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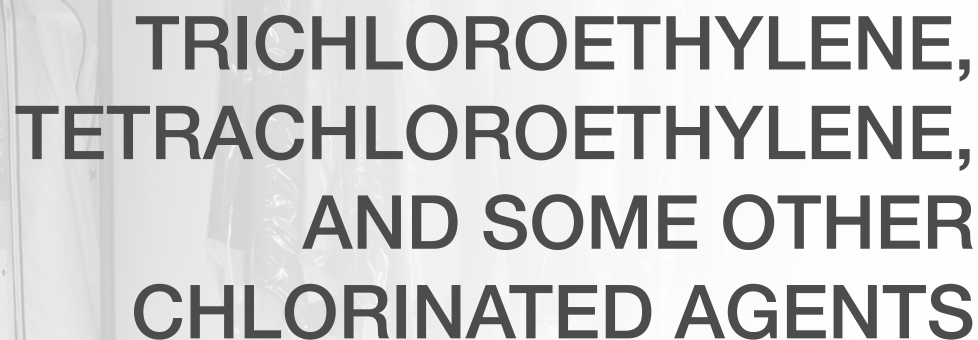

TRICHLOROETHYLENE, TETRACHLOROETHYLENE, AND SOME OTHER CHLORINATED AGENTS

VOLUME 106

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International Agency for Research on Cancer

World Health Organization

CHLORAL AND CHLORAL HYDRATE

Chloral and chloral hydrate were considered by previous IARC Working Groups in 1995 and 2004 (IARC, 1995, 2004). New data have since become available and these have been taken into consideration in the present evaluation. Chloral and chloral hydrate are considered together since the two substances exist in equilibrium in aqueous solution.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

(a) Chloral

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

Chem. Abstr. Serv. Name: Trichloroacetaldehyde

IUPAC Systematic Name: Chloral

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

(b) Chloral hydrate

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

Deleted Chem. Abstr. Serv. Reg. No.: 109128-19-0

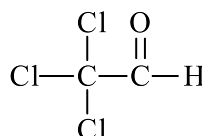
Chem. Abstr. Serv. Name: 2,2,2-Trichloro-1,1-ethanediol

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

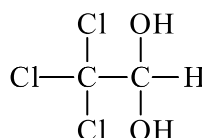
(a) Chloral



C_2HCl_3O

Relative molecular mass: 147.39

(b) Chloral hydrate



$C_2H_3Cl_3O_2$

Relative molecular mass: 165.40

1.1.3 Chemical and physical properties of the pure substances

(a) Chloral

Description: Oily liquid. Pungent, irritating odour (O'Neil *et al.*, 2006)

Boiling-point: 97.8 °C (O'Neil *et al.*, 2006)

Melting-point: –57.5 °C (O'Neil *et al.*, 2006)

Density: 1.510 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism [4626, 4426]), ultraviolet [5–3], nuclear magnetic resonance [8241] and mass [814] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)

Solubility: Freely soluble in water, in which it is converted to chloral hydrate; soluble in diethyl ether and ethanol (O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 10 kPa at 33.8 °C (Haynes, 2012)

Stability: Polymerizes under the influence of light and in presence of sulfuric acid to form a white solid trimer called metachloral (O'Neil *et al.*, 2006)

Conversion factor: mg/m^3 = 6.03 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa).

(b) Chloral hydrate

Description: Colourless, transparent, or white crystals with aromatic, penetrating and slightly acrid odour, slightly bitter, caustic taste. (O'Neil *et al.*, 2006)

Boiling-point: 96 °C, decomposes into chloral and water (Haynes, 2012)

Melting-point: 57 °C (O'Neil *et al.*, 2006)

Density: 1.9081 at 20 °C/4 °C (Haynes, 2012)

Spectroscopy data: Infrared (prism [5423]), nuclear magnetic resonance [10 362] and mass [1054] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)

Solubility: Very soluble in water, olive oil. Freely soluble in acetone, methyl ethyl ketone (O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 4.7 kPa at 20 °C; slowly evaporates on exposure to air (Jira *et al.*, 1986; O'Neil *et al.*, 2006)

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

Conversion factor: mg/m^3 = 6.76 × ppm, 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

Technical-grade chloral ranges in purity from 94% to 99% by weight, with water being the main impurity. Other impurities can include chloroform, hydrogen chloride, dichloroacetaldehyde and phosgene (Jira *et al.*, 1986).

Trade names for chloral include Grasex and Sporotal 100.

Trade names for chloral hydrate include: Ansopal, Aquachloral, Chloradorm, Chloraldurat, Chloralix, Dormel, Elix-nocte, Escre, Hydral, Lanchloral, Lorinal, Medianox, Nervifene, Noctec, Novochlorhydrate, Nycton, Phaldrone, Rectules, Somnos, Suppojuvent Sedante, Tosyl, Trawotox and Welldorm.

The United States Pharmacopeia (USP) specifies that USP-grade chloral hydrate must contain not less than 99.5% chloral hydrate (US Pharmacopeial Convention, 2012). Chloral hydrate is available as a liquid-filled capsule containing 500 mg of chloral hydrate and as a syrup containing 500 mg/5 mL (PDR Network, 2012).

Table 1.1 Methods for the analysis of chloral hydrate in water

Sample preparation	Assay procedure	Limit of detection	Reference
Extract with methyl-*t*-butyl ether or pentane	GC/ECD	0.002 µg/L	EPA (1995)
Extract with ethyl acetate at pH 3.2	GC/MS	0.006 µg/L	Serrano *et al.* (2011)

ECD, electron capture detection; GC, gas chromatography; MS, mass spectrometry

1.1.5 Analysis

Methods for the analysis of chloral hydrate have been reviewed by Delinsky *et al.* (2005) and Demeestere *et al.* (2007). Selected methods for the analysis of chloral hydrate in water are identified in Table 1.1. A biomonitoring method using gas chromatography-electron capture detection has been developed for measuring chloral hydrate in blood (Schmitt, 2002) and in urine (Garrett & Lambert, 1966).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Chloral was first synthesized by J. von Liebig in 1832 by chlorination of ethanol (Jira *et al.*, 2007).

Chloral is produced by chlorinating acetaldehyde or ethanol in acidic solution by gradually increasing the temperature from 0 °C to 90 °C (Jira *et al.*, 2007). Antimony trichloride is sometimes used as a catalyst. Chloral is distilled from the reaction mixture as the hydrate. The hydrate is then mixed with concentrated sulfuric acid, the heavier acid layer is drawn off, and chloral is distilled through a fractionating column of moderate height.

(b) Production volume

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).

Chloral (anhydrous) is known to have been produced by 14 companies in China, seven companies in India and one company each in Brazil, France, Japan, Mexico, the Russian Federation and the USA. Chloral hydrate was produced by four companies in China, three companies in Germany, two companies in Japan and one company each in Mexico, the Russian Federation and Spain (Chemical Information Services, 2002).

Chloral hydrate has been produced for use as a hypnotic drug in relatively low and gradually declining volume for many years. As an indication of the scale, production in the USA for this purpose was about 135 tonnes in 1978 (Jira *et al.*, 1986).

1.2.2 Use

(a) Chloral

The principal historical use of chloral has been in the production of the insecticide dichlorodiphenyltrichloroethane (DDT) and, to a lesser extent, other insecticides such as methoxychlor, naled, trichlorfon, and dichlorvos and the herbicide trichloroacetic acid (Jira *et al.*, 2007). In the USA in 1975, about 40% of chloral was used in the manufacture of DDT, about 10% in the manufacture of other pesticides and about 50% in other applications (IARC, 1995). After the banning of DDT in many countries, demand for chloral for this use has declined dramatically.

Chloral has also been used in the production of rigid polyurethane foam (IARC 1995; Boĭtsov *et al.*, 1970) and to induce swelling of starch

granules at room temperature (Whistler & Zysk, 1978).

(b) Chloral hydrate

Chloral hydrate has been used as a hypnotic drug since the 1870s, principally for the short-term treatment of insomnia. It was also used to allay anxiety and to induce sedation and/or sleep post-operatively, before electroencephalogram evaluations, and to treat the symptoms of withdrawal of alcohol and other drugs such as opiates and barbiturates. For many years chloral hydrate was widely used for the sedation of children before diagnostic, dental or medical procedures, but although still in use, it has largely been replaced by newer drugs with a lower risk of overdose (Pershad *et al.*, 1999).

After oral administration, chloral hydrate is converted rapidly to trichloroethanol, which is largely responsible for its hypnotic action. Externally, chloral hydrate has a rubefacient action (producing redness of the skin) and has been used as a counter-irritant. It is administered by mouth as a liquid or as gelatin capsules. It has also been dissolved in a bland fixed oil and given by enema or as suppositories (Gennaro, 2000; Royal Pharmaceutical Society of Great Britain, 2002).

Chloral hydrate is also an ingredient in Hoyer's solution, which is used in microscopy to mount organisms such as bryophytes, ferns, seeds, and arthropods (Anderson, 1954).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Chloral and chloral hydrate are not known to occur as natural products.

1.3.2 Environmental occurrence

(a) Air

No data are available on human exposure to chloral or chloral hydrate in air. The low volatility of chloral hydrate from a water solution precludes significant exposure by inhalation (EPA, 2000).

(b) Water

When raw water, containing natural organic material such as humic, tannic or amino acids, is treated by chlorination for use as drinking-water or in swimming pools, by-products resulting from disinfection can be formed, such as chloral, which is rapidly transformed into chloral hydrate (Miller & Uden, 1983; Sato *et al.*, 1985; Trehy *et al.*, 1986; Italia & Uden, 1988). Chloral hydrate is primarily produced by the treatment of water with chlorine or chloramine, but the use of pre-ozonation before chlorination or chloramination can favour its formation (Richardson *et al.*, 2007).

Table 1.2 summarizes recent measurements of chloral hydrate in drinking-water and swimming pool water in several countries.

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 11 278 employees in the USA were potentially exposed to chloral hydrate (NIOSH, 1994). The estimate is based on a survey of companies and did not involve measurement of actual exposures (IARC, 2004).

