This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organization, or the World Health Organization.

Concise International Chemical Assessment Document 25

CHLORAL HYDRATE

Note that the layout and pagination of this pdf file are not identical to the printed CICAD

First draft prepared by Dr R. Benson, Region VIII, Environmental Protection Agency, Denver, CO, USA

Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.

World Health Organization
Geneva, 2000

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

WHO Library Cataloguing-in-Publication Data

Chloral hydrate.

(Concise international chemical assessment document ; 25)

1.Chloral hydrate - toxicity 2.Risk assessment 3.Environmental exposure
I.International Programme on Chemical Safety II.Series

ISBN 92 4 153025 1 (NLM Classification: QV 85)
ISSN 1020-6167

The World Health Organization welcomes requests for permission to reproduce or translate its publications, in part or in full. Applications and enquiries should be addressed to the Office of Publications, World Health Organization, Geneva, Switzerland, which will be glad to provide the latest information on any changes made to the text, plans for new editions, and reprints and translations already available.

©World Health Organization 2000

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. All rights reserved.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, Germany, provided financial support for the printing of this publication.

Printed by Wissenschaftliche Verlagsgesellschaft mbH, D-70009 Stuttgart 10

TABLE OF CONTENTS

FOREWORD 1

1. EXECUTIVE SUMMARY 4

2. IDENTITY AND PHYSICAL/CHEMICAL PROPERTIES 5

3. ANALYTICAL METHODS 5

4. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE 6

5. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION 6

6. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE 6

6.1 Environmental levels 6
6.2 Human exposure 6

7. COMPARATIVE KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS 7

8. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS 8

8.1 Single exposure 8
8.1.1 Oral 8
8.1.2 Inhalation 9
8.2 Irritation and sensitization 9
8.3 Short-term exposure 9
8.4 Long-term exposure 9
8.4.1 Subchronic exposure 9
8.4.2 Chronic exposure and carcinogenicity 10
8.5 Genotoxicity and related end-points 11
8.5.1 Genotoxicity 11
8.5.2 Cell proliferation 12
8.5.3 Oncogene activation 13
8.5.4 Free radicals and DNA adduct formation 13
8.5.5 Cell communication 13
8.5.6 Peroxisome proliferation 13
8.6 Reproductive and developmental toxicity 13
8.7 Immunological and neurological effects 14

9. EFFECTS ON HUMANS 15

10. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD 16

11. EFFECTS EVALUATION 17

11.1 Evaluation of health effects 17
11.1.1 Hazard identification and dose–response assessment 17
11.1.2 Criteria for setting tolerable intakes or guidance values for chloral hydrate 20
11.1.3 Sample risk characterization 20
11.2 Evaluation of environmental effects 20

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES 20

REFERENCES 22

APPENDIX 1 — TOXICOKINETICS 25

APPENDIX 2 — CALCULATION OF BENCHMARK DOSE FOR TUMOUR INCIDENCE 26

APPENDIX 3 — SOURCE DOCUMENT 27

APPENDIX 4 — CICAD PEER REVIEW 27

APPENDIX 5 — CICAD FINAL REVIEW BOARD 28

APPENDIX 6 — INTERNATIONAL CHEMICAL SAFETY CARD 29

RÉSUMÉ D'ORIENTATION 31

RESUMEN DE ORIENTACIÓN 33

FOREWORD

Concise International Chemical Assessment Documents (CICADs) are the latest in a family of publications from the International Programme on Chemical Safety (IPCS) — a cooperative programme of the World Health Organization (WHO), the International Labour Organization (ILO), and the United Nations Environment Programme (UNEP). CICADs join the Environmental Health Criteria documents (EHCs) as authoritative documents on the risk assessment of chemicals.

CICADs are concise documents that provide summaries of the relevant scientific information concerning the potential effects of chemicals upon human health and/or the environment. They are based on selected national or regional evaluation documents or on existing EHCs. Before acceptance for publication as CICADs by IPCS, these documents undergo extensive peer review by internationally selected experts to ensure their completeness, accuracy in the way in which the original data are represented, and the validity of the conclusions drawn.

The primary objective of CICADs is characterization of hazard and dose–response from exposure to a chemical. CICADs are not a summary of all available data on a particular chemical; rather, they include only that information considered critical for characterization of the risk posed by the chemical. The critical studies are, however, presented in sufficient detail to support the conclusions drawn. For additional information, the reader should consult the identified source documents upon which the CICAD has been based.

Risks to human health and the environment will vary considerably depending upon the type and extent of exposure. Responsible authorities are strongly encouraged to characterize risk on the basis of locally measured or predicted exposure scenarios. To assist the reader, examples of exposure estimation and risk characterization are provided in CICADs, whenever possible. These examples cannot be considered as representing all possible exposure situations, but are provided as guidance only. The reader is referred to EHC 170[1] for advice on the derivation of health-based tolerable intakes and guidance values.

[1] International Programme on Chemical Safety (1994) *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits.* Geneva, World Health Organization (Environmental Health Criteria 170).

While every effort is made to ensure that CICADs represent the current status of knowledge, new information is being developed constantly. Unless otherwise stated, CICADs are based on a search of the scientific literature to the date shown in the executive summary. In the event that a reader becomes aware of new information that would change the conclusions drawn in a CICAD, the reader is requested to contact IPCS to inform it of the new information.

Procedures

The flow chart shows the procedures followed to produce a CICAD. These procedures are designed to take advantage of the expertise that exists around the world — expertise that is required to produce the high-quality evaluations of toxicological, exposure, and other data that are necessary for assessing risks to human health and/or the environment.

The first draft is based on an existing national, regional, or international review. Authors of the first draft are usually, but not necessarily, from the institution that developed the original review. A standard outline has been developed to encourage consistency in form. The first draft undergoes primary review by IPCS to ensure that it meets the specified criteria for CICADs.

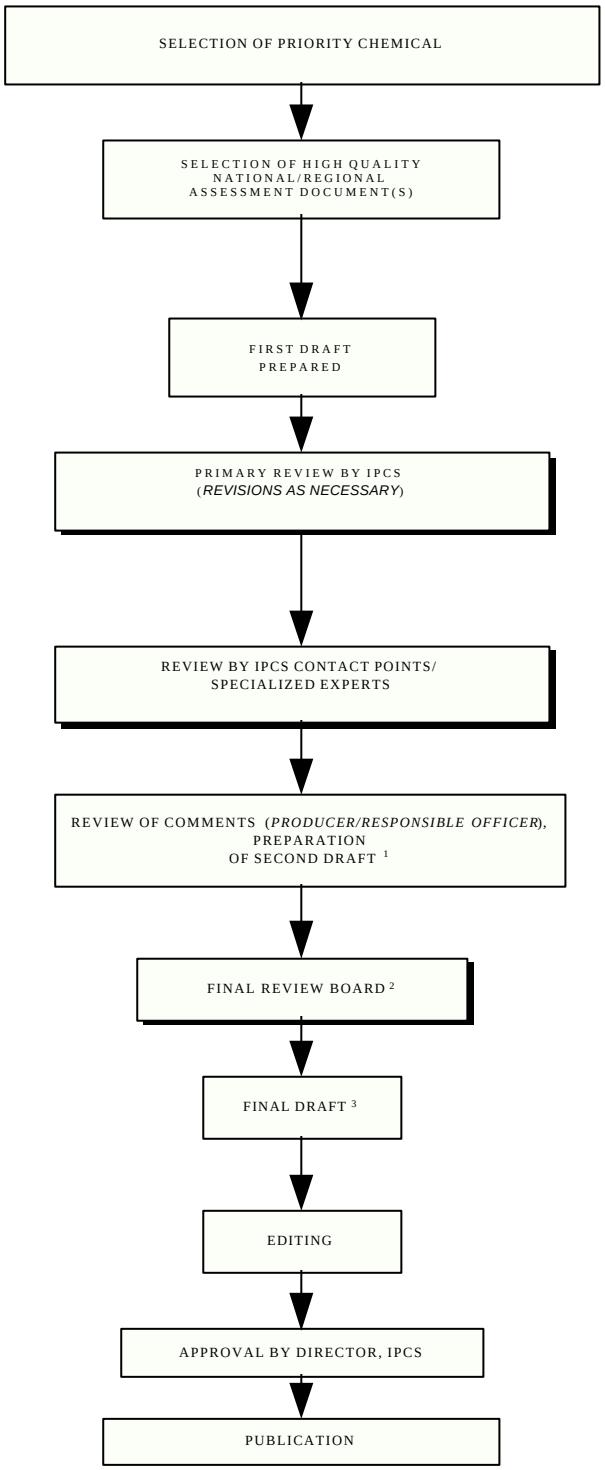
The second stage involves international peer review by scientists known for their particular expertise and by scientists selected from an international roster compiled by IPCS through recommendations from IPCS national Contact Points and from IPCS Participating Institutions. Adequate time is allowed for the selected experts to undertake a thorough review. Authors are required to take reviewers' comments into account and revise their draft, if necessary. The resulting second draft is submitted to a Final Review Board together with the reviewers' comments.

The CICAD Final Review Board has several important functions:

- to ensure that each CICAD has been subjected to an appropriate and thorough peer review;
- to verify that the peer reviewers' comments have been addressed appropriately;
- to provide guidance to those responsible for the preparation of CICADs on how to resolve any remaining issues if, in the opinion of the Board, the author has not adequately addressed all comments of the reviewers; and
- to approve CICADs as international assessments.

Board members serve in their personal capacity, not as representatives of any organization, government, or

CICAD PREPARATION FLOW CHART



1 Taking into account the comments from reviewers.
2 The second draft of documents is submitted to the Final Review Board together with the reviewers' comments.
3 Includes any revisions requested by the Final Review Board.

industry. They are selected because of their expertise in human and environmental toxicology or because of their experience in the regulation of chemicals. Boards are chosen according to the range of expertise required for a meeting and the need for balanced geographic representation.

Board members, authors, reviewers, consultants, and advisers who participate in the preparation of a CICAD are required to declare any real or potential conflict of interest in relation to the subjects under discussion at any stage of the process. Representatives of nongovernmental organizations may be invited to observe the proceedings of the Final Review Board. Observers may participate in Board discussions only at the invitation of the Chairperson, and they may not participate in the final decision-making process.

1. EXECUTIVE SUMMARY

This CICAD on chloral hydrate was prepared by the US Environmental Protection Agency (EPA) and is based on the US EPA's *Toxicological review on chloral hydrate* (US EPA, 2000). Scientific literature identified as of March 1999 was included. Information on the nature of the review processes and the availability of the source document is presented in Appendix 3. Information on the peer review of this CICAD is presented in Appendix 4. This CICAD was approved as an international assessment at a meeting of the Final Review Board, held in Sydney, Australia, on 21–24 November 1999. Participants at the Final Review Board meeting are listed in Appendix 5. The International Chemical Safety Card (ICSC 0234) for chloral hydrate, produced by the International Programme on Chemical Safety, has been reproduced in Appendix 6 (IPCS, 1993).

Chloral hydrate (CAS No. 302-17-0) is synthesized by the chlorination of ethanol. It is used in human and veterinary medicine as a sedative and hypnotic drug. The anhydrous chemical, chloral (CAS No. 75-87-6), is used as an intermediate in the synthesis of DDT, methoxychlor, naled, trichlorfon, dichlorvos, and trichloroacetic acid.

The major route of exposure of the general public is from drinking-water, as chloral hydrate is formed when drinking-water is disinfected with chlorine. A typical concentration of chloral hydrate in a public water supply in the USA is 5 µg/litre. Since chloral hydrate is a metabolite of trichloroethylene and tetrachloroethylene, people will be exposed to chloral hydrate if they are exposed to these chemicals. The public will be exposed to the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, as these chemicals are also formed when drinking-water is disinfected with chlorine. In its use as a sedative for people, the usual clinical dose is 250 mg, 3 times a day (equivalent to 10.7 mg/kg body weight per day). The metabolite trichloroethanol is responsible for the pharmacological effect. No quantitative information is available from occupational exposure.

Chloral hydrate is irritating to the skin and mucous membranes and often causes gastric distress, nausea, and vomiting at the recommended clinical dose. An acute overdose produces (in order of progression) ataxia, lethargy, deep coma, respiratory depression, hypotension, and cardiac arrhythmia. There is some evidence of hepatic injury in people surviving near-lethal, acute overdoses, but no convincing evidence that hepatic injury results from the recommended clinical dose. Several studies of the clinical use of chloral hydrate show a low incidence of minor side-effects. Despite its long use in human medicine, there is no published information on toxicity in controlled studies in humans following extended exposure.

Chloral hydrate is completely absorbed and rapidly metabolized following oral administration. The major metabolites are trichloroethanol and its glucuronide and trichloroacetic acid. Some data suggest that a small amount of dichloroacetic acid may be formed. In humans, the half-life of trichloroethanol and its glucuronide is about 8 h; the half-life of trichloroacetic acid is about 4 days. Some data suggest that the half-life of trichloroethanol is increased several-fold in pre-term and full-term infants compared with toddlers and adults. The major route of excretion of the metabolites of chloral hydrate is elimination in the urine. Chloral hydrate and its metabolites have been found in milk from women treated with chloral hydrate. The concentration of these chemicals, however, is too low to cause a pharmacological effect in the nursing infant.

Acute administration of chloral hydrate to mice causes loss of coordination (ataxia) at about the same exposure as in humans for the same effect. A 90-day study in mice shows no evidence of behavioural changes or other neurotoxicity. Chronic studies in rats and mice show no evidence of behavioural changes and no evidence of histopathological changes in nervous tissue. A slight decrement in humoral immunity was observed following exposure of mice for 90 days. Chloral hydrate has been tested for developmental effects in rats and mice. No structural abnormalities were observed. In a neurodevelopmental study in mice, there was a slight effect in passive avoidance learning. Although chloral hydrate has not been tested in a two-generation reproduction study, the data on reproductive performance and on effects on sperm and oocytes do not suggest that reproductive toxicity is likely to be a critical effect. In addition, no histopathological effects are observed in reproductive organs of rodents in subchronic or chronic studies. All of the studies in laboratory animals show non-cancer health effects at an exposure far in excess of the exposure that is effective for sedation in humans.

There are no carcinogenicity data from humans. Two bioassays in rats show no increase in tumours at any site. Three separate bioassays in male mice show an increased incidence of liver tumours. The most definitive of these studies shows an increased incidence and multiplicity of liver tumours at each of three exposures. These data provide suggestive evidence of carcinogenicity in male mice but are not considered appropriate for

conducting a human health risk assessment with a linear response at low exposure.[1]

There is an extensive database on genetic toxicity. A variety of results show that chloral hydrate is a weak gene mutagen and clastogen. Chloral hydrate induces aneuploidy in a wide variety of cell types. These latter effects are thought to arise by disruption of the spindle apparatus. High concentrations of chloral hydrate are required to cause observable effects. Although these data suggest that genotoxicity may play a role in the toxicity of chloral hydrate, the data indicate that these effects require concentrations that are unlikely to occur under physiological conditions at the exposures typically encountered in the environment. Some likely candidates for the induction of liver tumours in male mice include the formation of DNA adducts caused by free radicals generated by the metabolism of chloral hydrate by cytochrome P450 2E1 (CYP2E1) and through cytotoxicity leading to compensatory hyperplasia.

The tolerable intake for non-cancer health effects of 0.1 mg/kg body weight per day was estimated from the lowest-observed-adverse-effect level (LOAEL) for sedation in humans of 10.7 mg/kg body weight per day using a total uncertainty factor of 100.

Only limited data are available on environmental effects. Methanotrophs can convert chloral hydrate to trichloroethanol and trichloroacetic acid. Chloral hydrate also undergoes abiotic degradation under some conditions. Limited data are available on the inhibition of growth of bacteria, algae, and protozoa and developmental effects in sea urchins. Insufficient data are available with which to assess the risk to the environment from chloral hydrate.

2. IDENTITY AND PHYSICAL/CHEMICAL PROPERTIES

Chloral hydrate (CAS No. 302-17-0) is synthesized by the chlorination of ethanol. The structural formula is given in section 7. The CAS name is 2,2,2-trichloro-1,1-ethanediol. Synonyms include chloral monohydrate, trichloroacetaldehyde hydrate, trichloroacetaldehyde monohydrate, and 1,1,1-trichloro-2,2-dihydroxyethane. The relative molecular mass is 165.42; the solubility in water is 8.3 g/ml; the octanol/water partition coefficient (log K_{ow}) is 0.99; and the vapour pressure is 2 kPa at 25 °C. The chemical and physical properties of chloral hydrate are summarized in the International Chemical Safety Card included in this document (Appendix 6).

Chloral (CAS No. 75-87-6) is the anhydrous form of the chemical. The conversion from chloral to chloral hydrate occurs spontaneously when chloral is placed in aqueous media.

