 0.01 mg/m^3 in ambient air have been set for chloral in the Russian Federation (Environmental Chemicals Data and Information Network, 1993).

The United States Environmental Protection Agency (1994) has proposed that the maximum level of chloral hydrate in drinking-water be 0.04 mg/L. WHO (1993) recommends a provisional guideline value of 10 µg/L for chloral hydrate.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals[1]

Chloral

No data were available to the Working Group.

Chloral hydrate

Mouse: Groups of 25 and 20 male B6C3F1 mice, aged 15 days, were treated by gavage with a single dose of 5 or 10 mg/kg bw chloral hydrate (USP purity) in distilled water. A control group of 35 mice received distilled water only (0.01 ml/g bw). In order to study acute effects on the liver, 6–10 mice from each group were killed 24 h after treatment [numbers per group not specified]. Six mice at the high dose, seven at the low dose and 15 controls were killed when moribund and examined before termination. At week 92, all surviving mice were killed. From week 48 onwards, 19 controls, nine mice at the low dose and eight at the high dose were available for histological examination. Hepatic nodules described as 'hyperplastic', 'adenomatous' or 'trabecular' were found in two control mice, 3/9 at the low dose and 6/8 at the high

[1] The Working Group was aware of studies in progress by oral administration to rats (IARC, 1994b).

dose [$p < 0.002$, Fisher's exact test] (Rijhsinghani *et al.*, 1986). [The Working Group noted the poor reporting of survival and the unusual histological terminology and that only single low doses were tested.]

A group of 40 male B6C3F1 mice, four weeks of age, received 1 g/L chloral hydrate (purity, > 95%; impurities unspecified) in distilled drinking-water (mean dose, 166 mg/kg bw per day) for 104 weeks; 33 controls received distilled water only. Five mice per group were killed after 30 weeks and another five after 60 weeks, for interim evaluation. Three control and six treated mice died before week 104. All mice were subjected to complete necropsy. Hepatocellular carcinomas were found in 2/5 mice killed at 60 weeks and in none of five controls. Of those killed at 104 weeks, 11/24 and 2/20 controls [$p = 0.01$ Fisher's exact test] had hepatocellular carcinomas, 7/24 treated mice and 1/20 controls [$p = 0.04$] had hepatocellular adenomas and 17/24 treated mice and 3/20 controls [$p = 0.001$] had carcinomas or adenomas. One treated mouse had a hyperplastic liver nodule. The authors reported several non-neoplastic hepatic changes: 10/24 treated mice and 1/20 controls had hepatocellular necrosis and 19/24 treated mice and 1/20 controls had cytomegaly (Daniel *et al.*, 1992a).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

After oral administration, chloral hydrate is rapidly absorbed from the gastrointestinal tract. Its biotransformation to trichloroethanol must be rapid, since no parent compound could be detected in even the first samples taken 10 min after administration of 15 mg/kg bw to volunteers. Peak levels of trichloroethanol and trichloroethanol glucuronide were reached within 20–60 min after oral administration of aqueous solutions. The average half-life of trichloroethanol glucuronide was 6.7 h (Breimer *et al.*, 1974). The average plasma half-life for chloral hydrate metabolites was 8.2 h; the half-life of the third chloral hydrate metabolite, trichloroacetic acid, was about four days (Breimer *et al.*, 1974; Gorecki *et al.*, 1990), as it binds extensively to plasma proteins (Sellers & Koch-Weser, 1971). As < 50% of an administered dose of chloral hydrate was recovered as metabolites in urine, yet unknown biotransformation reactions may exist for chloral hydrate in humans (Müller *et al.*, 1974).

4.1.2 Experimental systems

In mammalian species, chloral hydrate is rapidly reduced to trichloroethanol, the metabolite that appears to be responsible for the hypnotic properties of the drug (Breimer, 1977). In dogs and horses, trichloroethanol is subsequently excreted with urine and bile as trichloroethanol glucuronide and in the urine after oxidation to trichloroacetic acid (Butler, 1948; Alexander, 1967). In rodents, a slightly different metabolic pattern is seen, as chloral hydrate is oxidized directly to trichloroacetic acid, and the oxidative pathway from trichloroethanol to trichloro-

acetate that is observed in humans seems to be absent (Daniel, 1963; Cabana & Gessner, 1970). A serum half-life of 0.2 h was reported for chloral hydrate in mice, but its rate of disappearance in dogs was two to seven times faster. Trichloroacetic acid formed by oxidation of chloral hydrate persisted in the serum of both mice and dogs (Butler, 1948; Cabana & Gessner, 1970; Breimer *et al.*, 1974).

