CHLORAL AND CHLORAL HYDRATE

These substances were considered by a previous Working Group, in February 1995 (IARC, 1995). Since that time, new data have become available and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

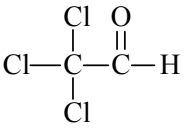
Chloral

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

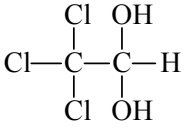
Chloral hydrate

Chem. Abstr. Serv. Reg. No.: 302-17-0
Deleted CAS Number: 109128-19-0
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: 165.40

1.1.3 *Chemical and physical properties of the pure substances*

Chloral

(*a*) *Description*: Liquid (Lide, 2000)
(*b*) *Boiling-point*: 97.8 °C (Lide, 2000)
(*c*) *Melting-point*: –57.5 ºC (Lide, 2000)
(*d*) *Density*: 1.512 at 20 °C/4 °C (Lide, 2000)
(*e*) *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)
(*f*) *Solubility*: Very soluble in water in which it is converted to chloral hydrate; soluble in diethyl ether and ethanol (Lide, 2000)
(*g*) *Volatility*: Vapour pressure, 6.66 kPa at 25 °C (Lide, 2000)
(*h*) *Stability*: Polymerizes under the influence of light and in presence of sulfuric acid to form a white solid trimer called metachloral (Budavari, 1998)
(*i*) *Conversion factor*: $mg/m^3 = 6.03 \times ppm$[a]

Chloral hydrate

(*a*) *Description*: Large, monoclinic plates with an aromatic, penetrating and slightly bitter odour and a slightly bitter, caustic taste (O'Neil, 2001)
(*b*) *Boiling-point*: 98 °C, decomposes into chloral and water (O'Neil, 2001)
(*c*) *Melting-point*: 57 °C (Lide, 2000)
(*d*) *Density*: 1.9081 at 20 °C/4 °C (Lide, 2000)

[a] Calculated from: mg/m^3 = (molecular weight/24.45) × ppm, assuming normal temperature (25 °C) and pressure (760 mm Hg)

(*e*) *Spectroscopy data*: Infrared (prism [5423]), nuclear magnetic resonance [10362] and mass [1054] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)

(*f*) *Solubility*: Freely soluble in water; freely soluble in acetone and methyl ethyl ketone; moderately or sparingly soluble in benzene, carbon tetrachloride, petroleum ether, toluene and turpentine (O'Neil, 2001)

(*g*) *Volatility*: Vapour pressure, 4.7 kPa at 20 °C; slowly evaporates on exposure to air (Jira *et al*., 1986; O'Neil, 2001)

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

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

1.1.4 *Technical products and impurities*

Trade names for chloral include Grasex and Sporotal 100; trade names for chloral hydrate include Ansopal, Aquachloral, Chloradorm, Chloraldurat, Chloralix, Dormel, Elixnocte, Escre, Hydral, Lanchloral, Lorinal, Medianox, Nervifene, Noctec, Novochlorhydrate, Nycton, Phaldrone, Rectules, Somnos, Suppojuvent Sedante, Tosyl, Trawotox and Welldorm (Royal Pharmaceutical Society of Great Britain, 2002).

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

US Pharmacopeia specifies that USP-grade chloral hydrate must contain not less than 99.5% $C_2H_3Cl_3O_2$ (Pharmacopeial Convention, 1990). Chloral hydrate is available as a 500-mg capsule, as a 325-, 500- and 650-mg suppository and as a 250- and 500-mg/mL syrup (Medical Economics Co., 1996).

1.1.5 *Analysis*

A common analytical method to determine chloral is to treat it for 2 min with 1 M sodium hydroxide, which cleaves chloral into chloroform and sodium formate; the excess alkali is then titrated with acid. Alternatively, chloral is treated with quinaldine ethyl iodide to form a blue cyanine dye, the quantity of which is measured spectrophotometrically. Gas chromatography (GC) can be used for quantitative analysis of chloral and its hydrate, which breaks down to chloral on vaporization (Jira *et al*., 1986).

Chloral hydrate has been determined in water using liquid–liquid extraction and GC with electron capture detection (GC–ECD). This method has been applied to drinking-water, water at intermediate stages of treatment and raw source water, and had a detection limit of 0.005 µg/L (Environmental Protection Agency, 1995; American Public Health Association/American Water Works Association/Water Environment Federation, 1999).

---

[a] Calculated from: mg/m^3 = (molecular weight/24.45) × ppm, assuming normal temperature (25 °C) and pressure (760 mm Hg)

In a national survey of chlorinated disinfection by-products in Canadian drinking-water conducted in 1993, the minimum quantifiable limit for this method was 0.1 μg/L (Health Canada, 1995).

An analytical survey of 16 drinking-water sources in various areas of Australia was conducted to determine the occurrence of disinfection by-products, including chloral hydrate, using a method based on a US Environmental Protection Agency standard method for the determination of chlorination disinfection by-products and chlorinated solvents in drinking-water by liquid–liquid extraction and GC–ECD (Simpson & Hayes, 1998).

The Association of Official Analytical Chemists has reported a spectrophotometric method for the determination of chloral hydrate in drugs, 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).

Mishchikhin and Felitsyn (1988) described a method for the GC determination of chloral hydrate in biological materials using four columns with different packings. The elution of the compounds was monitored with two flame ionization detectors, and the limit of detection was approximately 0.01 mg/sample.

Koppen *et al*. (1988) described the determination of trichloroethylene metabolites, including chloral hydrate, in rat liver homogenate. The method was based on selective thermal conversion of chloral hydrate into chloroform, which is determined using head-space-GC with ECD.

Liquid chromatographic methods have been developed for the determination of chloral hydrate. Optimal reversed-phase separations were achieved after derivatization of chloral hydrate with 1,2-benzenedithiol and ultraviolet detection at 220 nm. Alternatively, chloral hydrate can be reacted with sodium hydroxide to form sodium formate which is then analysed by anion-exchange liquid chromatography, with suppressed conductivity detection. Detection limits as low as 0.2 μg/L were reported (Bruzzoniti *et al*., 2001).

1.2 Production and use

1.2.1 *Production*

Chloral was first synthesized by J. von Liebig in 1832 by chlorinating ethanol; it was introduced as a hypnotic agent by Liebreich in 1869. Chloral is produced commercially by the chlorination of acetaldehyde or ethanol in hydrochloric acid, during which antimony trichloride may be used as a catalyst. Chloral hydrate is distilled from the reaction mixture and is then mixed with concentrated sulfuric acid, the heavier acid layer is drawn off, and chloral is fractionally distilled. Chloral hydrate is produced by adding water again to chloral (Jira *et al*., 1986; O'Neil, 2001).

Available information indicates that chloral (anhydrous) is produced by 14 companies in China, seven companies in India and one company each in Brazil, France, Japan, Mexico, Russia and the USA. Available information also indicates that chloral hydrate is produced by four companies in China, three companies in Germany, two companies in

Japan and one company each in Mexico, Russia and Spain (Chemical Information Services, 2002a).

Available information indicates that chloral (anhydrous) is formulated into pharmaceutical products by one company in the United Kingdom, and that chloral hydrate is formulated into pharmaceutical products by three companies each in Spain and the USA, two companies each in Belgium, France, Hungary, Switzerland and the United Kingdom and one company each in Argentina, Australia, Canada, Indonesia, Moldova and Singapore (Chemical Information Services, 2002b).

1.2.2 *Use*

Chloral was the first hypnotic drug and is a precursor in the commercial synthesis of the insecticide DDT, which was introduced in 1941. Chloral was an important chemical in the 1960s, but its importance has declined steadily since then because the use of DDT and other chlorinated insecticides has been restricted in many countries. Much smaller amounts are used to make other insecticides (methoxychlor, naled, trichlorfon and dichlorvos), a herbicide (trichloroacetic acid) and hypnotic drugs (chloral hydrate, chloral betaine, α-chloralose and triclofos sodium). Chloral hydrate also possesses anticonvulsant and muscle relaxant properties (Jira *et al*., 1986; Williams & Holladay, 1995).

As a hypnotic, chloral hydrate is principally used for the short-term (2-week) treatment of insomnia. It is used post-operatively to allay anxiety and to induce sedation and/or sleep. It is also used post-operatively as an adjunct to opiates and other analgesics to control pain. It is effective in reducing anxiety associated with the withdrawal of alcohol and other drugs such as opiates and barbiturates. It also has been used to produce sleep prior to electroencephalogram evaluations. In the USA, it has been widely used for sedation of children before diagnostic, dental or medical procedures. Following oral administration, chloral hydrate is converted rapidly to trichloroethanol, which is largely responsible for its hypnotic action; for oral use, it is sometimes given in a flavoured syrup. Externally, chloral hydrate has a rubefacient action 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).

The usual sedative dose of chloral hydrate for adults is 250 mg three times daily after meals. The usual hypnotic dose for adults is 500 mg–1 g 15–30 min before bedtime. When chloral hydrate is administered in the management of alcohol withdrawal symptoms, the usual dose is 500 mg–1 g repeated at 6-h intervals if needed. Generally, single doses or daily dosage for adults should not exceed 2 g. The sedative dose of chloral hydrate for children is 8 mg/kg bw or 250 mg/m^2 of body surface area three times a day, with a maximum dose of 500 mg three times a day. The hypnotic dose for children is 50 mg/kg bw or 1.5 g/m^2 of body surface area, with a maximum single dose of 1 g. As a premedication before electroencephalogram evaluation, children have been given chloral hydrate at a dose of 20–25 mg/kg bw (Medical Economics Co., 1996).

1.3 Occurrence

1.3.1 *Natural occurrence*

Chloral is not known to occur as a natural product.

1.3.2 *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 (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 in 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 and rapidly converted to chloral hydrate 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 (as chloral hydrate) in drinking-water as a result of chlorine-based disinfection of raw waters containing natural organic substances (see IARC, 1991). The concentrations of chloral hydrate measured in water are summarized in Table 1. Concentrations were higher in the summer than in the winter (LeBel *et al*., 1997; Williams *et al*., 1997). Kim *et al*. (2002) measured concentrations of chloral hydrate ranging from 0.19 to 30 μg/L in a model swimming pool system.

Chloral has also been detected in the spent chlorination liquor from the bleaching of sulfite pulp after oxygen treatment, at concentrations of < 0.1–0.5 g/ton of pulp (Carlberg *et al*., 1986). It has been found in trace amounts after photocatalytic degradation of trichloroethylene in water (Glaze *et al*., 1993).

1.3.5 *Other*

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

Table 1. Concentrations of chloral (as chloral hydrate) in water

Water type (location)	Concentration (μg/L)	Reference
Treatment plant (Canada)	< 0.1–15.1	Williams *et al.* (1997)
Treatment plant and distribution system (Canada)	< 0.1–23.4	LeBel *et al.* (1997)
Drinking-water (USA)[a]	7.2–18.2	Uden & Miller (1983)
Drinking-water (USA)[a]	0.01–5.0	Environmental Protection Agency (1988)
Drinking-water (USA)[a]	1.7–3.0	Krasner *et al*. (1989)
Drinking-water (USA)[a]	0.14–6.7	Koch & Krasner (1989)
Drinking-water (USA)[a]	6.3–28	Jacangelo *et al*. (1989)
Drinking-water and distribution system (USA)[a]	< 0.5–92	Blank *et al*. (2002)
Distribution system (Canada)	< 0.1–22.5	Williams *et al.* (1997)
Distribution system (Australia)	0.2–19	Simpson & Hayes (1998)
Swimming pool (Germany)	265	Baudisch *et al.* (1997)
Swimming pool (Germany)	0.3–67.5	Mannschott *et al.* (1995)

[a] Samples taken in water leaving the treatment plant

1.4 Regulations and guidelines

The WHO (1998) has established a provisional guideline of 10 μg/L for chloral and chloral hydrate in drinking-water. A provisional guideline is established when there is some evidence of a potential health hazard but where available data on health effects are limited, or where an uncertainty factor greater than 1000 has been used in the derivation of the tolerable daily intake.

The drinking-water guideline for chloral and chloral hydrate in Australia and New Zealand is 20 μg/L (National Health and Medical Research Council and Agriculture and Resource Management Council of Australia and New Zealand, 1996). This guideline also notes that the minimization of the concentration of all chlorination by-products is encouraged by reducing the amount of naturally occurring organic material in the source water, reducing the amount of chlorine added or using an alternative disinfectant, without compromising disinfection.

The European Union (European Commission, 1998) and Canada (Health Canada, 2003) have not set a guideline value but also encourage the reduction of concentrations of total disinfection by-products. The Environmental Protection Agency (1998) controls the formation of unregulated disinfection by-products in the USA, which would include chloral and chloral hydrate, with regulatory requirements for the reduction of the precursors, in this case, total organic carbon. The Stage 1 Disinfectants/Disinfection By-Product Rule mandates a reduction in the percentage of total organic carbon from source

water and to finished water on the basis of total organic carbon in source water and its alkalinity. 'Enhanced' coagulation or 'enhanced' softening are mandated as a treatment technique for this reduction, unless the levels of total organic carbon or disinfection by-products in source water are low.