3. ANALYTICAL METHODS

A method for the determination of trace amounts of chloral hydrate in environmental samples is available. Carbonyl compounds are converted to their 2,4-dinitrophenylhydrazone derivatives, separated with high-performance liquid chromatography, and detected by ultraviolet absorbance (Fung & Grosjean, 1981). The lowest quantifiable limit for a variety of carbonyls ranges from 1 to 6 ng.

Chloral hydrate and its metabolites (trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid) can be determined in rat liver homogenates using headspace gas chromatography and electron capture detection (Køppen & Dalgaard, 1988). The detection limits are 0.06 µg/ml for trichloroethanol and trichloroethanol glucuronide and 0.02 µg/ml for chloral hydrate and trichloroacetic acid. A comparable method for the determination of these chemicals in blood and urine is also available (Breimer et al., 1974). The detection limits are 0.5 µg/ml for chloral hydrate and trichloroethanol and 0.1 µg/ml for trichloroacetic acid.

Chloral hydrate and its metabolites can be measured in biological samples after conversion to the methyl esters and separation and detection with gas chromatography/mass spectrometry (Yan et al., 1999). The range for measurement is between 0.12 and 7.83 µmol/litre (equivalent to about 20–1290 µg/litre).

A method for determining trichloroethanol in plasma for use in a clinical laboratory with liquid chromatography has also been developed (Gupta, 1990). The method is useful for determining trichloroethanol in plasma in the pharmacologically active range (up to 12 mg/litre) and in the acutely toxic range (about 100 mg/litre). The method takes about 2 h to complete.

A spectrophotometric method for the determination of chloral hydrate in commercial drug products is based on the reaction of quinaldine ethyl

[1] In a National Toxicology Program carcinogenicity bioassay in mice that became available after the Final Review Board meeting, males had an increased incidence of hepatic tumours, and females had a low increased incidence of pituitary adenomas that was of borderline statistical significance.

iodide with chloral hydrate to produce a stable blue cyanine dye with an absorption maximum at about 605 nm (Helrich, 1990).

4. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

Chloral hydrate is not known to occur as a natural product. The major route of human exposure to chloral hydrate is from drinking-water. Chloral hydrate and its metabolites, trichloroacetic acid and dichloroacetic acid, are formed as by-products when water is disinfected with chlorine. The carbon is derived from natural organic matter (humic and fulvic substances) in the source water. The amount of chloral hydrate formed depends on the concentration of humic and fulvic substances and the conditions of chlorination. Additional chloral hydrate can be formed if water containing chlorine is mixed with food containing humic and fulvic acids (Wu et al., 1998). Chloral hydrate is also a metabolite of trichloroethylene and tetrachloroethylene. Humans will be exposed to chloral hydrate if they are exposed to these chemicals. Chloral hydrate has been widely used as a sedative and hypnotic drug in adult and pediatric medicine. Chloral is used as an intermediate in the synthesis of the insecticides DDT, methoxychlor, naled, trichlorfon, and dichlorvos and the herbicide trichloroacetic acid (IARC, 1995).

Chlorate hydrate could be released to the environment from wastewater treatment facilities, from the manufacture of pharmaceutical-grade chloral hydrate, and from the waste stream during the manufacture of insecticides and herbicides that use chloral as an intermediate.

In the USA, production of chloral hydrate/chloral was estimated at 590 tonnes in 1975, and imports were estimated at 47 tonnes in 1986 (HSDB, 1999). Production of chloral hydrate/chloral by Member States of the European Union was estimated at 2500 tonnes in 1984 (IARC, 1995).

5. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

Newman & Wackett (1991) reported the transformation of chloral hydrate to trichloroethanol and trichloroacetic acid by methanotrophic bacteria. These investigators also reported the abiotic breakdown of chloral hydrate to chloroform and formic acid. No detectable breakdown occurred at pH 7.0 and 30 °C for 24 h. At pH 9.0 and 60 °C, the half-time for breakdown was 16 min.

6. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

6.1 Environmental levels

No information is available.

6.2 Human exposure

The major route of exposure to chloral hydrate is from chlorinated drinking-water. A typical concentration of chloral hydrate in a public water supply in the USA is 5 µg/litre (US EPA, 1994). More than 200 million people in the USA are routinely exposed to chloral hydrate from this route. Assuming water consumption of 2 litres per day and a body weight of 70 kg, the exposure is 0.14 µg/kg body weight per day. Additional exposure could result from inhalation of aerolized water during showering. As these water droplets are typically not small enough to penetrate deep in the lung, they are deposited in the upper airways. Thus, the water droplets are an additional source of oral exposure to chloral hydrate. Some chloral hydrate from water used for showering/bathing would also be absorbed through the skin. Quantitative data on these additional sources of exposure are not available.

Simpson & Hayes (1998) reported the occurrence of chloral hydrate in the drinking-water of seven cities in Australia. The reported range was 0.2–19 µg/litre.

When chloral hydrate is used in clinical medicine, the recommended dose for an adult as a sedative is 250 mg, 3 times a day (equivalent to 10.7 mg/kg body weight per day); the recommended dose as a hypnotic drug is 500–1000 mg (equivalent to 7.1–14.3 mg/kg body weight) (Goodman & Gilman, 1985). The recommended dose for a child undergoing a medical or dental procedure is 50–100 mg/kg body weight (Badalaty et al., 1990; Fox et al., 1990). A child is typically given a higher dose than an adult because a deeper level of sedation is desired to obtain better cooperation from the child during the medical or dental procedure. There is no evidence that a child is less sensitive than an adult to the sedative effects of chloral hydrate.

No quantitative information is available from occupational exposure.

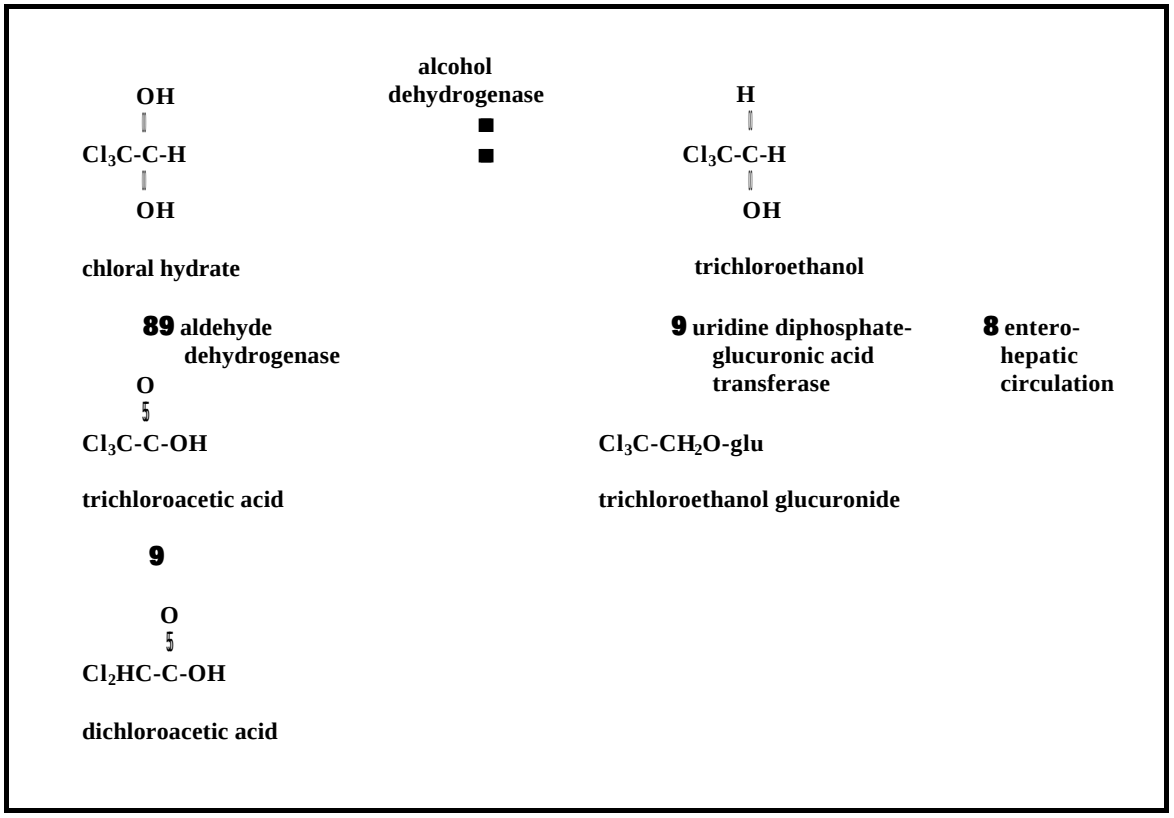


Figure 1: Metabolism of chloral hydrate.

7. COMPARATIVE KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Chloral hydrate is completely absorbed following oral administration; no information is available on dermal absorption. Qualitatively similar metabolism occurs in mice, rats, dogs, Japanese medaka (*Oryzias latipes*), and humans (Marshall & Owens, 1954; Owens & Marshall, 1955; Breimer, 1977; Gosselin et al., 1981; Goodman & Gilman, 1985; Hobara et al., 1986, 1987a,b, 1988a,b; Reimche et al., 1989; Gorecki et al., 1990; Hindmarsh et al., 1991; Mayers et al., 1991; Abbas et al., 1996; Lipscomb et al., 1996, 1998; Abbas & Fisher, 1997; Henderson et al., 1997; Stenner et al., 1997, 1998; Beland et al., 1998; Elfarra et al., 1998; Fisher et al., 1998; Merdink et al., 1998, 1999; Greenberg et al., 1999). The metabolic pathway is shown in Figure 1.

Chloral hydrate is rapidly metabolized in both hepatic and extrahepatic tissues to trichloroethanol and trichloroacetic acid. The alcohol dehydrogenase responsible for reducing it to trichloroethanol is located in both liver and erythrocytes. A portion of the trichloroethanol produced is conjugated with glucuronic acid. The majority of the trichloroethanol glucuronide is excreted in the urine. A portion of the trichloroethanol glucuronide is secreted into the bile and is subject to enterohepatic circulation. Oxidation of chloral hydrate to trichloracetic acid occurs primarily in the liver and kidney via an aldehyde dehydrogenase using nicotinamide adenine dinucleotide (NAD) as a cofactor. The major route of excretion of the metabolites of chloral hydrate is elimination in the urine. Chloral hydrate and its metabolites have been found in milk from women treated with chloral hydrate (Bernstine et al., 1954). The concentration of these chemicals, however, is too low to cause a pharmacological effect in the nursing infant (HSDB, 1999).

In mice and rats, 8% of the administered dose of chloral hydrate is directly eliminated in urine, 15% is converted to trichloroacetic acid (including the contribution from enterohepatic circulation), and 77% is converted to trichloroethanol (Beland et al., 1998). In humans, 92% of the administered dose of chloral hydrate is converted to trichloroethanol, and 8% is converted directly to trichloroacetic acid; additional trichloroacetic acid is formed during enterohepatic circulation of trichloroethanol, such that 35% of the initial dose of chloral hydrate is converted to trichloroacetic acid (Allen & Fisher, 1993).

Although earlier reports claimed the detection of substantial quantities of dichloroacetic acid in blood in studies with rodents (Abbas et al., 1996), data show that the dichloroacetic acid is most likely formed by an acid-catalysed dechlorination of trichloroacetic acid in the presence of reduced haemoglobin (Ketcha et al., 1996). Recent experimental data and pharmacokinetic model simulations in rodents suggest that dichloroacetic acid occurs only as a short-lived metabolite in the liver and is rapidly converted to two-carbon, non-chlorinated metabolites and carbon dioxide, with the chlorine atoms entering the chloride pool (Merdink et al., 1998). Using a different extraction procedure less likely to induce the artefactual formation of dichloroacetic acid, Henderson et al. (1997) showed the presence of dichloroacetic acid in children treated with chloral hydrate in a clinic.

Breimer (1977) administered an aqueous solution of chloral hydrate to five human volunteers. Each volunteer received a single oral dose of 15 mg/kg body weight. Chloral hydrate could not be detected in the plasma even at the first sampling time of 10 min. A method with a limit of detection of 0.5 mg/litre was used. Trichloroethanol and trichloroethanol glucuronide reached peak concentrations 20–60 min after administration of chloral hydrate. The maximum concentration of trichloroethanol in the plasma was about 5 mg/litre. The average half-lives of trichloroethanol and trichloroethanol glucuronide were 8 h (range 7–9.5 h) and 6.7 h (range 6–8 h), respectively. The half-life of trichloroacetic acid was about 4 days. Zimmermann et al. (1998) administered a single dose of 250 mg chloral hydrate in drinking-water to 18 healthy male volunteers (20–28 years of age). Chloral hydrate, trichloroethanol, and trichloroacetic acid were measured in plasma. Chloral hydrate could be detected 8–60 min after dosing in only some of the plasma samples. The measured concentration of chloral hydrate was not reported, but the limit of detection was stated as 0.1 mg/litre. The maximum plasma concentration of trichloroethanol of 3 mg/litre was achieved 0.67 h after dosing, and the maximum plasma concentration of trichloroacetic acid of 8 mg/litre was achieved 32 h after dosing. The terminal half-life was 9.3–10.2 h for trichloroethanol and 89–94 h for trichloroacetic acid.

Two toxicokinetic models are available for chloral hydrate in rats and mice (Abbas et al., 1996; Beland et al., 1998). Beland et al. (1998) treated rats and mice with chloral hydrate by gavage with 1 or 12 doses using 50 or 200 mg/kg body weight per dose. The maximum levels of chloral hydrate, trichloroethanol, and trichloroethanol glucuronide in the plasma were observed at the initial sampling time of 0.25 h. The half-life of chloral hydrate in the plasma was approximately 3 min. The half-lives of trichloroethanol and trichloroethanol glucuronide in the plasma were approximately 5 and 7 min, respectively. Trichloroacetic acid was the major metabolite found in the plasma, with the maximum level being reached 1–6 h after dosing. The half-life of trichloroacetic acid in the plasma was approximately 8–11 h. Comparable values were obtained for rats.

Estimates of the concentrations of trichloroacetic acid and trichloroethanol at steady state under various exposure conditions are in Appendix 1.

Several studies have investigated the age dependence of the metabolism of chloral hydrate (Reimche et al., 1989; Gorecki et al., 1990; Hindmarsh et al., 1991; Mayers et al., 1991). These studies were conducted in critically ill patients in neonatal and paediatric intensive care units and may not be representative of a population of healthy infants. The half-lives for trichloroethanol and its glucuronide were increased several-fold in pre-term and full-term infants compared with toddlers and adults. The half-lives for trichloroethanol in toddlers and adults were similar. These age-related differences likely are the result of the immaturity of hepatic metabolism, particularly glucuronidation, and decreased glomerular filtration.

Kaplan et al. (1967) investigated the effect of ethanol consumption on the metabolism of chloral hydrate in adults. Subjects ingested doses of ethanol (880 mg/kg body weight), chloral hydrate (9–14 mg/kg body weight), or both. In subjects consuming both ethanol and chloral hydrate, blood trichloroethanol levels rose more rapidly and reached higher values than in subjects consuming chloral hydrate only. Ethanol promotes the formation of trichloroethanol because the oxidation of ethanol provides NADH used for the reduction of chloral hydrate (Watanabe et al., 1998).

8. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

8.1 Single exposure

8.1.1 Oral

Sanders et al. (1982) studied the acute toxicity of chloral hydrate in CD-1 mice. Groups of eight male and eight female mice were given chloral hydrate by gavage in distilled water at 300, 600, 900, 1200, 1500, or 1800 mg/kg body weight. No deaths occurred at 900 mg/kg body weight or below in either sex. The

calculated LD_{50} for females was 1265 mg/kg body weight and for males was 1442 mg/kg body weight. Effects wereseen within 10 min of dosing. The mice became sedated at 300 mg/kg body weight. At 600 and 900 mg/kg body weight, the animals became lethargic and exhibited loss of righting reflex. Respiration was markedly inhibited at 1200, 1500, and 1800 mg/kg body weight. Inhibition of respiration appeared to be the immediate cause of death. Most deaths occurred within 4 h at 1800 mg/kg body weight. At 1200 and 1500 mg/kg body weight, some deaths occurred after 4 h, with all deaths occurring within 24 h.

Goldenthal (1971) reported an oral LD_{50} in rats of 480 mg/kg body weight.

8.1.2 Inhalation

Odum et al. (1992) exposed four female CD-1 mice to chloral for 6 h at a concentration of 100 ppm (603 mg/m^3). This exposure induced deep anaesthesia. The mice recovered normally after the exposure stopped. The effects in the lung included vacuolization of clara cells, alveolar necrosis, desquamination of the epithelium, and alveolar oedema. The lung to body weight ratio increased 1.5-fold, most likely due to the alveolar oedema.

8.2 Irritation and sensitization

There are no studies of irritation or sensitization in laboratory animals.