4.1.3 Comparison of humans and animals

Chloral hydrate is biotransformed along similar pathways in humans and all animal species tested. Trichloroethanol and its glucuronide are rapidly eliminated with urine, whereas trichloroacetate persists in both humans and animals. There appear to be no major quantitative differences in the kinetics of the metabolites.

4.2 Toxic effects

4.2.1 Humans

The lethal dose of chloral hydrate in humans is about 10 g; however, a fatal outcome was reported after ingestion of 4 g, and recovery has been seen after a dose of 30 g. The toxic effects that have been described after overdosing with chloral hydrate include irritation of the mucous membranes in the alimentary tract, depression of respiration and induction of cardiac arrhythmia. Habitual use of chloral hydrate is reported to cause unspecified hepatic and renal damage (Goodman Gilman *et al.*, 1991).

4.2.2 Experimental systems

The oral LD_{50} of chloral hydrate in rats was 480 mg/kg bw (Goldenthal, 1971); those in mice were reported as 1442 mg/kg bw in males and 1265 mg/kg bw in females (Sanders *et al.*, 1982). The cause of death after administration of lethal doses of chloral hydrate appeared to be inhibition of respiration.

The subchronic toxicity of chloral hydrate has been studied in CD1 mice and Sprague-Dawley rats. Administration of chloral hydrate to mice by gavage at daily doses of 14.4 and 144 mg/kg bw for 14 consecutive days resulted in an increase in relative liver weight and a decrease in spleen size. No other changes were seen. Administration of chloral hydrate to mice in drinking-water for 90 days at concentrations of 0.07 and 0.7 mg/ml resulted in dose-related hepatomegaly and significant changes in serum enzymes indicative of hepatic toxicity. In male mice, increased relative liver weights were also seen. After chloral hydrate was administered for 90 days in drinking-water to male and female Sprague-Dawley rats at a concentration of 0.3, 0.6, 1.2 or 2.4 mg/ml, the animals receiving the highest dose showed significant decreases in food and water consumption and weight gain. Males also had an apparent increase in the incidence of focal hepatocellular necrosis and increased activities of serum enzymes. No liver damage was seen in female rats (Daniel *et al.*, 1992b).

Exposure of female CD1 mice to 100 ppm [603 mg/m^3] chloral for 6 h induced deep anaesthesia, which was fully reversible on cessation of exposure. Vacuolation of lung Clara cells, alveolar necrosis, desquamation of the bronchiolar epithelium and alveolar oedema were

observed. Cytochrome P450 enzyme activity was reduced, although the activities of ethoxycoumarin *O*-diethylase and glutathione *S*-transferase were unaffected (Odum *et al.*, 1992).

Metabolism of chloral hydrate by male B6C3F1 mouse liver microsomes resulted in increased amounts of lipid peroxidation products (malonaldehyde and formaldehyde); the reactions could be inhibited by α-tocopherol or menadione (Ni *et al.*, 1994).

4.3 Reproductive and prenatal effects

4.3.1 Humans

Little information is available on the possible adverse effects of chloral on human pregnancy. Chloral hydrate is known to cross the human placenta at term (Bernstine *et al.*, 1954), but its use during relatively few pregnancies did not cause a detectable increase in abnormal outcomes (Heinonen *et al.*, 1977). Some data suggest that prolonged administration of sedative doses of chloral hydrate to newborns increases the likelihood of hyperbilirubinaemia (Lambert *et al.*, 1990).

Low levels of chloral hydrate have been found in breast milk. Although breast-feeding infants may be sedated by chloral hydrate in breast milk, the peak concentration measured (about 8 μg/ml) was considerably lower than the clinically active dose (Bernstine *et al.*, 1956; Wilson, 1981).

4.3.2 Experimental systems

Administration of one to five times the human therapeutic dose of chloral hydrate to pregnant mice (21.3 and 204.8 mg/kg per day in drinking-water during gestation) did not increase the incidence of gross external malformations in the offspring and did not impair normal development of pups (Kallman *et al.*, 1984).