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

Table 1.2 Concentrations of chloral hydrate in drinking-water

Country	Location	Concentration (μg/L)		Reference
		Mean	Range	
Drinking-water				
Australia	Seven cities	NR	0.2–19	Simpson & Hayes (1998)
Canada	Country-wide (summer 1993)	6.1	< 0.1–18.9	Koudjonou *et al.* (2008)
		3.6	0.3–13.6	
		8.4	0.2–23.4	
China	Beijing	0.93	NR–10.44	Wei *et al.* (2010)
Greece	Athens	NR	0.2–12.5	Golfinopoulos & Nikolaou (2005)
	Mytilene	NR	NR–0.5	Leivadara *et al.* (2008)
Spain	Eleven provinces	< 1[a]	< 1–12.1	Villanueva *et al.* (2012)
	Cordoba	NR	1.2–38	Serrano *et al.* (2011)
Swimming-pool water				
Republic of Korea	Seoul	16.9	5.1–34.9	Lee *et al.* (2010)
		3.6	ND–10.4	
		10.2	ND–23.4	
Spain	Cordoba	NR	53–340	Serrano *et al.* (2011)

[a] Median
ND, not detected; NR, not reported

In spite of the use of chloral as an intermediate in the synthesis of insecticides and herbicides, no specific measurement data from workers exposed during synthesis or formulation were available to the Working Group. Given the use of chloral hydrate as a sedative and hypnotic drug, workers could be exposed in the pharmaceutical industry during production. However no data on exposure measurement were identified by the Working Group.

1.3.4 Exposure in the general population

Chloral is an intermediate metabolite of trichloroethylene in humans, and chloral hydrate has been found in the plasma of people who have undergone anaesthesia with trichloroethylene (Cole *et al.*, 1975; Davidson & Beliles, 1991).

1.4 Regulations and guidelines

Chloral hydrate is a controlled substance in Canada and the USA, available by prescription only. Chloral hydrate is banned for marketing in India. There are no occupational limits for chloral or chloral hydrate.

2. Cancer in Humans

Chloral hydrate is a chemical that occurs in drinking-water and swimming pools as part of a mixture of by-products resulting from disinfection of drinking-water by chlorination. The chemicals in water-disinfection by-products do not occur in an isolated manner and there is no epidemiological evidence on risk of cancer associated specifically with these by-products. A detailed description of water-disinfection by-products and cancer risk is given in *IARC Monograph* Volume 101 (IARC, 2012a).

Only one epidemiological study has examined risk of cancer in humans exposed to chloral hydrate. Haselkorn *et al.* reported cancer morbidity among 2290 users of chloral hydrate within a cohort of 143 574 patients at Kaiser Permanente, USA, who had prescriptions filled

for the 215 most commonly used drugs between 1969 and 1973 (Haselkorn *et al.*, 2006). Study subjects in this cohort comprised an ethnically and socioeconomically diverse population, who had received both inpatient and outpatient care within the prepaid system. Cohort members were linked to the pharmacy records by each patient's unique medical record number. Cancer occurrence (1976–1998) for each study subject was ascertained via the local tumour registry and hospital records of all northern California hospitals in the Kaiser Permanente programme. Cancers diagnosed were verified by the review of medical records by a trained medical analyst. Expected numbers of overall and specific cancer were calculated based on age and sex standardized rates from the entire Kaiser Permanente cohort. By 1998, there were 285 cancer cases observed versus 258 expected [standardized incidence ratio, SIR, 1.1; 95% CI, 0.98–1.24]. Further, observed and expected numbers for cancers of mouth floor [SIR, 5.0; 95% CI, 1.03–14.6], stomach [SIR, 2.1; 95% CI, 1.2–3.3], lung [SIR, 1.3; 95% CI, 0.9–1.7], skin melanoma [SIR, 1.4; 95% CI, 0.7–2.4], prostate [SIR, 1.0; 95% CI, 0.7–1.4] and kidney [SIR, 0.2; 95% CI, 0.05–1.1] are reported. Relations between numbers of prescriptions of chloral hydrate and overall cancer, cancer of lung, stomach, prostate and melanomas were examined in a series of nested case–control studies, adjusted for potential confounders. No significant trends were observed. [The Working Group noted the relatively low power of this study to detect statistically significant deviations from unity for specific cancers].

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

Three groups of 20–35 male C57BL × C3HF$_1$ mice (age, 15 days) were given chloral hydrate in water intragastrically as a single dose at 0, 5, or 10 mg/kg body weight (bw). The mice were killed at various time intervals up to 92 weeks. Liver nodules were examined by histopathology and included hyperplastic nodules, hepatocellular adenoma and hepatocellular carcinoma [termed trabecular]. In mice killed between 48 and 92 weeks after dosing, a statistically significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) was observed in mice receiving chloral hydrate at a dose of 10 mg/kg bw (6 out of 8 versus 2 out of19 controls) (Rijhsinghani *et al.* (1986)). [The Working Group noted the small number of mice evaluated in this study and the low doses administered.]

The hepatocarcinogenicity of chloral hydrate was studied in two groups of 33–40 male B6C3F$_1$ mice given drinking-water containing chloral hydrate at a dose of 0 or 1 g/L (ingested dose, 166 mg/kg bw per day) for 104 weeks (Daniel *et al.*, 1992). Five animals per group were killed after 30 and 60 weeks of exposure. Microscopic examinations were performed on all gross lesions, liver, kidney, testis and spleen of mice surviving 104 weeks. Statistically significant increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were observed in treated mice surviving 104 weeks. Results are shown in Table 3.1. [The Working Group noted that a single dose was used, the group size was small, histopathological evaluation was limited to mice surviving at least 104 weeks, and the histopathological examinations were limited to the liver.]

The initial study by Daniel *et al.* (1992) was followed up with a study in groups of 72 male B6C3F$_1$ mice given repeated doses of chloral hydrate for 104 weeks (George *et al.*, 2000).

Table 3.1 Studies of carcinogenicity in experimental animals given chloral hydrate by oral administration

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C57BL×C3HF$_1$ (M) Up to 92 wk Rijhsinghani *et al.* (1986)	Single dose, by gavage at 0, 5, 10 mg/kg bw, to mice aged 15 days 35, 25, and 20/group	Hepatic nodules [hepatocellular adenoma or carcinoma (combined)]: 2/19, 2/9, 6/8*	*P < 0.05	Laboratory grade Small numbers of mice and low dose
Mouse, B6C3F$_1$ (M) 104 wk Daniel *et al.* (1992)	0, 1 g/L, in drinking-water 33, 40/group Interim kills of 10/group	Hepatocellular adenoma: 1/20, 7/24* Hepatocellular carcinoma: 2/20, 11/24** Hepatocellular adenoma or carcinoma (combined): 3/20, 17/24***	Fisher's exact test *P ≤ 0.05 **P ≤ 0.03 ***P ≤ 0.01	Purity, > 95% Histopathological data only for the liver
Mouse, B6C3F$_1$ (M) 104 wk George *et al.* (2000)	0, 0.12, 0.58, 1.28 g/L, in drinking-water 72/group	Prevalence in mice surviving > 78 wk: Hepatocellular adenoma: 21.4 (*n* = 42), 43.5 (*n* = 46)*, 51.3 (*n* = 39)*, 50.0% (*n* = 32)* Hepatocellular carcinoma: 54.8 (*n* = 42), 54.3 (*n* = 46), 59.0 (*n* = 39), 84.4% (*n* = 32)* Hepatocellular adenoma or carcinoma (combined): 64.3 (*n* = 42), 78.3 (*n* = 46), 79.5 (*n* = 39)*, 90.6% (*n* = 32)*	*P ≤ 0.05	Purity, > 99% Measured concentrations; histopathological data only for the liver; neoplasms observed in the kidney, spleen and testis reported not to exceed the incidences in control group or historical controls.
Mouse, B6C3F$_1$ (M) 12 or 20 months Von Tungeln *et al.* (2002)	*12 months* Two i.p. doses at age 8 and 15 days; total dose, 0 (DMSO control) or 2000 nmol per mouse 24, 24/group	*12 months* Hepatocellular adenoma: 1/24, 5/24	NS	Purity, NR Small numbers of mice; low total dose; histopathology restricted to the liver. Females were also injected with chloral hydrate and no hepatocellular tumours were observed.
	20 months Two i.p. doses at age 8 and 15 days; total dose, 0 (DMSO control) or 1000 nmol per mouse 23, 23/group	*20 months* Hepatocellular adenoma: 6/23, 9/23 Hepatocellular carcinoma: 2/23, 2/23 Hepatocellular adenoma or carcinoma (combined): 7/23, 10/23	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 104 wk NTP (2002a), Leakey *et al.* (2003)	Gavage 0, 25, 50, 100 mg/kg bw 5 days/wk by gavage to *ad libitum*-fed or dietary controlled mice. 48/group	*Fed* ad libitum Hepatocellular carcinoma: 4/48, 10/48, 10/47, 7/48 Hepatocellular adenoma or carcinoma (combined): 16/48, 25/48*, 23/47, 22/48 *Dietary controlled* Hepatocellular carcinoma: 2/48**, 5/48, 4/48, 8/48*** Hepatocellular adenoma or carcinoma (combined): 11/48****, 11/48, 14/48, 18/48	Poly-3 test *P = 0.0437 **P = 0.0371 (trend) ***P = 0.0422 ****P = 0.045 (trend)	Purity, > 99.5% No treatment-related reduction in survival
Mouse, B6C3F$_1$ (F) 104 wk NTP (2002b)	*Regimen A* Gavage; 0, 25, 50, 100 mg/kg bw, 5 days/wk 48/group	Pituitary gland (pars distalis) adenoma: 0/45*, 2/44, 0/47, 5/41** Malignant lymphoma: 9/48***, 7/48, 8/48, 15/48	Poly-3 test *P = 0.073 (trend) **P = 0.024 ***P = 0.0455 (trend)	Purity, 99% No exposure-related reduction in survival.
Rat, F344/N (M) 104 wk George *et al.* (2000)	0, 0.12, 0.58, 2.51 g/L, in drinking-water 78/group	Prevalence in rats surviving > 78 wk: Hepatocellular adenoma: 0 (n = 42), 7.1 (n = 44), 2.3 (n = 44), 4.5% (n = 42) Hepatocellular carcinoma: 2.4 (n = 42), 7.1 (n = 44), 0 (n = 44), 2.3% (n = 42) Hepatocellular adenoma or carcinoma (combined): 2.4 (n = 42), 14.3 (n = 44), 2.3 (n = 44), 6.8% (n = 42)	NS	Purity, > 99% Measured concentrations; histopathological data only for the liver; neoplasms observed in the kidney, spleen and testes reported not to exceed the incidences in control group or historical controls.

bw, body weight; F, female; i.p., intraperitoneal; M, male; mo, month; NR, not reported; NS, not significant; wk, week

Measured concentrations of chloral hydrate in the drinking-water were 0, 0.12, 0.58, and 1.28 g/L, corresponding to mean daily doses of 0, 13.5, 65, and 146.6 mg/kg bw. Six mice per group were killed after 26, 52, and 78 weeks. Histopathological examinations of the liver, kidney, spleen and testis were performed on mice surviving more than 78 weeks. Hepatocellular adenomas and carcinomas were observed as early as 52 weeks and incidence increased progressively with duration of treatment. In mice surviving more than 78 weeks, the incidence of hepatocellular adenoma was increased ($P \leq 0.05$) in all dose groups, the incidence of hepatocellular carcinoma was increased in the group at 1.28 g/L, and the incidence of hepatocellular adenoma or carcinoma (combined) was increased in the groups at 0.58 and 1.28 g/L. The incidences of neoplasms observed in the kidney, spleen, and testes were reported not to exceed those in the control group or in historical controls (data not shown). [The Working Group noted that the evaluations of tumour incidence were limited to the four organs examined microscopically.]