8.3 Short-term exposure

Sanders et al. (1982) studied the short-term toxicity of chloral hydrate in mice. Groups of male CD-1 mice were given chloral hydrate by gavage in distilled water at 14.4 or 144 mg/kg body weight per day for 14 days. No significant effect on body weight was observed. No changes in internal organs were noted from a gross examination. Groups of 11–12 mice were evaluated for several toxicological parameters. No significant effects on haematological or serum biochemical parameters were noted. There was a statistically significant ($P < 0.05$) increase in liver weight (17%) and a decrease in spleen weight (27%) at the high exposure. The no-observed-adverse-effect level (NOAEL) in this study is 14.4 mg/kg body weight per day; the LOAEL is 144 mg/kg body weight per day. The increase in liver weight, but not the decrease in spleen weight, was confirmed in a subsequent 90-day study by the same researchers.

8.4 Long-term exposure

8.4.1 Subchronic exposure

Sanders et al. (1982) administered chloral hydrate in drinking-water to CD-1 mice at 70 or 700 mg/litre (equivalent to 16 or 160 mg/kg body weight per day) for 90 days. In males, hepatomegaly (an increase in weight of 20% and 34% at the low and high exposure, respectively) and microsome proliferation (no increase in total microsomal protein, increase in cytochrome b_5 of 26% and 40%, increase in aminopyrine *N*-demethylase of 28% and 20%, and increase in aniline hydroxylase of 24% and 30% at the low and high exposures, respectively, when reported as mg of protein per mg of total liver protein) were observed. There were no biologically significant changes in serum enzymes. Hepatomegaly was not seen in females, but there were changes in hepatic microsomal parameters (increase in total microsomal protein of 10%, increase in aniline hydroxylase of 23%, and decrease in cytochrome b_5 of 12% when reported as mg of protein per mg of total liver protein), but only at the high exposure. No other significant toxicological changes were observed. Based on hepatomegaly and changes in microsomal parameters in males at the high exposure, this study identifies a LOAEL of 160 mg/kg body weight per day and a NOAEL of 16 mg/kg body weight per day.

Daniel et al. (1992b) exposed male and female Sprague-Dawley rats (10 per sex per dose) for 90 days to chloral hydrate in drinking-water at a concentration of 300, 600, 1200, or 2400 mg/litre (equivalent to an exposure of 24, 48, 96, or 168 mg/kg body weight per day in males and 33, 72, 132, or 288 mg/kg body weight per day in females). The tissues of animals from the high-exposure group and liver sections from all treated males were examined histopathologically. No mortality occurred in any groups prior to sacrifice. Organ weights, including liver weight, and clinical chemistry values in treated animals were only sporadically or inconsistently different from control animal values. Focal hepatocellular necrosis was observed in 2 of 10 males in each of the groups exposed to 96 and 168 mg/kg body weight per day. The necrotic lesion was minimal at 96 mg/kg body weight per day and was significantly more severe at 168 mg/kg body weight per day. Necrotic lesions were not reported in any treated females or in any control animals. While serum enzymes were generally increased in treated animals, dramatic increases were reported in males in the 168 mg/kg body weight per day group; mean aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels in this group were elevated 89%, 54%, and 127% above the corresponding control values, respectively.

8.4.2 Chronic exposure and carcinogenicity

Rijhsinghani et al. (1986) evaluated carcinogenic effects in male mice (C57BL × $C3HF_1$). Groups of 15-day-old mice received chloral hydrate by gavage in distilled water at 0, 5, or 10 mg/kg body weight (26, 15, and 14 mice per group, respectively). Animals were sacrificed when moribund or at week 78, at week 88, or between weeks 89 and 92. Livers were examined histopathologically using light and electron microscopy. In mice sacrificed 48–92 weeks after treatment, the incidence of hepatic nodules (adenomas or trabecular carcinomas) was 3/9 and 6/8 for animals from the 5 and 10 mg/kg 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 body weight group.[1]

Daniel et al. (1992a) exposed 40 male $B6C3F_1$ mice for 104 weeks to drinking-water containing chloral hydrate at 1 g/litre (equivalent to 166 mg/kg body weight per day). Untreated control animals (23 in one group and 10 in a second group) received distilled water. Interim sacrifices were conducted at 30 and 60 weeks of exposure (five animals per group at each sacrifice interval). Complete necropsy and microscopic examination were performed. There were no significant treatment-related effects on survival or body weight. There were no changes in spleen, kidney, or testis weights or histopathological changes in any tissue except the liver. The toxicity in the liver was characterized by increased absolute liver weight and liver to body weight ratio at all three sacrifice intervals. At week 104, liver weight was 37% higher than in controls, and liver to body weight ratio was 42% higher than in controls. Hepatocellular necrosis was noted in 10/24 (42%) treated animals; other pathological changes of mild severity reported in the livers of treated animals included cytoplasmic vacuolization, cytomegaly, and cytoplasmic alteration. The prevalence of liver tumours at terminal sacrifice was statistically significantly ($P < 0.05$) increased over controls, with hepatocellular carcinomas in 11/24 and hepatocellular adenomas in 7/24 animals; for carcinomas and adenomas combined, the prevalence was 17/24. In control animals, carcinomas, adenomas, and carcinomas and adenomas (combined) occurred in 2/20, 1/20, and 3/20, respectively. At the 60-week sacrifice, there were 2/5 treated animals with hepatocellular carcinomas, compared with 0/5 controls. No carcinomas, adenomas, or hyperplastic nodules were reported in animals sacrificed at week 30.

George et al. (2000) conducted a chronic bioassay for carcinogenicity in male $B6C3F_1$ mice. Mice were administered chloral hydrate in drinking-water for 104 weeks. Mice (72 in each group) had a mean exposure of 0, 13.5, 65, or 146.6 mg/kg body weight per day. There was no change in water consumption, survival, behaviour, body weight, or organ weights at any exposure. There was no evidence of hepatocellular necrosis at any exposure and only minimal changes in the levels of serum enzymes. This study identifies a NOAEL for non-cancer effects in mice of 146.6 mg/kg body weight per day (the highest exposure tested). There was no increase in the prevalence of neoplasia at sites other than the liver. Although the background response in this study is higher than normal for this strain of mice, the mice showed an increase in proliferative lesions in the liver (hyperplasia, adenoma, carcinoma, and combined adenoma and carcinoma) at all exposures. These data are summarized in Table 1. The calculated effective dose for a 10% tumour incidence (ED_{10}) is 1.98 mg/kg body weight per day, and its 95% lower confidence limit (LED_{10}) is 1.09 mg/kg body weight per day (see Appendix 2).

Leuschner & Beuscher (1998) conducted a chronic bioassay for carcinogenicity in Sprague-Dawley rats. Chloral hydrate was administered in drinking-water for 124 weeks (males) and 128 weeks (females). The rats (50 males and 50 females in each group) had an exposure of 15, 45, or 135 mg/kg body weight per day. There was no effect on survival, appearance, behaviour, body weight, food and water consumption, or organ weights. There was no evidence of an increased incidence of tumours in any organ. Histopathological examination revealed an increased incidence of hepatocellular hypertrophy at the highest exposure in males only (11% in controls versus 28% at the highest exposure; $P < 0.01$). This finding, graded as minimal to slight in severity, was characterized by a diffuse liver cell enlargement with slightly eosinophilic cytoplasm and was considered by the authors as a first sign of toxicity. The type, incidence, and severity of other non-neoplastic lesions were not increased in treated animals compared with controls. Based on the evidence of minimal toxicity in the liver, which is of doubtful biological significance, this study establishes a NOAEL of 45 mg/kg body weight per day and a LOAEL of 135 mg/kg body weight per day.

George et al. (2000) conducted a chronic bioassay for carcinogenicity in male F344 rats. Rats were administered chloral hydrate in drinking-water for 104 weeks. Rats (78 in each group) had a mean daily exposure of 0,

[1] After the Final Review Board meeting, a National Toxicology Program carcinogenicity bioassay became available. In this study, an up to 5 times higher single dose of chloral hydrate than that used in the Rijhsinghani et al. (1986) study administered to male or female $B6C3F_1$ mice failed to induce tumours in any organ (NTP, 2000a).

Table 1: Prevalence and multiplicity of hepatocellular proliferative lesions in mice at 104 weeks.[a]

Treatment group (mg/kg body weight per day)[b]	Number examined[c]	Hyperplasia	Adenoma	Carcinoma	Adenoma + carcinoma
0	42	7.1[d] 0.07 ± 0.04[e]	21.4[d] 0.21 ± 0.06[e]	54.8[d] 0.74 ± 0.12[e]	64.3[d] 0.95 ± 0.12[e]
13.5	46	32.6[f] 0.41 ± 0.10[f]	43.5[f] 0.65 ± 0.12[f]	54.3 0.72 ± 0.11	78.6[f] 1.37 ± 0.16[f]
65	39	33.3[f] 0.38 ± 0.09[f]	51.3[f] 0.95 ± 0.18[f]	59.0 1.03 ± 0.19	79.5[f] 1.97 ± 0.23[f]
146.6	32	37.5[f] 0.41 ± 0.10[f]	50.0[f] 0.72 ± 0.15[f]	84.4[f] 1.31 ± 0.17[f]	90.6[f] 2.03 ± 0.25[f]

[a] From George et al. (2000).
[b] Time-weighted mean daily dose.
[c] Animals surviving longer than 78 weeks.
[d] Prevalence (percentage of animals with at least one lesion).
[e] Multiplicity (number of lesions per animal ± SEM).
[f] Statistically different from the control value, $P < 0.05$.

7.4, 37.4, or 162.6 mg/kg body weight per day. There was no change in water consumption, survival, behaviour, body weight, or organ weights at any exposure. There was no indication of liver toxicity at any exposure as shown by the lack of liver necrosis, lack of hyperplasia, no increase in mitotic index, and only minimal changes in the levels of serum enzymes. There was no increase at any exposure in the prevalence or multiplicity of hepatocellular neoplasia or neoplasia at any other site. This study identifies a NOAEL of 162.6 mg/kg body weight per day (the highest exposure tested).[1]

Two of the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, have been shown to cause liver tumours in rodents. For example, trichloroacetic acid in drinking-water induced liver tumours in male and female mice when the exposure exceeded 200 mg/kg body weight per day (Herren-Freund et al., 1987; Bull et al., 1990; Pereira, 1996). There was no evidence of increased carcinogenicity, however, when male rats were exposed to trichloroacetic acid at 360 mg/kg body weight per day (DeAngelo et al., 1997). Dichloroacetic acid in drinking-water induced liver tumours in male and female mice when the exposure exceeded 160 mg/kg body weight per day (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991; Daniel et al., 1992a; Ferreira-Gonzalez et al., 1995; Pereira, 1996). Dichloroacetic acid also induced liver tumours in male rats when the exposure exceeded 40 mg/kg body weight per day (Richmond et al., 1995; DeAngelo et al., 1996).

A number of studies have shown that trichloroethylene is toxic to the mouse lung bronchiolar epithelium, causing a highly specific lesion to the clara cells of mice. Short-term exposure causes vacuolization of the clara cells; long-term exposure causes pulmonary adenomas and adenocarcinomas (Odum et al., 1992; Green et al., 1997). These effects are thought to be due to the accumulation of chloral within the clara cells. Trichloroethylene is efficiently metabolized to chloral, but the major pathway from chloral to trichloroethanol and its glucuronide is blocked, leading to an accumulation of chloral and the observed toxicity.

8.5 Genotoxicity and related end-points

8.5.1 Genotoxicity

There is an extensive database on the genotoxicity of chloral hydrate and its metabolites. A complete summary of these results is provided in US EPA (2000).

Chloral hydrate did not induce mutation in most strains of *Salmonella typhimurium*, but did in some studies with *S. typhimurium* TA100 and in a single study with *S. typhimurium* TA104. The latter response was inhibited by free-radical scavengers "-tocopherol and menadione (Ni et al., 1994).

[1] After the Final Review Board meeting, a National Toxicology Program carcinogenicity assay became available. In this study, lifetime gavage administration of chloral hydrate at similar dose levels induced hepatocellular tumours in male $B6C3F_1$ mice and a low frequency of pituitary hyperplasia and adenomas in females that was of borderline statistical significance (NTP, 2000b).

Chloral hydrate did not induce mitotic crossing-over in *Aspergillus nidulans* in the absence of metabolic activation. Chloral hydrate caused weak induction of meiotic recombination in the presence of metabolic activation and gene conversion in the absence of metabolic activation in *Saccharomyces cerevisiae*. It did not induce reverse mutation in *S. cerevisiae*. Chloral hydrate clearly induced aneuploidy in various fungi in the absence of metabolic activation.

Chloral hydrate induced somatic and germ cell mutations in *Drosophila melanogaster*.

Chloral hydrate did not produce DNA–protein cross-links in rat liver nuclei, DNA single-strand breaks/alkaline-labile sites in primary hepatocytes *in vitro*, or DNA repair in *Escherichia coli*. One study showed induction of single-strand breaks in liver DNA of both rats and mice treated *in vivo*; another study in both species using higher concentrations of chloral hydrate found no such effect.

Chloral hydrate was weakly mutagenic, but did not induce micronuclei in mouse lymphoma cells *in vitro*. Chloral hydrate increased the frequency of micronuclei in Chinese hamster cell lines. Although a single study suggested that chloral hydrate induces chromosomal aberrations in Chinese hamster CHED cells *in vitro*, the micronuclei produced probably contained whole chromosomes and not chromosome fragments, as the micronuclei could all be labelled with antikinetochore antibodies.

In 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, chromosomal dislocation from the mitotic spindle, and a total lack of mitotic spindles in Chinese hamster DON:Wg.3h cells.

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

Chloral hydrate increased the frequency of chromosomal aberrations in mouse bone marrow, spermatogonia, and primary and secondary spermatocytes, but not in oocytes, after *in vivo* treatment. Chloral hydrate induced chromosomal aberrations in mouse bone marrow erythrocytes after treatment *in vivo*. In one of these studies, the use of antikinetochore antibodies suggested induction of micronuclei containing both whole chromosomes and fragments. Chloral hydrate induced micronuclei in the spermatids of mice treated *in vivo* in some studies. Chloral hydrate induced aneuploidy in the bone marrow of mice treated *in vivo*. It increased the rate of aneuploidy in mouse secondary spermatocytes. It did not produce polyploidy in bone marrow, oocytes, or gonosomal or autosomal univalents in primary spermatocytes of mice treated *in vivo*. Chloral hydrate, however, induced polyploidy and meiotic delay when a synchronized population of mouse oocytes was exposed *in vitro* prior to the resumption of maturation.

Trichloroethanol, a reduction product of chloral hydrate, did not induce 8 prophage in *E. coli* or mutation in *S. typhimurium* TA100. Trichloroethanol caused spindle aberrations when mouse oocytes were treated *in vitro*.

Trichloroacetic acid did not induce 8 prophage in *E. coli* and was not mutagenic to *S. typhimurium* in the presence or absence of metabolic activation. Trichloroacetic acid was weakly positive in the mouse lymphoma assay with metabolic activation. Trichloroacetic acid also did not induce chromosomal damage in human lymphocytes or micronuclei in bone marrow *in vitro*. It is unclear whether trichloroacetic acid can induce chromosomal damage *in vivo*, because some studies have been positive and others negative.

Dichloroacetic acid did not induce differential toxicity in DNA repair-deficient strains of *S. typhimurium* but did induce 8 prophage in *E. coli*. Dichloroacetic acid gave equivocal results for gene mutation in *S. typhimurium* TA100 and TA98. Dichloroacetic acid was weakly mutagenic in the *in vitro* mouse lymphoma assay and induced chromosomal aberrations but not micronuclei or aneuploidy in that test system. Dichloroacetic acid induced micronuclei in mouse polychromatic erythrocytes *in vivo* and mutations at the *lacI* locus in the transgenic $B6C3F_1$ mouse (the Big Blue Mouse) *in vivo* at an exposure that induces liver tumours in male mice. It is unclear whether dichloroacetic acid can induce primary DNA damage, as some assays are positive and others negative.

8.5.2 Cell proliferation

Rijhsinghani et al. (1986) evaluated the acute effects of chloral hydrate on liver cell proliferation in 15-day-old male mice ($C57BL \times C3HF_1$). Mice were given 0, 5, or 10 mg chloral hydrate/kg body weight by gavage in distilled water (9, 10, and 6 mice per group, respectively) and sacrificed after 24 h. Cell proliferation was evaluated by calculating the mitotic index (number of mitoses per

100 nuclei) from liver sections. The mitotic index in liver cells was significantly increased (0.9235) in mice receiving 5 mg/kg body weight when compared with the control value (0.3382), and elevated (0.7433) (although not statistically significantly) in mice receiving 10 mg/kg body weight. Hepatic necrosis was not observed in mice from either treatment group at autopsy.

