

Chloral hydrate; CASRN 302-17-0

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the [IRIS assessment development process](#). Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the [guidance documents located on the IRIS website](#).

STATUS OF DATA FOR Chloral hydrate

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Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	09/15/2000
Inhalation RfC (I.B.)	qualitative discussion	09/15/2000
Carcinogenicity Assessment (II.)	yes	09/15/2000

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Chloral hydrate
CASRN — 302-17-0
Last Revised — 09/15/2000

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is

essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
CNS depression and GI irritation in humans	NOAEL: None	100	1	0.1 mg/kg-day
	LOAEL: 10.7 mg/kg-day			
Goodman and Gilman, 1985				

*Conversion Factors and Assumptions — The LOAEL is based on an exposure of 250 mg, three times a day, causing CNS depression (sedation) and GI irritation (nausea, vomiting), and on an average body weight of 70 kg.

I.A.2. Principal and Supporting Studies (Oral RfD)

Although the reference value of 0.1 mg/kg-day derived from the pharmacologically active dose in humans is an acute RfD, keeping the exposure below this level will also be protective for any noncancer health effect from chronic exposure. For example, chronic exposure to chloral hydrate does not cause adverse effects in the liver of rats or mice until the exposure approaches 135 or 160 mg/kg-day, respectively. Similarly, there are no reproductive, developmental, neurobehavioral, or immunological effects following long-term treatment of laboratory animals until the exposure approaches 160 mg/kg-day. Therefore, it is appropriate to use the acute RfD also as the chronic RfD.

Chloral hydrate has been widely used as a sedative/hypnotic drug in humans. The recommended dose for an adult as a sedative is 250 mg, three times a day (equivalent to 10.7 mg/kg-day); the recommended dose as an hypnotic is 500-1,000 mg (equivalent to 7-14 mg/kg) (Goodman and Gilman, 1985). The recommended dose for a child as a sedative is 9 mg/kg, three times a day, to 25 mg/kg in single dose (Hindmarsh et al., 1991). The recommended dose for a child undergoing a medical or dental procedure is 50 to 100 mg/kg (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. Because of the rapid metabolism of chloral hydrate,

trichloroethanol is responsible for the majority of the pharmacological activity (Marshall and Owens, 1954; Breimer, 1977; Goodman and Gilman, 1985). The concentration of trichloroethanol in the plasma in the pharmacologically active range is approximately 5 mg/L and above and in the toxic range is 100 mg/L and above.

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 Anyebuno and Rosenfeld (1991), Ludwigs et al. (1996), Marshall (1977), and Sing et al. (1996). A potentially life-threatening oral dose for humans is approximately 10 g (143 mg/kg), although death has been reported from as little as 4 g, and some individuals have survived ingesting 30 g or more. Extended abuse of chloral hydrate may result in development of paranoid behavior, in tolerance to the pharmacological effect, and in physical dependence or addiction. Sudden withdrawal after habituation can precipitate seizure, delirium, and death in untreated individuals.

Shapiro et al. (1969) reviewed the medical records of 1,618 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, four received 1 g, one received 0.75 g, and 33 received 0.5 g. Reported adverse reactions included gastrointestinal symptoms in 10 patients, depression of the central nervous system (CNS) 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 and Greenblatt (1979) reviewed medical records of 5,435 hospital patients who received chloral hydrate at a dose of either 0.5 g (about 7 to 8 mg/kg) or 1 g (about 14 to 16 mg/kg). 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 (flapping tremor characterized by an intermittent lapse of assumed posture due to involuntary sustained contractions of groups of muscles), or hypotension. The data show that adverse reactions involving the CNS became more frequent with increasing dosage in patients older than 50 years, in patients who died during hospitalization, in patients who received concurrently benzodiazepine antianxiety 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) that received a single dose of 80 to 100 mg/kg 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 to 75 mg/kg chloral hydrate. 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 hyperbilirubinemia (DHB), an increase in the concentration of unconjugated bilirubin in the serum, in neonates following prolonged administration of chloral hydrate (25 to 50 mg/kg administered for up to 20 days). 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 that developed DHB had received a mean cumulative dose of 1,035 mg/kg. In contrast, 34 patients whose direct bilirubin levels were within normal ranges received a mean cumulative dose of 183 mg/kg. As the total bilirubin levels (free plus conjugated bilirubin) were the same in both groups and within the normal range, the increased direct bilirubin could result from competition between trichloroethanol and bilirubin in the glucuronidation pathway, known to function suboptimally in neonates.

