

WHO/SDE/WSH/05.08/49
English only

Chloral Hydrate in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

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Preface

One of the primary goals of WHO and its member states is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water.” A major WHO function to achieve such goals is the responsibility “to propose ... regulations, and to make recommendations with respect to international health matters”

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published on selected chemicals in 1998 and on microbial aspects in 2002. The third edition of the GDWQ was published in 2004, and the first addendum to the third edition was published in 2005.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the documents for the third edition and addenda.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried

out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

Acknowledgements

The first draft of Chloral Hydrate in Drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality*, was prepared by the Water Quality and Health Bureau, Health Canada, to whom special thanks are due.

The work of the following working group coordinators was crucial in the development of this document and others contributing to the first addendum to the third edition:

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The draft text was discussed at the Working Group Meeting for the first addendum to the third edition of the GDWQ, held on 17–21 May 2004. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinator was Dr J. Bartram, Coordinator, Water, Sanitation and Health Programme, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr Robert Bos, Water, Sanitation and Health Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms Penny Ward provided invaluable administrative support at the Working Group Meeting and throughout the review and publication process. Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

AFC	antibody-forming cell
ALDH	aldehyde dehydrogenase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CAS	Chemical Abstracts Service
CoA	coenzyme A
CYP	cytochrome P-450
DCA	dichloroacetic acid
DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid
ECD	electron capture detection
EPA	Environmental Protection Agency (USA)
FAO	Food and Agriculture Organization of the United Nations
GAC	granular activated carbon
GC	gas chromatography
GDWQ	<i>Guidelines for Drinking-water Quality</i>
IC ₅₀	median inhibitory concentration
ICR	Information Collection Rule (USA)
LD ₅₀	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MTBE	methyl <i>tert</i> -butyl ether
NCTR	National Center for Toxicological Research (USA)
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Co-operation and Development
PBPK	physiologically based pharmacokinetic
ppm	part per million
SDH	sorbitol dehydrogenase
TCA	trichloroacetic acid
TCE	trichloroethylene
TCOG	trichloroethanol glucuronide
TCOH	trichloroethanol
TDI	tolerable daily intake
THM	trihalomethane
USA	United States of America

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1. GENERAL DESCRIPTION

1.1 Identity

CAS No.:	302-17-0
Molecular formula:	C ₂ H ₃ Cl ₃ O ₂

Chloral hydrate is also known as trichloroacetaldehyde monohydrate and 2,2,2-trichloro-1,1-ethanediol.

1.2 Physicochemical properties¹

<i>Property</i>	<i>Value</i>	<i>Reference</i>
Melting point	57 °C	Hansch et al., 1995
Boiling point	96 °C	Hansch et al., 1995
Vapour pressure	2 kPa at 25 °C	Reynolds & Prasad, 1982; Hansch et al., 1995
Water solubility	9.3 × 10 ⁶ mg/litre at 25 °C	McEvoy, 1999
Log octanol–water partition coefficient	0.99	IPCS, 2000a

1.3 Major uses and sources in drinking-water

Chloral hydrate is used as a sedative and hypnotic in human and veterinary medicine. It is also used in the manufacture of DDT (Budavari et al., 1996) and dichloroacetic acid (DCA) (Kirk-Othmer, 1991). In addition, chloral hydrate is used as an intermediate in the production of the insecticides methoxychlor, naled, trichlorfon and dichlorvos, the herbicide trichloroacetic acid (TCA) and the hypnotic drugs chloral betaine, chloralose and trichlorfos sodium (IARC, 1995).

Chloral hydrate can be formed as a by-product of the chlorination of water containing organic precursor molecules, such as fulvic and humic acids. Chloral hydrate can also be released in the environment from wastewater treatment facilities following chlorination, from the manufacture of pharmaceutical-grade chloral hydrate and from the waste stream during the manufacture of insecticides and herbicides that use chloral hydrate as an intermediate (US EPA, 2000).

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

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

¹ Conversion factor in air: 1 ppm = 6.77 mg/m³.

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2.2 Water

According to surveys conducted in Canada in 1995 and 1997, the mean level of chloral hydrate in drinking-water ranged from 1.2 to 3.8 µg/litre in winter and from 3.6 to 8.4 µg/litre in summer, with a maximum level of 22.5 µg/litre observed in winter from a sampling of 53 sites (Health Canada, 1995; Edsall & Charlton, 1997; Williams et al., 1997). The concentration of chloral hydrate in water supplies in the USA averages 5 µg/litre (US EPA, 1994).

The median chloral hydrate concentration reported under the US Information Collection Rule (ICR) in finished water (1.7 µg/litre) was similar to the values reported in other studies (Krasner et al., 1989, 2.1 µg/litre; US EPA, 1992, 2.5 µg/litre), whereas the maximum reported chloral hydrate concentration (46 µg/litre) exceeded the highest values reported in these other studies (Krasner et al., 1989, 22 µg/litre; US EPA, 1992, 25 µg/litre).

The reported chloral hydrate concentration in distribution system samples ranged from the method reporting limit of 0.5 µg/litre up to 92 µg/litre (one sample). Surface water showed higher concentrations (median value of 4.0 µg/litre) than groundwater (median value of 0.5 µg/litre).

The chloral hydrate concentrations reported under the ICR in the distribution systems of surface water plants (median value of 4.0 µg/litre) were generally higher than those in the finished water at the water plants (median value of 2.4 µg/litre), suggesting that chloral hydrate concentrations increase somewhat across the distribution system (K. Ozelin, personal communication, 2004).

2.3 Food

No data are available on human exposure to chloral hydrate in food (IARC, 1995).

2.4 Pharmaceuticals

For adults, the usual hypnotic dose of chloral hydrate is 0.5–1 g given 15–30 min before retiring or, when used as a preoperative medication, 30 min before surgery; the usual sedative dosage is 250 mg 3 times daily after meals. When chloral hydrate is administered in the management of alcohol withdrawal symptoms, the usual dosage is 0.5–1 g repeated at 6-h intervals if needed (McEvoy, 1999). Generally, single doses or daily dosages for adults should not exceed 2 g.

For children, the hypnotic dose of chloral hydrate is 50 mg/kg of body weight or 1.5 g/m², with a maximum dose of 1 g. The sedative dosage for children is 8 mg/kg of body weight or 250 mg/m² 3 times daily, with a maximum dosage of 500 mg 3 times a day. As a premedication before electroencephalogram evaluation, children have been given chloral hydrate at a dose of 20–25 mg/kg of body weight (McEvoy, 1999).

2.5 Estimated total exposure and relative contribution of drinking-water

The intake of chloral hydrate from pharmaceutical exposure is approximately 17 mg/kg of body weight for a 60-kg adult and 29 mg/kg of body weight for a 35-kg child (assuming a dose of 1 g). These doses are much higher than the doses from drinking-water (<1 µg/kg of body weight per day) using the maximum concentration of 46 µg/litre as above and a body weight of 60 kg. In other words, clinical exposure is considerably greater than exposure from drinking-water, but of shorter duration. However, the general population is exposed to chloral hydrate mainly (more than 80% of total exposure) from drinking-water. Occupational exposure during manufacturing may also occur (IARC, 1995).

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

In humans, chloral hydrate is rapidly absorbed and then either oxidized to TCA (8%) or reduced to trichloroethanol (TCOH) (92%), mainly by the liver, but also by the kidney. TCOH may be conjugated with glucuronic acid to form trichloroethanol glucuronide (TCOG; urochloralic acid), an inactive metabolite (Ogino et al., 1990; McEvoy, 1999). Additional TCA is formed during enterohepatic circulation of TCOH, such that 35% of the initial dose of chloral hydrate is converted to TCA (Allen & Fisher, 1993). The erythrocytes also metabolize chloral hydrate to TCOH, mainly via alcohol dehydrogenase.