As part of the chronic bioassay for carcinogenicity, George et al. (2000) evaluated hepatocyte proliferation in male F344 rats and male $B6C3F_1$ mice. Exposures are given in section 8.4.2. Five days prior to sacrifice at 13, 26, 52, or 72 weeks in rats and 26, 52, or 78 weeks in mice, animals were given bromodeoxyuridine. Labelled nuclei were identified by chromogen pigment over the nuclei, and the labelling index was calculated. Outside of the areas with tumours in the liver of mice, there was no significant evidence of increased hepatocyte proliferation in rats or mice.

8.5.3 Oncogene activation

Velazquez (1994) investigated the induction of H-*ras* proto-oncogene mutations in mice. DNA from normal liver and tumour tissue was obtained from male $B6C3F_1$ mice administered 1 g chloral hydrate/litre (166 mg/kg body weight per day) in drinking-water for 2 years. H-*ras* mutations were present in one out of seven (14%) tumours. The spectrum of mutations was the same as that of spontaneous liver tumours. Based on these data, it is unlikely that H-*ras* activation is a mechanism of carcinogenicity relevant to chloral hydrate.

8.5.4 Free radicals and DNA adduct formation

Ni et al. (1994, 1995, 1996) studied the metabolism of chloral hydrate in an *in vitro* system using microsomes from male $B6C3F_1$ mice. The metabolism of chloral hydrate generated free radicals as detected by electron spin resonance spectroscopy and caused endogenous lipid peroxidation, resulting in the production of malondialdehyde, formaldehyde, and acetaldehyde, all of which are known to produce liver tumours in rodents. Trichloroacetic acid and trichloroethanol also produced free radicals and induced lipid peroxidation when tested in this system. The authors speculated that the free radicals were $Cl_3CCO_2^{\bullet}$ and/or $Cl_3C^{\bullet}$. Incubation of chloral hydrate, trichloroethanol, or trichloroacetic acid in the presence of microsomes and calf thymus DNA resulted in the formation of a malondialdehyde-modified DNA adduct. This research group further showed that chloral hydrate induced an increase in mutations at the *hprt* and *tk* loci in transgenic human lymphoblastoid cells containing CYP2E1. In contrast, when the parental cell line lacking CYP2E1 was treated with the same concentration of chloral hydrate, no mutations were found at either locus. These data implicate CYP2E1 as the primary cytochrome subfamily involved in the metabolism of chloral hydrate to reactive intermediates.

8.5.5 Cell communication

The effects of 1-, 4-, 6-, 24-, 48-, and 168-h exposures to chloral hydrate (0, 1, 5, or 10 mmol/litre) on gap junction intercellular communication in Clone 9 cell cultures (normal rat hepatocytes) were reported by Benane et al. (1996). No differences in intercellular communication were seen between the groups treated with 1 mmol/litre at 1, 4, and 6 h of exposure and controls, as measured by a dye transfer protocol. There were significant differences between all other groups and the controls. The shortest exposure time and lowest exposure concentration that reduced dye transfer significantly were in the group treated with 1 mmol/litre for 24 h.

8.5.6 Peroxisome proliferation

As part of the chronic bioassay for carcinogenicity in mice, George et al. (2000) found no evidence of peroxisome proliferation using cyanide-insensitive palmitoyl CoA oxidase in the livers of male mice treated with chloral hydrate for 26 weeks.

8.6 Reproductive and developmental toxicity

Klinefelter et al. (1995) evaluated effects on sperm morphology and motility in F344 rats administered chloral hydrate in drinking-water for 52 weeks at levels of 0, 55, or 188 mg/kg body weight per day. The researchers examined cauda epididymal sperm motion parameters and testicular and epididymal histopathology. Chloral hydrate did not cause any visible systemic toxicity and had no effects on epididymal or testicular histopathology. However, the percentage of motile sperm was significantly decreased ($P < 0.01$) from 68% in controls to 58% in rats exposed to 188 mg/kg body weight per day. The percentage of progressively motile sperm was also significantly decreased ($P < 0.01$) from 63% in controls to 53% in this group. In addition, the frequency distribution of the average straight-line velocities of sperm at this exposure was significantly shifted ($P < 0.01$) to the lower ranges when compared with controls. In this study, the NOAEL is 55 mg/kg body weight per day; the LOAEL is 188 mg/kg body weight per day.

Kallman et al. (1984) exposed male and female CD-1 mice to chloral hydrate in drinking-water at 21.3 or 204.8 mg/kg body weight per day. Animals were exposed for 3 weeks prior to breeding. Exposure of females (5 per group) continued during gestation and until pups were weaned at 21 days of age. No gross malformations were

noted, and no significant effects were observed in duration of gestation, number of pups delivered, pup weight, or number of stillborn pups. All pups (15 per group) showed the same rate of development and level of performance on several neurobehavioural tests, except that pups exposed to 204.8 mg/kg body weight per day when tested at 23 days of age showed impaired retention of passive avoidance learning on both the 1-h and 24-h retention tests ($P < 0.05$). This study identified a NOAEL for neurodevelopmental toxicity of 21.3 mg/kg body weight per day and a LOAEL of 204.8 mg/kg body weight per day based on the impairment in passive avoidance learning. This study also identifies a NOAEL for reproductive and other developmental effects of 204.8 mg/kg body weight per day (the highest exposure tested).

Johnson et al. (1998) tested the potential for chloral hydrate to cause developmental toxicity in Sprague-Dawley rats. Chloral hydrate was administered in drinking-water to 20 rats from gestational day 1 to gestational day 22 at an average exposure of 151 mg/kg body weight per day. Control animals were given distilled water. There was no evidence of maternal toxicity, no change in the number of implantation or resorption sites, 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. A detailed examination found no evidence of cardiac anomalies. Based on this study, the NOAEL for developmental toxicity is 151 mg/kg body weight per day (the highest exposure tested).

Johnson et al. (1998) also tested the potential for trichloroethanol and trichloroacetic acid to cause developmental toxicity in Sprague-Dawley rats. The protocol was identical to the study with chloral hydrate. Trichloroethanol was administered to 10 rats at an average exposure of 153 mg/kg body weight per day. No evidence of developmental toxicity was found. In contrast, when trichloroacetic acid was administered to 11 rats at an average exposure of 291 mg/kg body weight per day, developmental toxicity was observed. The effects included statistically significant ($P < 0.05$) increases in average resorptions, in average implantations, and in cardiac anomalies. Although the specific cardiac anomalies found were different, the results with trichloroacetic acid are generally consistent with those reported by Smith et al. (1989), who observed adverse developmental effects from trichloroacetic acid at an exposure of 330 mg/kg body weight per day and above.

Saillenfait et al. (1995) tested the potential of chloral hydrate to cause developmental toxicity using a rat whole-embryo culture system. Embryos (20 per dose) from Sprague-Dawley rats were explanted on gestational day 10 and exposed to chloral hydrate at a concentration of 0, 0.5, 1, 1.5, 2, or 2.5 mmol/litre (equivalent to 0, 83, 165, 248, 331, or 414 mg/litre) for 46 h. At 2.5 mmol/litre, all embryos died. No lethality was seen at lower exposures. Chloral hydrate caused concentration-dependent decreases in growth and differentiation and increases in the incidence of morphologically abnormal embryos. No effects were observed in any parameter at 0.5 mmol/litre. Decreases in crown–rump length, somite (embryonic segment) number, and the protein or DNA content of embryos were seen at 1 mmol/litre and above. At 1, 1.5, and 2 mmol chloral hydrate/litre, respectively, 18%, 68%, and 100% of embryos were malformed. Brain, eye, and ear malformations were the most prominent effects at these concentrations. Abnormalities in the trunk and pericardial dilation also occurred at 2 mmol/litre. In this *in vitro* test system, chloral hydrate was a slightly more potent teratogen than trichloroacetic acid or dichloroacetic acid.

Although chloral hydrate did not cause meiotic delay in the oocytes of adult mice when administered at the time of resumption of maturation induced by hormones (Mailhes & Marchetti, 1994), it did cause adverse effects *in vitro* when a synchronized population of oocytes was exposed prior to resumption of maturation (Eichenlaub-Ritter & Betzendahl, 1995; Eichenlaub-Ritter et al., 1996). In this test system, chloral hydrate induced lagging of chromosomes during telophase I, inhibited spindle elongation in anaphase B, and caused chromosome displacement from the spindle equator in metaphase I and II. Oocytes became irreversibly arrested in maturation when exposed to chloral hydrate prior to resumption of maturation or when chloral hydrate was present during the first or second 8 h of maturation. Spindle aberrations were observed when oocytes were treated with trichloroethanol (Eichenlaub-Ritter et al., 1996).

8.7 Immunological and neurological effects

Kauffmann et al. (1982) administered chloral hydrate by gavage in distilled water at 14.4 or 144 mg/kg body weight per day to groups of 11–12 male CD-1 mice for 14 days. No effects on humoral or cell-mediated immunity were detected at either exposure.

Kauffmann et al. (1982) administered chloral hydrate to male and female CD-1 mice in drinking-water at 70 or 700 mg/litre (equivalent to 16 or 160 mg/kg body weight per day) for 90 days. Humoral immunity was assessed by the number of splenic antibody-forming cells produced against sheep red blood cells (12 mice in the control group and 8 mice in the exposed groups) and haemagglutination titres (20–21 mice in the control group and 13–16 mice in the exposed groups). Cell-mediated

immunity was assessed by delayed-type hypersensitivity to sheep red blood cells (17–20 mice in the control group and 15–16 mice in the exposed groups). Lymphocyte response was assessed using a T-cell mitogen (Con A) and a B-cell mitogen (LPS) (17–22 animals in the control group and 13–16 mice in the exposed groups). In males, no effects were detected in either humoral or cell-mediated immunity at either exposure. No effects on cell-mediated immunity were noted in females at either exposure. In females, both exposures resulted in a statistically significant decrease ($P < 0.05$) in humoral immune function (36% and 40% at the low and high exposures, respectively) when expressed as antibody-forming cells per spleen. The decrease, however, was statistically significant only at the higher exposure when expressed as antibody-forming cells per million spleen cells (a 32% decrease). There was no effect on haemagglutination titres or on spleen cell response to the B-cell mitogen at either exposure. The decrease in antibody-forming cells per million spleen cells at the higher exposure in female mice is regarded as an adverse response in this study. Accordingly, the NOAEL for immunotoxicity is 16 mg/kg body weight per day; the LOAEL is 160 mg/kg body weight per day.

Kallman et al. (1984) administered chloral hydrate by gavage in distilled water at 50, 100, 200, 300, or 400 mg/kg body weight to groups of 12 male CD-1 mice. All doses resulted in the rapid onset of ataxia, with an ED_{50} (maximal effect seen in 50% of animals) of 84.2 mg/kg body weight at 5 min (the time of maximal effect). Animals recovered within 2–3 h. No delayed changes in muscular coordination were detectable when the mice were tested 24 h after treatment.

Kallman et al. (1984) evaluated behavioural toxicity in groups of 12 male CD-1 mice administered chloral hydrate by gavage in distilled water at 14.4 or 144 mg/kg body weight per day for 14 days. When measured 24–48 h after exposure was terminated, no significant effects on body weight, motor activity, physical appearance, behaviour, muscular coordination, or endurance were observed.

Kallman et al. (1984) exposed groups of 12 male CD-1 mice to drinking-water containing chloral hydrate at a concentration of 70 or 700 mg/litre (equivalent to 16 or 160 mg/kg body weight per day) for 90 days. When measured 24 h after exposure was terminated, no treatment-related effects on mortality, body weight, physical appearance, behaviour, locomotor activity, learning in repetitive tests of coordination, response to painful stimuli, strength, endurance, or passive avoidance learning were observed. Both exposures resulted in a decrease of about 1 °C in mean body temperature ($P < 0.05$). Because of the lack of increased effect with a 10-fold increase in exposure and because hypothermia as a side-effect of chloral hydrate or from an overdose of chloral hydrate has not been reported in humans, the decrease in body temperature is not considered an adverse effect. This study identifies a NOAEL for neurobehavioural toxicity of 160 mg/kg body weight per day (the highest exposure tested).

A condensation product of tryptamine and chloral hydrate, 1-trichloromethyl-1,2,3,4-tetrahydro-$-carboline (TaClo), has been found in the blood of elderly patients administered chloral hydrate for 2–7 days (Bringmann et al., 1999). This metabolite initiated a slowly progressive neurodegeneration when administered to rats in a subchronic study (Gerlach et al., 1998). There is, however, no evidence of neurodegeneration in the chronic studies with chloral hydrate in rats and mice.

9. EFFECTS ON HUMANS

Chloral hydrate has been widely used as a sedative and hypnotic drug in humans. Trichloroethanol is responsible for the pharmacological activity (Marshall & Owens, 1954; Breimer, 1977; Goodman & Gilman, 1985). Exposure information is discussed in section 6.2.

Chloral hydrate is irritating to the skin and mucous membranes and often causes gastric distress, nausea, and vomiting at recommended doses. There are no reports of sensitization in humans. Overdoses produce (in order of progression) ataxia, lethargy, deep coma, respiratory depression, hypotension, and cardiac arrhythmias. The life-threatening effects are from severe respiratory depression, hypotension, and cardiac arrhythmias. For some representative case reports, see Marshall (1977), Anyebuno & Rosenfeld (1991), Ludwigs et al. (1996), and Sing et al. (1996). A potentially life-threatening oral dose for humans is approximately 10 g (143 mg/kg body weight), although death has been reported from as little as 4 g, and some individuals have survived ingesting 30 g or more. Extended use of chloral hydrate may result in development of tolerance to the pharmacological effect and physical dependence on or addiction to chloral hydrate.

Shapiro et al. (1969) reviewed the medical records of 1618 patients who had received chloral hydrate at 1 g (213 patients, 13%), 0.5 g (1345 patients, 83%), or various other doses (60 patients, 4%). Adverse reactions were reported in 38 patients (2.3%). Of these patients, 4 received 1 g, 1 received 0.75 g, and 33 received 0.5 g. Reported adverse reactions included gastrointestinal symptoms in 10 patients, central nervous system (CNS) depression in 20 patients, skin rash in 5 patients,

prolonged prothrombin time in 1 patient, and bradycardia in 1 patient. In all patients, the side-effects disappeared when chloral hydrate therapy was stopped. There was no evidence of association between adverse side-effects and age, weight, or sex.

Miller & Greenblatt (1979) reviewed the medical records of 5435 hospital patients who received chloral hydrate at a dose of either 0.5 g (about 7–8 mg/kg body weight) or 1 g (about 14–16 mg/kg body weight). Adverse reactions were noted in 119 cases (2.2%). CNS depression was most common (58 patients, or 1.1%), with minor sensitivity reactions, including rash, pruritus, fever, and eosinophilia, second most common (19 patients, or 0.35%). Other adverse reactions included gastrointestinal disturbances (0.28%) and CNS excitement (0.22%). Three individuals (0.05%) were judged to have life-threatening reactions involving CNS depression, asterixis (involuntary jerking movements), or hypotension. The data show that adverse reactions involving the CNS become more frequent with increasing dosage in patients older than 50 years, in patients who died during hospitalization, in patients who received concurrently benzodiazepine anti-anxiety drugs, and in patients with elevated levels of blood urea nitrogen.

Greenberg et al. (1991) reported various side-effects experienced by children receiving chloral hydrate sedation in preparation for computer tomography (CT) procedures. In a “high-dose” group, composed of 295 children (average age 2.18 years) who received a single dose of 80–100 mg/kg body weight and a maximum total dose of 2 g, adverse reactions occurred in 23 of the patients (7%) and included vomiting (14 patients), hyperactivity (5 patients), and respiratory symptoms, such as wheezing and secretion aspiration (4 patients). Cardiac monitoring did not reveal any abnormalities or arrhythmias in any of the children. A second “lower-dose” cohort of 111 children (average age 1.9 years) received 40–75 mg chloral hydrate/kg body weight. These patients received the lower dose because of existing liver or renal impairment, respiratory insufficiency, or CNS depression. There were no adverse side-effects or complications reported in this group. Children with severe liver or renal disease or affected by severe CNS depression were not treated with chloral hydrate.

Lambert et al. (1990) conducted a retrospective analysis of hospital medical records to investigate a possible link between chloral hydrate administration and direct hyperbilirubinaemia (DHB) in neonates following prolonged administration of chloral hydrate (25–50 mg/kg body weight for up to 20 days). Direct bilirubin is a measure of the free, unconjugated bilirubin in the serum. In the first study, the DHB was of unknown etiology in 10 of the 14 newborns with DHB; all 10 of these DHB patients had received chloral hydrate. In the second study, among 44 newborns who had received chloral hydrate, 10 patients who developed DHB had received a mean cumulative dose of 1035 mg/kg body weight. In contrast, 34 patients whose direct bilirubin levels were within normal ranges received a mean cumulative dose of 183 mg/kg body weight. The total bilirubin levels (free plus conjugated) were the same in both groups and within the normal range.