2. Studies of Cancer in Humans

See Introduction to the monographs on chloramine, chloral and chloral hydrate, dichloroacetic acid, trichloroacetic acid and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of male C57BL × $C3HF_1$ mice [$B6C3F_1$ mice], 15 days of age, were fasted for 3 h and then administered a single intragastric dose of 5 (25 animals) or 10 (20 animals) μg/g bw crystalline chloral hydrate (USP grade) dissolved in distilled water. A group of 35 controls was administered distilled water only. Groups of 6–10 mice used for cell proliferation studies were killed 24 h after receiving chloral hydrate. The remainder were weaned at 4 weeks of age, and animals were killed when moribund or at intervals up to 92 weeks after treatment. The student *t*-test was used for evaluating significance between the treatment and control groups. [Data on survival and body weight were not presented.] Liver tumours [identified as nodules] were found in mice killed between 48 and 92 weeks following treatment, were classified as hyperplastic, adenomatous or trabecular and were grouped for statistical analysis. [The authors did not account for all animals.] The incidences were: control, 2/19 (10.5%; two trabecular); 5 μg/kg, 3/9 (33.3%; one hyperplastic, one adenomatous and one trabecular); and 10 μg/kg, 6/8 (75%; three adenomatous and three trabecular; $p < 0.05$) (Rijhsinghani *et al.*, 1986).

A group of 40 male $B6C3F_1$ mice, 28 days of age, was exposed to a target concentration of 1 g/L chloral hydrate (measured dose, 0.95 ± 0.14 g/L; 95% pure with no identifiable impurities) in distilled water for 104 weeks. Five mice were examined at 30 and another five at 60 weeks. A group of 33 control animals was given distilled water alone. Statistical methods included a one-factor analysis of variance (performed as two-tailed tests) for continuous variables (e.g. body and organ weights, water consumption). Data on survival and tumour counts were analysed using the log rank test and tumour prevalence was analysed by Fisher's exact test. Statistical significance was set conservatively ($p \leq 0.03$). Marginal significance was set at $0.03 < p < 0.05$. Time-weighted water consumption was 175.5 ± 18.6

for animals administered chloral hydrate versus 197.0 ± 16.4 mL/kg bw per day for those in the control group, which yielded a mean daily dose of 166 mg/kg bw per day. The slight increase in the mortality observed in the chloral hydrate-treated group was not statistically different; tissues were examined at various intervals in five animals from the control and treated groups. No tumours were found in either control or chloral hydrate-treated animals at 30 weeks. At 60 weeks, no tumours were observed in the control group (0/5); hepatocellular carcinomas (2/5 [40%]) were found in chloral hydrate-treated animals. At 104 weeks, treatment with chloral hydrate marginally increased the prevalence (7/24 [29%] versus 1/20 [5%]) and multiplicity (0.29 versus 0.10 per animal) of hepatocellular adenomas and significantly increased the prevalence (11/24 [46%] versus 2/20 [10%]; $p < 0.03$) and multiplicity (0.75 versus 0.25 per animal) of hepatocellular carcinoma in comparison with controls. The values for animals bearing both adenomas and carcinomas were 17/24 (71%) versus 3/20 (15%) ($p \leq 0.01$) and 1.04 versus 0.25 per animal ($p \leq 0.01$) in comparison with controls (Daniel *et al.*, 1992a).

Groups of 72 male B6C3F$_1$ mice, 28–30 days of age, were exposed to chloral hydrate (> 99% pure) dissolved in deionized water for 104 weeks. Target concentrations were 0.05, 0.5 and 1.0 g/L, and the measured concentrations of chloral hydrate were 0.12 ± 0.02, 0.58 ± 0.04 and 1.28 ± 0.20 g/L. Water consumption, time-weighted over 104 weeks, was 111.7 (control), 112.8 (0.12 g/L), 112.1 (0.58 g/L) and 114.5 (1.28 g/L) mL/kg bw per day, which yielded mean daily doses of 0, 13.5, 65.0 and 146.6 mg/kg bw per day, respectively, time-weighted over the duration of the study. Continuous variables were analysed using a one-way analysis of variance. Parametric and non-parametric tests were used where appropriate. Tumour prevalence (number of animals with a lesion per number of animals examined) was analysed using Fisher's exact and Fisher-Irwin (for trends with dose) tests. Tumour multiplicity (number of lesions per animal) was analysed using log-rank tests and a log-rank monotone trend test. Using one-sided tests, $p \leq 0.05$ was considered to be significant and $0.05 < p < 0.1$ was considered to be of marginal significance. No alterations in body weight were seen in any of the chloral hydrate-treated groups compared with controls. Six animals from each group were killed at 26, 52 and 78 weeks, and all remaining animals were killed at 104 weeks. Unscheduled deaths were: vehicle control, 16/72; 13.5 mg/kg per day, 9/72; 65.0 mg/kg per day, 19/72; and 146.6 mg/kg per day, 28/72. The increased number of unscheduled deaths in the high-dose group was not significant when compared with survival in the control group (log-rank test). The total number of animals examined for pathology over the course of the study was: 65/72, 69/72, 62/72 and 60/72 for the control, low, mid and high doses, respectively. Tumour prevalence and multiplicity were calculated in animals that survived longer than 78 weeks; the numbers of animals in the analysis were 42 controls, 46 low-dose, 39 mid-dose and 32 high-dose. The prevalence and multiplicity of hepatocellular adenomas were 21.4% and 0.21 ± 0.06 for controls, 43.5% and 0.65 ± 0.12 ($p \leq 0.05$) for the low dose, 51.3% and 0.95 ± 0.18 ($p \leq 0.05$) for the mid dose and 50.0% and 0.72 ± 0.15 ($p \leq 0.05$) for the high dose. The values for hepatocellular carcinoma were 54.8% and 0.74 ± 0.12 for controls, 54.3% and 0.72 ± 0.11 for the low dose, 59.0% and 1.03 ± 0.19 for the mid dose and 84.4% and 1.31 ± 0.17 ($p \leq 0.05$) for the high dose. The

prevalence and multiplicity values for animals bearing either adenoma or carcinoma were 64.5% and 0.95 ± 0.13 for controls, 78.3% and 1.37 ± 0.16 ($p \leq 0.05$, multiplicity only) for the low dose, 79.5% and 1.97 ± 0.25 ($p \leq 0.05$) for the mid dose and 90.6% and 2.03 ± 0.24 ($p \leq 0.05$) for the high dose. One adenoma occurred in the mid-dose group at 26 weeks (George *et al.*, 2000).

Groups of 48 female B6C3F_1 mice, 28 days of age, received 0 (control), 25, 50 and 100 mg/kg bw chloral hydrate (purity ~99.5%) in distilled water by gavage for 104 weeks. No differences in survival or mean body weights were observed among the treated and control animals. The incidence of hyperplasia of the pituitary gland pars distalis was 3/45 (6.7%) control, 6/44 (13.6%) low-dose, 4/47 (8.5%) mid-dose and 0/41 (0%) high-dose animals. The incidence of pars distalis adenoma was 0/45 control, 2/44 (5%) low-dose, 0/47 mid-dose and 5/41 (12%) high-dose mice ($p = 0.0237$). The historical incidence of control groups was 15/308 (4.9%) with a range of 0–6%. The incidences of malignant lymphoma were: 9/48 (19%) control, 7/48 (15%) low-dose, 8/48 (17%) mid-dose and 15/48 (31%) high-dose mice. The historical incidence for malignant lymphoma in control groups was 92/374 (24.6%) with a range of 21–43%. In a second experiment, groups of 48 female mice, 28 days of age, received 0 or 100 mg/kg bw chloral hydrate by gavage on 5 days per week. Eight mice from each group were killed at 3, 6 or 12 months, after which treatment was discontinued for the remaining animals for the duration of the study (104 weeks). Survival was similar among all treatment groups. The mean body weights of animals receiving 100 mg/kg bw chloral hydrate for 3 and 6 months tended to be greater than those of vehicle controls between 52 and 104 weeks. Incidences of pars distalis adenoma were 0/45 controls, 3/36 (8%) at 3 months, 1/36 (3%) at 6 months, 1/33 (3%) at 12 months and 5/41 (12%) at 24 months ($p = 0.0237$). In a third experiment, groups of 48 female mice, 28 days of age, received a single dose of 0, 10, 25 or 50 mg/kg bw chloral hydrate by gavage and were held for 104 weeks. No differences were observed in survival, body weight gains or neoplasia among the control and chloral hydrate-treated groups. In a fourth experiment, groups of 48 male and 48 female mice, 15 days of age, received a single oral dose of 0, 10, 25 or 50 mg/kg bw chloral hydrate by gavage and were held for 104 weeks. No differences were observed in survival, body weight or neoplasia among the control and chloral hydrate-treated groups. The authors concluded that under the conditions of the 2-year gavage study, there was equivocal evidence of carcinogenic activity for chloral hydrate based on an increased incidence of pituitary gland par distalis adenoma. No increased carcinogenicity was found for single-dose administration of chloral hydrate to male or female mice. No increase in hepatocarcinogenicity was observed under any dose regimen (National Toxicology Program, 2002a).

Groups of 120 male B6C3F_1 mice, 6 weeks of age, received chloral hydrate (99% pure) in distilled water at doses of 0, 25, 50 or 100 mg/kg bw on 5 days per week for 104–105 weeks. Animals in each dose group were divided into two dietary groups: *ad libitum* and dietary controlled. Twelve animals per group were killed for interim evaluation at 15 months. Survival of chloral hydrate-treated mice was similar to that of vehicle control animals. Mean body weights of chloral hydrate-treated animals in each dietary group did

not differ from those of the controls. Liver weights of all treated groups were greater than those of the controls, but not statistically significantly so. In the groups fed *ad libitum*, the incidence of combined hepatocellular adenomas and carcinomas was significantly greater in low-dose animals than in controls: control, 16/48 (33%); low-dose, 25/48 (52%; $p = 0.0437$); mid-dose, 23/47 (49%); and high-dose, 22/48 (46%). The incidences of hepatocellular carcinoma and of combined neoplasms occurred with a positive dose trend: control, 11/48 (23%); low-dose, 11/48 (23%); mid-dose, 14/48 (29%); and high-dose, 18/48 (38%; $p = 0.0450$); and the incidence of hepatocellular carcinomas in the high-dose dietary-controlled mice was significantly increased: control, 2/48 (4%); low-dose, 5/48 (10%); mid-dose, 4/48 (8%); and high-dose, 8/48 (17%; $p = 0.0422$) (National Toxicology Program, 2002b).

3.1.2 *Rat*

Groups of 50 male and 50 female Sprague-Dawley rats, 25–29 days of age [age at the start of treatment not specified], were administered chloral hydrate [purity not specified] dissolved in drinking-water [not specified] at concentrations that had been found to give doses of 15, 45 and 135 mg/kg bw per day for 124 weeks (males) and 128 weeks (females). Two control groups received drinking-water only. Concentrations of chloral hydrate were adjusted to the mean drinking-water intake of the previous week for each group to maintain a constant dose level relative to the body weights of the animals. None of the treatments with chloral hydrate had a significant effect on survival (Fisher's exact *t*-test) or on body weight gain, or feed and water consumption (Dunnett's multiple *t*-test). No chloral hydrate-related tumours (Peto test) or other lesions were observed at any organ site [complete histopathology performed] in the animals treated for 124 and 128 weeks. [The authors stated that chloral hydrate was ineffective in increasing neoplasia in rats but the data were not provided.] (Leuschner & Beuscher, 1998).

Groups of 78 male Fischer 344/N rats, 28–30 days of age, were administered chloral hydrate (> 99% pure) dissolved in deionized water at target concentrations of 0, 0.05, 0.5 and 2.0 g/L. Parametric and non-parametric tests were used where appropriate. Tumour prevalence (number of animals with a lesion per number of animals examined) was analysed using Fisher's exact and Fisher-Irwin (for trends with dose) tests. Tumour multiplicity (number of lesions per animal) was analysed using log-rank tests and a log-rank monotone trend test. Using one-sided tests, $p \leq 0.05$ was considered to be significant and $0.05 < p < 0.1$ was considered to be of marginal significance. The measured concentrations for the low-dose and mid-dose animals were the same as those in the experiment with mice. The measured concentrations of chloral hydrate were 0.12 ± 0.02, 0.58 ± 0.04 and 2.51 ± 0.13 g/L, respectively. Water consumption, time-weighted over 104 weeks, was 61.7 (control), 61.9 (0.12 g/L), 64.5 (0.58 g/L) and 64.8 (2.51 g/L) mL/kg bw per day, which yielded mean daily doses of 0, 7.4, 37.4, and 162.6 mg/kg bw per day time-weighted over the duration of the study, respectively. No alterations in body weight were seen in any of the chloral hydrate-treated groups compared with controls. Six animals

from each group were killed at 13, 26, 52 and 78 weeks, and all remaining animals were killed at 104 weeks. Unscheduled deaths were: vehicle control, 18/78; 7.4 mg/kg, 14/78; 37.4 mg/kg, 16/78; and 162.6 mg/kg, 18/78. The total number of animals examined for pathology over the course of the study was 71/78, 70/78, 73/78 and 74/78 for the control, low-dose, mid-dose and high-dose groups, respectively. Prevalence and multiplicity were calculated in animals that survived longer than 78 weeks. The number of animals examined were 42 control, 44 low-dose, 44 mid-dose and 42 high-dose. The prevalence and multiplicity of hepatocellular adenomas, respectively, were 0% and 0 for controls, 7.1% and 0.07 ± 0.04 per animal for the low dose, 2.3% and 0.02 ± 0.02 per animal for the mid dose, and 4.5% and 0.05 ± 0.03 per animal for the high dose. The values for hepatocellular carcinoma were 2.4% and 0.02 ± 0.02 for controls, 7.1% and 0.07 ± 0.04 for the low dose, 0 for the mid dose and 2.3% and 0.02 ± 0.02 for the high dose. The values for animals bearing either adenoma or carcinoma were 2.4% and 0.02 ± 0.02 for controls, 14.3% and 0.14 ± 0.05 for the low dose, 2.3% and 0.02 ± 0.02 for the mid dose and 6.8% and 0.07 ± 0.04 for the high dose (George *et al.*, 2000).