The plasma half-lives in humans for therapeutic doses of chloral hydrate, TCOH and TCA are about 4–5 min, 8–12 h and 67 h, respectively (Ellenhorn & Barceloux, 1988).

DCA was identified in infants and children aged 3 months to 18 years diagnosed with congenital lactic acidosis who had received chloral hydrate as a sedative. Patients treated with chloral hydrate showed the presence of TCA (after 10.15 min), DCA (after 10.76 min) and TCOH (after 15.95 min). For patients receiving chloral hydrate at 50 mg/kg of body weight, the plasma TCOH level rapidly increased to a maximum of 115 µg/ml 25 min after chloral hydrate administration, with a half-life of 9.7 h. In contrast, plasma DCA and TCA levels increased slowly, reaching a maximum of 22 µg/ml and 65 µg/ml at 7.5 h and 11.5 h, respectively. This study showed that TCA and DCA are formed from chloral hydrate, but also indicate that TCA's half-life is very long in children (Henderson et al., 1997). There is evidence that TCA may be converted to DCA in samples of blood taken for analysis unless appropriate steps are taken, raising concerns about whether the reported levels of DCA in humans are too high (Ketcha et al., 1996).

Healthy male volunteers ($n = 18$) were administered a single dose of 250 mg of chloral hydrate in drinking-water. Chloral hydrate, TCOH and TCA were measured in the plasma. Chloral hydrate could be detected in only some of the plasma samples, 8–60 min after dosing. No concentration was reported, but the limit of detection was stated as 0.1 mg/litre. The maximum plasma concentrations of TCOH and TCA, 3

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mg/litre and 8 mg/litre, respectively, were achieved 0.67 h and 32 h after dosing, respectively. The terminal half-life was 9.3–10.2 h for TCOH and 89–94 h for TCA (Zimmermann et al., 1998).

Based on pharmacokinetic information, chloral hydrate and the pharmacologically active metabolite TCOH will not accumulate in the human body (Gilman et al., 1985). As infants have an immature hepatic metabolism, particularly the glucuronidation pathway, with decreased glomerular filtration, they have a longer TCOH half-life than their adult counterparts. In contrast, toddlers have a TCOH half-life that is similar to that of adults, indicating maturation of liver metabolism in toddlers (IPCS, 2000a).

Chloral hydrate was rapidly metabolized by rats and mice, producing both TCOH and TCA as the major metabolites, with a higher concentration of TCA in mice than in rats. The plasma concentration of chloral hydrate was dose-dependent. The metabolic rates were, however, not affected by the dose or the sex. The metabolism of chloral hydrate, TCA and TCOH was shown *in vitro* to give rise to free radical intermediates that cause lipid peroxidation and the formation of malondialdehyde (Beland, 1999). Lipscomb et al. (1996) found TCOH to be the first major metabolite of chloral hydrate *in vivo* in the blood and liver of Fischer 344 rats, B6C3F1 mice and humans.

Male B6C3F1 mice were given chloral hydrate intravenously at dose levels of 67.8, 678 or 2034 $\mu\text{mol/kg}$ of body weight (10, 100 or 300 mg/kg of body weight) to investigate body clearance. Chloral hydrate disappeared from the blood with a plasma half-life ranging from 5 to 24 min. It was cleared rapidly from systemic circulation (36.0 litres/h per kg of body weight), with a plasma terminal half-life of 5 min after intravenous administration of 67.8 $\mu\text{mol/kg}$ of body weight. After intravenous administration of 678 and 2034 $\mu\text{mol/kg}$ of body weight, however, the clearance decreased to 20 and 7.6 litres/h per kg of body weight, respectively, indicating saturable kinetics; the plasma terminal half-life increased 4-fold to 17 and 22 min, respectively. The following metabolites were detected in a dose-dependent fashion over the period of the study: TCOH, TCOG, TCA and DCA. The terminal half-lives of TCOH and TCOG were similar, ranging from 0.2 to 0.7 h. Both TCA and DCA were formed rapidly from chloral hydrate but were slowly cleared from systemic circulation. Based on the results of this study, it was suggested that DCA was formed as a metabolite of TCA (Abbas et al., 1996).

B6C3F1 mice (80 per sex) and Fischer F344 rats (80 per sex) were randomly assigned to treatment groups with 1 or 12 doses of chloral hydrate (50 or 500 mg/kg of body weight). The animals receiving multiple doses were treated daily, except weekends, and had at least two consecutive dose days before the last day of the treatment. Concentrations of chloral hydrate and its metabolites were determined in plasma at 0.25, 1, 3, 6 and 24 h and 2, 4, 8 and 16 days after the last treatment. Chloral hydrate was observed at maximum levels at the initial sampling time of 0.25 h. After 1 h, levels dropped substantially; by 3 h, chloral hydrate could not be detected. TCA was the major metabolite detected in the plasma 1–6 h after dosing; it then decreased slowly and could not be detected after 2 days. TCOH was also detected after 0.25 h at maximum levels and by 1–3 h approached the limits of detection. The plasma half-life

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values of chloral hydrate were similar in both species. The rate of TCA elimination was increased in mice following multiple doses, but not in rats. The half-lives of TCOH and TCOG were significantly greater in rats than in mice (Beland et al., 1998).

In order to examine the effects of the enterohepatic circulation on the kinetics of chloral hydrate and the formation of TCA and DCA, F344 rats were infused via jugular vein cannulae with chloral hydrate at doses of 12, 48 or 192 mg/kg of body weight (Merdink et al., 1999). TCOH and TCA were both dosed as an intravenous bolus at 100 mg/kg of body weight. The rats were divided into two groups: a bile-interrupted group (without enterohepatic circulation) and a bile-intact group. Blood and bile were collected at regular intervals. Chloral hydrate was rapidly cleared by the rats, with body clearance values of 3060 and 4108 ml/h per kg of body weight for bile-interrupted and bile-intact animals, respectively. It was rapidly metabolized to TCOH and TCA; TCOH was further conjugated with glucuronide and excreted in urine, while TCA was slowly excreted in the urine. Regardless of treatment, TCA demonstrated no difference with regard to any of its kinetic parameters. In contrast, TCOH was affected by the bile removal, showing a decrease in half-life from 1.98 to 0.79 h. The blood concentration/time profile for chloral hydrate elimination was biphasic at all doses in both bile-interrupted and bile-intact animals. The first phase had an elimination half-life of 0.09 h, and the second phase had a half-life of 0.75 h; therefore, chloral hydrate has a half-life similar to that of free TCOH (0.75 h for chloral hydrate versus 0.71 h for free TCOH). The removal of the enterohepatic circulation had little effect on most of the kinetic parameters for chloral hydrate and its metabolites at low doses; however, differences were noted at the highest dose (i.e., 192 mg/kg of body weight), resulting in a 44% and 17% decrease for TCA and TCOH, respectively. DCA was not detected in rats dosed with chloral hydrate and was not detected above the limit of quantification of 2 $\mu\text{mol/litre}$ in rats dosed intravenously with either TCOH or TCA. Renal clearance of chloral hydrate, free TCOH and TCA of 2, 2.7 and 38 ml/h per kg of body weight, respectively, clearly indicated an efficient renal tubular reabsorption mechanism for these compounds. In addition, there is evidence that TCOG is processed and reabsorbed from the gut based on the significant amount in the bile. Some of it is converted back to chloral hydrate and oxidized to TCA, therefore accounting for the longer half-life of the latter (Merdink et al., 1999).