Kaplan et al. (1967) investigated whether ethanol ingestion increased the effects of chloral hydrate. Five male volunteers weighing 70–107 kg consumed ethanol (880 mg/kg body weight), chloral hydrate (1 g, 9–14 mg/kg body weight), or both. Blood pressure and cardiac rate did not vary significantly among treatments. In the presence of ethanol, the concentration of trichloroethanol in the blood rose more rapidly and reached a higher value, but the rate of depletion was not significantly changed. The increase in the concentration of trichloroethanol was not sufficient to produce a marked enhancement of the hypnotic effect. The volunteers reported symptoms (drowsiness, dizziness, blurred vision) and their severity during the 6-h observation period. At all time points, the rank order of effects was ethanol plus chloral hydrate > ethanol > chloral hydrate.

No long-term studies of chloral hydrate in humans were located. Chloral hydrate is addictive and is a controlled substance (Schedule IV) in the USA.

10. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

Some data are available from cell multiplication inhibition tests (toxic thresholds) in bacteria, algae, and protozoa. These data are summarized in Table 2.

Schatten & Chakrabarti (1998) showed that chloral hydrate at 0.1% (only concentration tested) causes alteration of centrosomal material and abnormal microtubule configurations in California sea urchins (*Strongylocentrotus purpuratus* and *Lytechinus pictus*). Chakrabarti et al. (1998) also showed that chloral hydrate at 4 mmol/litre (660 mg/litre, only concentration tested) induced ciliary loss in the early embryo phase of *Lytechinus pictus*. Exposure in this study was for 30 h at the blastula stage (14 h after fertilization).

Table 2: Effects of chloral hydrate on bacteria, algae, and protozoa.

Test system	Effect	Reference
Bacteria (*Pseudomonas putida*)	16-h EC_3 at 1.6 mg/litre	Bringmann & Kuehn, 1980a
Green alga (*Scenedesmus quadricaudata*)	7-day EC_3 at 2.8 mg/litre	Bringmann & Kuehn, 1980a
Blue-green alga (cyanobacterium) (*Microcystis aeruginosa*)	8-day EC_3 at 78 mg/litre	Bringmann & Kuehn, 1976
Protozoan (*Enterosiphon sulcatum*)	72-h EC_5 at 79 mg/litre	Bringmann & Kuehn, 1980a
Protozoan (*Uronema parduczi*)	EC_5 at 86 mg/litre	Bringmann & Kuehn, 1980b

11. EFFECTS EVALUATION

11.1 Evaluation of health effects

11.1.1 Hazard identification and dose–response assessment

Chloral hydrate has been extensively used as a sedative and hypnotic drug in human and veterinary medicine. The metabolite trichloroethanol is responsible for the pharmacological effect. Chloral hydrate is irritating to the skin and mucous membranes and often causes gastric distress, nausea, and vomiting at recommended doses. Acute overdoses produce (in order of progression) ataxia, lethargy, deep coma, respiratory depression, hypotension, and cardiac arrhythmias. There is some evidence of hepatic injury in people surviving near-lethal, acute overdoses, but no convincing evidence that hepatic injury results from the recommended clinical dose. Despite its long use in human medicine, there is no published information on toxicity in controlled studies in humans following extended exposure.

Chloral hydrate is completely absorbed and rapidly metabolized following oral administration. The major metabolites are trichloroethanol and its glucuronide and trichloroacetic acid. Some data suggest that a small amount of dichloroacetic acid may be formed. In humans, the half-life of trichloroethanol and its glucuronide is about 8 h; the half-life of trichloroacetic acid is about 4 days. Some data suggest that the half-life of trichloroethanol is increased several-fold in pre-term and full-term infants compared with toddlers and adults. The major route of excretion of the metabolites of chloral hydrate is elimination in the urine. Chloral hydrate and its metabolites have been found in milk from women treated with chloral hydrate. The concentration of these chemicals, however, is too low to cause a pharmacological effect in the nursing infant.

Acute administration of chloral hydrate to mice causes loss of coordination (ataxia) at about the same exposure as in humans for the same effect. A 90-day study in mice shows no evidence of behavioural changes or other neurotoxicity. Chronic studies in rats and mice show no evidence of behavioural changes and no evidence of histopathological changes in nervous tissue. These studies used an exposure approximately 15 times the recommended clinical dose in humans. There is some evidence of mild liver toxicity following chronic exposure in rats and mice. A slight decrement in humoral immunity was observed in female mice following exposure for 90 days. The antibody-forming cell response is considered an excellent indicator of the status of humoral immunity because of the complex cellular cooperation required to produce antibody and because the number of cells that produce antibody can be quantified. A depression in the number of these cells is considered an adverse response because the production of antibodies is important to the defence strategy of the organism. However, the quantitative relationship between the depression in antibody-forming cells in the spleen and the concentration of circulating antibody is unknown. In this study, because there was no depression in circulating antibodies measured by the haemagglutination titre, there might be no significant depression in the ability of the host to mount a protective antibody response. Chloral hydrate has been tested for developmental effects in rats and mice. No structural abnormalities were observed. A slight effect was observed in mice in passive avoidance learning when dams were exposed prior to breeding, during gestation, and during nursing and pups were tested at 23 days of age. Although chloral hydrate has not been tested in a two-generation reproduction study, the data on reproductive performance and on effects on sperm and oocytes do not suggest that reproductive toxicity is likely to be a critical effect. In addition, no histopathological effects are observed in reproductive organs of rodents in subchronic or chronic studies. Some *in vitro* data, however, suggest that chloral hydrate administered to young female children might have a latent effect on fertility. All of the studies in laboratory animals show non-cancer health effects at an exposure far in excess of the exposure that is effective for sedation in humans. A complete summary of the exposure–response data is presented in Table 3.

Table 3: Summary of non-neoplastic effects.

Species	Duration	End-point	NOAEL (mg/kg body weight per day)	LOAEL (mg/kg body weight per day)	Reference
Human	1 day, 3 doses	Sedation	–	10.7	Goodman & Gilman, 1985
Rat	90 days	Mild liver necrosis and increase in serum enzymes	96	168	Daniel et al., 1992b
Rat	104 weeks	–	162.6	–	George et al., 2000
Rat	124 weeks	Liver hypertrophy	45	135	Leuschner & Beuscher, 1998
Rat	52 weeks	Sperm motility	55	188	Klinefelter et al., 1995
Rat	gestation days 1–22	Development	151	–	Johnson et al., 1998
Mouse	14 days	Increased liver weight	14.4	144	Sanders et al., 1982
Mouse	90 days	Increased liver weight	16	160	Sanders et al., 1982
Mouse	104 weeks	Increased liver weight and necrosis	–	166[a]	Daniel et al., 1992a
Mouse	104 weeks	–	146.6[b]	–	George et al., 2000
Mouse	3 weeks pre-breeding and during gestation	Reproduction and development	204.8	–	Kallman et al., 1984
Mouse	Pre-breeding, gestation, and nursing	Passive avoidance learning in pups	21.3	204.8	Kallman et al., 1984
Mouse	1 day	Ataxia	–	50	Kallman et al., 1984
Mouse	14 days	Neurobehaviour	144	–	Kallman et al., 1984
Mouse	90 days	Neurobehaviour	160	–	Kallman et al., 1984
Mouse	14 days	Immunotoxicity	144	–	Kauffmann et al., 1982
Mouse	90 days	Humoral immunity	16	160	Kauffmann et al., 1982

[a] Tumours at 166 mg/kg body weight per day.
[b] Hyperplasia and tumours at 13.5, 65, and 146.6 mg/kg body weight per day.

Simultaneous ingestion of ethanol and chloral hydrate increases the sedative effects and side-effects of chloral hydrate. The mechanism is the increase in the concentration of the pharmacologically active metabolite, trichloroethanol, in the presence of ethanol. Chronic users of ethanol are, therefore, somewhat more sensitive to the adverse effects of chloral hydrate.

Because of the immaturity of hepatic metabolism, particularly the glucuronidation pathway, and decreased glomerular filtration in infants, the half-life of trichloroethanol is longer in pre-term and full-term infants. This group is therefore somewhat more sensitive to the adverse effects of chloral hydrate. Toddlers and adults are likely to show similar sensitivity to chloral hydrate.

Although male laboratory rodents seem to be more sensitive than female laboratory rodents to hepatic effects, there is no evidence of a gender effect in humans with respect to the sedative effects or side-effects of chloral hydrate at the recommended clinical dose.

There are no carcinogenicity data from humans. Two bioassays in rats show no increase in tumours at any site. These studies were limited, because only minimal toxicity was observed in the livers of the rats in these bioassays. In one study, only slight hypertrophy was observed at the highest exposure; in the other study, no effects were observed at the highest exposure. No data are available in female mice. There are three separate bioassays showing an increased incidence of liver tumours in male mice. One study, conducted in a very limited number of animals, showed an increase in tumours following a single exposure. The second study tested only one exposure level but used an adequate number of animals. The third study shows an increase in incidence and multiplicity of liver tumours at each of three exposures. There are no data identifying a lesion that is a precursor to the tumours. The strain of mice used has a very high spontaneous incidence of liver tumours. Two of the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, have been shown to cause liver tumours in rodents. Trichloroacetic acid causes liver tumours only in mice. Dichloroacetic acid causes tumours in both rats and mice.[1]

Chloral hydrate has been extensively studied as a genotoxic agent. Chloral hydrate was positive in some bacterial mutation tests, indicating that it may be capable of inducing point mutations. It was also positive in the mouse lymphoma assay for mutations at the *tk* locus. Chloral hydrate also induced somatic and germ cell mutations in *D. melanogaster*. Some data also show chloral hydrate to be a very weak clastogen in mammalian cells.

Chloral hydrate has been shown to induce aneuploidy in a variety of cells, including *S. cerevisiae*, *A. nidulans*, Chinese hamster embryonic fibroblasts, Chinese hamster primary cell lines LUC2 and DON:Wg3h, human peripheral blood lymphocytes, mouse spermatocytes, and mouse spermatids. Because there is a mixture of positive and negative *in vivo* data, with no reason to weigh some studies more than others, it is not clear whether chloral hydrate is capable of inducing genetic damage *in vivo*. Additional *in vivo* studies using standard protocols would help clarify the relevance of genetic damage to a human health risk assessment.

The effects on aneuploidy are thought to arise via disruption of the mitotic spindle structure or function by inhibition of tubulin and/or microtubule-associated proteins; both substances are components of the spindle apparatus. Some data also suggest that chloral hydrate may act on the spindle apparatus through an increase in the concentration of intracellular free calcium.

Several other mechanisms may play a role in the induction of tumours in the liver of male mice. There is no convincing evidence that chloral hydrate causes direct damage to DNA. *In vitro* studies with chloral hydrate, trichloroethanol, and trichloroacetic acid and mouse microsomes, however, show lipid peroxidation and the formation of covalently bound DNA adducts. These effects appear to be mediated by the formation of free radicals by CYP2E1. Another possibility is cytotoxicity leading to compensatory hyperplasia. A single treatment of mice with chloral hydrate caused an increase in the mitotic index in liver cells. The increased cell division is hypothesized to either provide additional opportunities for errors in DNA replication or allow initiated cells to progress to a tumour. Another potentially contributing mechanism of carcinogenesis is disruption of intercellular communication, which has been shown in one experiment to be influenced by chloral hydrate.

The mechanism of chloral hydrate-induced carcinogenicity in male mice is unclear. Two mechanisms that appear ruled out are H-*ras* proto-oncogene activation and peroxisome proliferation.

[1] In a National Toxicology Program carcinogenicity bioassay that became available after the Final Review Board meeting, a carcinogenic effect was not observed after a single dose of chloral hydrate; after lifetime exposure, males had an increased incidence of hepatic tumours, and females had a low increased incidence of pituitary adenomas that was of borderline statistical significance.

Although there is suggestive evidence of carcinogenicity in male mice, the weight of evidence is not sufficient to consider tumour induction as the critical effect.

11.1.2 Criteria for setting tolerable intakes or guidance values for chloral hydrate

The effect that occurs at the lowest exposure is mild sedation in humans. As this effect would not be intended or desirable in the general population outside of the clinical setting, this response is considered an adverse effect and is used to derive the tolerable intake.

Acute gavage exposure in mice shows neurological effects (ataxia) at about the same exposure for the comparable effect in humans. A subchronic study in mice using sensitive tests for neurobehavioural changes found none. Chronic studies in rats and mice show no evidence of neurobehavioural changes and no evidence of histopathological changes in nervous tissue. As with other chlorinated chemicals, there is some evidence of carcinogenic effects in the liver of male mice following chronic exposure.

Although the tolerable intake derived from the pharmacologically active dose in humans is an acute tolerable intake, keeping the exposure below this level will also be protective for any non-cancer health effect from chronic exposure. Therefore, it is appropriate to use the acute tolerable intake as the chronic tolerable intake as well.

No data are available to determine a NOAEL in humans. The recommended clinical dose for sedation in adults is 250 mg, taken 3 times a day (Goodman & Gilman, 1985). A low incidence of side-effects also occurs at this exposure. The LOAEL is 10.7 mg/kg body weight per day (assuming a 70-kg body weight). The pharmacokinetic information shows that chloral hydrate and the pharmacologically active metabolite, trichloroethanol, will not bioaccumulate.

The tolerable intake (IPCS, 1994) of 0.1 mg/kg body weight per day was derived from the LOAEL of 10.7 mg/kg body weight per day using a total uncertainty factor of 100. An uncertainty factor of 10 was used to extrapolate from a LOAEL to a NOAEL, and an uncertainty factor of 10 was used for intraspecies variability. An uncertainty factor for chronic duration was not used. Chloral hydrate and the active metabolite, trichloroethanol, do not bioaccumulate. The half-life of chloral hydrate is a few minutes, and the half-life of trichloroethanol is a few hours. Therefore, an enhanced effect from continuous, daily exposure is not possible. Finally, there is information from clinical use that long-term exposure to chloral hydrate results in tolerance to the sedative effect. Developmental toxicity, including developmental neurotoxicity, and immunotoxicity are not critical effects. Although there is no two-generation reproduction study, an uncertainty factor for database limitations is not needed, as there is evidence from several studies that reproductive toxicity is not likely to be a critical effect.

There are no inhalation studies adequate for setting a guidance value or tolerable intake.

There are data in male mice showing that chloral hydrate causes tumours in the liver. It is not known whether this response is relevant for humans.

11.1.3 Sample risk characterization

The quantitative estimate of human risk for non-cancer effects is based on the recommended clinical dose for sedation in humans and the minor incidence of side-effects at this dose. The tolerable intake is 0.1 mg/kg body weight per day. This is 1% of the recommended single dose for sedation in humans.

Although there is suggestive evidence of formation of tumours in the liver of male mice and there are some data showing genotoxicity, the mode of action for the formation of tumours is not known. It is also not known whether this response is relevant for humans.

Millions of people are exposed to chloral hydrate on a daily basis because it is formed during the disinfection of drinking-water with chlorine. The typical concentration in a public water supply in the USA is 5 µg/litre. Assuming a water consumption of 2 litres per day and a body weight of 70 kg, the exposure is 0.14 µg/kg body weight per day. This exposure is approximately 700 times lower than the tolerable intake.

11.2 Evaluation of environmental effects

Insufficient data are available with which to assess the risk to the environment from chloral hydrate.

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

IARC (1995) evaluated the carcinogenicity data for chloral hydrate. It was concluded that there is inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of chloral hydrate. Chloral hydrate is therefore not classifiable as to its carcinogenicity to humans (Group 3).

IPCS (2000) recently evaluated the toxicological data on water disinfectants and disinfectant by-products, including chloral hydrate. Considering the dose level of 16 mg/kg body weight per day in the 90-day study in mice (Sanders et al., 1982; see section 8.4.1) as a LOAEL (rather than as a NOAEL, as was done in the present document) and using an uncertainty factor of 10 for intra- and interspecies extrapolation and another factor of 10 for the use of a LOAEL rather than a NOAEL, the Task Group calculated a tolerable daily intake (TDI) for chloral hydrate of 16 µg/kg body weight per day. (As the present document considered the increase in liver weight at 16 mg/kg body weight to be a NOAEL rather than a LOAEL, the tolerable intake derived from the studies among humans was lower, as discussed in section 11.1.)

REFERENCES

Abbas R, Fisher JW (1997) A physiologically based pharmacokinetic model for trichloroethylene, and its metabolites, chloral hydrate, trichloroacetate, dichloroacetate, trichloroethanol, and trichloroethanol glucuronide in B6C3F$_1$ mice. *Toxicology and applied pharmacology*, 137:15–30.