Groups of 24 male and 21 female neonatal B6C3F$_1$ mice were given intraperitoneal injections of chloral hydrate in dimethyl sulfoxide (DMSO) on postnatal day 8 (three sevenths of the total dose) and day 15 (four sevenths of the total dose) (Von Tungeln *et al*., 2002). The total dose administered was 2000 nmol [0.33 mg] per mouse, and the mice were observed for 12 months. In a second experiment, groups of 23 males and 22 females were given intraperitoneal injections of chloral hydrate in DMSO on postnatal day 8 (one third of the total dose) and day 15 (the remaining two thirds of the total dose). In this experiment, the total dose administered was 1000 nmol [0.165 mg] per mouse and the groups were observed for 20 months. Control groups of 23–24 mice were injected with the vehicle (DMSO) only. Histopathological examination was restricted to the liver. In males, a non-statistically significant increase in the incidence of hepatocellular adenoma was observed after 12 months and 20 months (5 out of 24 versus 1 out of 24, and 9 out of 23 versus 6 out of 23, respectively). No hepatocellular tumours were observed in the control or treated groups of female mice. [The Working Group noted that only the liver was examined microscopically and that the total doses administered were much lower than those used in other long-term studies of this chemical.]

The National Toxicology Program performed two studies of the carcinogenic effects of chloral hydrate in B6C3F$_1$ mice (NTP, 2002a, b). The first study (NTP, 2002a; Leakey *et al*., 2003) was an investigation of the influence of dietary restriction on the development of hepatocellular adenoma and hepatocellular carcinoma. Groups of 48 male B6C3F$_1$ mice were given chloral hydrate (dissolved in distilled water) by gavage at a dose of 0, 25, 50, or 100 mg/kg bw, 5 days per week for 104 weeks. The incidences of hepatocellular adenoma and hepatocellular carcinoma were much lower in mice with a controlled diet than in mice fed ad libitum. There was a statistically significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) in mice fed ad libitum at 25 mg/kg bw, but not at 50 or 100 mg/kg bw. There was a statistically significant increase in the incidence of hepatocellular carcinoma in mice with a controlled diet containing chloral hydrate at 100 mg/kg bw (Table 3.1). [The Working Group noted that the mice in this study were dosed for 5 days per week, while the doses in studies by Daniel *et al*. (1992) and George *et al*. (2000) were higher and were administered 7 days per week.]

In the second study (NTP, 2002b), female B6C3F$_1$ mice were given chloral hydrate (dissolved in distilled water) by gavage in two different regimens. In regimen A, groups of 48 mice were given chloral hydrate at a dose of 0, 25, 50, or 100 mg/kg bw, 5 days per week for 104 weeks (24 months). In regimen B, groups of 48 mice were given chloral hydrate at a dose of 0 (n = 24) or 100 mg/kg bw for 3, 6 or 12 months. Eight mice each from the

groups at 0 and 100 mg/kg bw were killed at 3, 6, or 12 months. The remaining mice were held until termination of the study at 24 months. For regimen A, an increasing trend in incidence of adenoma of the pituitary gland (pars distalis) in the group at the highest dose was observed in mice treated for 24 months. A statistically significant increasing trend in incidence of malignant lymphoma was also observed, but the incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatocellular adenoma or carcinoma (combined) were not increased. For regimen B, tumour incidences were not increased in female mice exposed to chloral hydrate for 3, 6, or 12 months. [The Working Group noted that mice in this study were dosed for 5 days per week, while the studies by Daniel *et al.* (1992) and George *et al.* (2000) used higher doses that were administered 7 days per week.]

3.2 Rat

Oral administration

Groups of 50 male and 50 female Sprague-Dawley rats were given drinking-water containing chloral hydrate at nominal doses of 0, 0, 15, 45, and 135 mg/kg bw (Leuschner & Beuscher, 1998). The rats were examined after 124 weeks (males) or 128 weeks (females) of exposure. A statistically significantly increase in hepatic hypertrophy was reported in the group of male rats at the highest dose. It was reported that the incidence of neoplastic lesions was not increased in treated males or females compared with rats in the control groups. [The Working Group noted that the lack of incidence data in this paper hampered evaluation of the study.]

Groups of 78 male F344/N rats were given drinking-water containing chloral hydrate at measured concentrations of 0, 0.12, 0.58, or 2.51 g/L (George *et al.*, 2000). These treatments provided mean daily doses of 0, 7.4, 37.4, and 162.6 mg/kg bw. Six rats per group were killed after 13, 26, 52, and 78 weeks. Survivors were maintained on treatment for 104 weeks. Histopathological examination of the liver, kidney, spleen and testis was performed on rats surviving more than 78 weeks. There were no treatment-related increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatocellular adenoma or carcinoma (combined). However, 3 out of 44 (7%) of rats receiving the lowest dose developed hepatocellular carcinomas, while the incidence of hepatocellular carcinomas for historical controls in this strain of rats was only 19 out of 2255 (0.8%) (Haseman *et al.*, 1998). The incidences of neoplasms of the kidney, spleen, and testis were reported not to exceed those observed in the control group or historical controls (data not shown). [The Working Group noted that the evaluations of tumour incidence were limited to the four organs examined microscopically.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Studies in humans have shown that chloral hydrate is rapidly absorbed after oral administration, with peak concentrations in blood occurring within 1 hour after dosing (Merdink *et al.*, 2008). The extent of oral absorption has not been measured precisely in humans, but is likely to be high due to the rapidity of absorption. Recovery of metabolites in urine, which represents a lower boundary on absorption, has been reported to be between 47% and 60% for subjects followed for 1 week after administration of a single oral dose (Müller *et al.*, 1974; Merdink *et al.*, 2008). It should be noted that 1 week is

insufficient for complete urinary excretion of the metabolites of chloral hydrate. With repeated oral dosing, Owens & Marshall (1955) reported high inter-individual variation in recovery, with average daily excretion ranging from 7% to 94% of the daily dose of chloral hydrate.

No data were available to the Working Group on absorption in humans via other routes of exposure.

(b) Experimental systems

Orally administered chloral hydrate is rapidly absorbed in rats and mice, with peak concentrations reported at 15 minutes after administration (Beland *et al.*, 1998). The extent of oral absorption has not been measured precisely in experimental systems, but is likely to be high due to the rapidity of absorption. The percentage absorption was not estimated since no studies on urinary excretion or mass balance for orally administered chloral hydrate were available to the Working Group.

No experimental data on absorption via other routes of exposure were available to the Working Group.

4.1.2 Distribution

(a) Humans

In humans, orally administered chloral hydrate enters the liver where it undergoes extensive metabolism (see Section 4.1.3); only a limited amount enters the systemic circulation (Merdink *et al.*, 2008). Chloral hydrate in the blood is rapidly eliminated by metabolism, as shown by Zimmermann *et al.* (1998), who reported a half-life of less than 1 hour, and Merdink *et al.* (2008), who reported a rapid initial decline with a terminal half-life of about 10 hours. [Although no data on distribution in human tissues were available, the Working Group noted that due to the extensive first-pass effect, it was likely that concentrations of chloral hydrate were much lower in extra-hepatic tissues than in the liver of humans exposed orally.]

No data on distribution via other routes of administration were available to the Working Group.

(b) Experimental systems

In rats and mice, orally administered chloral hydrate enters the liver where it undergoes extensive metabolism (see Section 4.1.3); only a limited amount enters the systemic circulation (Beland *et al.*, 1998). Chloral hydrate in the blood is rapidly eliminated, probably by metabolism, with levels reported to be below the limit of detection within 3 hours after oral administration (Beland *et al.*, 1998). [Although no data on tissue distribution were available, the Working Group noted that due to the extensive first-pass effect, it was likely that concentrations of chloral hydrate in extra-hepatic tissues were much lower than those in the liver of rodents exposed orally.]

The half-life of chloral hydrate after intravenous administration has been reported to be about 5–20 minutes in mice and rats (Abbas *et al.*, 1996; Merdink *et al.*, 1999), indicating rapid elimination from the systemic circulation. Abbas *et al.* (1996) reported concentrations of chloral hydrate in the liver to be about one quarter to one half of concentrations in the blood. No data on distribution to other tissues after intravenous administration were available to the Working Group.

No data on distribution via other routes of administration were available to the Working Group.