3.2 Intraperitoneal injection

3.2.1 *Mouse*

Groups of 22–24 male and 21–23 female neonatal B6C3F_1 mice were administered intraperitoneal injections of chloral hydrate [purity not specified] in fractionated doses at 8 and 15 days of age. A total dose of 1000 nmol was delivered in 30 μL dimethyl sulfoxide (DMSO) as one third and two thirds of the dose, and a total dose of 2000 nmol was delivered as three sevenths and four sevenths of the dose in 30 μL DMSO, respectively. As a positive control, 4-aminobiphenyl was used at total doses of 500 and 1000 nmol, respectively. The animals receiving 2000 nmol chloral hydrate were killed at 12 months of age while those receiving 1000 nmol were killed at 20 months of age. At 12 months, liver neoplasms (adenomas or carcinomas) were found in 5/24 (21% and 1.0 tumour per animal) male mice that received 2000 nmol chloral hydrate compared with 24/24 (100% and > 5.7 mean number of tumours per animal; $p < 0.0004$) mice treated with 4-aminobiphenyl. No neoplasms were observed in the DMSO controls. In male mice treated neonatally with 1000 nmol and kept for 20 months, 10/23 (43%) developed combined liver neoplasms and 1.4 tumours per animal compared with 7/23 (30%) and 1.4 tumours per animal for DMSO controls and 22/22 (100%; $p < 0.0004$) and 3.5 tumours per animal for the positive controls. No liver tumours were found in female mice that received chloral hydrate or DMSO alone. The incidence of liver adenomas in female mice treated with 500 nmol 4-aminobiphenyl was 9/23 (39%; $p < 0.006$), with 1.1 tumours per animal. The tumour incidence in male mice administered 2000 nmol chloral hydrate was one animal away from statistical significance; higher doses of chloral hydrate (2500 and 5000 nmol; one third and two thirds of the total dose injected intraperitoneally on days 8 and 15 of age, respectively) were evaluated in both male and female mice examined at 12 months of age;

1/24 male and 0/24 female mice had a liver adenoma in the low-dose treatment group. Liver adenomas were found in 2/22 males and 1/24 females in the high-dose group. One male developed a lung adenoma [no values were given either for the positive or vehicle control groups] (Von Tungeln *et al.*, 2002).

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

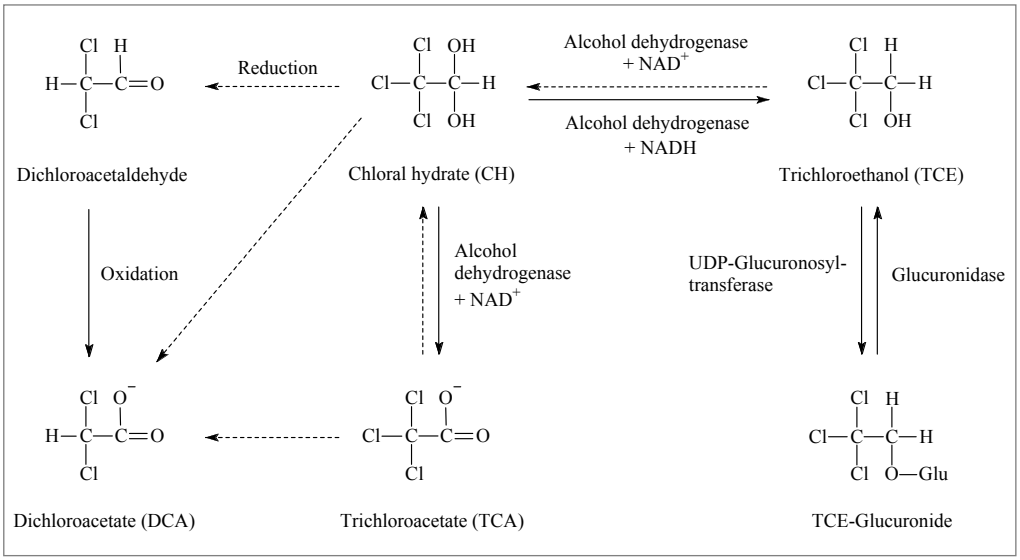
4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The metabolic pathways of chloral hydrate and its metabolites in humans are depicted in Figure 1.

The disposition of chloral hydrate has been studied in critically ill neonates and children (Mayers *et al*., 1991). Three groups of subjects (group 1, preterm infants; group 2, full-term infants; and group 3, toddler–child patients) were given a single oral dose of 50 mg/kg bw chloral hydrate. In contrast to data reported for adult humans (IARC, 1995), chloral hydrate was detected in plasma for several hours after administration. The half-lives for chloral hydrate were 1.0, 3.0, and 9.7 h and those for trichloroethanol were 39.8, 27.8 and 9.7 h in

Figure 1. Metabolic pathways of chloral hydrate and its metabolites



Dotted lines are proposed pathways and solid lines are established pathways.
NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide

groups 1, 2 and 3, respectively. The plasma concentration of trichloroacetic acid did not decline even 6 days after treatment with chloral hydrate.

The kinetics and metabolism of chloral hydrate have been studied in children aged 3 months to 18 years diagnosed with congenital lactic acidosis (Henderson *et al.*, 1997). The children received chloral hydrate, dichloroacetic acid or both, and chloral hydrate, trichloroacetic acid, dichloroacetic acid and trichloroethanol were measured in blood. In one child given 50 mg/kg bw chloral hydrate, the plasma half-life of this compound was 9.7 h. Dichloroacetic acid was also detected in this child, with a half-life of 8.0 h, compared with a half-life of 0.5–1.5 h in children given 25 mg/kg bw dichloroacetic acid. In a child given 12.5 mg/kg bw [1,2-^{13}C]dichloroacetic acid orally 15 min before administration of 50 mg/kg bw chloral hydrate, the half-lives of dichloroacetic acid and trichloroethanol were 0.34 h and 9.7 h, respectively. In a child given 50 mg/kg bw chloral hydrate 20 h before or immediately after administration of 12.5 mg/kg bw [1,2-^{13}C]dichloroacetic acid, the half-lives of dichloroacetic acid and trichloroethanol were 3.18 h and 7.2 h, respectively. The authors note that the half-life of dichloroacetic acid was greater when it was formed as a metabolite of chloral hydrate than when given directly and that the half-life of dichloroacetic acid was prolonged in a child given two doses of chloral hydrate. These data indicate that chloral hydrate or a metabolite of chloral hydrate, or both, inhibit the metabolism of dichloroacetic acid. [The Working Group noted that reported blood concentrations of dichloroacetic acid after administration of chloral hydrate may be confounded by the observation that trichloroacetic acid, a metabolite of chloral hydrate, may be converted to dichloroacetic acid in freshly drawn blood (Ketcha *et al.*, 1996).]

The bioavailability and pharmacokinetics of chloral hydrate have been studied in human volunteers (Zimmermann *et al.*, 1998). Eighteen healthy male subjects (aged 20–31 years) were given 250 or 500 mg chloral hydrate either in immediate-release or enteric-coated modified-release capsules or as a solution. Because of the extensive first-pass metabolism of chloral hydrate, the bioavailability of trichloroethanol was used as a surrogate for its absorption. The bioavailability of chloral hydrate given in capsules amounted to 94.8–101.6% of that given as a solution. The terminal half-lives for the elimination of trichloroethanol and trichloroacetic acid from plasma were 9.3–10.2 and 89–94 h, respectively.

1-Trichloromethyl-2,3,4,9-tetrahydro-1*H*-β-carboline (also called 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline), a dopaminergic neurotoxin formed by the endogenous reaction of chloral hydrate and tryptamine, was identified at concentrations of up to 20 ng/mL in the blood of five humans (65–82 years old) suffering from Parkinson's disease, who were given 750–3000 mg chloral hydrate orally over 2–7 days (Bringmann *et al.*, 1999).

4.1.2 *Experimental systems*

The pharmacokinetics and metabolism of chloral hydrate have been investigated in male B6C3F$_1$ mice (Abbas *et al.*, 1996). In mice given 11.2, 112 and 336 mg/kg bw

chloral hydrate intravenously, the clearances from the systemic circulation were 36, 20 and 7.6 L/h/kg and the half-lives were 0.09, 0.32, and 0.40 h, respectively, indicating saturable kinetics. Only 0.1–0.2% of the administered doses was eliminated unchanged in the urine. Trichloroethanol and trichloroethyl glucuronide were formed rapidly after treatment with chloral hydrate, with elimination half-lives of 0.26, 0.34, and 0.36 h for trichloroethanol and 0.21, 0.36, and 0.72 h for trichloroethyl glucuronide after the low, mid and high doses, respectively. Only a small amount of trichloroethanol was excreted in the urine (0.63–1.7% of the initial dose of chloral hydrate), but the excretion of trichloroethyl glucuronide amounted to 52–72% of the administered dose. Trichloroacetic acid was formed as a metabolite of chloral hydrate and was excreted in urine in amounts of 10–35% of the dose of the parent compound. Dichloroacetic acid was found in blood and liver, but not in the urine.

The effect of enterohepatic circulation on the pharmacokinetics of chloral hydrate was studied in male Fischer 344 rats (Merdink *et al.*, 1999). Cannulae were introduced into the bile duct and jugular vein of the rats, and chloral hydrate was infused into the jugular vein at doses of 12, 48 or 192 mg/kg bw. The elimination of chloral hydrate from the blood showed biphasic kinetics in both bile duct-cannulated and control rats; the half-lives for the first and second phases of elimination were estimated to be 0.09 and 0.75 h, respectively. There was no difference in the elimination half-lives of chloral hydrate from the plasma or its whole-body clearance between control and bile-duct-cannulated rats. Less than 1% of the total body clearance of chloral hydrate was attributed to renal or biliary clearance, and the remainder was attributed to its metabolism to trichloroacetic acid and trichloroethanol. The metabolism of chloral hydrate to trichloroacetic acid predominated at low doses (12 and 48 mg/kg bw), whereas metabolism to trichloroethanol predominated at higher doses (192 mg/kg bw).

The extrahepatic metabolism of chloral hydrate was studied in male and female mongrel dogs with a hepatic bypass and control animals that were given 25 mg/kg bw intravenously (Hobara *et al.*, 1987). The plasma and urine concentrations of unchanged chloral hydrate were higher in dogs with a hepatic bypass than in control animals (non-bypass dogs), as were serum concentrations of trichloroethanol. Trichloroethanol concentrations in urine were higher in bypass animals compared with controls except at 30 and 60 min after administration of chloral hydrate. Concentrations of trichloroethyl glucuronide and trichloroacetic acid in serum and urine were higher in controls than in bypass dogs at all sampling times. The authors concluded that there was significant extrahepatic conversion of chloral hydrate to trichloroethanol, but that formation of trichloroethyl glucuronide and trichloroacetic acid in extrahepatic tissues was lower than in animals with intact hepatic circulation.

The intestinal absorption of chloral hydrate was studied in male and female mongrel dogs fitted with jejunal, ileal and colonic loops that were perfused with a 264 µg/mL solution in phosphate buffer (pH 7.0) (Hobara *et al.*, 1988). The absorbed fraction of chloral hydrate was about 50% in the jejunum and ileum, and about 40% in the colon.

The metabolism of chloral hydrate was investigated in male and female B6C3F$_1$ mice and Fischer 344 rats given one dose or 12 doses (over a 16-day period) of 50 or 200 mg/kg bw by gavage (Beland *et al.*, 1998; Beland, 1999). Trichloroacetic acid was the major metabolite detected in the plasma of both rats and mice. Plasma concentrations of trichloroethanol were higher in female mice and rats than in males; in contrast, plasma concentrations of trichloroethyl glucuronide were higher in male than in female mice. The half-lives of trichloroethanol and trichloroethyl glucuronide were greater in mice than in rats. Pharmacokinetic analysis showed that the elimination half-life of chloral hydrate from plasma was similar in both rats and mice. In mice, but not in rats, the rate of elimination of trichloroacetic acid was greater in animals given 12 doses than in animals given one dose.

The metabolism of chloral hydrate to dichloroacetic acid was studied in male B6C3F$_1$ mice given 50 mg/kg bw intravenously, but dichloroacetic acid was not detected in the blood (limit of detection, 0.2 μg/mL) (Merdink *et al.*, 1998).

The kinetics of the dismutation of chloral hydrate by guinea-pig pulmonary carbonyl reductase has been studied (Hara *et al.*, 1991). The enzyme irreversibly converted choral hydrate into trichloroacetic acid and trichloroethanol in the presence of the reduced or oxidized cofactors, of which nicotinamide adenine dinucleotide (phosphate) ($NADP^+$) gave a higher reaction rate than did reduced $NADP^+$, and the concentration ratios of the two products to chloral hydrate utilized were 1:1. In the $NADP^+$-linked reaction trichloroacetic acid was the predominant product and its amount was compatible with that of trichloroethanol plus reduced $NADP^+$ produced, whereas in the reduced $NADP^+$-linked reaction equal amounts of trichloroacetic acid and trichloroethanol were formed and the cofactor was little oxidized. The steady-state kinetic measurements in the $NADP^+$-linked chloral hydrate oxidation were consistent with an ordered Bi-Bi mechanism which is the same as that for the secondary alcohol oxidation by the enzyme.

A comparison of the biotransformation of chloral hydrate to trichloroacetic acid and trichloroethanol in rat, mouse and human liver and blood is available (Lipscomb *et al.*, 1996). In hepatic 700 × *g* supernatant fractions (containing microsomes and mitochondria) from male B6C3F$_1$ mice, male Fischer 344 rats and humans, nicotinamide adenine dinucleotide (NAD^+) supported the oxidation of chloral hydrate to trichloroacetic acid and reduced nicotinamide adenine dinucleotide (NADH) supported the reduction of chloral hydrate to trichloroethanol. Kinetic analysis showed that the K_m for the reduction of chloral hydrate to trichloroethanol was at least 10-fold lower than that for the formation of trichloroacetic acid in all three species. Studies with lysed whole blood showed that chloral hydrate is biotransformed to both trichloroacetic acid and trichloroethanol, but that the latter metabolite predominates. Formation of trichloroacetic acid in blood was greater in humans and mice than in rats, and more trichloroethanol was formed in rodent blood than in human blood.