Abbas R, Seckel CS, Kidney JK, Fisher JW (1996) Pharmacokinetic analysis of chloral hydrate and its metabolism in B6C3F$_1$ mice. *Drug metabolism and disposition*, 24:1340–1346. See also Erratum, *Drug metabolism and disposition*, 25:1449 (1997).

Allen BC, Fisher JW (1993) Pharmacokinetic modeling of trichloroethylene and trichloroacetic acid in humans. *Risk analysis*, 13:71–86.

Anyebuno MA, Rosenfeld CR (1991) Chloral hydrate toxicity in a term infant. *Developments in pharmacological therapy*, 17:116–120.

Badalaty MM, Houpt MI, Koenigsberg SR, Maxwell KC, Desjardins PJ (1990) A comparison of chloral hydrate and diazepam sedation in young children. *Pediatric dentistry*, 12:33–37.

Beland FA, Schmitt TC, Fullerton NF, Young JF (1998) Metabolism of chloral hydrate in mice and rats after single and multiple doses. *Journal of toxicology and environmental health*, 54:209–226.

Benane SG, Blackman CF, House DE (1996) Effect of perchloroethylene and its metabolites on intercellular communication in clone 9 rat liver cells. *Journal of toxicology and environmental health*, 48:427–437.

Bernstine JB, Meyer AE, Bernstine RL (1954) Maternal blood and breast milk estimation following the administration of chloral hydrate during the puerperium. *Journal of obstetrics and gynaecology of the British Commonwealth*, 63:228–231.

Breimer DD (1977) Clinical pharmacokinetics of hypnotics. *Clinical pharmacokinetics*, 2:93–109.

Breimer DD, Ketelaars HCJ, Van Rossum JM (1974) Gas chromatographic determination of chloral hydrate, trichloroethanol, and trichloroacetic acid in blood and urine employing head-space analysis. *Journal of chromatography*, 88:55–63.

Bringmann G, Kuehn R (1976) Vergleichende Befunde der Schadwirkung wassergerfahrden der Stoffe gegen Bakterien (*Pseudomonas putida*) und Blaualgen (*Microcystis aeruginosa*). *Gas- und Wasserfach: Wasser/Abwasser*, 117:410–413.

Bringmann G, Kuehn R (1980a) Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in cell multiplication inhibition tests. *Water research*, 14:231–241.

Bringmann G, Kuehn R (1980b) Bestimmung der biologischen Schadwirkung wassergefahrdender Stoffe gegen Protozoen II Bakterienfressende Ciliaten. *Zeitschrift für Wasser und Abwasser Forschung*, 1:26–31.

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

Bull RJ, Sanchez IM, Nelson MA, Larson JL, Lansing AJ (1990) Liver tumor induction in B6C3F$_1$ mice by dichloroacetate and trichloroacetate. *Toxicology*, 63:341–359.

Chakrabarti A, Schatten H, Mitchell KD, Crosser M, Taylor M (1998) Chloral hydrate alters the organization of the ciliary basal apparatus and cell organelles in sea urchin embryos. *Cell and tissue research*, 293:453–462.

Daniel FB, DeAngelo AB, Stober JA, Olson GR, Page NP (1992a) Hepatocarcinogenicity of chloral hydrate, 2-chloroacetaldehyde, and dichloroacetic acid in the male B6C3F$_1$ mouse. *Fundamental and applied toxicology*, 19:159–168.

Daniel FB, Robinson M, Stober JA, Page NP, Olson GR (1992b) Ninety-day toxicity study of chloral hydrate in the Sprague-Dawley rat. *Drug and chemical toxicology*, 15:217–232.

DeAngelo AB, Daniel FB, Stober JA, Olson GR (1991) The carcinogenicity of dichloroacetic acid in the male B6C3F$_1$ mouse. *Fundamental and applied toxicology*, 16:337–347.

DeAngelo AB, Daniel FB, Most BM, Olson GR (1996) The carcinogenicity of dichloroacetic acid in the male Fischer 344 rat. *Toxicology*, 114:207–221.

DeAngelo AB, Daniel FB, Most BM, Olson GR (1997) The failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. *Journal of toxicology and environmental health*, 52:425–445.

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. *Mutation research*, 372:274–294.

Elfarra AA, Krause RJ, Last AR, Lash LH, Parker JC (1998) Species- and sex-related differences in metabolism of trichloroethylene to yield chloral and trichloroethanol in mouse, rat, and human liver microsomes. *Drug metabolism and disposition*, 26:779–785.

Ferreira-Gonzalez A, DeAngelo AB, Nasim S, Garrett CT (1995) Ras oncogene activation during hepatocarcinogenesis in B6C3F$_1$ male mice by dichloroacetic and trichloroacetic acid. *Carcinogenesis*, 16:495–500.

Fisher JW, Mahle D, Abbas R (1998) A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites: trichloroacetic acid and free trichloroethanol. *Toxicology and applied pharmacology*, 152:339–359.

Fox BE, O'Brien CO, Kangas KJ, Murphree AL, Wright KW (1990) Use of high dose chloral hydrate for ophthalmic exams in children: A retrospective review of 302 cases. *Journal of pediatric ophthalmology and strabismus*, 27:242–244.

Fung K, Grosjean D (1981) Determination of nanogram amounts of carbonyls as 2,4-dinitrophenylhydrazones by high-performance liquid chromatography. *Analytical chemistry*, 53:168–171.

George MH, Kilburn S, Moore T, DeAngelo AB (2000) The carcinogenicity of chloral hydrate administered in the drinking water to the male B6C3F$_1$ mouse and F344/N rat. *Toxicologic pathology* (in press).

Gerlach M, Xiao A-Y, Heim C, Lan J, God R, Feines D, Bringmann G, Riederer P, Sontag K-H (1998) 1-Trichloromethyl-1,2,3,4-tetrahydro-$-carboline increases extracellular serotonin and stimulates hydroxyl radical production in rats. *Neuroscience letters*, 257:17–20.

Goldenthal EI (1971) A compilation of LD$_{50}$ values in newborn and adult animals. *Toxicology and applied pharmacology*, 18:185–207.

Goodman LS, Gilman A (1985) *The pharmacological basis of therapeutics*, 7th ed. New York, NY, The Macmillan Co.

Gorecki DKJ, Hindmarsh KW, Hall CA, Mayers DJ, Sankaran K (1990) Determination of chloral hydrate metabolism in adult and neonate biological fluids after single-dose administration. *Journal of chromatography*, 528:333–341.

Gosselin RE, Smith RP, Hodge HC (1981) Clinical toxicology of commercial products. Baltimore, MD, Williams & Wilkins.

Green T, Mainwaring GW, Foster JR (1997) Trichloroethylene-induced mouse lung tumors: Studies of the mode of action and comparisons between species. *Fundamental and applied toxicology*, 37:125–130.

Greenberg MS, Burton GA Jr, Fisher FW (1999) Physiologically based pharmacokinetic modeling of inhaled trichloroethylene and its oxidative metabolites in B6C3F$_1$ mice. *Toxicology and applied pharmacology*, 154:264–278.

Greenberg SB, Faerber EN, Aspinall CL (1991) High dose chloral hydrate sedation for children undergoing CT. *Journal of computer assisted tomography*, 15:467–469.

Gupta RN (1990) Determination of trichloroethanol, the active metabolite of chloral hydrate, in plasma by liquid chromatography. *Journal of chromatography*, 500:655–659.

HSDB (1999) *Hazardous substances data bank*. Micromedex Inc. (CD-ROM version).

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 GN, Yan Z, James MO, Davydova N, Stacpoole PW (1997) Kinetics and metabolism of chloral hydrate in children: identification of dichloroacetate as a metabolite. *Biochemical and biophysical research communications*, 235:695–698.

Herren-Freund SL, Pereira MA, Khoury MD, Olson G (1987) The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid, in mouse liver. *Toxicology and applied pharmacology*, 90:183–189.

Hindmarsh KW, Gorecki DKJ, Sankaran K, Mayers DJ (1991) Chloral hydrate administration to neonates: Potential toxicological implications. *Canadian Society of Forensic Science*, 24:239–245.

Hobara T, Kobayashi H, Kawamoto T, Sato T, Iwamoto S, Hirota S, Sakai T (1986) Biliary excretion of trichloroethylene and its metabolites in dogs. *Toxicology letters*, 32:119–122.

Hobara T, Kobayashi H, Kawamoto T, Iwamoto S, Sakai T (1987a) The cholecystohepatic circulation of trichloroethylene and its metabolites in dogs. *Toxicology*, 44:283–295.

Hobara T, Kobayashi H, Kawamoto T, Iwamoto S, Hirota S, Sakai T (1987b) Extrahepatic metabolism of chloral hydrate, trichloroethanol, and trichloroacetic acid in dogs. *Pharmacology and toxicology*, 61:58–62.

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

Hobara T, Kobayashi H, Kawamoto T, Iwamoto S, Sakai T (1988b) The absorption of trichloroethylene and its metabolites from the urinary bladder of anesthetized dogs. *Toxicology*, 48:141–153.

IARC (1995) Chloral and chloral hydrate. *IARC (International Agency for Research on Cancer) monographs*, 63:245–269.

IPCS (1993) *International Chemical Safety Card — Chloral hydrate.* Geneva, World Health Organization, International Programme on Chemical Safety (ICSC 0234).

IPCS (1994) *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits*. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 170).

IPCS (2000) *Disinfectants and disinfectant by-products.* Geneva, World Health Organization (Environmental Health Criteria 216).

Johnson PD, Dawson BV, Goldberg SJ (1998) Cardiac teratogenicity of trichloroethylene metabolites. *Journal of the American College of Cardiology*, 32:540–545.

Kallman MJ, Kaempf GL, Balster RL (1984) Behavioral toxicity of chloral in mice: An approach to evaluation. *Neurobehavioral toxicology and teratology*, 6:137–146.

Kaplan HL, Forney RB, Hughes FW, Jain NC, Crim D (1967) Chloral hydrate and alcohol metabolism in human subjects. *Journal of forensic sciences*, 12:295–304.

Kauffmann BM, White KL, Sanders VM, Douglas KA, Sain LE, Borzelleca JF, Munson AE (1982) Humoral and cell-mediated immune status in mice exposed to chloral hydrate. *Environmental health perspectives*, 44:147–151.

Ketcha MM, Stevens DK, Warren DA, Bishop CT, Brashear WT (1996) Conversion of trichloroacetic acid to dichloroacetic acid in biological samples. *Journal of analytical toxicology*, 20:236–241.

Klinefelter GR, Suarez JD, Roberts NL (1995) Preliminary screening test for the potential of drinking water disinfectant by-products to alter male reproduction. *Reproductive toxicology*, 9:571–578.

Køppen B, Dalgaard L (1988) Determination of trichloroethylene metabolites in rat liver homogenates using headspace gas chromatography. *Journal of chromatography*, 442:325–332.

Lambert GH, Muraskas J, Anderson CL, Myers TF (1990) Direct hyperbilirubinemia associated with chloral hydrate administration in the newborn. *Pediatrics*, 86:277–281.

Leuschner J, Beuscher N (1998) Studies on the mutagenic and carcinogenic potential of chloral hydrate. *Arzneimittel-Forschung drug research*, 48:961–968.

Lipscomb JC, Mahle DA, Brashear WT, Garrett CM (1996) A species comparison of chloral hydrate metabolism in blood and liver. *Biochemical and biophysical research communications*, 227:340–350.

Lipscomb JC, Confer PD, Miller MR, Stamm SC, Snawder JE, Candiera SM (1998) Metabolism of trichloroethylene and chloral hydrate by the Japanese medaka (*Oryzias latipes*) *in vitro*. *Environmental toxicology and chemistry*, 17:325–332.

Ludwigs U, Divino-Fiiho JC, Magnusson N (1996) Suicidal chloral hydrate poisoning. *Journal of clinical toxicology*, 344:97–99.

Mailhes JB, Marchetti F (1994) Chemically induced aneuploidy in mammalian oocytes. *Mutation research*, 320:87–111.

Marshall AJ (1977) Cardiac arrhythmias caused by chloral hydrate. *British medical journal*, 2:994.

Marshall EK, Owens AH (1954) Absorption, excretion and metabolic fate of chloral hydrate and trichloroethanol. *Bulletin of the Johns Hopkins Hospital*, 95:1–18.

Mayers DJ, Hindmarsh KW, Sankaran K, Gorecki DKJ, Kasian GF (1991) Chloral hydrate disposition following single-dose administration to critically ill neonates and children. *Developments in pharmacological therapy*, 16:71–77.

Merdink JL, Conzalez-Leon A, Bull RJ, Schultz IR (1998) The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. *Toxicological sciences*, 45:33–41.

Merdink JL, Stenner RD, Stevens DK, Parker JC, Bull RJ (1999) Effect of enterohepatic circulation on the pharmacokinetics of chloral hydrate and its metabolites in F344 rats. *Journal of toxicology and environmental health*, 56:357–368.

Miller RR, Greenblatt DJ (1979) Clinical effects of chloral hydrate in hospitalized medical patients. *Journal of clinical pharmacology*, 19:669–674.

Newman LM, Wackett LP (1991) Fate of 2,2,2-trichloroacetaldehyde (chloral hydrate) produced during trichloroethylene oxidation by methanotrophs. *Applied and environmental microbiology*, 57:2399–2402.

Ni Y-C, Wong T-Y, Kadlubar FF, Fu PP (1994) Hepatic metabolism of chloral hydrate to free-radical(s) and induction of lipid peroxidation. *Biochemical and biophysical research communications*, 204:937–943.

Ni Y-C, Kadlubar FF, Fu FF (1995) Formation of malondialdehyde-modified 2'-deoxyguanosinyl adduct from metabolism of chloral hydrate by mouse liver microsomes. *Biochemical and biophysical research communications*, 205:1110–1117.

Ni Y-C, Wong T-Y, Lloyd RV, Heinze TM, Shelton S, Casciano D, Kadlubar FF, Fu PP (1996) Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol leading to induction of lipid peroxidation via a free radical mechanism. *Drug metabolism and disposition*, 24:81–90.

NTP (2000a) *Toxicology and carcinogenesis studies of chloral hydrate in B6C3F$_1$ mice (gavage studies).* Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP TR 502).

NTP (2000b) *Toxicology and carcinogenesis studies of chloral hydrate (*ad libitum *and dietary controlled) in male B6C3F1 mice (gavage study).* Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP TR 503).

Odum J, Foster JR, Green T (1992) A mechanism for the development of clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chemico-biological interactions*, 83:135–153.

Owens AH, Marshall EK (1955) Further studies on the metabolic fate of chloral hydrate and trichloroethanol. *Bulletin of the Johns Hopkins Hospital*, 97:320–326.

Pereira MA (1996) Carcinogenic activity of dichloroacetic acid and trichloroacetic acid in the liver of female B6C3F$_1$ mice. *Fundamental and applied toxicology*, 31:192–199.

Reimche LD, Sankaran K, Hindmarsh KW, Kasian GF, Gorecki DKJ, Tan L (1989) Chloral hydrate sedation in neonates and infants — clinical and pharmacologic considerations. *Developments in pharmacological therapy*, 12:57–64.

Richmond RE, Carter JH, Carter HW, Daniel FB, DeAngelo AB (1995) Immunohistochemical analysis of dichloroacetic acid (DCA)-induced hepatocarcinogenesis in male Fischer (F344) rats. *Cancer letters*, 92:67–76.

Rijhsinghani KS, Abrahams C, Swerdlow MA, Rao KVN, Ghose T (1986) Induction of neoplastic lesions in the livers of C57BL × C3HF$_1$ mice by chloral hydrate. *Cancer detection and prevention*, 9:279–288.

Saillenfait AM, Langonne I, Sabate JP (1995) Developmental toxicity of trichloroethylene, tetrachloroethylene and four of their metabolites in rat whole embryo culture. *Archives of toxicology*, 70:71–82.

Sanders VM, Kauffman BM, White KL, Douglas KA, Barnes DW, Sain LE, Bradshaw TJ, Borzelleca JF, Munson AE (1982) Toxicology of chloral hydrate in the mouse. *Environmental health perspectives*, 44:137–146.

Schatten H, Chakrabarti A (1998) Centrosome structure and function is altered by chloral hydrate and diazepam during the first reproductive cell cycles in sea urchin eggs. *European journal of cell biology*, 75:9–20.

Shapiro S, Stone D, Lewis GP, Jick H (1969) Clinical effects of hypnotics. II. An epidemiological study. *Journal of the American Medical Association*, 209:2016–2020.

Simpson KL, Hayes KP (1998) Drinking water disinfection by-products: an Australian perspective. *Water research*, 32:1522–1528.

Sing K, Erickson T, Amitai Y, Hryhorczuk D (1996) Chloral hydrate toxicity from oral and intravenous administration. *Journal of toxicology and clinical toxicology*, 34:101–106.