The conversion of TCA to DCA in biological samples taken from male B6C3F1 mice was observed in a study designed to analyse trichloroethylene (TCE) metabolites. These metabolites are also observed following chloral hydrate metabolism. TCA and DCA were converted to their methyl esters by dimethyl sulfate under acidic conditions and analysed by gas chromatography (GC) with electron capture detection (ECD). The conversion of TCA to DCA was observed in freshly drawn blood upon the addition of acid. The amount of TCA converted to DCA by the addition of the acid decreased with time. To prevent this conversion, it was recommended that blood samples be frozen prior to the addition of acid. Otherwise, this would lead to an overestimation of the amount of DCA formed as a metabolite of TCE or even chloral hydrate during analysis of metabolites (Ketcha et al., 1996).

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Chloral hydrate is an important metabolite of TCE and an intermediate in the formation of TCA. Based on the results of a number of studies, a physiologically based pharmacokinetic (PBPK) model for TCE was developed. This model includes enterohepatic recirculation of its metabolites. The model quantitatively predicts quite well the uptake, distribution and elimination of TCE, TCOH, TCOG and TCA. The PBPK model clearly shows that the formation of TCA is delayed following the enterohepatic recirculation, therefore accounting for the longer half-life of TCA observed in animal studies (Stenner et al., 1998).

Most of the chloral hydrate is excreted via the urine as TCOG, with small amounts excreted as free TCOH. The remainder is excreted as TCA (Butler, 1948; Marshall & Owens, 1954; Allen & Fisher, 1993). Chloral hydrate is not excreted unchanged (McEvoy, 1999).

4. EFFECTS ON LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

4.1 Acute exposure

The LD₅₀ for chloral hydrate in mice was 1265 mg/kg of body weight for females and 1442 mg/kg of body weight for males. Rats were more sensitive to chloral hydrate, with LD₅₀s of 285 and 479 mg/kg of body weight for newborn pups and adults, respectively (Sanders et al., 1982).

4.2 Short-term exposure

A 7-day study was performed with 28 male Sprague-Dawley rats (161–170 g) to investigate the biochemical and toxicological effects of chloral hydrate (Poon et al., 2000). The rats were administered chloral hydrate in drinking-water at dose levels of 5, 43 or 375 mg/kg of body weight per day. Control animals received phosphate-buffered water only. No gross changes in organ to body weight ratios for the brain, thymus, heart, liver, kidneys and spleen were observed. In the high-dose animals, TCA was found at significant levels in the serum (7.75 mg/dl). An increase of 36% in protein levels was found at 375 mg/kg of body weight per day in the liver homogenate only. In addition, a 3-fold increase ($P < 0.055$) in the hepatic peroxisomal enzyme palmitoyl CoA oxidase was observed at 375 mg/kg of body weight per day. A significant ($P < 0.05$) dose-related suppression in hepatic aldehyde dehydrogenase (ALDH) activity occurred in all treatment groups, ranging from 15% in the 5 mg/kg of body weight per day group to 68% in the 375 mg/kg of body weight per day group. An increase of 30% in glutathione-S-transferase accompanied by a 13% increase in glutathione was observed at 375 mg/kg of body weight per day. Among high-dose animals, decreases of 15% in liver cholesterol and liver triglyceride levels were observed. No treatment-related changes were observed in serum chemistry parameters, including cholesterol and triglyceride levels. The changes observed in the palmitoyl CoA oxidase enzymes, accompanied by changes in lipid homeostasis, were likely associated with TCA. A lowest-observed-adverse-effect level (LOAEL) of 5 mg/kg of body weight per day, the lowest dose tested, was determined based on ALDH suppression. However, this end-point is not generally viewed as a significant

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effect without histopathological effect in the liver, even though changes in the liver enzymes are an indication that the liver is the target organ of chloral hydrate exposure (Poon et al., 2000).

CD-1 mice (number not specified) were dosed by gavage with chloral hydrate at 0, 14.4 or 144 mg/kg of body weight per day for a period of 14 days. A dose-dependent increase in relative liver weights (18% compared with the control group) and a decrease in spleen weights (27% compared with the controls) as well as a decrease in blood lactate dehydrogenase (LDH) levels (20% compared with the control group) were observed in mice administered 144 mg/kg of body weight per day. A no-observed-adverse-effect level (NOAEL) of 14.4 mg/kg of body weight per day was identified in this study (Sanders et al., 1982).

CD-1 mice (48 per sex for control; 32 per sex for treatment groups) were administered chloral hydrate in drinking-water for 90 days at concentrations of 0, 70 or 700 mg/litre (corresponding to dose levels of 0, 16 or 160 mg/kg of body weight per day for males and 0, 18 or 173 mg/kg of body weight per day for females) (Sanders et al., 1982). Males exposed to chloral hydrate demonstrated a dose-related increase in body weight gain: 14.1 g, 15.1 g and 17.1 g for the control, low-dose and high-dose groups, respectively. Liver weight was significantly increased only in males: 12% at 16 mg/kg of body weight per day and 20% at 160 mg/kg of body weight per day. Fibrinogen levels were significantly different from control in males exposed to 160 mg/kg of body weight per day (9% increase) and in females exposed to 18 mg/kg of body weight per day (14% increase). Hepatomegaly and microsome proliferation were observed in males at both doses. Females, however, had a 10% increase in total microsomal protein at the higher dose only. In males, the cytochrome b₅ content increased ($P < 0.05$) from 0.402 nmol/mg protein for the control to 0.508 and 0.564 nmol/mg protein for the low- and high-dose groups, respectively. Aniline hydroxylase activity was significantly increased in males, from 1.35 nmol/mg per minute for the control to 1.68 and 1.75 nmol/mg per minute for the low- and high-dose groups, respectively. In females, the cytochrome b₅ content decreased significantly from 0.640 nmol/mg protein for the control group to 0.564 nmol/mg protein for the 173 mg/kg of body weight per day group. In females, aniline hydroxylase activity increased significantly from 1.70 nmol/mg per minute for the control group to 2.09 nmol/mg per minute for the 173 mg/kg of body weight per day group. The study identified a LOAEL of 16 mg/kg of body weight per day based on changes observed in the liver of males (Sanders et al., 1982).

Chloral hydrate was administered to CD-1 mice (both sexes, numbers not specified) in drinking-water at concentrations of 0, 70 or 700 mg/litre (corresponding to dose levels of 0, 16 and 160 mg/kg of body weight per day for males and 0, 18 and 173 mg/kg of body weight per day for females) for 90 days. Humoral immunity was assessed by verifying the number of splenic antibody-forming cells (AFC) produced in response to sheep red blood cells (for 12 mice in the control group and 8 in the treated group) and haemagglutination titres (20–21 mice in the control group and 13–16 in the treated group). A statistically significant decrease was observed in the humoral immune function (4.09×10^5 per AFC/spleen in the control compared with

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2.61×10^5 per AFC/spleen and 2.47×10^5 per AFC/spleen at 18 and 173 mg/kg of body weight per day, respectively, or 36% and 40% at the low and high exposures, respectively) in females. The decrease was also statistically significant when expressed as AFCs per million spleen cells (32% decrease at 173 mg/kg of body weight per day for females). The decrease in AFCs per million spleen cells at 173 mg/kg of body weight per day in female mice was considered an adverse response in this study. No effects were observed in males. The authors set a NOAEL at 16 mg/kg of body weight per day for humoral immunity and a LOAEL at 160 mg/kg of body weight per day (Kauffmann et al., 1982).