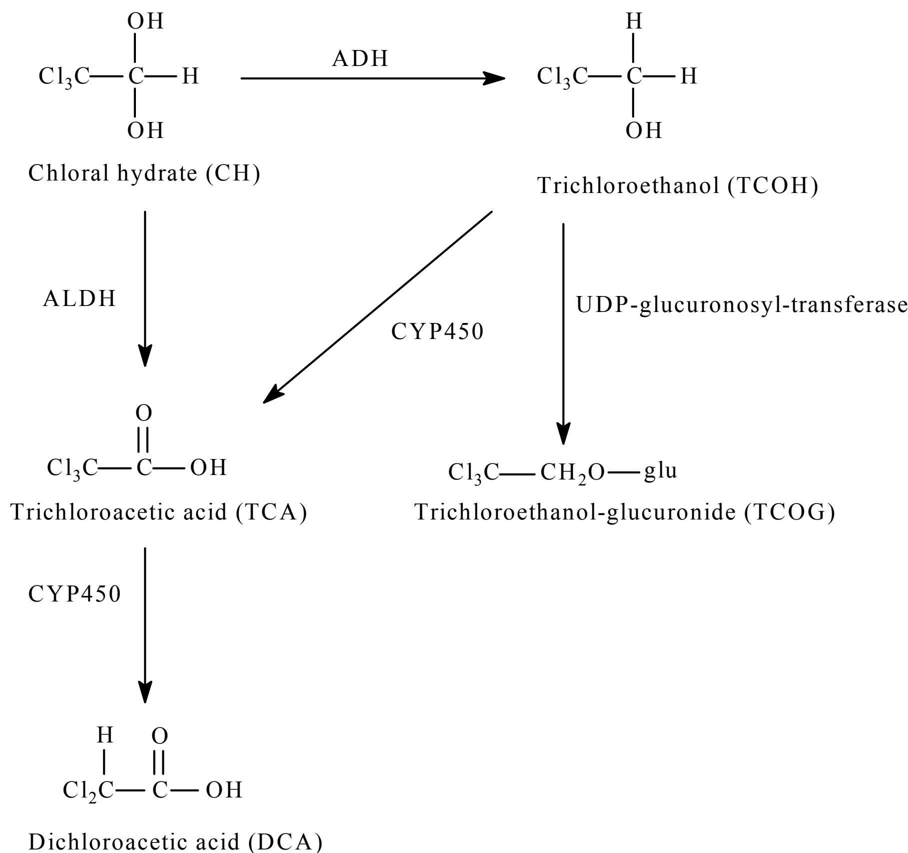
4.1.3 Metabolism

(a) Humans

The metabolic pathways of chloral hydrate and its metabolites in humans are depicted in Fig. 4.1.

Multiple studies in humans have reported that the metabolites of chloral hydrate include trichloroethanol, its glucuronide, and trichloroacetic acid. For instance, Owens & Marshall

Fig. 4.1 Metabolism of chloral hydrate



ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP450, enzyme of the cytochrome P450 family

(1955), Breimer *et al.* (1974), and Merdink *et al.* (2008) measured free trichloroethanol, total trichloroethanol (free plus glucuronidated), and trichloroacetic acid in blood and/or urine after administration of chloral hydrate to human volunteers. The terminal half-lives of trichloroethanol and trichloroacetic acid after oral exposure to chloral hydrate have been measured to be about 8–13 hours for trichloroethanol and 4–5 days for trichloroacetic acid (Breimer, 1977; Zimmermann *et al.*, 1998; Merdink *et al.*, 2008).

Merdink *et al.* (2008) also examined the importance of enterohepatic recirculation, by which trichloroethanol-glucuronide is excreted with bile into the intestine, where trichloroethanol is regenerated and reabsorbed in the gut. In this study, the subjects consumed a high-fat meal 4 hours after dosing with chloral hydrate, to “synchronize” the secretion of bile and subsequent excretion of trichloroethanol-glucuronide into the intestine. The resulting cyclic variation in the amount of trichloroethanol-glucuronide and the complex kinetic behaviour of plasma trichloroacetic acid (e.g. two distinct peak concentrations) are consistent with enterohepatic recirculation.

Considerable amounts of dichloroacetic acid were reported as a urinary metabolite of chloral hydrate in children (Henderson *et al.*, 1997a). However, only trace amounts were detected after administration of chloral hydrate to adults (Merdink *et al.*, 2008). [The Working Group noted that due to uncertainties as to artefactual (*ex vivo*) formation of dichloroacetic acid

in biological samples (Ketcha *et al.*, 1996), it was unclear whether this difference was due to life stage or to the analytical methodologies used.]

Bronley-DeLancey *et al.* (2006) used cryogenically preserved human hepatocytes to simultaneously evaluate the kinetics of the metabolism of chloral hydrate and alcohol dehydrogenase/aldehyde dehydrogenase (ADH/ALDH) genotype. Thirteen samples of human hepatocytes were examined, and large inter-individual variation in the V_{max} values for formation of trichloroethanol and trichloroacetic acid was reported. In this sample of limited size, no correlation with ADH/ALDH genotype was apparent. Furthermore, despite the large variation in V_{max} values between individuals, disposition of chloral hydrate into downstream metabolites was found to be relatively constant.

(b) Experimental systems

A similar spectrum of metabolites has been reported in experimental studies. Studies in rats, mice, and dogs have all identified trichloroacetic acid, trichloroethanol-glucuronide, and trichloroethanol as the major metabolites of chloral hydrate (Breimer *et al.*, 1974; Abbas *et al.*, 1996; Beland *et al.*, 1998; Merdink *et al.*, 1998, 1999).

The importance of enterohepatic recirculation in the kinetics of chloral hydrate metabolites has been examined by Merdink *et al.* (1999) through comparison between intact and bile-cannulated rats given chloral hydrate, trichloroethanol, and trichloroacetic acid via the jugular vein. A statistically significant difference in the kinetics of trichloroethanol and its glucuronide was reported for between intact and bile-cannulated rats given trichloroethanol. Kinetic differences for chloral hydrate, trichloroethanol and its glucuronide, or trichloroacetic acid after administration of chloral hydrate were evident only at the highest dose of chloral hydrate (192 mg/kg bw, compared with 12 and 48 mg/kg bw). Moreover, chloral hydrate, trichloroethanol and its glucuronide, and trichloroacetic acid were all detected in bile, with trichloroethanol-glucuronide exhibiting the highest peak concentrations. Therefore, while the data are consistent with enterohepatic recirculation of trichloroethanol and its glucuronide occurring in rats, this process appears to have little impact on the kinetics of chloral hydrate or its metabolites in rats, except at higher doses.

Abbas *et al.* (1996) reported detecting dichloroacetic acid in mice given chloral hydrate by oral administration, but it was later determined that these data were probably confounded by artefactual formation of dichloroacetic acid during sample preparation (Ketcha *et al.*, 1996; Abbas & Fisher, 1997; Merdink *et al.*, 1998). Later studies (Beland *et al.*, 1998; Merdink *et al.*, 1998, 1999) reported only trace or undetectable amounts of dichloroacetic acid in mice or rats given chloral hydrate. Merdink *et al.* (1998) have suggested that it is likely that some dichloroacetic acid is being formed as a short-lived intermediate, but that the extremely rapid elimination kinetics of dichloroacetic acid relative to its formation do not allow for accumulation (and detection) of dichloroacetic acid in the blood.

Chloral hydrate was shown to be an inhibitor of ALDH (Wang *et al.*, 1999), suggesting that production of trichloroacetic acid from chloral hydrate may not increase in a linear fashion with dose. An inhibitory effect of chloral hydrate on liver ADH was also reported in studies in mice (Sharkawi *et al.*, 1983). In a short-term study in rats, Poon *et al.* (Poon *et al.*, 2002) showed that exposure to drinking-water containing chloral hydrate led to statistically significant reduction in activity of liver ALDH, while the activity of liver aniline hydroxylase (a marker for CYP2E1) was significantly elevated in males and females receiving chloral hydrate at 200 ppm. In the same study, the findings of Wang *et al.* (1999) were confirmed, showing that chloral hydrate is a potent inhibitor of liver ALDH *in vitro*, with an IC_{50} of 8 μM, while trichloroacetic acid was weakly inhibitory, and trichloroethanol was without effect.

4.1.4 Excretion

(a) Humans

The primary known excretion route for chloral hydrate in humans is as the metabolites trichloroethanol-glucuronide and trichloroacetic acid in urine (Owens & Marshall, 1955; Merdink *et al.*, 2008). Owens & Marshall (1955) found that recovery of urinary metabolites was not complete, with average daily excretion ranging from 7% to 94% of daily doses of chloral hydrate, according to individual. Therefore, it is possible that other excretion routes exist that have not been well characterized. For instance, low concentrations of chloral hydrate have been found in breast milk. Although breastfeeding infants may be sedated by chloral hydrate in breast milk, the highest concentration measured in milk (about 15 µg/mL) is considerably lower than that measured in blood after administration of chloral hydrate at a clinically active dose (100 µg/mL) (Bernstine *et al.*, 1956; Wilson, 1981).

(b) Experimental systems

In mice and rats, chloral hydrate appears to be excreted primarily in urine as the metabolites trichloroethanol-glucuronide and trichloroacetic acid (Merdink *et al.*, 1998, 1999); however, there were no comprehensive studies of mass balance available to the Working Group. Therefore, the extent of recovery represented by urinary excretion is unknown, although it is often assumed to be 100% (Beland *et al.*, 1998).

Few notable differences have been found in the excretion of chloral hydrate in rats and mice. While Beland *et al.* (1998) noted statistically significant differences in the half-lives of trichloroethanol and its glucuronide in rats and mice, all estimated half-lives were very short (< 1 hour). Beland *et al.* (1998) also reported that with the same regime of repeated doses (12 doses of chloral hydrate at 50 or 200 mg/kg bw over 16 days), the area under the concentration–time curve (AUC) of trichloroacetic acid in rats was greater than that in mice.

4.2 Genotoxicity and related effects

4.2.1 Humans

Studies on the genotoxic effects of chloral hydrate in humans *in vivo* and *in vitro* are presented in Table 4.1.

Ikbal *et al.* (2004) assessed the frequencies of micronucleus formation and sister-chromatid exchange in cultured peripheral blood lymphocytes of 18 infants (age range, 31–55 days) before and after administration of a single dose (50 mg/kg bw) of chloral hydrate in breast milk or formula administered for the purposes of sedation before a hearing test. There was a statistically significant increase in the mean frequency of micronucleus formation (2.57 ± 0.20/1000 cells before treatment versus 3.56 ± 0.17/cell after treatment; $P = 0.004$), as well as in the mean frequency of sister-chromatid exchange (7.03 ± 0.18/cell before treatment versus 7.90 ± 0.19/cell after treatment; $P < 0.001$). On an individual level, 15 out of 18 individuals showed an increase in the frequency of micronucleus formation with treatment, and 18 out of 18 individuals showed an increase in the frequency of sister-chromatid exchange with treatment. Sister-chromatid exchange was also assessed by Gu *et al.* (1981) in human lymphocytes exposed *in vitro*, with inconclusive results.