The biotransformation of chloral hydrate was studied in hepatic microsomal fractions isolated from control and pyrazole-treated male B6C3F$_1$ mice (Ni *et al.*, 1994, 1996; Beland, 1999). Incubation of chloral hydrate in the presence of the free radical trapping agent, *N*-*tert*-butyl-α-phenylnitrone, with microsomes from control and pyrazole-treated

mice [oxygen concentration not stated] and analysis by electron spin resonance spectroscopy showed the formation of a carbon-centered free radical with a spectrum similar to that of carbon tetrachloride and trichloroacetic acid. Incubation of [1-^{14}C]chloral hydrate with horseradish peroxidase and prostaglandin H synthase led to the formation of [^{14}C]carbon dioxide (3.0 and 5.2%, respectively), indicating the formation of one-electron intermediates (Beland, 1999).

Trichloroethanol binds to bovine serum albumin with a K_D of 3.3 mmol/L, inducing a conformational change in this protein (Solt & Johansson, 2002).

4.1.3 *Comparison of humans and animals*

The metabolic fate of chloral hydrate is qualitatively similar in humans and animals: trichloroacetic acid, dichloroacetic acid and trichloroethanol have been detected in blood, and trichloroethyl glucuronide is the major urinary metabolite. In humans, the half-life of chloral hydrate ranged from ~1 to ~10 h (Mayers *et al.*, 1991; Zimmermann *et al.*, 1998), whereas in mice and rats, the half-life of chloral hydrate was < 1 h (Abbas *et al.*, 1996; Merdink *et al.*, 1999).

4.2 Toxic effects

4.2.1 *Humans*

Chloral hydrate is used clinically as a sedative or hypnotic.

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 overdoses of 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, 1996).

4.2.2 *Experimental systems*

The oral 50% lethal dose (LD_{50}) of chloral hydrate in rats was 480 mg/kg bw (Goldenthal, 1971); LD_{50s} in mice were reported to be 1442 mg/kg bw in males and 1265 mg/kg bw in females (Sanders *et al*., 1982). The immediate 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 in males only and significant changes in hepatic microsomal enzymes in both males and females, indicative of hepatic toxicity (Sanders *et al.*, 1982).

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, only male rats 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 µg/L] 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).

Male Sprague-Dawley rats were administered chloral hydrate in the drinking-water for 7 days at concentrations of 0.13, 1.35 or 13.5 mg/L (Poon *et al*., 2000). No changes were observed in body or organ weights. In the high-dose group, a threefold increase in palmitoyl-coenzyme A oxidase, a marker of peroxisome proliferation was observed. Hepatic, but not serum, cholesterol and triglycerides were decreased and glutathione *S*-transferases and glutathione levels were elevated at this dose. A significant increase in levels of serum trichloroacetic acid was also observed in the high-dose group; the authors concluded that the hepatic effects were probably attributable to this metabolite.

The effect of dietary restriction on the carcinogenicity of chloral hydrate was studied in male B6C3F$_1$ mice. In mice fed diets *ad libitum*, the only histopathological effect was an increase in the incidence of glomerulosclerosis with 25 and 100 mg/kg chloral hydrate. There appeared to be a small increase in the incidence of renal tubule degeneration at the high dose in the group fed controlled diets. Although a significant increase in hepatocellular carcinomas was observed at 100 mg/kg per day in the dietary-controlled groups (see Section 3), there was no associated increase in non-neoplastic pathology in the liver. However, there was evidence of peroxisome proliferation, as indicated by a significant induction of cytochrome P450 4A and lauric acid ω-hydroxylase activity, only at doses of 100 mg/kg chloral hydrate and above in dietary-controlled animals. Significant increases in palmitoyl coenzyme A fatty acid hydroxylase activity were observed in the dietary-controlled and calorically restricted animals, but only at doses of ≥ 250 mg/kg. The carcinogenesis bioassays in all three dietary groups were conducted at doses ≤ 100 mg/kg (National Toxicology Program, 2002b).

Incubation of chloral hydrate with male B6C3F$_1$ mouse liver microsomes resulted in increased amounts of lipid peroxidation products (malondialdehyde and formaldehyde). This effect was 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 hydrate 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 breastfeeding infants may be sedated by chloral hydrate in breast milk, the highest concentration measured in the milk (about 15 μg/mL) was considerably lower than that which would be measured in blood at a clinically active dose (100 μg/mL) (Bernstine *et al*., 1956; Wilson, 1981).

4.3.2 *Experimental systems*

In-vitro evidence indicates that chloral hydrate induces a meiotic delay in maturing mouse oocytes (Eichenlaub-Ritter & Betzendahl, 1995; Eichenlaub-Ritter *et al*., 1996). This effect on oocyte maturation apparently dominates the aneugenic effects. The concentrations required for meiotic delay (10 μg/mL) were two to three orders of magnitude below those required to affect tubulin polymerization (Brunner *et al.*, 1991; Wallin & Hartley-Asp, 1993). Meiotic arrest occurred at 125 μg/mL.

Chloral hydrate or a metabolite reached the testis and produced spermatid micronuclei of mice given single intraperitoneal injections of 41, 83 or 165 mg/kg bw and evaluated at 49 days following treatment (Allen *et al.*, 1994). The spermatogonial sperm-cell phase was significantly affected at all three doses. There was some evidence of increased frequency of micronucleated spermatids when treatment was in the preleptotene stage with the mid dose; the response was not dose-dependent. The leptotene–zygotene and the diakinesis-metaphase I phases appeared to be insensitive to chloral hydrate.

The parameters of reproduction were evaluated in male Fischer 344 rats administered 0.5 and 2 g/L chloral hydrate in the drinking-water for 52 weeks beginning at 28 days of age. At the high dose, corresponding to 188 mg/kg bw, chloral hydrate significantly decreased both the percentage of motil and progressively motil sperm, and shifted the straight-line velocity distribution of sperm to a lower modal velocity range (Klinefelter *et al*., 1995). Administration of doses of chloral hydrate that are somewhat higher than the human therapeutic dose to pregnant mice (21.3 and 204.8 mg/kg per day in drinking-water during the gestational period) 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

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 2 for details and references)

The results obtained with chloral hydrate in a collaborative European Union project on aneuploidy have been summarized (Adler, 1993; Natarajan, 1993; Parry, 1993), and the genetic and related effects of chloral hydrate have been reviewed (Leuschner & Beuscher, 1998; Beland, 1999; Moore & Harrington-Brock, 2000).

(*a*) *DNA binding*

In single studies in mice *in vivo*, chloral hydrate increased the level of malondialdehyde-derived adduct and 8-oxo-2′-deoxyguanosine in liver DNA (Von Tungeln *et al.*, 2002) but radioactively labelled chloral hydrate did not bind to liver DNA (Keller & Heck, 1988). Malondialdehyde adducts were formed when chloral hydrate was incubated with calf thymus DNA and microsomes from male B6C3F_1 mouse liver *in vitro* (Ni *et al.*, 1995).

(*b*) *Mutation and allied effects*

Chloral hydrate did not induce mutation in most strains of *Salmonella typhimurium*, but did in four of six studies with *S. typhimurium* TA100 and in two studies with *S. typhimurium* TA104. The latter response was inhibited by the free-radical scavengers α-tocopherol and menadione (Ni *et al.*, 1994; Beland, 1999). It did not induce reverse mutation in *Saccharomyces cerevisiae* D_7 in one study.

Chloral hydrate did not induce mitotic crossing over in *Aspergillus nidulans* in the absence of metabolic activation. In *S. cerevisiae*, weak induction of mitotic gene conversion after metabolic activation and of meiotic recombination in the absence of metabolic activation was seen, whereas no intrachromosomal recombination was observed.

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 negative with respect to induction of chromosome loss and gain.

In *Drosophila melanogaster,* chloral hydrate induced somatic mutations in a wing-spot test, but was either negative or inconclusive in sex-linked lethal mutation induction experiments.

In single studies, it was reported that chloral hydrate did not produce DNA–protein associations in rat liver nuclei or DNA single-strand breaks or alkaline-labile sites in rat primary hepatocytes *in vitro*.

In a single in-vitro study, a weak positive response was observed in the gene mutation test on a mouse lymphoma cell line.

In single in-vitro studies, a dose-dependent induction of sister chromatid exchanges was seen in chloral hydrate-treated Chinese hamster ovary cells.