Sprague-Dawley rats (10 per sex per dose) were exposed to chloral hydrate for 90 days in drinking-water at concentrations of 0, 300, 600, 1200 or 2400 mg/litre (corresponding to dose levels of 0, 24, 48, 96 and 168 mg/kg of body weight per day for males and 0, 33, 72, 132 and 288 mg/kg of body weight per day for females). Organ weight and clinical chemistry values in treated animals were found to be sporadically different from the control animal values. Focal hepatocellular necrosis was observed in the males exposed to 96 and 168 mg/kg of body weight per day. The lesions were minimal at 96 mg/kg of body weight per day and more severe at 168 mg/kg of body weight per day. Six male rats developed mild to moderate degeneration of the testicular seminiferous tubules at 48 mg/kg of body weight per day. This was not considered treatment-related, as it did not occur at higher doses. No lesions were observed in females or in any control animals. Changes in some serum chemistry values were observed in both sexes but were not dose-related, nor were they considered toxicologically significant. Based on the hepatotoxic effects and serum enzyme changes, the study identified a LOAEL of 96 mg/kg of body weight per day and a NOAEL of 48 mg/kg of body weight per day (Daniel et al., 1992b).

To assess the toxicity of chloral hydrate, Sprague-Dawley rats (10 per sex per dose) were administered chloral hydrate in drinking-water at 0, 0.2, 2, 20 or 200 mg/litre (corresponding to dose levels of 0, 0.02, 0.19, 1.9 or 19.8 mg/kg of body weight per day for males and 0, 0.03, 0.24, 2.6 or 23.6 mg/kg of body weight per day for females) for a period of 13 weeks. The control animals received distilled water only. The serum TCA level in the treated groups (both sexes) increased in a dose-related manner, from 0.010–0.011 mg/dl at doses of 0.19 and 0.24 mg/kg of body weight per day for males and females, respectively, to 0.4–0.6 mg/dl at 19.8 and 23.6 mg/kg of body weight per day for males and females, respectively. In female rats, uric acid was decreased in the 23.6 mg/kg of body weight per day group, and liver triglyceride was significantly lower in the 2.6 and 23.6 mg/kg of body weight per day groups, but serum triglyceride was not significantly elevated. Pooled liver homogenate from male rats in the control group was assayed for ALDH activity in the presence of various concentrations of TCOH, TCA and chloral hydrate. Chloral hydrate was a strong inhibitor of ALDH, causing an almost complete disappearance of enzyme activity at 0.2 mmol/litre (IC_{50} of 8 μ mol/litre). TCA at 0.4 mmol/litre produced a 20% decrease in enzyme activity, whereas TCOH had no effect on the enzyme at 0.4 mmol/litre. A minimal degree of vacuolation within the myelin sheath of the optic nerve was observed in 30–70% of the male rats in the highest dose group but was absent in the control males. ALDH was depressed in both sexes in the high-dose group only. The

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authors suggested that chloral hydrate and not TCA or TCOH was inhibitory towards ALDH. The present decrease in ALDH is consistent with the previous *in vitro* result in which a decrease of ALDH was observed. An increase in aniline hydroxylase levels at 19.8 and 23.6 mg/kg of body weight per day for males and females, respectively, without any other microsomal enzymes being affected, suggested that chloral hydrate had little effect on the phase I metabolizing enzymes. Because aniline hydroxylase activity is associated with CYP2E1, it may be postulated that there was induction of this enzyme by chloral hydrate or, more likely, by the immediate metabolite TCOH. The catalase level was significantly elevated in the male rats only starting at 0.19 mg/kg of body weight per day, suggesting a sex-dependent difference in regard to peroxisomal enzymes (Poon et al., 2002).

Based on the decrease of ALDH in both sexes at the highest dose, the increase in aniline hydroxylase in both sexes at the highest doses and the minimal vacuolation of the myelin sheath in males at the highest dose, the no-observed-effect level (NOEL) for chloral hydrate in drinking-water was identified by the authors as 1.9 mg/kg of body weight per day in males and 2.6 mg/kg of body weight per day in females. The LOAEL for males in this study was 19.8 mg/kg of body weight per day based on the mild vacuolation of the myelin sheath (Poon et al., 2002). (The authors stated that nervous tissue is particularly susceptible to inadequate fixation, with vacuolation being one of the most common histological artefacts.)

4.3 Long-term exposure

No long-term studies with animals exposed to chloral hydrate were found in the literature, other than the ones described in the section on carcinogenicity (see section 4.6 below).

4.4 Reproductive and developmental toxicity

The reproductive, embryo-fetotoxic and teratogenic effects of chloral hydrate have been studied in several species.

Male and female CD-1 mice (four per cage, total number not specified) were exposed to chloral hydrate in drinking-water at concentrations of 60 or 600 mg/litre. These chloral hydrate concentrations were selected to simulate daily exposure levels of approximately 14.4 and 144 mg/kg of body weight per day based upon body weight and estimated fluid consumption. However, actual exposure to chloral hydrate was slightly higher than intended due to the higher than expected fluid consumption by the mothers relative to the fluid intake generally observed in non-pregnant females. Therefore, the actual mean daily exposures for dams corresponded to 21.3 and 204.8 mg/kg of body weight per day. All animals were exposed for 3 weeks prior to breeding. Females were also exposed during gestation and until pups were weaned at 21 days of age. At 204.8 mg/kg of body weight per day, at 23 days of age, pups showed impaired retention of passive avoidance learning in both 1-h and 24-h retention tests. This study identified a NOAEL for neurodevelopmental toxicity of 21.3 mg/kg of body weight per day. A reproductive and developmental effects

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NOAEL was also identified at 204.8 mg/kg of body weight per day (Kallman et al., 1984).

Male F344 rats (two per cage, total number not specified) were administered chloral hydrate in drinking-water at concentrations of 0, 780 or 2700 mg/litre (corresponding to dose levels of 0, 55 and 188 mg/kg of body weight per day) for 52 weeks to evaluate the effects of chloral hydrate on sperm morphology and motility. A reduction in sperm motility was observed in rats exposed to 188 mg/kg of body weight per day (58%) compared with controls (68%). A shift in the frequency distribution of the average straight-line velocities of sperm also occurred at this dose compared with the controls. A NOAEL for effects on sperm motility was set at 55 mg/kg of body weight per day, and a LOAEL of 188 mg/kg of body weight per day was identified (Klinefelter et al., 1995).