The ability of chloral hydrate to induce aneuploidy and polyploidy was tested in human lymphocyte cultures established from blood samples obtained from two healthy nonsmokers (Sbrana *et al.*, 1993). Cells were exposed to chloral hydrate at doses of 50–250 µg/mL for 72 hours or 96 hours. No increases in the percentage of cells with hyperdiploidy, tetraploidy, or endoreduplication were observed when cells were exposed for 72 hours at any dose tested. Although no dose–response relationships was observed after 96 hours of exposure, there was a statistically

Table 4.1 Studies of genoxicity with chloral hydrate in humans *in vitro* and *in vivo*

Test system/end-point	Dose[a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
In vitro				
DNA damage (comet assay), TK6 cells	16.5	NT	+	Liviac *et al.* (2010)
DNA damage (comet assay), HepG2 cells	3.3	NT	+	Zhang *et al.* (2012)
DNA SSB, lymphoblastoid cells	1650	NT	–	Chang *et al.* (1992)
Gene mutation, *TK* and *HPRT* locus, lymphoblastoid cells	1000	NT	+	Beland (1999)
SCE, lymphocytes	54	NT	(+)	Gu *et al.* (1981)
Micronucleus formation, lymphocytes	100	–	+	Van Hummelen & Kirsch-Volders (1992)
Micronucleus formation, lymphoblastoid AHH-1 cell line	100	NT	+	Parry *et al.* (1996)
Micronucleus formation, lymphoblastoid maximum contaminant level-5 cell line	500	NT	–	Parry *et al.* (1996)
Micronucleus formation (kinetochore-positive), diploid LEO fibroblasts	120	NT	+	Bonatti *et al.* (1992)
Micronucleus formation, TK6 cells	413	NT	–	Liviac *et al.* (2010)
Aneuploidy (double Y induction), lymphocytes	250	NT	+	Vagnarelli *et al.* (1990)
Aneuploidy (hyperdiploidy and hypodiploidy), lymphocytes	50	NT	+	Sbrana *et al.* (1993)
Polyploidy, lymphocytes	137	NT	+	Sbrana *et al.* (1993)
C-Mitosis, lymphocytes	75	NT	+	Sbrana *et al.* (1993)
In vivo				
Micronucleus formation, infants, peripheral lymphocytes	50, oral	NT	+	Ikbal *et al.* (2004)

[a] Doses are in µg/mL for tests *in vitro*, and mg/kg bw for tests *in vivo*.

+, positive; (+), weakly positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; SCE, sister-chromatid exchange; SSBs, single strand breaks.

significant increase in the percentage of hyperdiploid cells at 150 µg/mL, and in the percentage of tetraploid cells at 137 µg/mL.

4.2.2 Experimental systems

Chloral hydrate has been evaluated for genotoxic potential in a variety of assays in experimental systems (see Table 4.2, 4.3 and 4.4).

(a) DNA binding and damage

There has been limited analysis of the DNA-binding potential of chloral hydrate (Keller & Heck, 1988; Ni *et al.*, 1995; Von Tungeln *et al.*, 2002). Keller & Heck (1988) conducted experiments in $B6C3F_1$ mice *in vitro* and in *vivo*. The mice were pretreated by gavage with trichloroethylene at a dose of 1500 mg/kg bw per day for 10 days, and then given [^{14}C]-labelled chloral intraperitoneally at a dose of 800 mg/kg bw. No detectable covalent binding of the radiolabel to DNA in the liver was observed.

Keller & Heck (1988) investigated the potential of chloral hydrate to form DNA–protein crosslinks in rat liver nuclei. No statistically significant increase in the frequency of DNA–protein crosslinks was observed with chloral hydrate at concentrations of 25, 100, or 250 mM [3.7, 14.7, 36.8 mg/mL]. DNA and RNA isolated from the nuclei treated with [C^{14}]-labelled chloral did not have any detectable bound radiolabel; however, concentration-dependent binding of the radiolabel to proteins from choral-treated nuclei was observed.

Incubation of chloral hydrate with liver microsomes from male $B6C3F_1$ mice resulted in increases in the amounts of lipid-peroxidation products (malondialdehyde and formaldehyde). This effect was inhibited by free radical scavengers, α-tocopherol or menadione (Ni *et al.*, 1994). Ni *et al.* (1995) subsequently observed malondialdehyde adducts in calf thymus DNA in the presence of chloral hydrate and liver microsomes from male $B6C3F_1$ mice. In another study in $B6C3F_1$ mice, exposure *in vivo* to nonradiolabelled chloral hydrate at a concentration of 2000 nmol [330 µg] resulted in an increase in malondialdehyde-derived adducts and 8-oxo-2′-deoxyguanosine adducts in liver DNA, indirect indicators of oxidative DNA damage (Von Tungeln *et al.*, 2002).

Kiffe *et al.* (2003) carried out the single-cell gel electrophoresis assay (comet assay) in Chinese hamster ovary K5 cells under standard assay conditions or with modifications involving collection of all cells, concurrent treatment with ethyl methanesulfonate (for detecting crosslinking properties) and/or analysis of subcellular DNA breakage. Chloral hydrate gave negative results except at the highest concentration (5000 µg/mL), at which cell viability was about 30%. Zhang *et al.* (2012) conducted the single-cell gel electrophoresis assay in HepG2 cells to assess the DNA-damaging potential of chloral hydrate. A statistically significant increase ($P < 0.01$) in DNA damage was reported after treatment with chloral hydrate at 20 µM [3.3 µg/mL] for 4 hours, with cell viability exceeding 75%. Cell viability decreased below 75% at concentrations of 80 µM [13.2 µg/mL] and higher. Liviac *et al.* (2010) carried out the single-cell gel electrophoresis assay in TK6 cells and reported statistically significant increases in DNA damage at concentrations of 100 µM [16.5 µg/mL] and higher, with no change in cell viability up to the highest concentration tested (10 mM) [1654 µg/mL]. Liviac *et al.* (2010) also examined DNA-repair kinetics, reporting that induced DNA damage was repaired after 45 minutes. Therefore, the degree of DNA damage appears to depend heavily on the type of cell used in the experimental system.

(b) Mutations

Chloral hydrate induced gene mutation in *Salmonella typhimurium* TA100 and TA104 strains, but not in most other strains assayed. Four out of six studies of reverse mutation in *S. typhimurium* TA100 and two out of two

Table 4.2 Genotoxicity of chloral hydrate in bacterial, yeast, and fungal systems

Test system/end-point	Doses[a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
SOS chromotest, *Escherichia coli* PQ37	10 000	–	–	Giller *et al.* (1995)
Salmonella typhimurium TA1535, TA98, reverse mutation	10 000	–	–	Waskell (1978)
S. typhimurium TA100, reverse mutation	2000 µg/plate	+	+	Ni *et al.* (1994)
S. typhimurium TA100, reverse mutation, liquid medium	300	+	–	Giller *et al.* (1995)
S. typhimurium TA100, TA104, reverse mutation	1000 µg/plate	+	+	Beland (1999)
S. typhimurium TA104, reverse mutation	1000 µg/plate	+	+	Ni *et al.* (1994)
S. typhimurium TA1535, reverse mutation	1850	–	–	Leuschner & Leuschner (1991)
S. typhimurium TA1535, TA1537 reverse mutation	6667	–	–	Haworth *et al.* (1983)
S. typhimurium TA1535, reverse mutation	10 000	–	–	Beland (1999)
S. typhimurium TA98, reverse mutation	7500	–	–	Haworth *et al.* (1983)
S. typhimurium TA98, reverse mutation	10 000 µg/plate	–	+	Beland (1999)
A.nidulans, diploid strain 35X17, mitotic crossover	1650	NT	–	Crebelli *et al.* (1985)
A. nidulans, diploid strain 30, mitotic crossover	6600	NT	–	Käfer (1986)
A. nidulans, diploid strain NH, mitotic crossover	1000	NT	–	Kappas (1989)
A. nidulans, diploid strain P1, mitotic crossover	990	NT	–	Crebelli *et al.* (1991)
A. nidulans, diploid strain 35X17, nondisjunction	825	NT	+	Crebelli *et al.* (1985)
A. nidulans, diploid strain 30, aneuploidy	825	NT	+	Käfer (1986)
A. nidulans, haploid conidia, aneuploidy, polyploidy	1650	NT	+	Käfer (1986)
A. nidulans, diploid strain NH, nondisjunction	450	NT	+	Kappas (1989)
A. nidulans, diploid strain P1, nondisjunction	660	NT	+	Crebelli *et al.* (1991)
A. nidulans, haploid strain 35, hyperploidy	2640	NT	+	Crebelli *et al.* (1991)
Saccharomyces cerevisiae, meiotic recombination	3300	NT	Inconclusive	Sora & Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	2500	NT	+	Sora & Agostini Carbone (1987)
S. cerevisiae, disomy in meiosis	3300	NT	+	Sora & Agostini Carbone (1987)
S. cerevisiae, D61.M, mitotic chromosomal malsegregation	1000	NT	+	Albertini (1990)
Drosophila melanogaster, somatic mutation wing spot test	825	NT	+	Zordan *et al.* (1994)
D. melanogaster, induction of sex-linked lethal mutation	37.2 (feed)	NT	Inconclusive	Beland (1999)
D. melanogaster, induction of sex-linked lethal mutation	67.5(injected)	NT	–	Beland (1999)

[a] Doses are in µg/mL for tests *in vitro* unless otherwise specified.
+, positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested

Table 4.3 Genotoxicity of chloral hydrate in mammalian systems *in vitro*

Test system/end-point	Dose [a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
DNA–protein crosslinks, rat nuclei	41 250	NT	–	Keller & Heck (1988)
DNA SSB, rat primary hepatocytes	1650	NT	–	Chang *et al.* (1992)
DNA damage (several variants of comet assay), Chinese hamster ovary (CHO K5) cells	5000	NT	–/+	Kiffe *et al.* (2003)
Gene mutation, mouse lymphoma L5178Y/$Tk^{+/-}$ cells	1000	NT	(+)	Harrington-Brock *et al.* (1998)
Gene mutation, mouse lymphoma L5178Y/$Tk^{+/-}$ cells	165	NT	–	Liviac *et al.* (2011)
Gene mutation, mouse lymphoma L5178Y/$Tk^{+/-}$ cells	562	NT	(+)	Fellows *et al.* (2011)
SCE, Chinese hamster ovary cells	100	+	+	Beland (1999)
Micronucleus formation (kinetochore-positive), Chinese hamster C1 cells	165	NT	+	Degrassi & Tanzarella (1988)
Micronucleus formation (kinetochore-negative), Chinese hamster C1 cells	250	NT	–	Degrassi & Tanzarella (1988)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells	400	NT	+	Parry *et al.* (1990)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells	400	NT	+	Lynch & Parry (1993)
Micronucleus formation, Chinese hamster V79 cells	316	NT	+	Seelbach *et al.* (1993)
Micronucleus formation, mouse lymphoma L5178Y/$Tk^{+/-}$ cells	1300	NT	–	Harrington-Brock *et al.* (1998)
Micronucleus formation, mouse lymphoma L5178Y/$Tk^{+/-}$ cells	500	NT	+	Nesslany & Marzin (1999)
Chromosomal aberration, Chinese hamster CHED cells	20	NT	+	Furnus *et al.* (1990)
Chromosomal aberration, Chinese hamster ovary cells	1000	+	+	Beland (1999)
Chromosomal aberration, mouse lymphoma L5178Y/$Tk^{+/-}$ cells	1250	NT	(+)	Harrington-Brock *et al.* (1998)
Aneuploidy, Chinese hamster CHED cells	10	NT	+	Furnus *et al.* (1990)