Smith MK, Randall JL, Read EJ, Stober JA (1989) Teratogenic activity of trichloroacetic acid in the rat. *Teratology*, 40:445–451.

Stenner RD, Merdink JL, Stevens DK, Springer DL, Bull RJ (1997) Enterohepatic recirculation of trichloroethanol glucuronide as a significant source of trichloroacetic acid. *Drug metabolism and disposition*, 25:529–535.

Stenner RD, Merdink JL, Fisher JW, Bull RJ (1998) Physiologically-based pharmacokinetic model for trichloroethylene considering enterohepatic recirculation of major metabolites. *Risk analysis*, 18:261–269.

US EPA (1994) National primary drinking water regulations; disinfectants and disinfection byproducts; proposed rule. US Environmental Protection Agency. *Federal register*, 59:38668–38829.

US EPA (2000) *Toxicological review on chloral hydrate*. Available from US Environmental Protection Agency's Risk Assessment Hotline [513-569-7254 (phone), 513-569-7159 (fax), rih.iris@epa.gov (e-mail address), or www.epa.gov/iris (Website)].

Velazquez SF (1994) *Activation of the H-*ras *oncogene by drinking water disinfection by-products*. Report submitted under the US Environmental Protection Agency's Small Grant Program. April 1994. 43 pp. (NTIS/PB95-200515).

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. *Mutation research*, 334:1–7.

Watanabe M, Takano T, Nakamura K (1998) Effect of ethanol on the metabolism of trichloroethylene in the perfused rat liver. *Journal of toxicology and environmental health*, 55:297–305.

Wu WW, Chadik PA, Davis WM, Powell DH, Delfino JJ (1998) Disinfection byproduct formation from the preparation of instant tea. *Journal of agricultural and food chemistry*, 46:3272–3279.

Yan Z, Henderson GN, James MO, Stacpoole P (1999) Determination of chloral hydrate in human plasma by gas chromatography–
mass spectrometry. *Journal of pharmaceutical and biomedical analysis*, 19:309–318.

Zimmermann T, Wehling M, Schulz HU (1998) Untersuchungen zur relativen Bioverfugbarkeit und Pharmakokinetik von Chloralhydrat und seinen Metaboliten. [The relative bioavailability and pharmacokinetics of chloral hydrate and its metabolites.] *Arzneimittelforschung*, 48:5–12.

APPENDIX 1 — TOXICOKINETICS

This toxicokinetic analysis is used to estimate the steady-state concentrations of trichloroacetic acid (TCA) and trichloroethanol (TCEOH) in mice and humans using a one-compartment model, assuming that the absorption of chloral hydrate (CH) from the gastrointestinal tract and its metabolism in the blood are very rapid compared with the rate of elimination of TCA and TCEOH. This assumption is supported by the data of Beland et al. (1998) in mice and Breimer (1977) and Zimmermann et al. (1998) in humans.

Beland et al. (1998) indicated that 15% of the dose of chloral hydrate is converted directly to TCA and 77% is converted to TCEOH. In humans, Allen & Fisher (1993) estimated that 8% of a dose of chloral hydrate is converted directly to TCA and 92% is converted to TCEOH. Additional TCA is formed from TCEOH. The total TCA formed in humans is approximately 35% of the dose of chloral hydrate.

Estimation of TCA concentration in mice at steady state at the clinically recommended dose for humans:

$[TCA]_{ss\text{-}blood} = PK_o/VK_{el} = 2.5$ mg/litre

$[TCA]_{ss\text{-}liver} = [TCA]_{ss\text{-}blood} \times PC = 3.0$ mg/litre

where:
- P is the proportion of CH converted to TCA = 0.15 (Beland et al., 1998)
- K_o is the dosing rate for CH = 10.7 mg/kg body weight per day, equivalent to 0.446 mg/kg body weight per hour
- V is the volume of distribution = 0.321 litre/kg (Beland et al., 1998)
- K_{el} is the first-order elimination constant for TCA = 0.0819/h (Beland et al., 1998)
- PC is the liver/blood partition coefficient = 1.18 (Abbas & Fisher, 1997)

Estimation of TCA concentration in humans at steady state at the clinically recommended dose:

$[TCA]_{ss\text{-}blood} = PK_o/VK_{el} = 55$ mg/litre

$[TCA]_{ss\text{-}liver} = [TCA]_{ss\text{-}blood} \times PC = 36$ mg/litre

where:
- P is the proportion of CH converted to TCA = 0.35 (Allen & Fisher, 1993)
- K_o is the dosing rate for CH = 10.7 mg/kg body weight per day, equivalent to 0.446 mg/kg body weight per hour
- V is the volume of distribution = 0.102 litre/kg (Allen & Fisher, 1993)
- K_{el} is the first-order elimination constant for TCA = 0.028/h (Allen & Fisher, 1993)
- PC is the liver/blood partition coefficient = 0.66 (Fisher et al., 1998)

Estimation of TCA concentration in humans at steady state at the tolerable intake:

$[TCA]_{ss\text{-}blood} = PK_o/VK_{el} = 1.8$ mg/litre

$[TCA]_{ss\text{-}liver} = [TCA]_{ss\text{-}blood} \times PC = 1.2$ mg/litre

where:
- P is the proportion of CH converted to TCA = 0.35 (Allen & Fisher, 1993)
- K_o is the dosing rate for CH = 0.1 mg/kg body weight per day, equivalent to 0.004 mg/kg body weight per hour
- V is the volume of distribution = 0.102 litre/kg (Allen & Fisher, 1993)
- K_{el} is the first-order elimination constant for TCA = 0.0078/h (Allen & Fisher, 1993)
- PC is the liver/blood partition coefficient = 0.66 (Fisher et al., 1998)

Estimation of TCEOH concentration in mice at steady state at 166 mg/kg body weight per day:

$[TCEOH]_{ss\text{-}blood} = PK_o/VK_{el} = 0.6$ mg/litre

where:
- P is the proportion of CH converted to TCEOH = 0.77 (Beland et al., 1998)
- K_o is the dosing rate for CH = 166 mg/kg body weight per day, equivalent to 6.917 mg/kg body weight per hour
- V is the volume of distribution = 1 litre/kg (cited in Beland et al., 1998)
- K_{el} is the first-order elimination constant for TCEOH = 9.24/h (Beland et al., 1998)

Chloral hydrate at 160 mg/kg body weight per day was the highest exposure used in the 90-day neurobehavioural study by Kallman et al. (1984); chloral hydrate at 166 mg/kg body weight per day was the highest exposure used in the 104-week bioassay of Daniel et al. (1992a). These exposures are a NOAEL for sedation in mice.

Estimation of TCEOH concentration in humans at steady state at the clinically recommended dose:

$[TCEOH]_{ss\text{-}blood} = PK_o/VK_{el} = 4.7$ mg/litre

where:
- P is the proportion of CH converted to TCEOH = 0.92 (Allen & Fisher, 1993)
- K_o is the dosing rate for CH = 10.7 mg/kg body weight per day, equivalent to 0.446 mg/kg body weight per hour
- V is the volume of distribution — not available, assumed 1 litre/kg
- K_{el} is the first-order elimination constant for TCEOH = 0.087/h (Breimer, 1977)

Estimation of TCEOH concentration in humans at steady state at the tolerable intake:

$[TCEOH]_{ss\text{-}blood} = PK_o/VK_{el} = 0.04$ mg/litre

where:
- P is the proportion of CH converted to TCEOH = 0.92 (Allen & Fisher, 1993)
- K_o is the dosing rate for CH = 0.1 mg/kg body weight per day, equivalent to 0.004 mg/kg body weight per hour
- V is the volume of distribution — not available, assumed 1 litre/kg
- K_{el} is the first-order elimination constant for TCEOH = 0.087/h (Breimer, 1977)

APPENDIX 2 — CALCULATION OF BENCHMARK DOSE FOR TUMOUR INCIDENCE

The Benchmark Dose (ED) for tumour incidence was derived from the incidence of adenoma plus carcinoma as reported by George et al. (2000). The human equivalent dose was calculated using (body weight)$^{3/4}$, assuming a human body weight of 70 kg and a mouse body weight of 0.035 kg. EPA Benchmark Dose Software was used to calculate the ED and its lower 95% confidence limit (LED) corresponding to a 10% increase in extra risk for tumour prevalence with the multistage model.

Multistage Model, Version Number 1.1.0b

The form of the probability function is:

P[response] =

$$(1 - background) \times \left[1 - (e^{-beta\ 1 \times dose^{1} - beta\ 2 \times dose^{2}})\right]$$

The parameter betas are restricted to be positive.

Dependent variable = Incidence
Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 250
Relative function convergence has been set to 2.220 45e^{-16}
Parameter convergence has been set to 1.490 12e^{-8}

Default initial parameter values
Background = 0.698 863
Beta(1) = 0.043 897
Beta(2) = 0.000 400 241

Parameter estimates

Variable	Estimate	Standard error
Background	0.691 141	0.073 072 3
Beta(1)	0.053 218 1	0.084 548 3
Beta(2)	0	0.004 035 19

Asymptotic correlation matrix of parameter estimates

	Background	Beta(1)	Beta(2)
Background	1	!0.6319	0.5007
Beta(1)	!0.6319	1	!0.9507
Beta(2)	0.5007	!0.9507	1

Analysis of deviance table

Model	Log(likelihood)	Deviance	DF	*P*-value
Full model	!81.2046			
Fitted model	!81.922	1.434 7	2	0.230 999
Reduced mode	!85.0504	6.256 83	2	0.043 787

Goodness of fit analysis

Administered dose (mg/kg body weight per day)	Human equivalent dose (mg/kg body weight per day)	Estimated probability	Expected	Observed	Size
0	0	0.6911	29.028	27	42
13.5	2.0000	0.7223	33.227	36	46
65	9.7	0.8157	31.812	31	39
146.6	21.9	0.9037	28.919	29	32

Chi-square = 1.41; DF = 2; *P*-value = 0.4949.

Benchmark dose computation

Specified effect	0.100 000
Risk type	Extra risk
Confidence level	0.950 000
ED	1.979 786
LED	1.090 1

APPENDIX 3 — SOURCE DOCUMENT

US Environmental Protection Agency (2000):
Toxicological review on chloral hydrate

Copies of the document may be obtained from:

EPA Risk Assessment Hotline
513-569-7254 (phone)
513-569-7159 (fax)
rih.iris@epa.gov (e-mail address)
www.epa.gov/iris (Website)

This document was prepared by R. Benson, Region VIII, Denver, CO.

The document and summary information on the Integrated Risk Information System (IRIS) have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

Internal EPA reviewers:

National Center for Environmental Assessment, Washington, DC
J. Cogliano
C. Siegel Scott
V. Vu

National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC
A. DeAngelo
R. Luebke

Office of Water, Washington, DC
A. Bathija

External peer reviewers:

P.E. Brubaker, Private Consultant
J.W. Fisher, Operational Toxicology Branch, Wright-Patterson Air Force Base
C.C. Willhite, Department of Toxic Substances Control, State of California

APPENDIX 4 — CICAD PEER REVIEW

The draft CICAD on chloral hydrate was sent for review to institutions and organizations identified by IPCS after contact with IPCS National Contact Points and Participating Institutions, as well as to identified experts. Comments were received from:

Centre of Industrial Hygiene and Occupational Diseases, Czech Republic

Department of Health, London, United Kingdom

Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany

Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, Germany

GSF Forschungszentrum für Umwelt und Gesundheit, GmbH, Oberschleissheim, Germany

Health and Safety Executive, Bootle, United Kingdom

Institut de Recherche en Santé et en Sécurité du Travail du Québec, Montreal, Canada

Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, People's Republic of China

National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC, USA

National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AK, USA

National Chemicals Inspectorate, Solna, Sweden

National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Sydney, Australia

National Institute for Occupational Safety and Health, Cincinnati, OH, USA

National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA

University of Bielefeld, Bielefeld, Germany

APPENDIX 5 — CICAD FINAL REVIEW BOARD

Sydney, Australia, 21–24 November 1999

Members

Dr R. Benson, Drinking Water Program, US Environmental Protection Agency, Region VIII, Denver, CO, USA

Dr T. Berzins, National Chemicals Inspectorate (KEMI), Solna, Sweden

Dr R.M. Bruce, National Center for Environmental Assessment, US Environmental Protection Agency, Cincinnati, OH, USA

Mr R. Cary, Health and Safety Executive, Merseyside, United Kingdom

Dr R.S. Chhabra, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA

Dr S. Chou, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA

Dr S. Dobson, Institute of Terrestrial Ecology, Monks Wood, Cambridgeshire, United Kingdom

Dr H. Gibb, National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC, USA

Dr R.F. Hertel, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany

Dr J. Kielhorn, Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, Germany

Dr S. Kristensen, National Occupational Health and Safety Commission (Worksafe), Sydney, NSW, Australia

Mr C. Lee-Steere, Environment Australia, Canberra, ACT, Australia

Ms M. Meek, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada

Ms F. Rice, National Institute for Occupational Safety and Health, Cincinnati, OH, USA

Dr J. Sekizawa, National Institute of Health Sciences, Tokyo, Japan

Dr D. Willcocks, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Sydney, NSW, Australia (*Chairperson*)

Professor P. Yao, Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, People's Republic of China

Observers

Mr P. Howe, Institute of Terrestrial Ecology, Huntingdon, Cambridgeshire, United Kingdom

Dr K. Ziegler-Skylakakis, GSF-Forschungszentrum für Umwelt und Gesundheit, GmbH, Oberschleissheim, Germany

Secretariat

Dr A. Aitio, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Ms M. Godden, Health and Safety Executive, Bootle, Merseyside, United Kingdom

Dr M. Younes, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

CHLORAL HYDRATE

0234
October 1999

CAS No: 302-17-0
RTECS No: FM8750000
UN No: 2811
EC No: 605-014-00-6

Trichloroacetaldehyde monohydrate
2,2,2-Trichloro-1,1-ethanediol
$C_2H_3Cl_3O_2$ / $Cl_3CCH(OH)_2$
Molecular mass: 165.4

TYPES OF HAZARD/ EXPOSURE	ACUTE HAZARDS/SYMPTOMS	PREVENTION	FIRST AID/FIRE FIGHTING
FIRE	Not combustible. Gives off irritating or toxic fumes (or gases) in a fire.		In case of fire in the surroundings: all extinguishing agents allowed.
EXPLOSION			In case of fire: keep drums, etc., cool by spraying with water.

EXPOSURE		**PREVENT DISPERSION OF DUST!**	
Inhalation	Confusion. Drowsiness. Nausea. Unconsciousness.	Local exhaust or breathing protection.	Fresh air, rest. Artificial respiration if indicated. Refer for medical attention.
Skin	Redness.	Protective gloves.	Rinse skin with plenty of water or shower.
Eyes	Redness.	Safety spectacles or eye protection in combination with breathing protection if powder.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
Ingestion	Abdominal pain. Vomiting (further see Inhalation).	Do not eat, drink, or smoke during work. Wash hands before eating.	Rinse mouth. Give a slurry of activated charcoal in water to drink. Refer for medical attention.

SPILLAGE DISPOSAL	**PACKAGING & LABELLING**	
Sweep spilled substance into containers; if appropriate, moisten first to prevent dusting. Carefully collect remainder, then remove to safe place. (Extra personal protection: P3 filter respirator for toxic particles).	T Symbol R: 25-36/38 S: (1/2-)25-45 UN Hazard Class: 6.1	Do not transport with food and feedstuffs.

EMERGENCY RESPONSE	**STORAGE**
Transport Emergency Card: TEC (R)-61G12b	Separated from strong bases, food and feedstuffs.

IPCS
International Programme on Chemical Safety

Prepared in the context of cooperation between the International Programme on Chemical Safety and the European Commission
© IPCS 2000

SEE IMPORTANT INFORMATION ON THE BACK.

IMPORTANT DATA

Physical State; Appearance
TRANSPARENT COLOURLESS CRYSTALS, WITH CHARACTERISTIC ODOUR.

Chemical dangers
The substance decomposes on heating producing toxic and corrosive fumes including hydrogen chloride. Reacts with strong bases producing chloroform.

Occupational exposure limits
TLV not established.

Routes of exposure
The substance can be absorbed into the body by inhalation of its aerosol and by ingestion.

Inhalation risk
A harmful contamination of the air will be reached rather slowly on evaporation of this substance at 20°C.

Effects of short-term exposure
The substance irritates the eyes, the skin and the respiratory tract. The substance may cause effects on the central nervous system, cardiovascular system, liver and kidneys, resulting in lowering of consciousness, cardiac disorders and impaired functions. Exposure at high levels may result in unconsciousness.

PHYSICAL PROPERTIES

Boiling point (decomposes): 97°C
Melting point: 57-60°C
Density: 1.9 g/cm^3

Solubility in water: very good
Octanol/water partition coefficient as log Pow: 0.99

ENVIRONMENTAL DATA

This substance may be hazardous to the environment; special attention should be given to water organisms.