An *in vitro* embryotoxicity study was performed with embryos from Sprague-Dawley rats exposed to chloral hydrate on gestational day 10 at 0, 0.5, 1.0, 1.5, 2.0 or 2.5 mmol/litre (equivalent to 0, 83, 165, 248, 331 and 414 mg/litre) for 46 h. At the highest dose, all embryos died, but no deaths were observed at lower doses. Chloral hydrate caused concentration-dependent decreases in growth and differentiation and increases in the incidence of morphologically abnormal embryos. At 1.0 mmol/litre, a decrease in crown-rump length, somite (embryonic segments) numbers and the protein or DNA content of embryos was observed. At 1.0, 1.5 and 2.0 mmol/litre, 18%, 68% and 100% of embryos, respectively, had malformations, malformations of the brain, eyes and ears being among the most frequent developmental effects encountered. At 2.0 mmol/litre, abnormalities were also observed in the trunk and in the optic and otic systems. At the higher concentrations, embryos exhibited severe alterations of the craniofacial region. Hypoplasia of the prosencephalon was also observed. Chloral hydrate caused pericardial dilation (45% of the embryos at 2 mmol/litre). Chloral hydrate produced a step increase in embryo lethality as concentrations increased. Based on this *in vitro* study, chloral hydrate was found to be more potent than TCA and DCA. A NOAEL of 0.5 mmol/litre (83 mg/litre) was identified for embryotoxicity (Saillenfait et al., 1995). Since this study is an *in vitro* study, it is difficult to compare the results with an *in vivo* study, nor is it possible to extrapolate the risk to human health. This study is unusual, because reproductive studies tend to involve exposure over the period of organogenesis (days 5–12) or over the entire gestation period.

Pregnant female Sprague-Dawley rats were exposed to chloral hydrate in drinking-water from gestational day 1 to day 22 at 1.232 mg/ml (corresponding to an average exposure of 151 mg/kg of body weight per day). There was no evidence of maternal toxicity, no change in the number of live or dead fetuses, no change in placental or fetal weight, no change in crown-rump length and no increase in the incidence of morphological changes. Heart malformations (not significant), such as atrial septal defects (2), mitral valve defects (2), ventricular septal defects (3) and pulmonary valve defects (1), were observed, compared with the control group, which had a total of 15 types of heart malformations. The chloral hydrate-exposed group had a 3.23% incidence of heart abnormalities, compared with 2.15% for the control group

(significantly different). In comparison, a group exposed to TCA at 291 mg/kg of body weight per day had a total of 15 heart defects (10.53% abnormal hearts). A LOAEL of 151 mg/kg of body weight per day for chloral hydrate was identified in this study based on development toxicity, and no NOAEL was identified (Johnson et al., 1998).

4.5 Mutagenicity and related end-points

Positive results were reported in *Salmonella typhimurium* in point mutation assays with or without microsomal activating enzymes (S9), with strain TA198 with or without S9 and with strain TA100 with or without S9, but negative results were obtained with strain TA1535 and in *Streptomyces coelicolor* and *Aspergillus nidulans*, but only with the anhydrous form (Waskell, 1978; Bruce & Heddle, 1979; Bignami et al., 1980; NTP, 2002a). Similar effects were observed in *A. nidulans* and *Saccharomyces cerevisiae* at doses between 0 and 25 mmol/litre, which induced diploid clones during meiosis I (Sora & Agostini Carbone, 1987). A positive dose trend was observed when chloral hydrate was tested at dose levels between 125 and 500 mg/kg of body weight in an *in vivo* mouse bone marrow micronucleus test (NTP, 2002b). Positive results in both sister chromatid exchanges and DNA strand breaks have been reported in human lymphocytes exposed *in vitro* to chloral hydrate (Gu et al., 1981). Positive results were found for aneuploidy and clastogenicity in several test systems using mammalian cells *in vitro* (Natarajan et al., 1993).

Chloral hydrate did not induce mitotic crossing-over in *A. nidulans* in the absence of metabolic activation. Chloral hydrate caused a weak induction of meiotic recombination in the presence of metabolic activation and gene conversion in the absence of metabolic activation in *S. cerevisiae*, but it did not induce reverse mutation in the same species. Chloral hydrate induced aneuploidy in various fungi in the absence of metabolic activation. Chloral hydrate did not produce DNA-protein crosslinks in rat liver nuclei, DNA single strand breaks/alkaline-labile sites in primary hepatocytes *in vitro* or DNA repair in *Escherichia coli*. Chloral hydrate increased the frequency of chromosomal aberrations in mouse bone marrow, spermatogonia and primary and secondary spermatocytes, but not in oocytes, after *in vivo* treatment. In mouse micronucleus assays, chloral hydrate increased the frequency of chromosomal aberrations in mouse bone marrow erythrocytes (US EPA, 2000).

Mouse lymphoma cells were treated with chloral hydrate at concentrations ranging from 0 to 1600 µg/ml to induce micronuclei and chromosomal aberrations. Chloral hydrate induced concentration-related cytotoxicity and a very weak mutagenic response. It was not positive for micronucleus induction and did not induce aneuploidy. Chloral hydrate was clastogenic, based on its chromosomal aberrations response: 6 aberrations/100 metaphases at 0 µg/ml and 14 aberrations/100 metaphases at 1250 µg/ml (Natarajan et al., 1993; Harrington-Brock et al., 1998).

Chloral hydrate induced aneuploidy in Chinese hamster embryonic fibroblasts *in vitro* without an exogenous metabolic system at 250 µg/ml (Harrington-Brock et al., 1998), in Chinese hamster pulmonary line LUC2 p4 cells *in vitro* at 250 µg/ml (Warr et al.,

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1993), in DON:Wg.3h Chinese hamster pulmonary line at 50 µg/ml (Warr et al., 1993) and in human peripheral blood lymphocytes at 50 µg/ml without an exogenous metabolic system (Sbrana et al., 1993).

Increases in micronuclei in mouse spermatids were observed when spermatogonia stem cells were exposed to chloral hydrate at 41, 83 or 165 mg/kg of body weight (Allen et al., 1994). Chloral hydrate gave negative test results in studies with ICR mouse metaphase II oocytes (Mailhes et al., 1993).

Chloral hydrate induced a significant increase in sister chromatid exchange at doses ranging from 1700 to 5000 µg/ml and in chromosomal aberrations in Chinese hamster ovary cells at doses ranging from 1000 to 3000 µg/ml with or without S9 (Bruce & Heddle, 1979; NTP, 2002a).

In *Drosophila melanogaster* (Canton S males), chloral hydrate induced a small increase in the frequency of sex-linked recessive lethal mutations in germ cells of male flies fed 5500 mg/kg of body weight (Yoon et al., 1985).

Male C57Bl/6J mice were given a single intraperitoneal injection of chloral hydrate at dose levels of 41, 83 or 165 mg/kg of body weight to characterize its potential to induce chromosome loss during germ cell division. Spermatids were analysed for micronuclei frequency and the presence or absence of kinetochore(s). Positive results were obtained for spermatid micronucleus induction with chloral hydrate when treatments corresponded to spermatogonial stem cell or preleptotene spermatocyte stages of development, which constituted the relevant cell populations damaged. Negative results were, however, observed after treatment during leptotene, zygotene or diakinesis metaphase stages (Rijhsinghani et al., 1986). The significantly increased levels of micronuclei observed were invariably of the kinetochore-negative type. It was not possible to determine whether these micronuclei derived from chromosome breakage or loss (Allen et al., 1994).

Chloral hydrate failed to induce DNA strand breaks in either rats or mice, in rodent hepatocytes in primary culture or in CCRF-CEM cells, a human lymphoblastic leukaemia cell line (Chang et al., 1992). Moore & Harrington-Brock (2000) found chloral hydrate and its metabolites to show evidence of some genotoxic activity, albeit at very high doses, indicating that chloral hydrate is a weak genotoxic chemical.

The positive effect of chloral hydrate in systems *in vivo* was less consistent following the oral route of exposure, although *in vivo* tests conducted following intraperitoneal administration were positive in the mouse germ cell, lymphocyte and micronuclear assay.

4.6 Carcinogenicity

Several drinking-water carcinogenicity studies have been conducted in mice and rats, as well as one feeding study in mice.