Table 2. Genetic and related effects of chloral hydrate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SOS chromotest, *Escherichia coli* PQ37	–	–	10 000	Giller *et al.* (1995)
Salmonella typhimurium TA100, TA1535, TA98, reverse mutation	–	–	10 000	Waskell (1978)
Salmonella typhimurium TA100, TA1537, TA1538, TA98, reverse mutation	+	+	1000	Haworth *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	–	–	5000 µg/plate	Leuschner & Leuschner (1991)
Salmonella typhimurium TA100, reverse mutation	+	+	2000 µg/plate	Ni *et al.* (1994)
Salmonella typhimurium TA100, reverse mutation, liquid medium	–	+	300	Giller *et al.* (1995)
Salmonella typhimurium TA100, reverse mutation	+	+	1000 µg/plate	Beland (1999)
Salmonella typhimurium TA104, reverse mutation	+	+	1000 µg/plate	Ni *et al.* (1994)
Salmonella typhimurium TA104, reverse mutation	+	+	1000	Beland (1999)
Salmonella typhimurium TA1535, reverse mutation	–	–	1850	Leuschner & Leuschner (1991)
Salmonella typhimurium TA1535, TA1537, reverse mutation	–	–	6667	Haworth *et al.* (1983)
Salmonella typhimurium TA1535, reverse mutation	–	–	10 000	Beland (1999)
Salmonella typhimurium TA98, reverse mutation	–	–	7500	Haworth *et al.* (1983)
Salmonella typhimurium TA98, reverse mutation	?	–	10 000 µg/plate	Beland (1999)
Saccharomyces cerevisiae D7, reverse mutation	–	–	3300	Bronzetti *et al.* (1984)
Saccharomyces cerevisiae RSY6, intrachromosomal recombination	–	NT	16.5	Howlett & Schiestl (2000)
Aspergillus nidulans, diploid strain 35 × 17, mitotic crossing-over	–	NT	1650	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid strain 30, mitotic crossing-over	–	NT	6600	Käfer (1986)
Aspergillus nidulans, diploid strain NH, mitotic crossing-over	–	NT	1000	Kappas (1989)
Aspergillus nidulans, diploid strain P1, mitotic crossing-over	–	NT	990	Crebelli *et al.* (1991)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae D7, mitotic gene conversion	–	(+)	2500	Bronzetti *et al.* (1984)
Aspergillus nidulans, diploid strain 35 × 17, haploids and nondisjunctional diploids	+	NT	825	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid strain 30 conidia, aneuploidy	+	NT	825	Käfer (1986)
Aspergillus nidulans, haploid conidia, aneuploidy and polyploidy	+	NT	1650	Käfer (1986)
Aspergillus nidulans, diploid strain NH, nondisjunctional mitotic segregants	+	NT	450	Kappas (1989)
Aspergillus nidulans, diploid strain P1, nondisjunctional diploids and haploids	+	NT	660	Crebelli *et al.* (1991)
Aspergillus nidulans, haploid strain 35, hyperploidy	+	NT	2640	Crebelli *et al.* (1991)
Saccharomyces cerevisiae, meiotic recombination	?	NT	3300	Sora & Agostini Carbone (1987)
Saccharomyces cerevisiae, disomy in meiosis	+	NT	2500	Sora & Agostini Carbone (1987)
Saccharomyces cerevisiae, diploids in meiosis	+	NT	3300	Sora & Agostini Carbone (1987)
Saccharomyces cerevisiae D61.M, mitotic chromosomal malsegregation	+	NT	1000	Albertini (1990)
Saccharomyces cerevisiae diploid strain D6, monosomy	+	NT	1000	Parry *et al.* (1990)
Seedlings of hexaploid Chinese spring wheat, Neatby's strain, chromosomal loss and gain	–	NT	5000	Sandhu *et al.* (1991)
Haemanthus katherinae endosperm, apolar mitosis, *in vitro*	+	NT	200	Molè-Bajer (1969)
Drosophila melanogaster, somatic mutation wing spot test	+		825	Zordan *et al*. (1994)
Drosophila melanogaster, induction of sex-linked lethal mutation	?		37.2 feed	Beland (1999)
Drosophila melanogaster, induction of sex-linked lethal mutation	–		67.5 inj	Beland (1999)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA–protein cross-links, rat liver nuclei *in vitro*	–	NT	41 250	Keller & Heck (1988)
DNA single-strand breaks (alkaline unwinding), rat primary hepatocytes *in vitro*	–	NT	1650	Chang *et al.* (1992)
Gene mutation, mouse lymphoma L5178Y/TK$^{+/-}$ -3.7.2C cell line *in vitro*	(+)		1000	Harrington-Brock *et al.* (1998)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	100	Beland (1999)
Micronucleus formation (kinetochore-positive), Chinese hamster C1-1 cells *in vitro*	+	NT	165	Degrassi & Tanzarella (1988)
Micronucleus formation (kinetochore-negative), Chinese hamster C1-1 cells *in vitro*	–	NT	250	Degrassi & Tanzarella (1988)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells *in vitro*	+	NT	400	Parry *et al.* (1990)
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells *in vitro*	+	NT	400	Lynch & Parry (1993)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	NT	316	Seelbach *et al.* (1993)
Micronucleus formation, mouse lymphoma L5178Y/TK$^{+/-}$ -3.7.2C cell line *in vitro*	–	NT	1300	Harrington-Brock *et al.* (1998)
Micronucleus formation, mouse lymphoma L5178Y/TK$^{+/-}$ -3.7.2C cell line *in vitro*	+	NT	500	Nesslany & Marzin (1999)
Chromosomal aberrations, Chinese hamster CHED cells *in vitro*	+	NT	20	Furnus *et al.* (1990)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	+	1000	Beland (1999)
Chromosomal aberrations, mouse lymphoma L5178Y/TK$^{+/-}$ -3.7.2C cell line *in vitro*	(+)	NT	1250	Harrington-Brock *et al.* (1998)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Multipolar mitotic spindles, Chinese hamster DON.Wg3H cells *in vitro*	+	NT	500	Parry *et al.* (1990)
Multipolar mitotic spindles, Chinese hamster DON.Wg3H cells *in vitro*	+	NT	50	Warr *et al.* (1993)
Aneuploidy, Chinese hamster CHED cells *in vitro*	+[c]	NT	10	Furnus *et al.* (1990)
Aneuploidy, primary Chinese hamster embryonic cells *in vitro*	+[c]	NT	250	Natarajan *et al.* (1993)
Aneuploidy (hypoploidy), Chinese hamster LUC2 p4 cells *in vitro*	+	NT	250	Warr *et al.* (1993)
Aneuploidy, mouse lymphoma L5178Y/TK$^{+/-}$ -3.7.2C cell line *in vitro*	–	NT	1300	Harrington-Brock *et al.* (1998)
Tetraploidy and endoreduplication, Chinese hamster LUC2 p4 cells *in vitro*	+	NT	500	Warr *et al.* (1993)
Lacking mitotic spindle, Chinese hamster DON.Wg3H cells *in vitro*	+	NT	250	Parry *et al.* (1990)
Lacking mitotic spindle, metaphase defects, Chinese hamster LUC1 cells *in vitro*	+	NT	50	Parry *et al.* (1990)
Chromosomal dislocation from mitotic spindle, Chinese hamster DON.Wg3H cells *in vitro*	+	NT	500	Parry *et al.* (1990); Warr *et al.* (1993)
Bivalent chromosomes in meiosis I, MF1 mouse oocytes *in vitro* (16 h treatment)	+	NT	10	Eichenlaub-Ritter *et al.* (1996)
Aberrant meiosis I spindle (fusiform poles), meiotic arrest, hypoploidy, MF1 mouse oocytes *in vitro* (16 h treatment)	+	NT	125	Eichenlaub-Ritter *et al.* (1996)
Inhibition of spindle elongation, PtK2 rat kangaroo kidney epithelial cells *in vitro*	+	NT	1000	Lee *et al.* (1987)
Inhibition of chromosome-to-pole movement, PtK2 rat kangaroo kidney epithelial cells *in vitro*	–	NT	1000	Lee *et al.* (1987)
Breakdown of mitotic microtubuli, PtK2 rat kangaroo kidney epithelial cells *in vitro*	+	NT	1000	Lee *et al.* (1987)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Porcine brain tubulin assembly inhibition *in vitro*	+	NT	9900	Brunner *et al.* (1991)
Porcine brain tubulin disassembly inhibition *in vitro*	+	NT	40	Brunner *et al.* (1991)
Bovine brain tubulin assembly inhibition *in vitro*	(+)	NT	165	Wallin & Hartley-Asp (1993)
Centriole migration block (kinetochore ultrastructure change), Chinese hamster cells (clone 237) *in vitro*	+	NT	1000	Alov & Lyubskii (1974)
Cell transformation, Syrian hamster embryo cells (24 h treatment)	+	NT	350	Gibson *et al.* (1995)
Cell transformation, Syrian hamster embryo cells (7 day treatment)	+	NT	1	Gibson *et al.* (1995)
Cell transformation, Syrian hamster dermal cell line 21NSR (24 h treatment)	+	NT	50	Parry *et al.* (1996)
Inhibition of intercellular communication, B6C3F1 mouse and Fischer 344 rat hepatocytes *in vitro*	–	NT	83	Klaunig *et al.* (1989)
Inhibition of intercellular communication, Sprague-Dawley rat liver Clone 9 cell *in vitro*	+	NT	165	Benane *et al.* (1996)
DNA single-strand breaks (alkaline unwinding), human lymphoblastoid CCRF-CEM cells *in vitro*	–	NT	1650	Chang *et al.* (1992)
Gene mutation, *tk* locus, human lymphoblastoid H2E1 V2[d] cell line *in vitro*	+	NT	1000	Beland (1999)
Gene mutation, *hprt* locus, human lymphoblastoid H2E1 V2[d] cell line *in vitro*	+	NT	1000	Beland (1999)
Sister chromatid exchange, human lymphocytes *in vitro*	(+)	NT	54	Gu *et al.* (1981)
Micronucleus formation, isolated human lymphocytes *in vitro*	–	–	1500	Vian *et al.* (1995)
Micronucleus formation, human lymphocytes in whole blood *in vitro*	+	NT	100	Migliore & Nieri (1991)
Micronucleus formation, human lymphocytes *in vitro*	(+)	NT	100	Ferguson *et al.* (1993)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, human lymphocytes *in vitro*	+	–	100	Van Hummelen & Kirsch-Volders (1992)
Micronucleus formation, human lymphoblastoid AHH-1[d] cell line *in vitro*	+	NT	100	Parry *et al.* (1996)
Micronucleus formation, human lymphoblastoid MCL-5[d] cell line *in vitro*	–	NT	500	Parry *et al.* (1996)
Micronucleus formation (kinetochore-positive), human diploid LEO fibroblasts *in vitro*	+	NT	120	Bonatti *et al.* (1992)
Aneuploidy (double Y induction), human lymphocytes *in vitro*	+	NT	250	Vagnarelli *et al.* (1990)
Aneuploidy (hyperdiploidy and hypodiploidy), human lymphocytes *in vitro*	+	NT	50	Sbrana *et al.* (1993)
Polyploidy, human lymphocytes *in vitro*	+	NT	137	Sbrana *et al.* (1993)
C-Mitosis, human lymphocytes *in vitro*	+	NT	75	Sbrana *et al.* (1993)
Host-mediated assay, *Saccharomyces cerevisiae* D7 recovered from CD1 mouse lungs, mitotic gene conversion, *in vivo*	(+)		500 po × 1	Bronzetti *et al.* (1984)
DNA single-strand breaks (alkaline unwinding), male Sprague-Dawley rat liver *in vivo*	+		300 po × 1	Nelson & Bull (1988)
DNA single-strand breaks (alkaline unwinding), male Fischer 344 rat liver *in vivo*	–		1650 po × 1	Chang *et al.* (1992)
DNA single-strand breaks (alkaline unwinding), male B6C3F$_1$ mouse liver *in vivo*	+		100 po × 1	Nelson & Bull (1988)
DNA single-strand breaks (alkaline unwinding), male B6C3F$_1$ mouse liver *in vivo*	–		825 po × 1	Chang *et al.* (1992)
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes *in vivo*	–		500 ip × 1	Leuschner & Leuschner (1991)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, BALB/c mouse spermatids *in vivo* (preleptotene spermatocytes treated)	–		83 ip × 1	Russo & Levis (1992a)
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids (diakinesis/metaphase I and metaphase II stages treated) *in vivo*	+		83 ip × 1	Russo & Levis (1992b)
Micronucleus formation (kinetochore-positive and -negative), male BALB/c mouse bone-marrow erythrocytes *in vivo*	+		200 ip × 1	Russo *et al.* (1992)
Micronucleus formation, male (C57Bl/Cne × C3H/Cne)F_1 mouse bone-marrow erythrocytes *in vivo*	–		400 ip × 1	Leopardi *et al.* (1993)
Micronucleus formation, C57B1 mouse spermatids *in vivo* (spermatogonial stem cells and preleptotene spermatocytes treated)	+		41 ip × 1	Allen *et al.* (1994)
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes *in vivo*	+		200 ip × 1	Marrazini *et al.* (1994)
Micronucleus formation, B6C3F_1 mouse spermatids after spermatogonial stem-cell treatment *in vivo*	+		165 ip × 1	Nutley *et al.* (1996)
Micronucleus formation, B6C3F_1 mouse spermatids after meiotic cell treatment *in vivo*	–		413 ip × 1	Nutley *et al.* (1996)
Micronucleus formation, male (102/E1×C3H/E1)F_1, BALB/c mouse peripheral-blood erythrocytes *in vivo*	–		200 ip × 1	Grawé *et al.* (1997)
Micronucleus formation, male B6C3F_1 mouse bone-marrow erythrocytes *in vivo*	+		500 ip × 3	Beland (1999)
Micronucleus formation, *Pleurodeles waltl newt larvae* peripheral erythrocytes *in vivo*	+		200[e]	Giller *et al.* (1995)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, male and female (102/E1 × C3H/E1)F_1 mouse bone-marrow cells *in vivo*	–		600 ip × 1	Xu & Adler (1990)
Chromosomal aberrations, male and female Sprague-Dawley rat bone-marrow cells *in vivo*	–		1000 po × 1	Leuschner & Leuschner (1991)
Chromosomal aberrations, BALB/c mouse spermatogonia treated, spermatogonia observed *in vivo*	–		83 ip × 1	Russo & Levis (1992b)
Chromosomal aberrations, (C57B1/Cne × C3H/Cne)F_1 mouse secondary spermatocytes (staminal gonia–pachytene treated), *in vivo*	+		82.7 ip × 1	Russo *et al.* (1984)
Chromosomal aberrations (translocations, breaks and fragments), (C57B1/Cne × C3H/Cne)F_1 mouse primary and secondary spermatocytes (from differentiating spermatogonia-pachytene stages treated), *in vivo*	–		413 ip × 1	Liang & Pacchierotti (1988)
Chromosomal aberrations, male Swiss CD-1 mouse bone-marrow erythrocytes *in vivo*	–		400 ip × 1	Marrazini *et al.* (1994)
Chromosomal aberrations, ICR mouse oocytes *in vivo*	–		600 ip × 1	Mailhes *et al.* (1993)
Gonosomal and autosomal univalents (C57B1/Cne × C3H/Cne)F_1 mouse primary spermatocytes (from differentiating spermatogonia–pachytene stages treated)	–	NT	413 ip × 1	Liang & Pacchierotti (1988)
Aneuploidy (C57Bl/Cne × C3H/Cne)F_1 mouse secondary spermatocytes (from differentiating spermatogonia-pachytene stages treated) *in vivo*	+		82.7 ip × 1	Russo *et al.* (1984)
Aneuploidy (C57B1/Cne × C3H/Cne)F_1 mouse secondary spermatocytes *in vivo* (from differentiating spermatogonia–pachytene stages treated) *in vivo*	(+)		165 ip × 1	Liang & Pacchierotti (1988)
Aneuploidy (hypoploidy and hyperploidy), ICR mouse oocytes *in vivo*	–[f]		200 ip × 1	Mailhes *et al.* (1988)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Polyploidy, male and female (102/E1 × C3H/E1)F_1 mouse bone-marrow cells *in vivo*	–		600 ip × 1	Xu & Adler (1990)
Aneuploidy (102/E1 × C3H/E1)F_1 mouse secondary spermatocytes *in vivo*	+		200 ip × 1	Miller & Adler (1992)
Aneuploidy, male (C57Bl/Cne × C3H/Cne)F_1 mouse bone marrow *in vivo*	+[c]		400 ip × 1	Leopardi *et al.* (1993)
Aneuploidy (C57Bl/Cne × C3H/Cne)F_1 mouse secondary spermatocytes *in vivo*	–		400 ip × 1	Leopardi *et al.* (1993)
Aneuploidy (hypoploidy and hyperploidy), ICR mouse oocytes *in vivo*	–[f]		600 ip × 1	Mailhes *et al.* (1993)
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes *in vivo*	+		200 ip x 1	Marrazini *et al.* (1994)
Trichloroethanol				
λ Prophage induction, *Escherichia coli* WP2	–	–	650 000[g]	DeMarini *et al*. (1994)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	7500 µg/plate	Waskell (1978)
Salmonella typhimurium TA100, reverse mutation	–	–	0.5 µg/cm^3 vapour[j]	DeMarini *et al*. (1994)
Salmonella typhimurium TA104, reverse mutation	–	+	2500 µg/plate	Beland (1999)
Inhibition of intercellular communication, male Sprague-Dawley rat liver Clone 9 cell *in vitro*	+	NT	150	Benane *et al.* (1996)

[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); NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw; feed, feeding; inj, injection; po, orally; ip, intraperitoneally

[c] Negative for induction of polyploidy

[d] AHH-1: native strain expressing CYP1A1; MCL-5 and H2E1 V2: engineered strains expressing other CYP

[e] Larvae reared in chloral hydrate-containing water

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

[g] Estimated from graph in paper

Chloral hydrate increased the frequency of micronuclei in Chinese hamster cell lines. The micronuclei produced probably contained whole chromosomes and not chromosome fragments, as the micronuclei could all be labelled with antikinetochore antibodies (Parry *et al.*, 1990; Lynch & Parry, 1993). When performed on mouse lymphoma cell line, the micronucleus test was negative in one study and positive in another.

Chloral hydrate also produced chromosomal aberrations *in vitro* in Chinese hamster cells and in mouse lymphoma cell line L5178Y/TK$^{+/-}$ -3.7.2C. It produced chromosomal dislocation from the mitotic spindle in Chinese hamster Don.Wg.3H cells *in vitro*.