NOTES

Use of alcoholic beverages enhances the harmful effect.

ADDITIONAL INFORMATION

LEGAL NOTICE Neither the EC nor the IPCS nor any person acting on behalf of the EC or the IPCS is responsible for the use which might be made of this information

©IPCS 2000

RÉSUMÉ D'ORIENTATION

Le présent CICAD relatif à l'hydrate de chloral a été préparé par l'Environmental Protection Agency des États-Unis (EPA) sur la base d'un de ses documents intitulé *Toxicological review on chloral hydrate* (US EPA, 2000). Les données qu'il contient proviennent d'un dépouillement de la littérature scientifique jusqu'en mars 1999. On trouvera à l'appendice 3 des renseignements sur la manière dont l'étude bibliographique a été effectuée et sur les sources de données disponibles. L'appendice 4 donne des indications sur les modalités d'examen du présent CICAD par des pairs. Ce CICAD a été approuvé en tant qu'évaluation internationale lors d'une réunion du Comité d'évaluation finale qui s'est tenue à Sydney (Australie) du 21 au 24 novembre 1999. La liste des participants à cette réunion figure à l'appendice 5. La Fiche internationale sur la sécurité chimique (ICSC 0234) de l'hydrate de chloral établie par le Programme international sur la sécurité chimique est reproduite à l'appendice 6 (IPCS, 1993).

La synthèse de l'hydrate de chloral (No CAS 302-17-0) s'effectue par chloration de l'éthanol. On l'utilise en médécine humaine et vétérinaire comme sédatif et hypnotique. Le chloral, qui en est la forme anhydre (No CAS 75-87-6) est utilisé comme intermédiaire dans la synthèse du DDT, du méthoxychlore, du naled, du trichlorfon, du dichlorvos et de l'acide trichloracétique.

La principale voie d'exposition de la population générale est l'eau de boisson, car il se forme de l'hydrate de chloral lors de la désinfection de l'eau par le chlore. Aux États-Unis, la concentration habituelle d'hydrate de chloral dans l'eau des réseaux publics de distribution est de 5 µg/litre. Comme ce composé est un métabolite du trichloréthylène et du tétrachloréthylène, la population se trouve exposée à l'hydrate de chloral si elle l'est à ces deux composés. Par ailleurs, il y a également exposition à deux métabolites de l'hydrate de chloral, les acides dichlor- et trichloracétique, du fait que ces deux composés se forment également dans l'eau de consommation lors de sa désinfection par le chlore. Lorsque l'hydrate de chloral est utilisé comme sédatif, la dose habituelle est de 250 mg trois fois par jour (soit l'équivalent de 10,7 mg/kg de poids corporel par jour). C'est un métabolite, le trichloréthanol, qui est responsable de l'effet pharmacologique. On ne dispose d'aucune donnée quantitative sur l'exposition professionnelle.

L'hydrate de chloral est irritant pour la peau et les muqueuses et il provoque souvent des troubles gastriques, des nausées et des vomissements lorsqu'on l'utilise à la dose recommandée dans la pratique clinique. Une surdose aiguë entraîne progressivement ataxie, léthargie, coma profond, dépression respiratoire, hypotension et arrythmie cardiaque. On a trouvé des signes de lésions hépatiques chez des sujets ayant échappé de peu à la mort par intoxication aiguë due une surdose, mais rien ne prouve par contre de façon convaincante qu'à la dose clinique recommandée, le composé entraîne des lésions hépatiques. Plusieurs études portant sur l'utilisation clinique de l'hydrate de chloral ont mis en évidence des effets secondaires mineurs et peu fréquents. Bien que ce produit soit utilisé depuis longtemps en médecine, aucune étude toxicologique contrôlée sur des sujets humains n'a été publiée.

L'hydrate de chloral est intégralement absorbé et rapidement métabolisé après administration par la voie orale. Ses principaux métabolites sont le trichloréthanol et son glucuronide ainsi que l'acide trichloracétique. D'après certaines données, il pourrait se former également un peu d'acide dichloracétique. Chez l'Homme, la demi-vie du trichloréthanol et de son glucuronide est d'environ 8 h; celle de l'acide trichloracétique est à peu près égale à 4 jours. Un certain nombre de données incitent à penser que la demi-vie du trichloréthanol est plus de deux fois plus longue chez les prématurés et les nouveau-nés à terme que chez les enfants en bas âge et les adultes. La principale voie d'excrétion des métabolites de l'hydrate de chloral est la voie urinaire. On peut le retrouver, accompagné de ses métabolites, dans le lait de mères traitées par ce produit. Toutefois leur concentration est trop faible pour avoir des effets pharmacologiques chez les nourrissons alimentés au sein.

Administré à des souris, le composé provoque une perte de coordination (ataxie) à une dose comparable à celle qui produit le même effet chez l'Homme. Une étude de 90 jours sur des souris n'a révélé aucun signe d'altération du comportement ni de neurotoxicité. Des études au long cours sur des rats et des souris n'ont pas non plus permis de constater d'anomalies comportementales ni de modifications histopathologiques touchant les tissus nerveux. Après exposition de souris pendant 90 jours, on a observé une légère diminution de l'immunité humorale. D'autres études n'ont mis en évidence aucun effet sur le développement des souris et des rats. Aucune anomalie structurale n'a été relevée. Une étude consacrée à l'action de l'hydrate de chloral sur le développement nerveux de la souris n'a mis en évidence qu'un léger effet sur l'apprentissage de l'évitement passif. Le composé n'a pas fait l'objet d'études de toxicité génésique sur deux générations, mais les données dont on dispose sur l'activité génésique des animaux et les effets sur les spermatozoïdes et les ovocytes ne permettent pas de penser que l'hydrate de chloral puisse avoir des effets majeurs sur la reproduction. Par ailleurs, les études

chroniques et subchroniques effectuées sur des rongeurs n'ont pas mis en évidence d'effets histopathologiques au niveau de l'appareil reproducteur. Toutes les études effectuées sur des animaux de laboratoire mettent en évidence un certain nombre d'effets, mais à l'exclusion de tout effet cancérogène et à des doses qui sont très supérieures à celle qui provoque la sédation chez l'Homme.

En ce qui concerne l'Homme, on ne possède aucune donnée de cancérogénicité. Deux tests biologiques effectués sur le rat ne révèlent aucune augmentation de la fréquence des tumeurs, quelle que soit la localisation. Par contre, dans trois autres tests distincts effectués sur des souris mâles, on constate une augmentation de l'incidence des tumeurs hépatiques. Celle de ces études dont le caractère est le plus définitif indique une augmentation de l'incidence et de la multiplicité des tumeurs pour chacune des trois doses utilisées. Ces données semblent indiquer que le produit est cancérogène chez la souris mâle mais on estime qu'elles ne permettent pas d'évaluer le risque pour l'Homme avec une réponse linéaire aux faibles doses.[1]

Il existe une importante base de données sur les effets génotoxiques. Divers résultats indiquent que l'hydrate de chloral est faiblement mutagène et clastogène. Il provoque une aneuploïdie chez des cellules très diverses. On pense que cet effet est dû à destruction de l'appareil fusorial. Des concentrations élevées sont nécessaires pour que ces effets soient observables. Même si ces résultats donnent à penser que la toxicité de l'hydrate de chloral s'exerce notamment au niveau des gènes, ils montrent également que ces effets ne se produisent qu'à des concentrations qui ont peu de chances d'exister dans les conditions physiologiques, compte tenu de l'exposition habituelle à ce produit dans l'environnement. La formation des tumeurs hépatiques chez la souris mâle peut s'expliquer par la formation d'adduits de l'ADN avec des radicaux libres produits lors de la métabolisation de l'hydrate de chloral par les enzymes du cytochrome P450 2E1 (CYP 2E1) ou par une cytotoxicité conduisant à une hyperplasie compensatoire.

La dose journalière tolérable pour les effets non cancérogènes a été estimée à 0,1 mg/kg pc à partir de la dose la plus faible produisant un effet sédatif observable chez l'Homme (LOAEL), dose qui est égale à 10,7 mg/kg par jour, avec un facteur d'incertitude de 100.

On ne possède que des données limitées sur les effets environnementaux. Les méthanotrophes sont capables de transformer l'hydrate de chloral en trichloréthanol et en acide trichloracétique. Le composé subit également une dégradation abiotique dans certaines conditions. On dispose de données limitées sur l'inhibition de la croissance des bactéries, des algues et des protozoaires. Des résultats sont également disponibles concernant l'effet du composé sur le développement des oursins. On ne possède pas assez de données pour pouvoir évaluer le risque que l'hydrate de chloral représente pour l'environnement.

[1] Un test biologique effectué dans le cadre du Programme national de toxicologie et dont les résultats n'ont été connus qu'après la réunion du Comité d'évaluation finale, a montré que l'incidence des tumeurs hépatiques était en augmentation chez les souris mâles et que chez les femelles, il y avait une faible augmentation des adénomes hypophysaires, augmentation dont la signification statistique était limite.

RESUMEN DE ORIENTACIÓN

Este CICAD sobre el hidrato de cloral, preparado por la Agencia para la Protección del Medio Ambiente (EPA), se basó en el *Examen toxicológico sobre el hidrato de cloral* de la EPA de los Estados Unidos (US EPA, 2000). Se incluyó la bibliografía científica localizada hasta marzo de 1999. La información relativa al carácter de los procesos de examen y a la disponibilidad del documento original figura en el apéndice 3. La información sobre el examen colegiado de este CICAD se presenta en el apéndice 4. Este CICAD se aprobó como evaluación internacional en una reunión de la Junta de Evaluación Final celebrada en Sydney, Australia, los días 21-24 de noviembre de 1999. En el apéndice 5 figura la lista de participantes en esta reunión. La Ficha internacional de seguridad química (ICSC 0234) para el hidrato de cloral, preparada por el Programa Internacional de Seguridad de la Sustancias Químicas, se reproduce en el apéndice 6 (IPCS, 1993).

El hidrato de cloral (CAS Nº 302-17-0) se sintetiza mediante la cloración de etanol. Se utiliza en la medicina humana y veterinaria como sedante e hipnótico. El cloral (CAS Nº 75-87-6), producto químico anhidro, se utiliza como intermediario en la síntesis de DDT, metoxicloro, naled, triclorfon, diclorvos y ácido tricloroacético.

La vía principal de exposición del público general es el agua de bebida, puesto que al desinfectar dicha agua con cloro se forma hidrato de cloral. La concentración normal de hidrato de cloral en el sistema público de abastecimiento de agua de los Estados Unidos es 5 µg/litro. Debido a que el hidrato de cloral es un metabolito del tricloroetileno y el tetracloroetileno, el público estará expuesto al hidrato de cloral si lo está a estos productos químicos. La población está expuesta a los ácidos tricloroacético y dicloroacético, metabolitos del hidrato de cloral, porque también se forman cuando se desinfecta el agua de bebida con cloro. En su uso como sedante humano, la dosis clínica normal es de 250 mg tres veces al día (equivalente a 10,7 mg/kg de peso corporal al día). El metabolito tricloroetanol es el responsable del efecto farmacológico. No se dispone de información cuantitativa relativa a la exposición ocupacional.

El hidrato de cloral es irritante de la piel y las membranas mucosas y con frecuencia provoca trastornos gástricos, náuseas y vómitos con la dosis clínica recomendada. Una sobredosis aguda produce (en orden de progresión) ataxia, letargo, coma profundo, depresión respiratoria, hipotensión y arritmia cardíaca. Hay algunas pruebas de lesiones hepáticas en personas que sobreviven a sobredosis agudas casi letales, pero no hay pruebas convincentes de que se produzcan tales lesiones con la dosis clínica recomendada. En varios estudios sobre el uso clínico del hidrato de cloral se ha puesto de manifiesto una incidencia baja de efectos secundarios menores. A pesar de utilizarse desde hace mucho tiempo en la medicina humana, no hay información publicada sobre la toxicidad en estudios controlados realizados con personas después de una exposición prolongada.

Tras la administración oral, el hidrato de cloral se absorbe completamente y se metaboliza con rapidez. Los principales metabolitos son el tricloroetanol y su glucurónido y el ácido tricloroacético. Algunos datos parecen indicar que se puede formar una pequeña cantidad de ácido dicloroacético. En el ser humano, la semivida del tricloroetanol y su glucurónido es de unas ocho horas; la semivida del ácido tricloroacético es de alrededor de cuatro días. Algunos datos indican que la semivida del tricloroetanol aumenta varias veces en los niños prematuros y los nacidos a término en comparación con los niños que empiezan a caminar y los adultos. La vía principal de excreción de los metabolitos del hidrato de cloral es la orina. Se han detectado hidrato de cloral y sus metabolitos en la leche de mujeres tratadas con este producto. Sin embargo, su concentración es demasiado baja para provocar un efecto farmacológico en los niños lactantes.

La administración aguda de hidrato de cloral a ratones provoca la pérdida de la coordinación (ataxia) con una exposición prácticamente semejante a la de las personas para el mismo efecto. En un estudio de 90 días en ratones no se obtuvieron pruebas de cambios de comportamiento u otros signos de neurotoxicidad. En estudios crónicos con ratas y ratones no se detectaron cambios de comportamiento ni cambios histopatológicos en el tejido nervioso. Tras la exposición de ratones durante 90 días al hidrato de cloral se observó una ligera disminución en la inmunidad humoral. Se han realizado pruebas con hidrato de cloral para estudiar sus efectos en el desarrollo de ratas y ratones. No se observaron anomalías estructurales. En un estudio del neurodesarrollo en ratones, se observó un ligero efecto en el aprendizaje de la evitación pasiva. Aunque no se ha realizado ningún estudio de reproducción de dos generaciones con hidrato de cloral, los datos sobre el rendimiento reproductivo y sobre sus efectos en el esperma y los oocitos no indican que haya probabilidad de que la toxicidad reproductiva sea un efecto crítico. Además, en estudios subcrónicos o crónicos no se observaron efectos histopatológicos en los órganos reproductores de roedores. En todos los estudios realizados con animales de laboratorio se detectaron efectos en la salud distintos del cáncer con una exposición muy superior a la eficaz para la sedación humana.

No hay datos de carcinogenicidad en el ser humano. En dos biovaloraciones en ratas no se observó un aumento de tumores en ninguna parte. En tres biovaloraciones separadas en ratones machos se detectó un aumento de la incidencia de tumores hepáticos. El más definitivo de estos estudios demostró una mayor incidencia y multiplicidad de tumores hepáticos en cada una de las tres exposiciones. Estos datos parecen indicar la existencia de carcinogenicidad en ratones machos, pero no se consideran adecuados para realizar una evaluación del riesgo en la salud humana con una respuesta lineal para una exposición baja.[1]

Hay una amplia base de datos sobre la toxicidad genética. Diversos resultados ponen de manifiesto que el hidrato de cloral tiene una actividad mutagénica de los genes y clastogénica débil. El hidrato de cloral induce aneuploidía en una gran variedad de tipos de células. Se considera que estos últimos efectos se deben a una perturbación del huso acromático. Se necesitan concentraciones altas de hidrato de cloral para provocar efectos observables. Aunque estos datos parecen indicar que la genotoxicidad puede desempeñar una función en la toxicidad del hidrato de cloral, también ponen de manifiesto que estos efectos requieren concentraciones que no es probable que se alcancen en condiciones fisiológicas con las exposiciones que se producen normalmente a partir del medio ambiente. Algunos mecanismos probables para la inducción de tumores hepáticos en ratones machos son la formación de aductos de ADN mediante radicales libres generados en el metabolismo del hidrato de cloral en el citocromo P450 2E1 (CYP2E1) y la citotoxicidad que da lugar a una hiperplasia compensatoria.

Se estimó una ingesta tolerable para los efectos en la salud distintos del cáncer de 0,1 mg/kg de peso corporal al día a partir de la concentración más baja con efectos adversos observados (LOAEL) para la sedación en las personas de 10,7 mg/kg, utilizando un factor de incertidumbre total de 100.

Sólo se dispone de datos limitados sobre los efectos en el medio ambiente. Los organismos metanotróficos pueden convertir el hidrato de cloral en tricloroetanol y ácido tricloroacético. El hidrato de cloral experimenta asimismo degradación abiótica en algunas condiciones. Hay datos limitados sobre la inhibición del crecimiento de bacterias, algas y protozoos y sobre los efectos en el desarrollo de los erizos de mar. No hay datos disponibles suficientes que permitan evaluar el riesgo para el medio ambiente derivado del hidrato de cloral.

[1] En una biovaloración de la carcinogenicidad en ratones del Programa Nacional de Toxicología, disponible después de la reunión de la Junta de Evaluación Final, los machos presentaban una mayor incidencia de tumores hepáticos y las hembras un pequeño aumento de la incidencia de adenomas hipofisiarios, en el límite de la significación estadística.