In the first drinking-water study in mice (Rijhsinghani et al., 1986), chloral hydrate was given to 15-day-old male C57BL × C3HF1 mice as a single dose in distilled water at two dose levels, 5 mg/kg of body weight in group 1 (25 mice) and 10 mg/kg of body weight in group 2 (20 mice). The control group (group 3) (35 mice) was given distilled water only for 92 weeks. To study short-term effects, 6–10 mice from each group were sacrificed 24 h after receiving chloral hydrate. The remaining mice were weaned at 4 weeks of age. To study long-term effects, animals were sacrificed when found moribund or at intervals up to 92 weeks. A few mice died at different intervals, and others were killed for examination at fixed intervals. In mice sacrificed 48–92 weeks after treatment, the incidence of hepatic nodules (adenomas and trabecular carcinomas) was 3/9 and 6/8 for animals from the 5 and 10 mg/kg of body weight per day dose groups, respectively, compared with 2/19 in controls. The increase in tumours was statistically significant ($P < 0.05$) only in the 10 mg/kg of body weight per day group. There was an increase in the relative weight of the liver in mice given 10 mg of chloral hydrate per kg of body weight per day in the long-term study. A hepatic nodule was first observed in a mouse 48 weeks after the administration of chloral hydrate at 10 mg/kg of body weight per day. In mice sacrificed between weeks 48 and 92 after chloral hydrate administration, gross hepatic nodules were found in 3/9 animals in group 1, 6/8 in group 2 and 2/19 in group 3. Compared with the control group, there was a significant increase ($P < 0.05$) in the incidence of hepatic nodules in mice given chloral hydrate at a dose of 10 mg/kg of body weight per day only. The hepatic nodular lesions ranged from hyperplastic foci of clear or acidophilic cells to hepatocellular adenomas and trabecular carcinomas containing eosinophilic hepatocytes. An increase in liver to body weight ratio was also observed in the 10 mg/kg of body weight per day group but not in the 5 mg/kg of body weight per day group (Rijhsinghani et al., 1986). It is important to note that this study is almost 20 years old and that the protocol used was not based on Organisation for Economic Co-operation and Development (OECD) guidelines regarding the use of two sexes, a required number of animals and a sufficient number of doses for a carcinogenicity study. The increased incidence of hepatic tumours in this study is believed to have been due to normal variation in mice and not a result of chloral hydrate treatment (NTP, 2002b).

In the second drinking-water study in mice (Daniel et al., 1992a), 40 male B6C3F1 mice received 1 g of chloral hydrate per litre (166 mg/kg of body weight per day) in drinking-water for 104 weeks. Two control groups, totalling 33 animals, received plain water. Interim sacrifices were made at 30 and 60 weeks (five controls and five chloral hydrate-exposed mice) for biochemical and interim pathological analysis. Those animals that died during the study (three in the control group and six in the chloral hydrate group) were not counted as being at risk for tumour development. Only mild histopathological changes were observed in the liver, and no changes were noted in other organs. At the 60-week sacrifice, hepatocellular carcinomas were found

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in two chloral hydrate-treated mice, compared with zero in the controls. No lesions were found in the control group at this time. At the end of the study, 11 out of 24 surviving mice exposed to chloral hydrate had hepatocellular carcinomas, and 7 had hepatocellular adenomas. Hepatocellular lesions in controls included 2 animals with carcinomas and 1 with adenomas out of 20 survivors examined. The incidence of both adenomas and carcinomas was significantly greater than in the control group. The increase in the combined incidence of the two lesions, adenomas and carcinomas, was highly significant (Daniel et al., 1992a). Although this study indicated that the liver is the target organ following exposure to chloral hydrate, it is considered inadequate based on OECD guideline standard protocols, because only one dose was used.

In the third drinking-water study in mice (NTP, 2002a), B6C3F1 mice, divided into five groups, received chloral hydrate in distilled water by gavage for 105 weeks; the control groups received distilled water only. In group A, 48 female mice (28 days old) received 0, 25, 50 or 100 mg of chloral hydrate per kg of body weight per day, 5 days per week (0, 17.9, 35.7 or 71.4 mg/kg of body weight per day adjusted for 7 days per week dosing). In group B, 24 females (28 days old) received 0 mg/kg of body weight per day, and three groups of 48 females received 100 mg/kg of body weight per day 5 days per week. Eight mice from the 0 and 100 mg/kg of body weight per day groups were killed at 3, 6 and 12 months. In group C, 48 females (28 days old) received a single dose of 0, 10, 25 or 50 mg/kg of body weight and were held for 105 weeks. In groups D and E, respectively, 48 female mice and 48 male mice received a single dose of 0, 10, 25 or 50 mg/kg of body weight when they were 15 days old and were held for 105 weeks. Four mice from groups C, D and E were killed at 3 or 6 months, and eight mice from group B were designated for the hepatic cell proliferation analyses; mice killed at 3 or 6 months in group B were also designated for apoptosis analyses. Survival of all dosed mice in all the groups was similar to that of the vehicle control group. Slight variations in mean body weight were observed in some groups. The incidence of pituitary pars distalis adenomas occurred with a positive dose-related trend in group A, and the incidence in the 71.4 mg/kg of body weight per day group was significantly greater than that in the control group. There was also a significant positive time-related trend in the incidence of adenoma in female mice administered 71.4 mg/kg of body weight per day for up to 24 months. There was a significant increase in the severity of pars distalis hyperplasia in the first group at 71.4 mg/kg of body weight per day. The authors concluded that there was equivocal evidence of carcinogenic activity of chloral hydrate in female mice treated continuously for 2 years, based on increased incidences of pituitary gland pars distalis adenoma. No increased incidences of neoplasms were seen in female B6C3F1 mice that received a single dose of chloral hydrate at 15 or 28 days of age or in male B6C3F1 mice that received a single dose of chloral hydrate at 15 days of age. No hepatic carcinogenicity was seen under all dosing conditions. The NOAEL for non-neoplastic effects was determined to be 71.4 mg/kg of body weight per day (NTP, 2002a).

In another mouse drinking-water study (George et al., 2000), male B6C3F1 mice (72 per dose) were exposed to chloral hydrate in drinking-water at concentrations of 0, 120, 580 or 1280 mg/litre (corresponding to dose levels of 0, 13.5, 65 or 146.6 mg/kg

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of body weight per day) for 104 weeks. The mice showed an increase of proliferative lesions in the liver (hyperplasia, adenomas, carcinoma and combined adenoma and carcinoma) at all exposure levels. The prevalence of hyperplasia was increased in all dose groups (32.6%, 33.3% and 37.5% for the low-, mid- and high-dose groups) compared with the controls (7.1%). The prevalence of hepatocellular carcinomas was increased in the high-dose group (84.4%) compared with 54.8%, 54.3% and 59.0% in the control, low-dose and mid-dose groups. The prevalence of hepatadenomas was significantly increased in all dose groups: 43.5%, 51.3% and 50.0% for the low-, mid- and high-dose groups, respectively, compared with 21.4% in the control group. The combined hepatocellular adenomas and carcinomas incidence was increased in the low- (78.6%), mid- (79.5%) and high-dose (90.6%) groups, compared with 64.3% for the controls, although the increases were significant only at the mid- and high-dose levels. Serum LDH, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and sorbitol dehydrogenase (SDH) activities and total antioxidant levels reflected the minimal degree of hepatocellular damage observed microscopically. None of these parameters in the chloral hydrate-treated groups was altered compared with the control values after 52 and 78 weeks of exposure. Palmitoyl CoA oxidase activities in the homogenates of livers were not significantly increased above the control value, indicating that chloral hydrate did not induce peroxisome proliferation. Enhanced liver neoplasia occurred at the lowest dose, 13.5 mg/kg of body weight per day; therefore, a NOAEL could not be determined. However, a LOAEL can be set at 13.5 mg/kg of body weight per day. Results for combined neoplasms were significantly increased in the mid- and high-dose groups for prevalence and in all dose groups for multiplicity. This study indicated that the incidences of hepatocellular adenoma were increased at all doses levels, but the incidence of hepatocellular carcinoma increased at the high dose only (George et al., 2000).