Table 4.3 (continued)

Test system/end-point	Dose [a] (LED or HID)	Results		Reference
		With exogenous metabolic activation	Without exogenous metabolic activation	
Aneuploidy, primary Chinese hamster embryonic cells	250	NT	+	Natarajan *et al.* (1993)
Aneuploidy, Chinese hamster LUC2p4 cells	250	NT	+	Warr *et al.* (1993)
Aneuploidy, mouse lymphoma L5178Y/*Tk*$^{+/-}$	1300	NT	–	Harrington-Brock *et al.* (1998)
Tetraploidy and endoreduplіation, Chinese hamster LUC2p4cells	500	NT	+	Warr *et al.* (1993)
Cell transformation, Syrian hamster embryo cells (24-hour treatment)	350	NT	+	Gibson *et al.* (1995)
Cell transformation, Syrian hamster embryo cells (7-day treatment, conditioned media without X-ray irradiated feeder layer)	5	NT	+	Pant *et al.* (2008)
Cell transformation, Syrian hamster dermal cell line (24-hour treatment)	50	NT	+	Parry *et al.* (1996)

[a] Doses are in μg/mL for tests *in vitro*.
+, positive; (+), weakly positive; –, negative; –/+, some variants of test gave negative results, some gave positive results; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested; SCE, sister-chromatid exchange; SSB, single-strand break

Table 4.4 Genotoxicity of chloral hydrate in mammalian systems *in vivo*

Test system/end-point	Doses[a] (LED or HID)	Results	Reference
DNA SSB, male Sprague-Dawley rat liver	300, oral	+	Nelson & Bull (1988)
DNA SSB, male F344 rat liver	1650, oral	–	Chang *et al.* (1992)
DNA SSB, male B6C3F$_1$ mouse liver	100, oral	+	Nelson & Bull (1988)
DNA SSB, male B6C3F$_1$ mouse liver	825, oral	–	Chang *et al.* (1992)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes (raised in water containing chloral hydrate)	200	+	Giller *et al.* (1995)
Micronucleus formation, *Carassius auratus gibelio* (crucian carp) blood, gill, and fin cells (raised in water containing chloral hydrate)	400	+	Arkhipchuk & Garanko (2005)
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	–	Leuschner & Leuschner (1991)
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	–	Russo & Levis (1992a)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	+	Russo & Levis (1992b)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	+	Russo *et al.* (1992)
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	–	Leopardi *et al.* (1993)
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	+	Allen *et al.* 1994
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini *et al.* (1994)
Micronucleus formation, B6C3F$_1$ mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	+	Nutley *et al.* (1996)
Micronucleus formation, B6C3F$_1$ mouse spermatids after meiotic cell treatment	413, i.p.	–	Nutley *et al.* (1996)
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	–	Grawé *et al.* (1997)
Micronucleus formation, male B6C3F$_1$ mouse bone-marrow erythrocytes	500, i.p., × 3	+	Beland (1999)
Chromosomal aberration, male and female F1 mouse bone-marrow cells	600, i.p.	–	Xu & Adler (1990)
Chromosomal aberration, male and female Sprague-Dawley rat bone-marrow cells	1000, oral	–	Leuschner & Leuschner (1991)
Chromosomal aberration, BALB/c mouse spermatogonia treated	83, i.p.	–	Russo & Levis (1992b)

Table 4.4 (continued)

Test system/end-point	Doses[a] (LED or HID)	Results	Reference
Chromosomal aberration, F1 mouse secondary spermatocytes	82.7, i.p.	+	Russo *et al.* (1984)
Chromosomal aberration, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	–	Marrazzini *et al.* (1994)
Chromosomal aberration, ICR mouse oocytes	600, i.p.	–	Mailhes *et al.* (1993)
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	–	Xu & Adler (1990)
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	+	Miller & Adler (1992)
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	–	Leopardi *et al.* (1993)
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazzini *et al.* (1994)

[a] Doses are in mg/kg bw for tests *in vivo*.

+, positive; –, negative; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; SSB, single-strand breaks

studies in *S. typhimurium* TA104 gave positive results (Haworth *et al.*, 1983; Ni *et al.*, 1994; Giller *et al.*, 1995; Beland, 1999). Waskell (1978) studied the effect of chloral hydrate (dose range, 1.0–13 mg/plate) on gene mutation in different *S. typhimurium* strains (TA98, TA100, TA1535) in the Ames assay. No revertant colonies were observed in strains TA98 or TA1535 either in the presence and absence of metabolic activation by S9 (9000 × *g* rat liver supernatant). Similar results were obtained by Leuschner & Leuschner (1991); however, in TA100, a dose-dependent statistically significant increase in the frequency of revertant colonies was obtained in the presence and absence of metabolic activation. It should be noted that the chloral hydrate used (obtained from Sigma), recrystallized one to six times from chloroform, was described as "crude." However, this positive result was consistent with those of other studies in this strain, as noted above (Waskell, 1978). Giller *et al.* (1995) studied the genotoxicity of chloral hydrate in three short-term tests. Chloral induced mutations in strain TA100 of *S. typhimurium* (fluctuation test). Similar results were obtained by Haworth *et al.* (1983). [The Working Group noted that these results were consistent with those of several studies with trichloroethylene, in which low, but positive, responses were observed in the TA100 strain in the presence of metabolic activation from S9, even when genotoxic stabilizers were not present.]

A statistically significant increase in the frequency of mitotic segregation was observed in *Aspergillus nidulans* treated with chloral hydrate at a concentration of 5 [827 μg/mL] or 10 mM [1654 μg/mL] (Crebelli *et al.*, 1985). Studies of mitotic crossing-over in *A. nidulans* gave negative results, while these same studies gave positive results for aneuploidy (Crebelli *et al.*, 1985, 1991; Käfer, 1986; Kappas, 1989).

Two studies in *Saccharomyces cerevisiae* investigated chromosomal malsegregation after exposure to chloral hydrate (Sora & Agostini Carbone, 1987; Albertini, 1990). Chloral hydrate (1–25 mM) [165–4135 μg/mL] was dissolved in the sporulation medium and the frequencies of various meiotic events such as recombination and disomy were analysed. Chloral hydrate inhibited sporulation as a function of dose and increased the frequency of diploid and disomic clones. Chloral hydrate was also tested for mitotic chromosome malsegregation in *S. cerevisiae* D61.M (Albertini, 1990). The test strain was exposed at a dose range of 1–8 mg/mL. An increase in the frequency of chromosomal malsegregation was observed after exposure to chloral hydrate.

Two studies of mutagenicity with chloral hydrate were performed in *Drosophila* (Zordan *et al.*, 1994; Beland, 1999). In these two studies, chloral hydrate gave positive results in the wing spot test for somatic mutation (Zordan *et al.*, 1994), while the results of a test for induction of sex-linked lethal mutation were equivocal when chloral hydrate was administered in the feed, but negative when chloral hydrate was administered by injection (Beland, 1999).

In a mammalian system, Harrington-Brock *et al.* (1998) noted that increases in mutant frequency observed in *Tk* $^{+/-}$ mouse lymphoma cell lines treated with chloral hydrate were not statistically significant. The mutants were primarily small-colony *Tk* mutants, indicating that most mutants induced by chloral hydrate resulted from chromosomal mutation rather than point mutation. It should be noted that cytotoxicity was observed at most concentrations tested (350–1600 μg/mL). Percentage cell survival ranged from 96% to 4%. Fellows *et al.* (2011) and Liviac *et al.* (2011) also tested for mutagenicity with chloral hydrate in *Tk* $^{+/-}$ mouse lymphoma cell lines without metabolic activation from S9. Fellows *et al.* (2011) reported a statistically significant increase in mutant frequency at the highest tested concentration of 3.4 mM [562 μg/mL], at which a high degree of cytotoxicity was observed. Liviac *et al.* (2011) tested chloral hydrate at lower concentrations of 1 μM to 1 mM [0.16–165 μg/mL], at which

relative total growth was at least 66%, and did not observe any statistically significant increases in mutant frequency. [The Working Group noted that reported increases in mutant frequencies in mammalian systems were not statistically significant, except at highly cytotoxic concentrations.]

(c) Chromosomal effects

Exposure to chloral hydrate induces the formation of micronuclei in most test systems, including assays *in vitro* and *in vivo* in mammalian species (Degrassi & Tanzarella, 1988; Leuschner & Leuschner, 1991; Bonatti *et al.*, 1992; Russo *et al.*, 1992; Russo & Levis, 1992a, b; Van Hummelen & Kirsch-Volders, 1992; Leopardi *et al.*, 1993; Lynch & Parry, 1993; Seelbach *et al.*, 1993; Allen *et al.*, 1994; Marrazzini *et al.*, 1994; Giller *et al.*, 1995; Nutley *et al.*, 1996; Parry *et al.*, 1996; Grawé *et al.*, 1997; Harrington-Brock *et al.*, 1998; Beland, 1999; Nesslany & Marzin, 1999; Ikbal *et al.*, 2004; Arkhipchuk & Garanko, 2005; Liviac *et al.*, 2010). For instance, chloral hydrate has been shown to induce micronucleus formation, but not structural chromosomal aberrations in mouse bone-marrow cells. Micronuclei induced by non-clastogenic agents are generally believed to represent intact chromosomes that have failed to segregate into either daughter-cell nucleus at cell division (Xu & Adler, 1990; Russo *et al.*, 1992). Furthermore, micronuclei induced by chloral hydrate in mouse bone-marrow cells (Russo *et al.*, 1992) and in cultured mammalian cells (Degrassi & Tanzarella, 1988; Bonatti *et al.*, 1992) have been shown to be predominantly kinetochore-positive in composition upon analysis with immunofluorescence methods. The presence of a kinetochore in a micronucleus is considered evidence that the micronucleus contains a whole chromosome lost at cell division (Degrassi & Tanzarella, 1988; Hennig *et al.*, 1988; Eastmond & Tucker, 1989). In contrast, Allen *et al.* (1994) harvested spermatids from male C57B1/6J mice given chloral hydrate by intraperitoneal administration 49 days before harvesting, and found statistically significantly increased frequencies of kinetochore-negative micronuclei; however, no dose–response relationship was observed. The results of this study contrast with those of studies described above (Degrassi & Tanzarella, 1988; Bonatti *et al.*, 1992) which demonstrated predominantly kinetochore-positive micronuclei.