Aneuploidy was induced *in vitro* by chloral hydrate in Chinese hamster cells but not in mouse lymphoma cell line L5178Y/TK$^{+/-}$-3.7.2C. In a study on kangaroo rat 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 and a total lack of mitotic spindles in Chinese hamster Don.Wg.3H cells. In a study on cultured mouse oocytes, chloral hydrate induced bivalent chromosomes, aberrant spindle, hypoploidy and meiotic arrest.

Cell transformation was observed in Syrian hamster embryo and dermal cells exposed to chloral hydrate *in vitro*.

Chloral hydrate induced DNA single-strand breaks and *TK* and *HPRT* loci gene mutations in human lymphoblastoid cells and weakly induced sister chromatid exchange in cultured human lymphocytes. It did not inhibit cell-to-cell communication in mouse or rat hepatocytes *in vitro*, but did in a study on Sprague-Dawley rat liver clone 9 cells.

In human diploid fibroblasts *in vitro*, chloral hydrate induced micronuclei that 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 were attributed to differences between whole blood and purified lymphocyte cultures (Vian *et al.*, 1995), but this hypothesis has not been tested. Moreover, micronuclei were induced *in vitro* in human lymphoblastoid cell line AHH-1 (native strain expressing CYP1A1), but not in human lymphoblastoid cell line MCL-5 (engineered strain expressing other CYPs).

In human lymphocytes *in vitro*, it induced aneuploidy, C-mitosis and polyploidy.

Chloral hydrate increased the rate of mitotic gene conversion in a host-mediated assay with *S. cerevisiae* D_7 recovered from CD_1 mouse lungs.

One study showed induction of single-strand breaks in liver DNA of both rats and mice treated with chloral hydrate *in vivo*; another study in both species found no such effect.

In vivo, micronuclei were induced in mouse bone-marrow erythrocytes in four of six studies after treatment with chloral hydrate; in one of these studies, the use of antikinetochore antibodies suggested induction of micronuclei containing both whole chromosomes and fragments. Micronuclei were not produced in peripheral erythrocytes of two strains of male mice treated *in vivo*. Chloral hydrate induced micronuclei in the spermatids of mice treated *in vivo* in three of five studies. The cell-cycle stage chosen for treatment is crucial, as micronuclei are induced after spermatogonial treatment of stem cells and not meiotic cells; 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, which may lose their normal kinetochore protein composition and structure (Allen *et al.*, 1994).

In a single study, chloral hydrate induced micronuclei in erythrocytes of newt larvae.

In vivo, the frequency of chromosomal aberrations in spermatogonia, primary and secondary spermatocytes and oocytes was not increased in single studies after treatment with chloral hydrate. Chloral hydrate induced chromosomal aberrations in mouse secondary spermatocytes in one study after treatment *in vivo*, but failed to induce chromosomal aberration in mouse and rat bone marrow treated *in vivo*.

In single studies, chloral hydrate induced aneuploidy and hyperploidy in the bone marrow of mice treated *in vivo*. It increased the rate of aneuploidy in mouse secondary spermatocytes in three of four studies, but failed to show increased frequency of numerical or structural chromosome changes in mouse oocytes. It did not produce polyploidy in oocytes and in bone marrow or gonosomal or autosomal univalents in primary spermatocytes of mice treated *in vivo*.

Genetic effects of trichloroethanol

Trichloroethanol, a reduction product of chloral hydrate, induced formation of stable DNA adducts when incubated with calf thymus DNA and microsomes from male $B6C3F_1$ mouse liver *in vitro* (Ni *et al.*, 1995), but caused mutation in *S. typhimurium* strain TA104 in the presence of metabolic system but not in strain TA100 or TA98. It failed to induce λ prophage in *E. coli*. It inhibited gap-junctional intercellular communication in rat hepatocytes *in vitro*.

4.5 Mechanistic considerations

Chloral hydrate is genotoxic *in vivo* and *in vitro*, and its induction of liver tumours in mice appears to be associated with peroxisome proliferation.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Chloral is a chlorinated aldehyde that found extensive use, beginning in the 1940s, as a precursor in the production of the insecticide DDT and, to a lesser extent, of other insecticides and pharmaceuticals. This use of chloral has declined steadily since the 1960s, especially in those countries where the use of DDT has been restricted. Chloral is readily

converted to chloral hydrate in the presence of water. Chloral hydrate is used as a sedative before medical procedures and to reduce anxiety related to withdrawal from drugs. Wider exposure to chloral hydrate occurs at microgram-per-litre levels in drinking-water and swimming pools as a result of chlorination.

5.2 Human carcinogenicity data

Several studies were identified that analysed risk with respect to one or more measures of exposure to complex mixtures of disinfection by-products that are found in most chlorinated and chloraminated drinking-water. No data specifically on chloral or chloral hydrate were available to the Working Group.

5.3 Animal carcinogenicity data

Following administration in drinking-water, chloral hydrate increased the incidence of hepatocellular neoplasms in male mice in two studies. An increased trend with dose for hepatocellular carcinoma in one study with dietary-restricted male mice was reported. Chloral hydrate was not active in male or female rats in two studies. Chloral hydrate increased the incidence of adenomas in the pars distalis of the pituitary gland in female mice only at the highest dose in one gavage study, but did not induce tumours of the pars distalis in male mice.

5.4 Other relevant data

Chloral hydrate is a sedative and hypnotic with some clinical uses. These late effects are also evident in animal studies.

Chloral hydrate is metabolized to trichloroacetic acid, trichloroethanol (which is converted to trichloroethyl glucuronide) and dichloroacetic acid in humans and in rodents.

There is limited evidence from a single study that chloral hydrate affects sperm, but no evidence of actual reproductive or developmental toxicity has been shown.

Chloral hydrate is a well-established aneuploidogenic agent that also has some mutagenic activity. *In vivo*, it clearly induced aneuploidy and micronuclei in mammals, whereas chromosomal aberrations were not found in most studies. Conflicting results were obtained with regard to the induction of DNA damage in chloral hydrate-treated mammals. In human cells *in vitro*, chloral hydrate induced aneuploidy, micronuclei and gene mutations. Sister chromatid exchange and DNA strand-break studies yielded inconclusive and negative results, respectively. Chloral hydrate clearly induced micronuclei in Chinese hamster cells, whereas findings in mouse lymphoma cells were conflicting. It failed to induce DNA damage, but caused weak mutagenicity and a clear induction of aneuploidy, chromosomal aberrations and sister chromatid exchange in rodent cells *in vitro*. It induced the formation of micronuclei in erythrocytes of newt larvae *in vivo*. In fungi, chloral hydrate clearly induced aneuploidy, while mitotic recombination and gene conversion assays were incon-

clusive. Induction of somatic mutation (but not sex-linked mutation) by chloral hydrate was demonstrated in insects. In bacteria, the compound induced base-pair substitution mutations. When incubated with calf thymus DNA, chloral hydrate induced the formation of malonaldehyde-related DNA adducts.

5.5 Evaluation

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

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

Overall evaluation

Chloral hydrate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Abbas, R.R., Seckel, C.S., Kidney, J.K. & Fisher, J.W. (1996) Pharmacokinetic analysis of chloral hydrate and its metabolism in B6C3F1 mice. *Drug. Metab. Dispos.*, **24**, 1340–1346

Adler, I.-D. (1993) Synopsis of the in vivo results obtained with the 10 known or suspected aneugens tested in the CEC collaborative study. *Mutat. Res.*, **287**, 131–137

Albertini, S. (1990) Analysis of nine known or suspected spindle poisons for mitotic chromosome malsegregation using *Saccharomyces cerevisiae* D61.M. *Mutagenesis*, **5**, 453–459

Allen, J.W., Collins, B.W. & Evansky, P.A. (1994) Spermatid micronucleus analyses of trichloroethylene and chloral hydrate effects in mice. *Mutat. Res*., **323**, 81–88

Alov, I.A. & Lyubskii, S.L. (1974) Experimental study of the functional morphology of the kinetochore in mitosis. *Byull. éksp. Biol. Med.*, **78**, 91–94

American Public Health Association/American Water Works Association/Water Environment Federation (1999) *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., Washington, DC [CD-ROM]

Baudisch, C., Pansch, G., Prösch, J. & Puchert, W. (1997) [*Determination of Volatile Halogenated Hydrocarbons in Chlorinated Swimming Pool Water. Research Report*], Aussnstelle Schwerin, Landeshygieneinstitut Mecklenburg-Vorpommern (in German) [cited in WHO (2000) *Guidelines for Safe Recreational-Water Environments*, Vol. 2, *Swimming Pools, Spas and Similar Recreational-Water Environments*, Geneva]

Beland, F.A. (1999) NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F$_1$ mice. *Toxic. Rep. Ser.*, **59**, 1–66, A1–E7

Beland, F.A., Schmitt, T.C., Fullerton, N.F. & Young, J.F. (1998) Metabolism of chloral hydrate in mice and rats after single and multiple doses. *J. Toxicol. environ. Health*, **A54**, 209–226

Benane, S.G., Blackman, C.F. & House, D.E. (1996) Effect of perchloroethylene and its metabolites on intercellular communication in clone 9 rat liver cells. *J. Toxicol. environ. Health*, **48**, 427–437

Bernstine, J.B., Meyer, A.E. & Hayman, H. (1954) Maternal and foetal blood estimation following the administration of chloral hydrate during labour. *J. Obstet. Gynaecol.*, **61**, 683–685

Bernstine, J.B., Meyer, A.E. & Bernstine, R.L. (1956) Maternal blood and breast milk estimation following the administration of chloral hydrate during the puerperium. *J. Obstet. Gynaecol.*, **63**, 228–231

Blank, V., Shukairy, H.M. & McLain, J. (2002) Unregulated organic DBPs in ICR finished water and distribution systems. In: McGuire, M.J., McLain, J. & Obdensky, A., eds, *Information Collection Rule Analysis*, Denver, CO, American Water Works Research Foundation

Boitsov, A.N., Rotenberg, Y.S. & Mulenkova, V.G. (1970) [On the toxicological evaluation of chloral in the process of its liberation during filling and pouring of foam polyurethanes.] *Gig. Tr. prof. Zabol*., **14**, 26–29 (in Russian)

Bonatti, S., Cavalieri, Z., Viaggi, S. & Abbondandolo, A. (1992) The analysis of 10 potential spindle poisons for their ability to induce CREST-positive micronuclei in human diploid fibroblasts. *Mutagenesis*, **7**, 111–114

Bringmann, G., God, R., Fähr, S., Feineis, D., Fornadi, K. & Fornadi, F. (1999) Identification of the dopaminergic neurotoxin 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline in human blood after intake of the hypnotic chloral hydrate. *Anal. Biochem.*, **270**, 167–175

Bronzetti, G., Galli, A., Corsi, C., Cundari, E., Del Carratore, R., Nieri, R. & Paolini, M. (1984) Genetic and biochemical investigation on chloral hydrate in vitro and in vivo. *Mutat. Res.*, **141**, 19–22

Brunner, M., Albertini, S. & Würgler, F.E. (1991) Effects of 10 known or suspected spindle poisons in the *in vitro* porcine brain tubulin assembly assay. *Mutagenesis*, **6**, 65–70

Bruzzoniti, M.C., Mentasti, E., Sarzanini, C. & Tarasco, E. (2001) Liquid chromatographic methods for chloral hydrate determination. *J. Chromatogr.*, **A920**, 283–289

Budavari, S., ed. (1998) *The Merck Index*, 12th Ed., Version 12:2, Whitehouse Station, NJ, Merck & Co. [CD-ROM]

Carlberg, G.E., Drangsholt, H. & Gjøs, N. (1986) Identification of chlorinated compounds in the spent chlorination liquor from differently treated sulphite pulps with special emphasis on mutagenic compounds. *Sci. total Environ*., **48**, 157–167

Chang, L.W., Daniel, F.B. & DeAngelo, A.B. (1992) Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes. *Environ. mol. Mutag.*, **20**, 277–288

Chemical Information Services (2002a) *Directory of World Chemical Producers (Version 2002.1)*, Dallas, TX

Chemical Information Services (2002b) *Worldwide Bulk Drug Users Directory (Version 2002)*, Dallas, TX [http://www.chemical.info.com]

Cole, W.J., Mitchell, R.G. & Salamonsen, R.F. (1975) Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. *J. Pharm. Pharmacol*., **27**, 167–171

Crebelli, R., Conti, G., Conti, L. & Carere, A. (1985) Mutagenicity of trichloroethylene, trichloroethanol and chloral hydrate in *Aspergillus nidulans*. *Mutat. Res.*, **155**, 105–111

Crebelli, R., Conti, G., Conti, L. & Carere, A. (1991) *In vitro* studies with nine known or suspected spindle poisons: Results in tests for chromosome malsegregation in *Aspergillus nidulans*. *Mutagenesis*, **6**, 131–136

Daniel, F.B., DeAngelo, A.B., Stober, J.A., Olson, G.R. & Page, N.P. (1992a) Hepatocarcinogenicity of chloral hydrate, 2-chloroacetaldehyde, and dichloroacetic acid in the male B6C3F1 mouse. *Fundam. appl. Toxicol.*, **19**, 159–168

Daniel, F.B., Robinson, M., Stober, J.A., Page, N.P. & Olson, G.R. (1992b) Ninety-day toxicity study of chloral hydrate in the Sprague-Dawley rat. *Drug chem. Toxicol*., **15**, 217–232

Davidson, I.W.F. & Beliles, R.P. (1991) Consideration on the target organ toxicity of trichloroethylene in terms of metabolite toxicity and pharmacokinetics. *Drug Metab. Rev*., **23**, 493–599

Degrassi, F. & Tanzarella, C. (1988) Immunofluorescent staining of kinetochores in micronuclei: A new assay for the detection of aneuploidy. *Mutat. Res.*, **203**, 339–345

DeMarini, D.M., Perry, E. & Shelton, M.L. (1994) Dichloroacetic acid and related compounds: Induction of prophage in *E. coli* and mutagenicity and mutation spectra in Salmonella TA100. *Mutagenesis*, **9**, 429–437

Eichenlaub-Ritter, U. & Betzendahl, I. (1995) Chloral hydrate induced spindle aberrations, metaphase I arrest and aneuploidy in mouse oocytes. *Mutagenesis*, **10**, 477–486

Eichenlaub-Ritter, U., Baart, E., Yin, H. & Betzendahl, I. (1996) Mechanisms of spontaneous and chemically-induced aneuploidy in mammalian oogenesis: Basis of sex-specific differences in response to aneugens and the necessity for further tests. *Mutat. Res*., **372**, 279–294

Environmental Protection Agency (1988) *Health and Environmental Effects Document for Chloral*, Washington, DC, Office of Solid Waste and Emergency Response, Office of Health and Environmental Assessment, 65 pp.