4.4 Genetic and related effects (see also Table 2 and Appendices 1 and 2)

4.4.1 Humans

No data were available to the Working Group.

4.4.2 Experimental systems

The results obtained with chloral hydrate in a collaborative European Union project on aneuploidy have been summarized (Adler, 1993; Natarajan, 1993; Parry, 1993).

(a) DNA binding

In a single study in mice *in vivo*, radioactively labelled chloral hydrate did not bind to liver DNA.

(b) Mutation and allied effects

Chloral hydrate did not induce mutation in most strains of *Salmonella typhimurium*, but did in two of four studies with *S. typhimurium* TA100 and in a single study with *S. typhimurium*

Table 2. Genetic and related effects of chloral hydrate

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	2500	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	500	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	1850	Leuschner & Leuschner (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2000	Ni *et al.* (1994)
SA4, *Salmonella typhimurium* TA104, reverse mutation	+	+	1000	Ni *et al.* (1994)
SA5, *Salmonella typhimurium* TA1535 reverse mutation	-	-	5000	Waskell (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	1850	Leuschner & Leuschner (1991)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	1850	Leuschner & Leuschner (1991)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	1850	Leuschner & Leuschner (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	5000	Waskell (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	5000	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	1850	Leuschner & Leuschner (1991)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	-	-	3300	Bronzetti *et al.* (1984)
ANG, *Aspergillus nidulans*, diploid strain 35×17, mitotic crossing-over	-	0	1650	Crebelli *et al.* (1985)
ANG, *Aspergillus nidulans*, diploid strain 30, mitotic crossing-over	-	0	6600	Käfer (1986)
ANG, *Aspergillus nidulans*, diploid strain NH, mitotic crossing-over	-	0	1000	Kappas (1989)
ANG, *Aspergillus nidulans*, diploid strain P1, mitotic crossing-over	-	0	990	Crebelli *et al.* (1991)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	-	(+)	2500	Bronzetti *et al.* (1984)
ANN, *Aspergillus nidulans*, diploid strain 35×17, haploids and nondisjunctional diploids	+	0	825	Crebelli *et al.* (1985)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ANN, *Aspergillus nidulans*, diploid strain 30 conidia, aneuploidy	+	0	825	Käfer (1986)
ANN, *Aspergillus nidulans*, haploid conidia, aneuploidy and polyploidy	+	0	1650	Käfer (1986)
ANN, *Aspergillus nidulans*, diploid strain NH, nondisjunctional mitotic segregants	+	0	450	Kappas (1989)
ANN, *Aspergillus nidulans*, diploid strain P1, nondisjunctional diploids and haploids	+	0	660	Crebelli *et al.* (1991)
ANN, *Aspergillus nidulans*, haploid strain 35, hyperploidy	+	0	2640	Crebelli *et al.* (1991)
SCN, *Saccharomyces cerevisiae*, meiotic recombination	?	0	3300	Sora & Agostini Carbone (1987)
SCN, *Saccharomyces cerevisiae*, disomy in meiosis	+	0	2500	Sora & Agostini Carbone (1987)
SCN, *Saccharomyces cerevisiae*, diploids in meiosis	+	0	3300	Sora & Agostini Carbone (1987)
SCN, *Saccharomyces cerevisiae* D61.M, mitotic chromosomal malsegregation	+	0	1000	Albertini (1990)
SCN, *Saccharomyces cerevisiae* diploid strain D6, monosomy	+	0	1000	Parry *et al.* (1990)
***Seedlings of hexaploid Chinese spring wheat, Neatby's strain, chromosomal loss and gain	-	0	5000	Sandhu *et al.* (1991)
DMM, *Drosophila melanogaster,* somatic mutation wing spot test	+		825	Zordan *et al.* (1994)
DIA, DNA–protein cross-links, rat liver nuclei *in vitro*	-	0	41 250	Keller & Heck (1988)
DIA, DNA single-strand breaks (alkaline unwinding), rat primary hepatocytes *in vitro*	-	0	1650	Chang *et al.* (1992)
MIA, Kinetochore-positive micronuclei, Chinese hamster C1-1 cells *in vitro*, with antikinetochore antibodies	+	0	165	Degrassi & Tanzarella (1988)