Chloral hydrate induced aneuploidy *in vitro* in multiple Chinese hamster cell lines (Furnus *et al.*, 1990; Natarajan *et al.*, 1993; Warr *et al.*, 1993) and in human lymphocytes (Vagnarelli *et al.*, 1990; Sbrana *et al.*, 1993), but not in mouse lymphoma cells (Harrington-Brock *et al.*, 1998). Studies performed *in vivo* in various mouse strains led to increased aneuploidy in spermatocytes (Russo *et al.*, 1984; Liang & Pacchierotti, 1988; Miller & Adler, 1992), but not in oocytes (Mailhes *et al.*, 1993) or bone-marrow cells (Xu & Adler, 1990; Leopardi *et al.*, 1993).

The potential of chloral hydrate to induce aneuploidy in mammalian germ cells has been of particular interest since Russo *et al.* (1984) first demonstrated that treatment of male mice with chloral hydrate results in statistically significant increases in the frequencies of hyperploidy in metaphase II cells. This hyperploidy was thought to have arisen from chromosomal nondisjunction in premeiotic/meiotic cell division, and may be a consequence of chloral hydrate interfering with spindle formation [reviewed by Russo *et al.* (1984) and Liang & Brinkley (1985)]. Chloral hydrate also causes meiotic delay, which may be associated with aneuploidy (Miller & Adler, 1992).

Several studies have included analysis of chromosomal aberration *in vitro* and *in vivo* after exposure to chloral hydrate; there have been some positive results *in vitro* (Furnus *et al.*, 1990; Harrington-Brock *et al.*, 1998; Beland, 1999). In mouse lymphoma cell lines and Chinese hamster embryo cells treated with chloral hydrate, there was no significant increase in chromosomal aberration (Harrington-Brock *et al.*, 1998; Furnus

et al., 1990). Other studies of chromosome aberration *in vivo* have mostly reported negative results (Liang & Pacchierotti, 1988; Xu & Adler, 1990; Leuschner & Leuschner, 1991; Russo & Levis, 1992a, b; Mailhes *et al.*, 1993), with the exception of one study (Russo *et al.*, 1984) in mice of an F1 cross, C57B1/Cne × C3H/Cne.

Positive results for sister-chromatid exchange were observed by Beland (1999) in Chinese hamster ovary cells exposed *in vitro* with and without an exogenous metabolic activation system.

(d) Cell transformation

Chloral hydrate gave positive results in three studies designed to measure cellular transformation in Syrian hamster cells (dermal and/or embryo) exposed to chloral hydrate (Gibson *et al.*, 1995; Parry *et al.*, 1996; Pant *et al.*, 2008).

4.3 Non-genotoxic mechanisms of carcinogenesis

4.3.1 Mechanisms related to liver carcinogenesis

(a) Cell proliferation

(i) Humans

No data in humans were available to the Working Group.

(ii) Experimental systems

Rijhsinghani *et al.* (1986) reported increases in the mitotic index in liver cells of male C56BL × C3HF1 mice (age, 15 days), 24 hours after receiving chloral hydrate as a single dose at 5 or 10 mg/kg bw by gavage. The increase was only statistically significant at 5 mg/kg bw. No necrosis was observed. George *et al.* (2000) measured the hepatocyte labelling index in male F344 rats and male B6C3F$_1$ mice given drinking-water containing chloral hydrate for 13 (rats only), 26, 52, and 78 weeks. Except for the intermediate dose (0.58 g/L) at 26 weeks, no increases in labelling index were reported in either species. No changes in liver necrosis were observed in either species at any dose or time-point. [The Working Group noted that the lack of significant liver necrosis suggests that the small, transient proliferative responses observed were not due to cytotoxicity. The transient nature of any proliferative responses suggested that cell proliferation induced by chloral hydrate is not an important mechanistic contributor to carcinogenicity caused by chloral hydrate.]

(b) Cell communication

(i) Humans

No data in humans were available to the Working Group.

(ii) Experimental systems

Chloral hydrate has been reported to reduce gap-junction communication in rat clone 9 cells *in vitro* (Benane *et al.*, 1996; Zhang *et al.*, 2011). There are no data as to whether cell communication is altered *in vivo*.

(c) Activation of peroxisome proliferator-activated receptor α (PPARα)

(i) Humans

No data in humans were available to the Working Group.

(ii) Experimental systems

Male Sprague-Dawley rats given drinking-water containing chloral hydrate for 7 days exhibited increased activity of the hepatic peroxisomal enzyme palmitoyl-coenzyme A oxidase, which is indicative of PPARα activation (Poon *et al.*, 2000). Male B6C3F$_1$ mice given drinking-water containing chloral hydrate for 26 weeks did not show evidence of PPARα activation, as measured by activity of hepatic cyanide-insensitive palmitoyl-coenzyme A oxidase (George *et al.*, 2000). Similarly, Leakey *et al.* (2003) reported that male B6C3F$_1$ mice given chloral hydrate by

gavage for 15 months showed no induction of the markers of PPARα activation lauric acid ω-hydroxylase activity and CYP4A immunoreactive protein when fed *ad libitum*, but increases in these markers were reported at the highest dose, (100 mg/kg bw) in dietary-controlled mice.

There were increases in the incidence of liver tumours in mice fed *ad libitum* and dietary-controlled mice (George *et al.*, 2000; Leakey *et al.*, 2003). Sprague-Dawley rats have not been tested for carcinogenicity, but a long-term bioassay in male F344 rats did not report increased incidences of liver tumours (George *et al.*, 2000). The lack of association between PPARα activation and liver tumorigenicity across experiments suggests that PPARα activation is not an important mechanistic contributor to carcinogenicity attributable to chloral hydrate.

4.3.2 Mechanisms related to lung carcinogenesis

(a) Cytotoxicity

(i) Humans

No data on mechanisms for lung carcinogenesis of chloral hydrate in humans were available to the Working Group.

(ii) Experimental systems

No data on mechanisms of lung carcinogenesis of chloral hydrate in animals were available to the Working Group. Odum *et al.* (1992) reported that exposure of mice to chloral hydrate at a concentration of 100 ppm [100 µg/mL] by inhalation for 6 hours led to pulmonary cytotoxicity in the form of bronchiolar lesions, alveolar necrosis, desquamation of the epithelium, and alveolar oedema. These effects appeared to be a direct effect of chloral hydrate, rather than a result of metabolism, since exposures to trichloroethanol and trichloroacetic acid did not result in any pulmonary effects. Odum *et al.* (1992) reported that the bronchiolar lesions appeared identical to those caused by exposure to trichloroethylene by inhalation.

4.4 Susceptibility data

4.4.1 Inter-individual variability

Early studies of sedative effects of chloral hydrate have demonstrated that its effects on the central nervous system are strain-dependent in rats (Riley *et al.*, 1979) and mice (McIntyre & Alpern, 1985), similar to that reported for ethyl alcohol. These observations suggested similarities in pathways of metabolism for chloral hydrate and ethyl alcohol. Indeed, Lipscomb *et al.* (Lipscomb *et al.*, 1997) showed that CYP2E1 is a key pathway for oxidative metabolism of trichloroethylene to chloral hydrate, that there exists up to 10-fold difference in CYP2E1 activity among humans, and that human inter-individual variability in microsomal formation of chloral hydrate correlates with CYP2E1 activity and protein levels. This study did not address the potential for these inter-individual differences in formation of chloral hydrate to affect its toxicity or carcinogenicity.

Chloral hydrate is further metabolized to trichloroacetic acid by ALDH, and to trichloroethanol by ADH. ALDH and ADH are known to be polymorphic in humans, and these polymorphisms are well known to have a major impact on cancer susceptibility in people who consume beverages containing ethanol, especially in Asian countries (IARC, 2010, 2012b, 2012c; Chang *et al.*, 2012). It has therefore been suggested that subpopulations with certain polymorphisms in the ALDH and ADH metabolic pathways may have greater than expected formation of trichloroacetic acid and thus an enhanced risk of adverse health effects after exposure to chloral hydrate or other chlorinated solvents.

The effects of chloral hydrate on the metabolism of alcohol and acetaldehyde have been suggested as a mechanism for the dramatic

effects of coexposure to chloral hydrate (as well as other chlorinated solvents) and ethyl alcohol. Firstly, such coexposures lead to more than additive sedative effects in rodents (Sharkawi *et al.*, 1983) and humans (Sellers *et al.*, 1972). Secondly, adverse health effects indicative of elevated blood levels of acetaldehyde have been described as "degreaser's flush" (Stewart *et al.*, 1974). Thus, an additional factor for individual susceptibility to adverse health effects of chloral hydrate may be coexposure to ethyl alcohol.

4.4.2 Life-stage susceptibility

Several clinical reports document adverse health effects in neonates or infants who underwent anaesthesia by chloral hydrate (Reimche *et al.*, 1989). It has been suggested that accumulation of trichloroacetic acid and trichloroethanol in the tissues of infants may be the reason for the enhanced frequency of toxic effects. Because of the immaturity of hepatic metabolism, particularly the glucuronidation pathway, and decreased glomerular filtration, the half-life of trichloroethanol is longer in infants (pre-term and full-term) than in adults. There is indirect evidence of competition for hepatic glucuronidation for bilirubin with trichloroethanol in pre-term infants. In addition, it has been shown that enzymes participating in alcohol metabolism are expressed at lower levels in infants than in adults (Tran *et al.*, 2007).