In another component of the above drinking-water study using rats (George et al., 2000), male F344 rats (78 per dose) were administered chloral hydrate in drinking-water at concentrations of 0, 120, 580 or 2510 mg/litre (corresponding to dose levels of 0, 7.4, 37.4 or 162.6 mg/kg of body weight per day) for 104 weeks. No changes were noted in water consumption, survival, behaviour or body weight at any exposure level. Serum LDH, ALT, AST and SDH activities and total antioxidant levels reflected the minimal degree of hepatocellular damage observed microscopically. The authors did not indicate the nature of the “activities.” None of these parameters in any of the chloral hydrate-treated groups was altered compared with the control values after 52 and 78 weeks of exposure. The NOAEL for this study was set at 162.6 mg/kg of body weight per day (George et al., 2000). It should be noted that this study did not achieve the maximum tolerated dose (US EPA, 2000), and only male rats were used.

In a second drinking-water study with rats (Leuschner & Beuscher, 1998), Sprague-Dawley rats (50 per sex per group) were administered chloral hydrate at doses of 0 (untreated drinking-water), 15, 45 or 135 mg/kg of body weight per day in drinking-water for 124 weeks for males and 128 weeks for females. There was no evidence of an increased incidence of tumours in any organs. An increase in the incidence of hepatocellular hypertrophy was observed at the highest dose (28%) compared with the control (11%). The finding was characterized by diffuse liver cell enlargement with

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slightly eosinophilic cytoplasm. The increase in hepatocellular hypertrophy was seen only in the male rats and was graded as minimal to slight in severity. The type, incidence and organ distribution of the neoplastic lesions in the chloral hydrate-treated rats did not differ from the control rats, and the lesions were therefore regarded as random events. No change was observed in body weight or organ weight. A NOAEL of 45 mg/kg of body weight per day and a LOAEL of 135 mg/kg of body weight per day were set based on the increased incidence of hepatocellular hypertrophy (Leuschner & Beuscher, 1998). As with the previous study, there are indications that this study did not achieve the maximum tolerated dose (US EPA, 2000).

In a feeding study with mice (NTP, 2002b), a group of 120 male B6C3F1 mice was fed 0, 25, 50 or 100 mg of chloral hydrate per kg of body weight per day 5 days per week (0, 17.9, 35.7 or 71.4 mg/kg of body weight per day adjusted for 7 days per week dosing) for 2 years. The male mice were divided into two groups of 60 mice: one received feed *ad libitum*, while the other received feed in a measured daily amount (gavage). Evaluation of 12 mice per dose group and diet was performed after 15 months, and the other 48 mice per dose group and diet were evaluated after 2 years. Histopathological changes were observed only in the liver. The incidence of hepatocellular adenomas or carcinomas was significantly greater in the 17.9 mg/kg of body weight per day group only in the *ad libitum* group. In the dietary controlled study, the incidence of hepatocellular carcinomas was significantly different at 71.4 mg/kg of body weight per day only. The NOAEL for non-neoplastic effects is 71.4 mg/kg of body weight per day. The incidences of hepatocellular adenoma/carcinoma for the *ad libitum* group in male B6C3F1 mice were 33%, 52%, 48% and 46% at dose levels of 0, 17.9, 35.7 and 71.4 mg/kg of body weight per day, respectively, compared with 42% for the historical data. In the controlled diet, the incidences of combined adenoma and carcinoma were lower than the historical control: 23%, 23%, 29% and 38% at dose levels of 0, 17.9, 35.7 and 71.4 mg/kg of body weight per day, respectively, compared with 42.2% for the historical control with the same strain of mice. No female mice were treated in this study (NTP, 2002b).

4.7 Mode of action

In the Poon et al. (2002) subchronic toxicity study described above, it was postulated that the biological effects observed were attributable to TCA, a known peroxisomal proliferator. Triglyceride depression may also be a TCA effect. The presence of TCA in the serum and increased liver catalase lend support to a peroxisomal proliferation effect of chloral hydrate. However, this is of limited relevance in humans, since humans and other primates are less responsive than rats and mice in terms of peroxisomal proliferation. In contrast, hepatic hypotriglyceridaemia is of relevance to humans, because the hypolipidaemic effect of peroxisome proliferators is common to both experimental animals and humans.

In an *in vitro* study using male B6C3F1 mouse liver microsomes, it was found that chloral hydrate generated free radical intermediates that resulted in endogenous lipid peroxidation, thus forming malondialdehyde, formaldehyde, acetaldehyde, acetone

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and propionaldehyde, substances that are known to be tumorigenic. Both TCA and TCOH also induced lipid peroxidation, but TCA had a stronger effect than TCOH, suggesting that TCA formation is the predominant pathway leading to lipid peroxidation. Cytochrome P-450 (possibly the isoenzyme CYP2E1) was the enzyme responsible for the metabolic activation of chloral hydrate and its metabolites to TCA and TCOH, leading to tumorigenic lipid peroxidation (Ni et al., 1996).

5. EFFECTS ON HUMANS

Chloral hydrate was introduced into therapeutics more than 100 years ago and has been used as a sedative/hypnotic agent in children, adults and animals since its introduction (Henderson et al., 1997). No data are available at the present time to determine a NOAEL in humans. The LOAEL is 12.5 mg/kg of body weight per day (assuming a body weight of 60 kg), based on the recommended dose for an adult as a sedative at 250 mg, 3 times a day.

Oral administration of chloral hydrate at high doses causes gastric irritation, with nausea, vomiting and diarrhoea as the most frequent adverse effects. Other adverse effects of chloral hydrate may include leukopenia, eosinophilia and, rarely, ketonuria (McEvoy, 1999).

The toxic blood level for chloral hydrate was estimated at 10 mg/100 ml, and the lethal blood level at 25 mg/100 ml (Ellenhorn et al., 1997).

While a lethal oral dose of 10 g has been reported for adults, death has occurred after ingestion of 4 g, and some patients have survived ingestion of as much as 30 g (McEvoy, 1999). Ingestion of 20 g by a patient, who later became comatose, resulted in gastric perforation that was detected 4 days post-ingestion. Gastrointestinal haemorrhage followed by the development of oesophageal strictures has been observed with a dose of 18 g. Hepatic (jaundice, aminotransferase elevation) and renal (albuminuria) dysfunction may occur several days after ingestion, but are rarely serious or prolonged (Abbas et al., 1996).