MIA, Kinetochore-negative micronuclei, Chinese hamster C1-1 cells *in vitro*, with antikinetochore antibodies	-	0	250	Degrassi & Tanzarella (1988)
MIA, Kinetochore-positive micronuclei, Chinese hamster LUC2 cells *in vitro*	+	0	400	Parry *et al.* (1990)
MIA, Kinetochore-positive micronuclei, Chinese hamster LUC2 cells *in vitro*	+	0	400	Lynch & Parry (1993)
MIA, Micronuclei, Chinese hamster V79 cells *in vitro*	+	0	316	Seelbach *et al.* (1993)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ICR, Inhibition of intercellular communication, B6C3F1 mouse hepatocytes *in vitro*	-	0	83	Klaunig *et al.* (1989)
ICR, Inhibition of intercellular communication, F344 rat hepatocytes *in vitro*	-	0	83	Klaunig *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster CHED cells *in vitro*	+	0	20	Furnus *et al.* (1990)
AIA, Aneuploidy, Chinese hamster CHED cells *in vitro*	+[c]	0	10	Furnus *et al.* (1990)
AIA, Aneuploidy, primary Chinese hamster embryonic cells *in vitro*	+[c]	0	250	Natarajan *et al.* (1993)
AIA, Aneuploidy (hypoploidy), Chinese hamster LUC2 p4 cells *in vitro*	+	0	250	Warr *et al.* (1993)
***, Tetraploidy and endoreduplication, Chinese hamster LUC2 p4 cells *in vitro*	+	0	500	Warr *et al.* (1993)
***, Apolar mitosis, *Haemanthus katherinae* endosperm *in vitro*	+	0	200	Molè-Bajer (1969)
***, Inhibition of spindle elongation, PtK2 rat kangaroo kidney epithelial cells *in vitro*	+	0	1000	Lee *et al.* (1987)
***, Inhibition of chromosome-to-pole movement, PtK2 rat kangaroo kidney epithelial cells *in vitro*	-	0	1000	Lee *et al.* (1987)
***, Breakdown of mitotic microtubuli, PtK2 rat kangaroo kidney epithelial cells *in vitro*	+	0	1000	Lee *et al.* (1987)
***, Multipolar mitotic spindles, Chinese hamster DON.Wg.3H cells *in vitro*	+	0	500	Parry *et al.* (1990)
***, Chromosomal dislocation from mitotic spindle, Chinese hamster DON.Wg.3H cells *in vitro*	+	0	500	Parry *et al.* (1990)
***, Lacking mitotic spindle, Chinese hamster DON.Wg.3H cells *in vitro*	+	0	250	Parry *et al.* (1990)
***, Metaphase defects, lacking mitotic spindle, Chinese hamster LUC1 cells *in vitro*	+	0	50	Parry *et al.* (1990)
***Multipolar mitotic spindles, Chinese hamster DON.Wg.3H cells *in vitro*	+	0	50	Warr *et al.* (1993)
***, Chromosomal dislocation from mitotic spindle, Chinese hamster DON.Wg.3H cells *in vitro*	+	0	500	Warr *et al.* (1993)
DIH, DNA single–strand breaks (alkaline unwinding), human lymphoblastoid CCRF-CEM cells *in vitro*	-	0	1650	Chang *et al.* (1992)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	0	54	Gu *et al.* (1981)
MIH, Micronucleus induction, isolated human lymphocytes *in vitro*	-	-	1500	Vian *et al.* (1995)
MIH, Micronucleus induction, human lymphocytes in whole blood *in vitro*	+	0	100	Migliore & Nieri (1991)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MIH, Micronucleus induction, human lymphocytes *in vitro*	(+)	0	100	Ferguson *et al.* (1993)
MIH, Micronucleus induction, human lymphocytes *in vitro*	+	-	100	Van Hummelen & Kirsch-Volders (1992)
MIH, Kinetochore-positive micronuclei, human diploid LEO fibroblasts *in vitro*	+	0	120	Bonatti *et al.* (1992)
***, Aneuploidy (double Y induction), human lymphocytes *in vitro*	+	0	250	Vagnarelli *et al.* (1990)
AIH, Aneuploidy (hyperdiploidy and hypoploidy), human lymphocytes *in vitro*	+	0	50	Sbrana *et al.* (1993)
AIH, Polyploidy, human lymphocytes *in vitro*	+	0	137	Sbrana *et al.* (1993)
***, C-Mitosis, human lymphocytes *in vitro*	+	0	75	Sbrana *et al.* (1993)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* D7 recovered from CD1 mouse lungs	(+)	0	500 po × 1	Bronzetti *et al.* (1984)
DVA, DNA single-strand breaks (alkaline unwinding), rat liver *in vivo*	+		300 po × 1	Nelson & Bull (1988)
DVA, DNA single-strand breaks (alkaline unwinding), mouse liver *in vivo*	+		100 po × 1	Nelson & Bull (1988)