Neuroapoptosis has been detected in the infant mouse brain after exposure to chloral hydrate (Cattano *et al.*, 2008).

4.4.3 Sex differences

With regard to differences in tumour incidence between males and females in studies of long-term exposure to chloral hydrate, some studies reported differential susceptibility in males and females (see Section 3). However, major inconsistencies exist between studies for target tissue and tumour incidence, which makes it difficult to conclude with certainty that either males or females may be more susceptible to carcinogenesis induced by chloral hydrate.

[The Working Group noted that although male laboratory rodents seem to be more sensitive than females to the hepatic effects of chloral hydrate, there was no evidence for differences between men and women in their sensitivity to the sedative or adverse health effects of chloral hydrate at the recommended clinical doses.]

4.5 Toxic non-cancer effects

4.5.1 Liver toxicity, including hepatomegaly, necrosis, and enzyme changes

(a) Humans

One study has shown that sedative doses of chloral hydrate to newborns increase the likelihood of hyperbilirubinaemia (Lambert *et al.*, 1990).

(b) Experimental systems

Long-term (104 weeks) exposure of male mice to chloral hydrate at a dose equivalent to 166 mg/kg bw per day resulted in no changes in organ weights except in the liver, which showed an approximate 40% increase in absolute liver weight and in the liver-to-body weight ratio (Daniel *et al.*, 1992a). The same type of study was conducted in male and female rats with the only non-cancer effect reported as focal hepatocellular necrosis in 2 out of 10 male rats (Daniel *et al.*, 1992b).

Similar studies in mice and rats by George *et al.* (2000) also showed no effects in rats and only proliferative lesions in the liver of mice at doses up to approximately 150 mg/kg bw per day. The National Toxicology Program (NTP, 2002a) conducted studies of long-term exposure in male and female mice given chloral hydrate at doses of up to 71 mg/kg bw per day, and

observed no non-neoplastic effects. A long-term (approximately 125 weeks) study by Leuschner & Beuscher (1998) in rats given chloral hydrate at doses of up to 135 mg/kg bw per day showed no increase in non-neoplastic lesions.

The short-term toxicity of chloral hydrate has been studied in CD1 mice and Sprague-Dawley rats. In mice, administration of chloral hydrate at daily doses of 14.4 or 144 mg/kg bw by gavage for 14 consecutive days resulted in an increase in relative liver weight and a decrease in spleen size. No other changes were seen. Mice given drinking-water containing chloral hydrate at a concentration of 0.07 or 0.7 mg/mL for 90 days showed dose-related hepatomegaly in males only and significant changes in hepatic microsomal enzymes in both males and females, indicative of hepatic toxicity (Sanders *et al.*, 1982).

Male Sprague-Dawley rats were given drinking-water containing chloral hydrate at a concentration of 0.13, 1.35, or 13.5 mg/L for 7 days (Poon *et al.*, 2000). No changes were observed in body or organ weights.

4.5.2 Central nervous system

(a) Humans

Choral hydrate has long been used in both human and veterinary medicine as a sedative and hypnotic drug. The effects on the central nervous system are due to the metabolite, trichloroethanol (Shapiro *et al.*, 1969; Miller & Greenblatt, 1979).

(b) Experimental systems

Exposure of female CD1 mice to chloral at a dose of 100 ppm [603 µg/L] for 6 hours induced deep anaesthesia, which was fully reversible on cessation of exposure (Odum *et al.*, 1992).

4.5.3 Kidney toxicity

(a) Humans

No other data in humans were available to the Working Group.

(b) Experimental systems

The National Toxicity Program (NTP, 2002a) reported an increase in the incidence of glomerulosclerosis in mice fed *ad libitum* with diets containing chloral hydrate at a concentration of 25 or 100 mg/kg. Other long-term studies did not report any lesions in the kidney (Daniel *et al.*, 1992a; Leuschner & Beuscher, 1998).

4.6 Synthesis of mechanistic considerations

A comprehensive database exists to characterize the absorption, distribution, metabolism and excretion of chloral hydrate in humans and experimental animals; this database supports the conclusion that major similarities are evident between humans and rodents. Chloral hydrate is rapidly metabolized to trichloroethanol and trichloroacetic acid, which have been detected in blood and urine. Trichloroacetic acid that is formed from the metabolism of chloral hydrate is slowly metabolized and has a longer plasma half-life in humans (2–4 days) than in rodents (5–6 hours). The major excretion pathway for metabolites of chloral hydrate is in the urine. The major enzymes responsible for the metabolism of chloral hydrate are ADH and ALDH. This metabolic pathway is very similar in humans and rodents.

Strong evidence suggests that chloral hydrate is genotoxic, both *in vivo* and *in vitro*, in mammalian and other test systems (including the standard genotoxicity battery, with and without metabolic activation). The types of genotoxic damage detected encompassed mutations, chromosomal aberrations, and micronucleus

formation. One study found that in infants exposed orally to chloral hydrate, a significant increase in micronucleus formation in peripheral blood lymphocytes was observed.

Adverse health outcomes of chloral hydrate have been reported for the liver, kidney, and central nervous system, which suggests that these organs are potential targets for this chemical.

The strength of evidence for liver as a target tissue is strong; in addition to cancer in mice, some evidence of liver toxicity (hyperbilirubinaemia) has been reported in infants exposed to chloral hydrate. Available data on non-genotoxic mechanisms in the liver are almost exclusively from studies in animals. Multiple mechanisms have been suggested to operate, including a transient increase in cell proliferation, induction of the peroxisome proliferation response (inconclusive evidence for activation of PPARα in both studies in rodents, with poor correlation with tumours), and disruption of gap-junction intercellular communications (limited evidence from one study *in vitro*). The strength of evidence for non-genotoxic mechanisms in liver cancer is weak.

The strength of evidence for the central nervous system as a target tissue is strong. Chloral hydrate is a sedative in humans and in animals. The relevance of neurotoxicity to cancer is unknown.

The strength of evidence for kidney as a target tissue is weak. Some evidence of kidney toxicity (glomerular sclerosis) has been reported in studies in animals.

There is strong potential for inter-individual variability in the adverse effects of chloral hydrate. ADH and ALDH are the major metabolic enzymes for chloral hydrate, and common polymorphisms that result in differences in alcohol metabolism have been reported in humans. Consumption of alcoholic beverages may also be a susceptibility factor because of effects on alcohol metabolism enzymes that are important for the biotransformation of chloral hydrate.

5. Summary of Data Reported

5.1 Exposure data

Chloral is a chlorinated aldehyde that is readily converted to chloral hydrate in the presence of water. It can be formed as a disinfection by-product as a result of chlorine-based disinfection of water. Chloral has been used historically in the production of dichlorodiphenyltrichloroethane (DDT) and, to a lesser extent, other insecticides and pharmaceuticals. The use of chloral has declined steadily since the use of DDT has been restricted. Chloral hydrate is used as a sedative during medical procedures and to reduce anxiety related to withdrawal from drugs. Exposure to chloral hydrate may occur at microgram-per-litre levels through consumption of chlorinated drinking-water and ingestion of swimming-pool water.

5.2 Human carcinogenicity data

Only one epidemiological study has examined risk of cancer in humans after exposure to chloral hydrate. Relationships between the number of prescriptions for chloral hydrate and morbidity for all cancers, and cancers of the lung, stomach, prostate, and melanoma were examined in nested case–control analyses based on records from a health plan in California, USA, and no significant associations were observed. The single available study of carcinogenicity in humans provides no support for relationship between exposure to chloral hydrate and risk of cancer.

5.3 Animal carcinogenicity data

Chloral hydrate was evaluated for carcinogenicity in male mice in two studies with drinking-water and in two gavage studies. In the studies with drinking-water, chloral hydrate

induced a significant increase in the incidence of hepatocellular adenoma and hepatocellular carcinoma. In the gavage studies, an increased incidence in hepatocellular adenoma or carcinoma (combined) was observed.

In another study in male dietary-controlled mice treated by gavage, chloral hydrate increased the incidence and the trend in incidence of hepatocellular carcinoma.

In the single study in female mice treated by gavage, exposure to chloral hydrate was associated with increases in the trend and in the incidence of malignant lymphoma and in the incidence of adenoma of the pituitary gland.

Chloral hydrate in drinking-water was evaluated for carcinogenicity in one study in male and female rats, and in a second study in male rats. No dose-related increases in tumour incidence were reported. In one of the studies, however, the incidence of hepatocellular carcinoma in one treatment group of male rats was higher than that in historical controls. Several design and reporting deficiencies limited the power of both studies to adequately evaluate the carcinogenic potential of chloral hydrate in rats.

5.4 Mechanistic and other relevant data

Major similarities exist between humans and experimental animals with regard to the absorption, distribution and metabolism of chloral hydrate. In all species studied, rapid metabolism of chloral hydrate by alcohol and aldehyde dehydrogenases results in formation of trichloroacetic acid and trichloroethanol). However, trichloroacetic acid has a much longer plasma half-life in humans (2–4 days) than in rodents (5–6 hours), which is indicative of much slower excretion or metabolism of this metabolite in humans. Strong evidence is available from studies in humans *in vivo*, animals *in vivo* and *in vitro*, and from bacterial and other test systems, to support the conclusion that chloral hydrate is a genotoxic agent. Cancer findings in animals and toxicity findings in humans and animals designate liver as a major target organ for chloral hydrate. In the liver, non-genotoxic mechanisms may participate in carcinogenesis. Some data on kidney toxicity in rodents suggest that kidney may also be a target organ. The central nervous system is also a potential target organ because chloral hydate has a sedative effect in humans and animals. The relevance of the apparent effects on the kidney and central nervous system to the potential cancer hazard of chloral hydrate in these organs is unknown. There is potential for inter-individual variability in the adverse effects of chloral hydrate because it is metabolized through alcohol and aldehyde dehydrogenases; polymorphisms in these enzymes have been shown to have a major impact on their function. Consumption of beverages containing alcohol may also be a susceptibility factor due effects on these same enzymes.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Chloral and chloral hydrate are *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In reaching the evaluation, the Working Group considered the following:

- Chloral hydrate is absorbed, distributed and metabolized similarly in humans and in rodents;
- Chloral hydrate is genotoxic in numerous assays for genotoxicity;
- Evidence exists that chloral hydrate is genotoxic in exposed humans, supporting the conclusion that a genotoxic mechanism also operates in humans.

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