Kaplan et al. (1967) investigated whether ethanol ingestion altered the metabolism of chloral hydrate or increased subjective symptoms. Five male volunteers weighing 70 to 107 kg consumed ethanol (880 mg/kg), chloral hydrate (1 g, 9 to 14 mg/kg), 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 concentration, 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-hour 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 in the published literature. Chloral hydrate is addictive and is a controlled substance (Schedule IV) in the United States.

The effect that occurs at the lowest exposure is CNS depression and gastrointestinal irritation in humans. As these effects would not be intended or desirable in the general population, EPA considers these responses as adverse effects and are used to derive the reference dose.

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 neurobehavioral changes found none. Chronic studies in rats and mice show no evidence of neurobehavioral changes and no evidence of histopathological changes in nervous tissue. As with other chlorinated chemicals, there is some evidence of hepatotoxicity in rodent liver following chronic oral exposure. These effects are of minimal severity, may be related to precancerous lesions, and occur at an exposure greater than that required for CNS depression and gastrointestinal irritation following an acute bolus dose.

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 and Gilman, 1985). The LOAEL is 10.7 mg/kg-day (assuming a 70 kg body weight). The pharmacokinetic information shows that chloral hydrate and the pharmacologically active metabolite, trichloroethanol, will not bioaccumulate.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF = 100.

An uncertainty factor of 10 was used to extrapolate from a LOAEL to NOAEL. An uncertainty factor of 10 was used for intraspecies variability. An uncertainty factor for chronic duration is not used. Chloral hydrate and the active metabolite, trichloroethanol, do not bioaccumulate. Therefore, continuous daily exposure to chloral hydrate at the reference dose will not result in a concentration of trichloroethanol in the blood required for the pharmacological 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.

Although the reference value derived from the pharmacologically active dose in humans is an acute RfD, keeping the exposure below this level will also be protective for any noncancer health effect from chronic exposure. For example, chronic exposure to chloral hydrate does not cause adverse effects in the liver of rats or mice until the exposure approaches 135 or 160 mg/kg-day, respectively. Similarly, there are no reproductive, developmental, neurobehavioral, or immunological effects following long-term treatment of laboratory

animals until exposure approaches 160 mg/kg-day. Therefore, it is appropriate to use the acute RfD also as the chronic RfD.

Simultaneous ingestion of ethanol and chloral hydrate increases the sedative 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 abusers 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, the half-life of trichloroethanol is longer in infants (pre-term and full term) than in adults. The half-life of trichloroethanol in toddlers and adults is similar. Because of the longer half-life of trichloroethanol, pre-term and full term infants will experience prolonged effects when chloral hydrate is administered. However, at the reference dose for chloral hydrate, the steady-state concentration of trichloroethanol in these groups is far below the concentration required for the pharmacological effect.

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 to the sedative or side-effects of chloral hydrate at the recommended clinical dose.

MF = 1

I.A.4. Additional Studies/Comments (Oral RfD)

Metabolism and Toxicokinetics

Chloral hydrate is completely absorbed following oral administration. Qualitatively similar metabolism occurs in mice, rats, dogs, Japanese Medaka, and humans (Abbas et al., 1996; Abbas and Fisher, 1997; Beland et al., 1998; Breimer, 1977; Elfarra et al., 1998; Fisher et al., 1998; Goodman and Gilman, 1985; Gorecki et al., 1990; Gosselin et al., 1981; Greenberg et al., 1999; Henderson et al., 1997; Hindmarsh et al., 1991; Hobara et al., 1986, 1987a,b, 1988a,b; Lipscomb et al., 1996, 1998; Marshall and Owens, 1954; Mayers et al., 1991; Merdink et al., 1998, 1999; Owens and Marshall, 1955; Reimche et al., 1989; Stenner et al., 1997, 1998).

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 to form trichloroethanol- β -glucuronide, which 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 trichloroacetic 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 the urine.