DVA, DNA single-strand breaks (alkaline unwinding), male Fischer 344 rat liver *in vivo*	-		1650 po × 1	Chang *et al.* (1992)
DVA, DNA single-strand breaks (alkaline unwinding), male B6C3F$_1$ mouse liver *in vivo*	-		825 po × 1	Chang *et al.* (1992)
CGC, Chromosomal aberrations, (C57B1/Cne×C3H/Cne)F$_1$ mouse secondary spermatocytes (staminal gonia–pachytene treated)	+		82.7 ip × 1	Russo *et al.* (1984)
CGC, Chromosomal aberrations (translocations, breaks and fragments), (C57B1/Cne × C3H/Cne)F$_1$ mouse primary and secondary spermatocytes (from differentiating spermatogonia–pachytene stages treated)	-		413 ip × 1	Liang & Pacchierotti (1988)
CBA, Chromosomal aberrations, male and female (102/E1 × C3H/E1)F$_1$ mouse bone-marrow cells *in vivo*	-		600 ip × 1	Xu & Adler (1990)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	-		1000 po × 1	Leuschner & Leuschner (1991)
CGG, Chromosomal aberrations, BALB/c mouse spermatogonia treated, spermatogonia observed *in vivo*	-		83 ip × 1	Russo & Levis (1992a)
COE, Chromosomal aberrations, ICR mouse oocytes treated *in vivo*	-		600 ip × 1	Mailhes *et al.* (1993)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronuclei, male and female NMRI mice, bone-marrow erythrocytes *in vivo*	-		500 ip × 1	Leuschner & Leuschner (1991)
MVM, Micronuclei, mouse spermatids *in vivo* (preleptotene spermatocytes treated)	-		83 ip × 1	Russo & Levis (1992b)
MVM, Micronuclei, male BALB/c mouse bone marrow erythrocytes *in vivo*	+		83 ip × 1	Russo & Levis (1992a)
MVM, Micronuclei, BALB/c mouse early spermatids *in vivo* (diakinesis/metaphase I and metaphase II stages treated)	+		83 ip × 1	Russo & Levis (1992a)
MVM, Kinetochore-positive and -negative micronuclei, male BALB/c mouse bone-marrow erythrocytes *in vivo*	+		200 ip × 1	Russo *et al.* (1992)
MVM, Micronuclei, male (C57Bl/Nce × C3H/Cne)F_1 mouse bone–marrow erythrocytes *in vivo*	-		400 ip × 1	Leopardi *et al.* (1993)
MVM, Micronuclei, mouse spermatids *in vivo* (spermatogonial stem cells and preleptotene spermatocytes treated)	+		41 ip × 1	Allen *et al.* (1994)
AVA, Aneuploidy, (C57Bl/Nce × C3H/Cne)F_1 mouse secondary spermatocytes *in vivo*	+		82.7 ip × 1	Russo *et al.* (1984)
AVA, Aneuploidy (C57B1/Cne × C3H/Cne)F_1 mouse secondary spermatocytes (from differentiating spermatogonia–pachytene stages treated)	(+)		165 ip × 1	Liang & Pacchierotti (1988)
AVA, Aneuploidy (hypoploidy), ICR mouse oocytes *in vivo*	-[d]		200 ip × 1	Mailhes *et al.* (1988)
AVA, Polyploidy, male and female 102/E1 × C3H/E1)F_1 mouse bone-marrow cells *in vivo*	-		600 ip × 1	Xu & Adler (1990)
AVA, Aneuploidy, (102/E1 × C3H/E1)F_1 mouse secondary spermatocytes *in vivo*	+		200 ip × 1	Miller & Adler (1992)
AVA, Aneuploidy, male (C57Bl/Nce × C3H/Cne)F_1 mouse bone marrow *in vivo*	+[c]		400 ip × 1	Leopardi *et al.* (1993)
AVA, Aneuploidy, (C57Bl/Nce × C3H/Cne)F_1 mouse secondary spermatocytes *in vivo*	-		400 ip × 1	Leopardi *et al.* (1993)
AVA, Hypoploidy, ICR mouse oocytes *in vivo*	-[d]		600 ip × 1	Mailhes *et al.* (1993)
BVD, Binding to DNA, male B6C3F1 mouse liver *in vivo*	-		800 ip × 1	Keller & Heck (1988)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***, Gonosomal and autosomal univalents (C57B1/Cne × C3H/Cne)F_1 mouse primary spermatocytes (from differentiating spermatogonia–pachytene stages treated)	-		413 ip × 1	Liang & Pacchierotti (1988)
***, Porcine brain tubulin assembly inhibition *in vitro*	+	0	9900	Brunner *et al.* (1991)
***, Porcine brain tubulin disassembly inhibition *in vitro*	+	0	40	Brunner *et al.* (1991)
***, Bovine brain tubulin assembly inhibition *in vitro*	(+)	0	165	Wallin & Hartley-Asp (1993)
***, Centriole migration block, Chinese hamster cells clone 237 *in vitro*	+	0	1000	Alov & Lyubskii (1974)
Trichloroethanol				
***, λ Prophage induction, WP2 in *Escherichia coli*	-	-	155 000	DeMarini *et al.* (1994)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	0.5 vapour	DeMarini *et al.* (1994)