Environmental Protection Agency (1995) *Method 551.1. Determination of chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid–liquid Extraction and Gas Chromatography with Electron Capture Detection* (Revision 1.0), Cincinnati, OH, National Exposure Research Laboratory, Office of Research and Development

Environmental Protection Agency (1998) National primary drinking water regulations; disinfectants and disinfection byproducts; final rule. *Fed. Regist*., December 16

European Commission (1998) *Council Directive 98/83/EC of 3 November 1998 on the Quality of Water Intended for Human Consumption*, Luxemburg, Office for Official Publications of the European Communities

Ferguson, L.R., Morcombe, P. & Triggs, C.N. (1993) The size of cytokinesis-blocked micronuclei in human peripheral blood lymphocytes as a measure of aneuploidy induction by set A compounds in the EEC trial. *Mutat. Res.*, **287**, 101–112

Furnus, C.C., Ulrich, M.A., Terreros, M.C. & Dulout, F.N. (1990) The induction of aneuploidy in cultured Chinese hamster cells by propionaldehyde and chloral hydrate. *Mutagenesis*, **5**, 323–326

Gennaro, A.R., ed. (2000) *Remington: The Science and Practice of Pharmacy*, 20th Ed., Baltimore, MD, Lippincott Williams & Wilkins, pp. 1417–1418

George, M.H., Moore, T., Kilburn, S., Olson, G.R. & DeAngelo, A.B. (2000) Carcinogenicity of chloral hydrate administered in drinking water to the male F344/N rat and male B6C3F$_1$ mouse. *Toxicol. Pathol*., **28**, 610–618

Gibson, D.P., Aardema, M.J., Kerckaert, G.A., Carr, G.J., Brauninger, R.M. & LeBoeuf, R.A. (1995) Detection of aneuploidy-inducing carcinogens in the Syrian hamster embryo (SHE) cell transformation assay. *Mutat. Res.*, **343**, 7–24

Giller, S., Le Curieux, F., Gauthier, L., Erb, F. & Marzin, D. (1995) Genotoxicity assay of chloral hydrate and chloropicrine. *Mutat. Res.*, **348**, 147–152

Glaze, W.H., Kenneke, J.F. & Ferry, L.J. (1993) Chlorinated byproducts from the TiO_2-mediated photodegradation of trichloroethylene and tetrachloroethylene in water. *Environ. Sci. Technol.*, **27**, 177–184

Goldenthal, E.I. (1971) A compilation of LD50 values in newborn and adult animals. *Toxicol. appl. Pharmacol*., **18**, 185–207

Goodman Gilman, A., Rall, T.W., Nies, A.S. & Taylor, P., eds (1991) *The Pharmacological Basis of Therapeutics*, New York, Pergamon Press, pp. 357–364

Goodman Gilman, A., Hardman, J.G., Limbird, L.E., Molinoff, P.B. & Ruddon, R.W., eds (1996) *The Pharmacological Basis of Therapeutics*, 9th Ed., New York, McGraw-Hill, p. 381

Grawé, J., Nüsse, M. & Adler, I.-D. (1997) Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry. I. Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity. *Mutagenesis*, **12**, 1–8

Gu, Z.W., Sele, B., Jalbert, P., Vincent, M., Vincent, F., Marka, C., Chmara, D. & Faure, J. (1981) [Induction of sister chromatid exchange by trichloroethylene and its metabolites.] *Toxicol. Eur. Res.*, **3**, 63–67 (in French)

Hansch, C., Leo, A. & Hoekman, D. (1995) *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*, Washington, DC, American Chemical Society, p. 4

Hara, A., Yamamoto, H., Deyashiki, Y., Nakayama, T., Oritani, H. & Sawada, H. (1991) Aldehyde dismutation catalyzed by pulmonary carbonyl reductase: Kinetic studies of chloral hydrate metabolism to trichloroacetic acid and trichloroethanol. *Biochim. biophys. Acta.*, **1075**, 61–67

Harrington-Brock, K., Doerr, C.L. & Moore, M.M. (1998) Mutagenicity of three disinfection by-products: Di- and trichloroacetic acid and chloral hydrate in L5178Y/TK$^{+/-}$ -3.7.2C mouse lymphoma cells. *Mutat. Res.*, **413**, 265–276

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutag.*, **Suppl. 1**, 3–142

Health Canada (1995) *A National Survey of Chlorinated Disinfection By-products in Canadian Drinking Water* (95-EHD-197), Ottawa, Ontario, Minister of Supply and Services Canada

Health Canada (2003) *Summary of Guidelines for Canadian Drinking Water Quality*, Ottawa

Heinonen, O.P., Slone, D. & Shapiro, S. (1977) *Birth Defects and Drugs in Pregnancy*, Littleton, MA, PSG Publishing Co.

Helrich, K., ed. (1990) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed., Vol. 1, Arlington, VA, Association of Official Analytical Chemists, p. 562

Henderson, G.N., Yan, Z., James, M.O., Davydova, N. & Stacpoole, P.W. (1997) Kinetics and metabolism of chloral hydrate in children: Identification of dichloroacetate as a metabolite. *Biochem. biophys. Res. Commun.*, **235**, 695–698

Hobara, T., Kobayashi, H., Kawamoto, T., Iwamoto, S. & Sakai, T. (1987) Extrahepatic metabolism of chloral hydrate, trichloroethanol and trichloroacetic acid in dogs. *Pharmacol. Toxicol.*, **61**, 58–62

Hobara, T., Kobayashi, H., Kawamoto, T., Iwamoto, S. & Sakai, T. (1988) Intestinal absorption of chloral hydrate, free trichloroethanol and trichloroacetic acid in dogs. *Pharmacol. Toxicol.*, **62**, 250–258

Howlett, N.G. & Schiestl, R.H. (2000) Simultaneous measurement of the frequencies of intrachromosomal recombination and chromosome gain using the yeast DEL assay. *Mutat. Res.*, **454**, 53–62

IARC (1991) *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans*, Vol. 52, *Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*, Lyon, pp. 55–141

IARC (1995) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 63, *Dry Cleaning, Some Chlorinate Solvents and Other Industrial Chemicals*, Lyon, pp. 245–269

Italia, M.P. & Uden P.C. (1988) Multiple element emission spectral detection gas chromatographic profiles of halogenated products from chlorination of humic acid and drinking water. *J. Chromatogr.*, **438**, 35–43

Jacangelo, J.G., Patania, N.L., Reagan, K.M., Aieta, E.M., Krasner, S.W. & McGuire, M.J. (1989) Ozonation: Assessing its role in the formation and control of disinfection by-products. *J. Am. Water Works Assoc.*, **81**, 74–84

Jira, R., Kopp, E. & McKusick, B.C. (1986) Chloroacetaldehydes. In: Gerhartz, W., Yamamoto, Y.S., Campbell, F.T., Pfefferkorn, R. & Rounsaville, J.F., eds., *Ullmann's Encyclopedia of Industrial Chemistry*, 5th rev. Ed., Volume A6, New York, VCH Publishers, pp. 527–536

Käfer, E. (1986) Tests which distinguish induced crossing-over and aneuploidy from secondary segregation in Aspergillus treated with chloral hydrate and γ-rays. *Mutat. Res.*, **164**, 145–166

Kallman, M.J., Kaempf, G.L. & Balster, R.L. (1984) Behavioral toxicity of chloral in mice: An approach to evaluation. *Neurobehav. Toxicol. Teratol.*, **6**, 137–146

Kappas, A. (1989) On the mechanisms of induced aneuploidy in *Aspergillus nidulans* and validation of tests for genomic mutations. *Prog. clin. Biol. Res.*, **318**, 377–384

Keller, D.A. & Heck, H.d'A. (1988) Mechanistic studies on chloral toxicity: Relationship to trichloroethylene carcinogenesis. *Toxicol. Lett.*, **42**, 183–191

Ketcha, M.M., Stevens, D.K., Warren, D.A., Bishop, C.T. & Brashear, W.T. (1996) Conversion of trichloroacetic acid to dichloroacetic acid in biological samples. *J. anal. Toxicol.*, **20**, 236–241

Kim, H., Shim, J. & Lee, S. (2002) Formation of disinfection by-products in chlorinated swimming pool water. *Chemosphere*, **46**, 123–130

Klaunig, J.E., Ruch, R.J. & Lin, E.L.C. (1989) Effects of trichloroethylene and its metabolites on rodent hepatocyte intercellular communication. *Toxicol. appl. Pharmacol.*, **99**, 454–465

Klinefelter, G.R., Suarez, J.D., Roberts, N.L. & DeAngelo, A.B. (1995) Preliminary screening for the potential of drinking water disinfection byproducts to alter male reproduction. *Reprod. Toxicol.*, **9**, 571–578

Koch, B. & Krasner, S.W. (1989) Occurrence of disinfection by-products in a distribution system. In: *Proceedings of the AWWA, Annual Conference*, Part 2, Chicago, IL, American Water Works Association, pp. 1203–1230

Kojima, T., Suanhara, T. & Kuroda, H. (1990) [Determination of chloroacetic acids in tap water.] *Kagawa-ken Eisei Kenkyushoho*, (17), 122–128 [Analytical Abstract: 54(4):H124] (in Japanese)

Koppen, B., Dalgaard, L. & Christensen, J.M. (1988) Determination of trichloroethylene metabolites in rat liver homogenate using headspace gas chromatography. *J. Chromatogr.*, **442**, 325–332

Krasner, S.W., McGuire, M.J., Jacangelo, J.G., Patania, N.L., Reagan, K.M. & Aieta, E.M. (1989) The occurrence of disinfection by-products in US drinking water. *J. Am. Water Works Assoc.*, **81**, 41–53

Lambert, G.H., Muraskas, J., Anderson, C.L. & Myers, T.F. (1990) Direct hyperbilirubinemia associated with chloral hydrate administration in the newborn. *Pediatrics*, **86**, 277–281

LeBel, G.L., Benoit, F.M. & Williams, D.T. (1997) A one-year survey of halogenated disinfection by-products in the distribution system of treatment plants using three different disinfection processes. *Chemosphere*, **34**, 2301–2317

Lee, G.M., Diguiseppi, J., Gawdi, G.M. & Herman, B. (1987) Chloral hydrate disrupts mitosis by increasing intracellular free calcium. *J. Cell Sci.*, **88**, 603–612

Leopardi, P., Zijno, A., Bassani, B. & Pacchierotti, F. (1993) In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells. *Mutat. Res.*, **287**, 119–130

Leuschner, J. & Beuscher, N. (1998) Studies on the mutagenic and carcinogenic potential of chloral hydrate. *Arzneim.-Forsch./Drug Res.*, **48**, 961–968

Leuschner, J. & Leuschner, F. (1991) Evaluation of the mutagenicity of chloral hydrate in vitro and in vivo. *Arzneim.-Forsch./Drug Res.,* **41**, 1101–1103

Liang, J.C. & Pacchierotti, F. (1988) Cytogenetic investigation of chemically-induced aneuploidy in mouse spermatocytes. *Mutat. Res.*, **201**, 325–335

Lide (2000) Properties of Organic Compounds on CD-ROM, Version 6, Chapman & Hall/CRC, London

Lipscomb, J.C., Mahle, D.A., Brashear, W.T. & Garrett, C.M. (1996) A species comparison of chloral hydrate metabolism in blood and liver. *Biochem. biophys. Res. Commun.*, **227**, 340–350

Lynch, A.M. & Parry, J.M. (1993) The cytochalasin-B micronucleus/kinetochore assay *in vitro*: Studies with 10 suspected aneugens. *Mutat. Res.*, **287**, 71–86

Mailhes, J.B., Preston, R.J., Yuan, Z.P. & Payne, H.S. (1988) Analysis of mouse metaphase II oocytes as an assay for chemically induced aneuploidy. *Mutat. Res.*, **198**, 145–152

Mailhes, J.B., Aardema, M.J. & Marchetti, F. (1993) Investigation of aneuploidy induction in mouse oocytes following exposure to vinblastine-sulfate, pyrimethamine, diethylstilbestrol diphosphate, or chloral hydrate. *Environ. mol. Mutag.*, **22**, 107–114

Mannschott, P., Erdinger, L. & Sonntag, H.-G. (1995) [Halogenated organic compounds in swimming pool water.] *Zbl. Hyg.*, **197**, 516–533 (in German)

Marrazzini, A., Betti, C., Bernacchi, F., Barrai, I. & Barale, R. (1994) Micronucleus test and metaphase analyses in mice exposed to known and suspected spindle poisons. *Mutagenesis*, **9**, 505–515

Mayers, D.J., Hindmarsh, K.W., Sankaran, K., Gorecki, D.K.J. & Kasian, G.F. (1991) Chloral hydrate disposition following single-dose administration to critically ill neonates and children. *Dev. Pharmacol. Ther.*, **16**, 71–77