The medical records of 1618 patients who had received chloral hydrate at 1 g (14.3 mg/kg of body weight), 0.5 g (7.6 mg/kg of body weight) or various other doses (not specified) were reviewed. The study was carried out within the context of a comprehensive drug surveillance programme operating in three Boston, Massachusetts, USA, hospitals for a period of up to 3 years. The results indicated that cirrhosis of the liver was the most common diagnosis (15%), while chronic obstructive respiratory tract disease (7%), carcinoma of the breast (7%) and congestive cardiac failure (7%) were also reported, although a causal association could not be determined. In this study, it was not clear if the patients had the identified clinical effects prior to being exposed to chloral hydrate or if they developed the clinical effects after being exposed to chloral hydrate. Other adverse reactions included gastrointestinal symptoms (10 patients), depression of the central nervous system (20 patients), skin rash (5 patients), prolonged prothrombin time (1 patient), worsened hepatic encephalopathy (1 patient) and bradycardia (1 patient). The

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latter adverse effects disappeared soon after the end of chloral hydrate administration (exposure). No associations were found between adverse side effects and age, weight or sex in this study (Shapiro et al., 1969).

Only 2.2% of 5435 hospitalized patients receiving chloral hydrate at doses of 0.5 or 1 g for insomnia experienced adverse reactions. Central nervous system depression was the predominant adverse effect (1.1%), followed by sensitivity reaction (0.35%), gastrointestinal disturbances (0.28%), central nervous system excitation (0.22%) and other (0.28%) (Miller & Greenblatt, 1979).

Greenberg et al. (1991) observed the effects of chloral hydrate sedation in children receiving computer tomography procedures. Adverse effects observed in children receiving a high dose (80–100 mg/kg of body weight and a maximum total dose of 2 g) included vomiting, hyperactivity and respiratory symptoms such as wheezing and secretion aspiration. No abnormalities were observed following cardiac monitoring, nor was any arrhythmia observed in any of the children. In contrast, no adverse effects were observed in children receiving a low dose of chloral hydrate (40–75 mg/kg of body weight). However, it should be noted that these children received lower doses because of existing liver or renal impairment, respiratory insufficiency or central nervous system depression.

No long-term clinical studies of chloral hydrate exposure in humans were available in the published literature.

6. PRACTICAL ASPECTS

6.1 Analytical methods and analytical achievability

A solvent extraction procedure (EPA Method 551.1) was developed by the US Environmental Protection Agency (EPA) for the analysis of chloral hydrate using methyl *tert*-butyl ether (MTBE) as a solvent for the extraction (US EPA, 1995), with a detection limit of 0.5 µg/litre (AWWARF, 1991). Chloral hydrate is analysed using GC with ECD. The limit of detection is approximately 30 ng/litre (Department of Agriculture, Fisheries and Forestry Australia, 1996).

A solvent extraction method (MTBE) adapted from EPA Method 551 (US EPA, 1990) for the analysis of chloral hydrate was developed by Health Canada (LeBel & Williams, 1996, 1997; Koudjonou & LeBel, 2003). The sampling protocol requires field pH adjustment (pH 4.5) and use of ascorbic acid to quench the residual chlorine. The water extracts are analysed by GC with ECD. The minimum quantification limit for chloral hydrate using this method is 0.1 µg/litre.

Method 5710 D of the 20th edition of the *Standard Methods for the Examination of Water and Wastewater* is used to analyse chloral hydrate. This method stipulates that chloral hydrate may be analysed with trihalomethanes (THMs) using a sulfite reducing solution to quench the reaction. Chloral hydrate is also analysed using

liquid–liquid extraction and capillary column GC with ECD, with a detection limit of 0.5 µg/litre (APHA et al., 1998).

6.2 Treatment and control methods and technical achievability

Chloral hydrate concentrations may be reduced by changes to disinfection practice (e.g., enhanced coagulation and softening to remove organic precursor compounds, moving the point of disinfection to reduce the reaction between chlorine and precursor compounds, and using chloramines for residual disinfection instead of chlorine) or by granular activated carbon (GAC) treatment. Complete removal of 4.5 µg of chloral hydrate per litre by GAC over 1 year's operation was reported from a pilot plant study (Lykins et al., 1991).

7. CONCLUSION

No epidemiological or carcinogenic studies were found in humans associating exposure to chloral hydrate with cancer, despite the fact that chloral hydrate has been used for many decades (and still is used) as a sedative and hypnotic drug in adults and children (specifically for dental procedures).

IARC (1995) classified chloral hydrate as not classifiable as to its carcinogenicity to humans (Group 3) in 1995, based on inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of chloral hydrate. There is equivocal evidence of genotoxicity for chloral hydrate.

For compounds that are possibly carcinogenic to humans, the guideline value is based upon a TDI derived by the division of the lowest NOAEL or LOAEL by appropriate uncertainty factors.

The non-cancer end-point of histopathology in the liver, as derived in George et al. (2000), was chosen for the risk assessment. Male B6C3F1 mice were treated in a lifetime study with chloral hydrate in drinking-water at concentrations of 0, 120, 580 or 1280 mg/litre (corresponding to dose levels of 0, 13.5, 65 or 146.6 mg/kg of body weight per day). The study showed that chloral hydrate induced an increase in the incidence of proliferative lesions (hyperplasia, adenoma, carcinoma and combined adenoma and carcinoma) at all exposures, except for carcinoma at the two lower exposures. The background response in this study is higher than normal for this strain of mice; however, the mice at all exposures showed an increase in proliferative lesions in the liver. At 120 mg/litre (13.5 mg/kg of body weight per day and above), significant increases in the incidence of proliferative lesions were observed. This increase in proliferative lesions is an important end-point. Since these lesions were observed at all dose levels, no NOAEL could be derived; therefore, a LOAEL of 120 mg/litre (13.5 mg/kg of body weight per day) was set. An additional uncertainty factor of 3 was added to account for the limitations of the database in regards to evidence of carcinogenicity in animals.

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A TDI of 0.0045 mg/kg of body weight is derived by applying an uncertainty factor of 3000 ($\times 10$ for interspecies variability; $\times 10$ for intraspecies variability; $\times 10$ to account for the use of a LOAEL instead of a NOAEL, as no NOAEL was observed in the relevant study; and $\times 3$ to account for limited evidence of carcinogenicity) to the LOAEL of 13.5 mg/kg of body weight per day, based on an increased incidence of liver histopathology in B6C3F1 mice.

It should be noted that this TDI is considerably more conservative than the TDI derived by IPCS (2000a) in an evaluation of disinfectants and disinfection by-products, on the basis of the recommended clinical dose for sedation in adults (Gilman et al., 1985). This TDI, based on a therapeutic dose, is judged to be inappropriate for the derivation of a health-based value for lifetime exposure in drinking-water. It should also be noted that the LOAEL used here (13.5 mg/kg of body weight per day) is similar to the LOAEL used to derive a TDI in IPCS (2000b), a more comprehensive evaluation of chloral hydrate, based on an older study in which hepatomegaly and changes in microsomal parameters were observed in male CD-1 mice at 16 mg/kg of body weight per day (Sanders et al., 1982). However, the IPCS (2000b) derivation used an uncertainty factor of 1000, rather than 3000, as has been used here to account for the limitations of the database in regards to evidence of carcinogenicity in animals. The TDI used here is, therefore, more conservative.

A health-based value of 0.1 mg/litre (rounded figure) can be calculated on the basis of the TDI of 0.0045 mg/kg of body weight, assuming an allocation of 80% of the TDI to drinking-water and a 60-kg adult consuming 2 litres of water per day. The health-based value is expected to be protective of non-cancer end-points, including neurodevelopmental toxicity. However, because chloral hydrate usually occurs in drinking-water at concentrations well below this health-based value (i.e., generally below 10 $\mu\text{g/litre}$), it is not considered necessary to derive a guideline value.

It should be noted that the allocation factor of 80% of the TDI was used rather than the 20% that was used previously, since exposure to chloral hydrate comes mostly from drinking-water.

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