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; -, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study); 0, not tested

[b] LED, lowest effective dose; HID, highest effective dose. In-vitro tests, μg/ml; in-vivo tests, mg/kg bw; ip, intraperitoneally; po, orally

[c] Negative for induction of polyploidy

[d] Slight induction of hypoploid cells may have been due to technical artefacts.

***, Not included on profile

TA104. The latter response was inhibited by the free-radical scavangers α-tocopherol and menadione (Ni *et al.*, 1994).

Chloral hydrate did not induce mitotic crossing over in *Aspergillus nidulans* in the absence of metabolic activation, but weak induction of meiotic recombination in the presence of metabolic activation and of gene conversion in the absence of metabolic activation were seen in *Saccharomyces cerevisiae*. It did not induce reverse mutation in *S. cerevisiae* in one study. Chloral hydrate clearly induced aneuploidy in various fungi in the absence of metabolic activation. The results of a single study in Chinese spring wheat were inconclusive with respect to induction of chromosome loss and gain.

Chloral hydrate induced somatic mutations in *Drosophila melanogaster* in a wing-spot test.

In single studies, chloral hydrate did not produce DNA–protein cross-links in rat liver nuclei or DNA single-strand breaks/alkaline-labile sites in rat primary hepatocytes *in vitro*. It increased the frequency of micronuclei in Chinese hamster cell lines. Although a single study suggested that chloral hydrate induces chromosomal aberrations in Chinese hamster CHED cells *in vitro*, the micronuclei produced probably contained whole chromosomes and not chromosome fragments, as the micronuclei could all be labelled with antikinetochore antibodies. In a study of rat kangaroo kidney epithelial cells, chloral hydrate inhibited spindle elongation and broke down mitotic microtubuli, although it did not inhibit pole-to-pole movement of chromosomes. It produced multipolar spindles, chromosomal dislocation from the mitotic spindle and a total lack of mitotic spindles in Chinese hamster Don.Wg.3H cells. It did not inhibit cell-to-cell communication in mouse or rat hepatocytes *in vitro*.

In a single study, chloral hydrate weakly induced sister chromatid exchange in cultured human lymphocytes. It induced micronuclei, aneuploidy, C-mitosis and polyploidy in human cells *in vitro*. In human diploid fibroblasts, the micronuclei contained kinetochores. Micronuclei were induced in studies with human whole blood cultures but not in one study with isolated lymphocytes. The differences seen in the micronucleus test have been attributed to differences between whole blood and purified lymphocyte cultures (Vian *et al.*, 1995), but this hypothesis has not been tested.