McKinney, L.L., Uhing, E.H., White, J.L. & Picken, J.C., Jr (1955) Vegetable oil extraction. Autoxidation products of trichloroethylene. *Agric. Food Chem.*, **3**, 413–419

Medical Economics Co. (1996) *PDR®: Generics*, 2nd Ed., Montvale, NJ, Medical Economics Data Production Co., pp. 584–586

Merdink, J.L., Gonzalez-Leon, A., Bull, R.J. & Schultz, I.R. (1998) The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. *Toxicol. Sci.*, **45**, 33–41

Merdink, J.L., Stenner, R.D., Stevens, D.K., Parker, J.C. & Bull, R.J. (1999) Effect of enterohepatic circulation on the pharmacokinetics of chloral hydrate and its metabolites in F344 rats. *J. Toxicol. environ. Health*, **A56**, 357–368

Migliore, L. & Nieri, M. (1991) Evaluation of twelve potential aneuploidogenic chemicals by the *in vitro* human lymphocyte micronucleus assay. *Toxicol. in Vitro*, **5**, 325–336

Miller, B.M. & Adler, I.-D. (1992) Aneuploidy induction in mouse spermatocytes. *Mutagenesis*, **7**, 69–76

Miller, J.W. & Uden, P.C. (1983) Characterization of nonvolatile aqueous chlorination products of humic substances. *Environ. Sci. Technol.*, **17**, 150–157

Mishchikhin, V.A. & Felitsin, F.P. (1988) [Gas-chromatographic detection of chloroform, carbon tetrachloride, dichloroethane, trichloroethylene and chloralhydrate in biological material.] *Sud. med. Ekspert.*, **31**, 30–33 (in Russian)

Molè-Bajer, J. (1969) Fine structural studies of apolar mitosis. *Chromosoma*, **26**, 427–448

Moore, M.M. & Harrington-Brock, K. (2000) Mutagenicity of trichloroethylene and its metabolites: Implications for the risk assessment of trichloroethylene. *Environ. Health Perspect.*, **108**, Suppl. 2, 215–223

Natarajan, A.T. (1993) An overview of the results of testing of known or suspected aneugens using mammalian cells *in vitro*. *Mutat. Res.*, **287**, 113–118

Natarajan, A.T., Duivenvoorden, W.C.M., Meijers, M. & Zwanenburg, T.S.B. (1993) Induction of mitotic aneuploidy using Chinese hamster primary embryonic cells. Test results of 10 chemicals. *Mutat. Res.*, **287**, 47–56

National Health and Medical Research Council and Agriculture and Resource Management Council of Australia and New Zealand (1996) *Australian Drinking Water Guidelines Summary*, Canberra

National Institute for Occupational Safety and Health (1994) *National Occupational Exposure Survey (1981–1983)*, Cincinnati, OH

National Toxicology Program (2002a) *Toxicology and Carcinogenesis Studies of Chloral Hydrate* (NTP Technical Report 502), Research Triangle Park, NC

National Toxicology Program (2002b) *Toxicology and Carcinogenesis Studies of Chloral Hydrate (*ad libitum *and dietary controlled)* (NTP Technical Report 503), Research Triangle Park, NC

Nelson, M.A. & Bull, R.J. (1988) Induction of strand breaks in DNA by trichloroethylene and metabolites in rat and mouse liver *in vivo*. *Toxicol. appl. Pharmacol.*, **94**, 45–54

Nesslany, F. & Marzin, D. (1999) A micromethod for the *in vitro* micronucleus assay. *Mutagenesis*, **14**, 403–410

Ni, Y.-C., Wong, T.-Y., Kadlubar, F.F. & Fu, P.P. (1994) Hepatic metabolism of chloral hydrate to free radical(s) and induction of lipid peroxidation. *Biochem. biophys. Res. Commun.*, **204**, 937–943

Ni, Y.-C., Kadlubar, F.F. & Fu, P.P. (1995) Formation of malondialdehyde-modified 2′-deoxyguanosinyl adduct from metabolism of chloral hydrate by mouse liver microsomes. *Biochem. biophys. Res. Commun.*, 216, 1110–1117

Ni, Y.-C., Wong, T.-Y., Lloyd, R.V., Heinze, T.M., Shelton, S., Casciano, D., Kadlubar, F.F. & Fu, P.P. (1996) Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol leading to induction of lipid peroxidation *via* a free radical mechanism. *Drug Metab. Dispos.*, **24**, 81–90

Nutley, E.V., Tcheong, A.C., Allen, J.W., Collins, B.W., Ma, M., Lowe, X.R., Bishop, J.B., Moore, D.H., II & Wyrobek, A.J. (1996) Micronuclei induced in round spermatids of mice after stem-

cell treatment with chloral hydrate: Evaluations with centromeric DNA probes and kinetochore antibodies. *Environ. mol. Mutag.*, **28**, 80–89

Odum, J., Foster, J.R. & Green, T. (1992) A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chem.-biol. Interact*., **83**, 135–153

Ohlson, J. (1986) Dry etch chemical safety. *Solid State Technol.*, **July**, 69–73

O'Neil, M.J., ed. (2001) *The Merck Index*, 13th Ed., Whitehouse Station, NJ, Merck & Co, p. 355

Parry, J.M. (1993) An evaluation of the use of in vitro tubulin polymerisation, fungal and wheat assays to detect the activity of potential chemical aneugens. *Mutat. Res.*, **287**, 23–28

Parry, J.M., Parry, E.M., Warr, T., Lynch, A. & James, S. (1990) The detection of aneugens using yeasts and cultured mammalian cells. In: Mendelsohn, M.L. & Albertini, R.J., eds, *Mutation and the Environment. Part B, Metabolism, Testing Methods, and Chromosomes*, New York, Wiley-Liss, pp. 247–266

Parry, J.M., Parry, E.M., Bourner, R., Doherty, A., Ellard, S., O'Donovan, J., Hoebee, B., de Stoppelaar, J.M., Mohn, G.R., Önfelt, A., Renglin, A., Schultz, N., Söderpalm-Berndes, C., Jensen, K.G., Kirsch-Volders, M., Elhajouji, A., Van Hummelen, P., Degrassi, F., Antoccia, A., Cimini, D., Izzo, M., Tanzarella, C., Adler, I.-D., Kliesch, U., Schriever-Schwemmer, G., Gasser, P., Crebelli, R., Carere, A., Andreoli, C., Benigni, R., Leopardi, P., Marcon, F., Zinjo, Z., Natarajan, A.T., Boei, J.J.W.A., Kappas, A., Voutsinas, G., Zarani, F.E., Patrinelli, A., Pachierotti, F., Tiveron, C. & Hess, P. (1996) The detection and evaluation of aneugenic chemicals. *Mutat. Res.*, **353**, 11–46

Pharmacopeial Convention (1990) *The United States Pharmacopeia, 22nd rev./The National Formulary*, 17th rev., Rockville, MD, pp. 269–270

Poon, R., Nadeau, B. & Chu, I. (2000) Biochemical effects of chloral hydrate on male rats following 7-day drinking water exposure. *J. appl. Toxicol*., **20**, 455–461

Rijhsinghani, K.S., Swerdlow, M.A., Ghose, T., Abrahams, C. & Rao, K.V.N. (1986) Induction of neoplastic lesions in the livers of $C_{57}BL \times C_3HF_1$ mice by chloral hydrate. *Cancer Detect. Prev.*, **9**, 279–288

Royal Pharmaceutical Society of Great Britain (2002) *Martindale, The Complete Drug Reference,* 33rd Ed., London, The Pharmaceutical Press [MicroMedex online]

Russo, A. & Levis, A.G. (1992a) Detection of aneuploidy in male germ cells of mice by means of a meiotic micronucleus assay. *Mutat. Res.*, **281**, 187–191

Russo, A. & Levis, A.G. (1992b) Further evidence for the aneuploidogenic properties of chelating agents: Induction of micronuclei in mouse male germ cells by EDTA. *Environ. mol. Mutag.*, **19**, 125–131

Russo, A., Pacchierotti, F. & Metalli, P. (1984) Nondisjunction induced in mouse spermatogenesis by chloral hydrate, a metabolite of trichloroethylene. *Environ. Mutag.*, **6**, 695–703

Russo, A., Stocco, A. & Majone, F. (1992) Identification of kinetochore-containing ($CREST^+$) micronuclei in mouse bone marrow erythrocytes. *Mutagenesis*, **7**, 195–197

Sadtler Research Laboratories (1991) *Sadtler Standard Spectra, 1981–1991 Supplementary Index*, Philadelphia, PA

Sanders, V.M., Kauffmann, B.M., White, K.L., Jr, Douglas, K.A., Barnes, D.W., Sain, L.E., Bradshaw, T.J., Borzelleca, J.F. & Munson, A.E. (1982) Toxicology of chloral hydrate in the mouse. *Environ. Health Perspect*., **44**, 137–146

Sandhu, S.S., Dhesi, J.S., Gill, B.S. & Svendsgaard, D. (1991) Evaluation of 10 chemicals for aneuploidy induction in the hexaploid wheat assay. *Mutagenesis*, **6**, 369–373

Sato, T., Mukaida, M., Ose, Y., Nagase, H. & Ishikawa, T. (1985) Chlorinated products from structural compounds of soil humic substances. *Sci. total Environ*., **43**, 127–140

Sbrana, I., Di Sibio, A., Lomi, A. & Scarcelli, V. (1993) C-Mitosis and numerical chromosome aberration analyses in human lymphocytes: 10 known or suspected spindle poisons. *Mutat. Res.*, **287**, 57–70

Seelbach, A., Fissler, B. & Madle, S. (1993) Further evaluation of a modified micronucleus assay with V79 cells for detection of aneugenic effects. *Mutat. Res.*, **303**, 163–169

Simpson, K.L. & Hayes, K.P. (1998) Drinking water disinfection by-products: An Australian perspective. *Water Res*., **32**, 1522–1528

Solt, K. & Johansson, J.S. (2002) Binding of the active metabolite of chloral hydrate, 2,2,2-trichloroethanol, to serum albumin demonstrated using tryptophan fluorescence quenching. *Pharmacology*, **64**, 152–159

Sora, S. & Agostini Carbone, M.L. (1987) Chloral hydrate, methylmercury hydroxide and ethidium bromide affect chromosomal segregation during meiosis of *Saccharomyces cerevisiae*. *Mutat. Res.*, **190**, 13–17

Trehy, M.L., Yost, R.A. & Miles, C.J. (1986) Chlorination byproducts of amino acids in natural waters. *Environ. Sci. Technol.*, **20**, 1117–1122

Uden, P.C. & Miller, J.W. (1983) Chlorinated acids and chloral in drinking water. *J. Am. Water Works Assoc.*, **75**, 524–527

Vagnarelli, P., De Sario, A. & De Carli, L. (1990) Aneuploidy induced by chloral hydrate detected in human lymphocytes with the Y97 probe. *Mutagenesis*, **5**, 591–592

Van Hummelen, P. & Kirsch-Volders, M. (1992) Analysis of eight known or suspected aneugens by the *in vitro* human lymphocyte micronucleus test. *Mutagenesis*, **7**, 447–455

Vian, L., Van Hummelen, P., Bichet, N., Gouy, D. & Kirsch-Volders, M. (1995) Evaluation of hydroquinone and chloral hydrate on the in vitro micronucleus test on isolated lymphocytes. *Mutat. Res.*, **334**, 1–7

Von Tungeln, L.S., Yi, P., Bucci, T.J., Samokyszyn, V.M., Chou, M.W., Kadlubar, F.F. & Fu, P.P. (2002) Tumorigenicity of chloral hydrate, trichloroacetic acid, trichloroethanol, malondialdehyde, 4-hydroxy-2-nonenal, crotonaldehyde, and acrolein in the $B6C3F_1$ neonatal mouse. *Cancer Lett.*, **185**, 13–19

Wallin, M. & Hartley-Asp, B. (1993) Effects of potential aneuploidy inducing agents on microtubule assembly *in vitro*. *Mutat. Res.*, **287**, 17–22

Warr, T.J., Parry, E.M. & Parry, J.M. (1993) A comparison of two in vitro mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens. *Mutat. Res.*, **287**, 29–46

Waskell, L. (1978) A study of the mutagenicity of anesthetics and their metabolites. *Mutat. Res.*, **57**, 141–153

Weast, R.C. & Astle, M.J. (1985) *CRC Handbook of Data on Organic Compounds*, Volumes I and II, Boca Raton, Florida, CRC Press

WHO (1998) *Guidelines for Drinking-water Quality*, 2nd Ed., Vol. 2, *Health Criteria and Other Supporting Information*, Geneva (addendum to Vol. 2, pp. 281–283)

Williams, M. & Holladay, M.W. (1995) Hypnotics, sedatives, anticonvulsants, and anxiolytics. In: Kroschwitz, J.I. & Howe-Grant, M., eds., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th Ed., Vol. 13, New York, John Wiley & Sons, pp. 1082–1100

Williams, D.T., LeBel, G.L. & Benoit, F.M. (1997) Disinfection by-products in Canadian drinking water. *Chemosphere*, **34**, 299–316

Wilson, J.T. (1981) *Drugs in Breast Milk*, Lancaster, MTP

Xu, W. & Adler, I.-D. (1990) Clastogenic effects of known and suspect spindle poisons studied by chromosome analysis in mouse bone marrow cells. *Mutagenesis*, **5**, 371–374

Zimmermann, T., Wehling, M. & Schulz, H.-U. (1998) [The relative bioavailability and pharmacokinetics of chloral hydrate and its metabolites.] *Arzneim.-Forsch./Drug Res.*, **48**, 5–12 (in German)

Zordan, M., Osti, M., Pesce, M. & Costa, R. (1994) Chloral hydrate is recombinogenic in the wing spot test in *Drosophila melanogaster*. *Mutat. Res.*, **322**, 111–116