Chloral hydrate increased the rate of mitotic gene conversion in a host-mediated assay with *S. cerevisiae* recovered from mouse lungs. One study showed induction of single-strand breaks in liver DNA of both rats and mice treated *in vivo*; another study in both species found no such effect. The frequency of chromosomal aberrations in mouse bone marrow, spermatogonia, primary and secondary spermatocytes and oocytes was not increased in single studies after treatment with chloral hydrate *in vivo*. In one study, it induced chromosomal aberrations in mouse secondary spermatocytes after treatment of animals *in vivo*. Micronuclei were induced in mouse bone-marrow erythrocytes in two of four studies after treatment with chloral hydrate *in vivo*; in one of these studies, the use of antikinetochore antibodies suggested induction of micronuclei containing both whole chromosomes and fragments. Chloral hydrate induced micronuclei in the spermatids of mice treated *in vivo* in two studies but not in a third in which the stage of spermatogenesis studied, the premeiotic S-phase (preleptotene), was concluded to be sensitive only to clastogenic agents. In one of the studies that showed an effect, only kinetochore-negative micronuclei were induced, but kinetochore-negative micronuclei were also produced by another established aneuploidogen, vincristine sulfate. The finding may therefore

suggest not induction of fragments harbouring micronuclei but an inability of the antibody to label kinetochores in the micronuclei. Chloral hydrate induced aneuploidy in the bone marrow of mice treated *in vivo* in one study. It increased the rate of aneuploidy in mouse secondary spermatocytes in three of four studies, and one study also suggested increased hypodiploidy in mouse oocytes. It did not produce polyploidy in bone marrow or oocytes or gonosomal or autosomal univalents in primary spermatocytes of mice treated *in vivo*.

Trichloroethanol, a reduction product of chloral hydrate, did not induce λ prophage in *E. coli* or mutation in *S. typhimurium* TA100.

5. Summary and Evaluation

5.1 Exposure data

Chloral has been produced commercially since the 1940s by chlorination of ethanol. Until the early 1970s, its major use was in the production of the insecticide DDT. Chloral is also used as an intermediate in the production of the insecticides methoxychlor, naled, trichlorfon and dichlorvos, the herbicide trichloroacetic acid and the hypnotic drugs chloral hydrate, chloral betaine, α-chloralose and triclofos sodium.

Human exposure to chloral (or its hydrate) can occur during its production and use, from drinking chlorinated water and from pharmaceutical use.

Chloral is rapidly converted to its hydrate in contact with aqueous solutions.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Chloral hydrate was tested for carcinogenicity in one adequate study in male mice by oral administration. It increased the incidence of hepatocellular adenomas and carcinomas.

5.4 Other relevant data

Chloral hydrate is metabolized rapidly in both humans and experimental animals to trichloroethanol and trichloroacetate. Its main acute toxic effects in humans are inhibition of respiration and induction of cardiac arrhythmia. Repeated administration of chloral hydrate damages the liver in mice and in male rats. Exposure of mice by inhalation results in damage to Clara cells in the lung.

Chloral hydrate crosses the human placenta, but there have been no reports of adverse results other than an increased likelihood of hyperbilirubinaemia in infants. No malformations and no effect on development were observed in the offspring of mice administered chloral throughout gestation.

Chloral hydrate is a well-established aneuploidogenic agent. It clearly induced aneuploidy and micronuclei in mammals treated *in vivo*, whereas chromosomal aberrations were not found in most studies. Conflicting results were obtained with regard to the induction of DNA damage in mammals treated with chloral hydrate *in vivo*.

Chloral hydrate induced aneuploidy and micronuclei in cultured human cells *in vitro*, but the results with regard to the induction of sister chromatid exchange were inconclusive. In rodent cells *in vitro*, chloral hydrate increased the induction of micronuclei but did not induce DNA damage; chromosomal aberrations were induced in a single study *in vitro*. In fungi, chloral hydrate clearly induced aneuploidy, while the results of studies on mitotic recombination and gene conversion were inconclusive. A single study showed induction of somatic mutation by chloral hydrate in insects. The results of assays for mutagenicity in bacteria were inconsistent.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of chloral and chloral hydrate.

There is *inadequate evidence* in experimental animals for the carcinogenicity of chloral.

There is *limited evidence* in experimental animals for the carcinogenicity of chloral hydrate.

Overall evaluation

Chloral and chloral hydrate are *not classifiable as to their carcinogenicity to humans (Group 3).*

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[1]For definition of the italicized terms, see Preamble, pp. 22–26.

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