Chloral hydrate and its metabolites have been found in milk (Bernstine et al., 1956). As soon as lactation started, mothers (n=50) were treated with a 1.33 g rectal suppository of chloral hydrate. Samples of maternal blood and breast milk were taken for analysis from 15 minutes and at varying intervals up to 24 hours following administration of the drug. The maximum concentration of the sum of chloral hydrate, trichloroethanol, and trichloroethanol-glucuronide (the potential pharmacologically active species) in milk occurred within 1 hour after administration of the drug and averaged 53 mg/L (n=11). The amount of chloral hydrate required for sedation in infants is 10 mg in a single feeding of 100 mL of milk.

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 and Fisher, 1993).

Although earlier reports claimed the detection of substantial quantities of dichloroacetic acid in blood from studies in rodents (Abbas et al., 1996), data show that the dichloroacetic acid is most likely formed by an acid-catalyzed dechlorination of trichloroacetic acid in the presence of reduced hemoglobin (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, nonchlorinated metabolites and carbon dioxide (Merdink et al., 1998). Using a different extraction procedure less likely to induce the artifactual 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. Chloral hydrate could not be detected in the plasma even at the first sampling time of 10 minutes. A method with a limit of detection of 0.5 mg/L was used. Trichloroethanol and trichloroethanol-glucuronide reached peak concentrations 20 to 60 minutes after administration of chloral hydrate. The maximum concentration of trichloroethanol in the plasma was about 5 mg/L. The average half-lives of

trichloroethanol and trichloroethanol-glucuronide were 8 hours (range 7-9.5 hours) and 6.7 hours (range 6-8 hours), 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 150 mL of drinking water to 18 healthy male volunteers (20 to 28 years of age). Chloral hydrate, trichloroethanol, and trichloroacetic acid were measured in plasma. Chloral hydrate could only be detected 8 to 60 minutes after dosing in 15 of 18 plasma samples. The measured concentration of chloral hydrate in plasma ranged from 0.1 mg/L (the limit of detection) to 1 mg/L. The mean maximum plasma concentration of trichloroethanol of 3 mg/L was achieved 0.67 hours after dosing. The mean maximum plasma concentration of trichloroacetic acid of 8 mg/L was achieved 32 hours after dosing. The terminal half-life for trichloroethanol was 9.3 to 10.2 hours and for trichloroacetic acid was 89 to 94 hours.

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

Several studies have investigated the age-dependence of the metabolism of chloral hydrate (Gorecki et al., 1990; Hindmarsh et al., 1991; Mayers et al., 1991; Reimche et al., 1989). These studies were conducted in critically ill patients in neonatal and pediatric intensive care units and may not be representative of a population of healthy infants. The half-lives for trichloroethanol and its glucuronide were increased fourfold in preterm and threefold in full-term infants. The half-life for trichloroethanol in toddlers was similar to that reported for adults. The reported half-lives for elimination of trichloroethanol were 39.8 hours, 27.8 hours, and 9.67 hours for pre-term infants, full-term infants, and toddlers, respectively (Mayers et al., 1991), compared with 7-9.5 hours reported by Breimer (1977) and 9.3-10.2 hours reported by Zimmermann et al. (1998). 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), chloral hydrate (9 to 14 mg/kg), or both. In subjects consuming both ethanol and chloral hydrate, the concentration

of trichloroethanol in blood rose more rapidly and reached a higher concentration 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).

Chronic Bioassays

Daniel et al. (1992a) exposed 40 male B6C3F₁ mice for 104 weeks to drinking water containing chloral hydrate at 1 g/L (equivalent to 166 mg/kg-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 (5 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. With the exception of changes in the liver, there were no changes in organ weight (spleen, kidneys, or testes) or histopathological changes in any tissues. 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 controls, and liver-to-body weight ratio was 42% higher than 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. This study shows a LOAEL at 166 mg/kg-day (the only exposure tested).

George et al. (2000) conducted a chronic bioassay for carcinogenicity in male B6C3F₁ 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-day. At the termination of the study, a complete necropsy and histopathological examination of liver, kidney, spleen, and testes from all animals was conducted. In addition a complete histopathological examination was conducted on five animals from the high-dose group. There was no change in water consumption, survival, behavior, body weight, or organ weights (liver, kidney, spleen, and testes) 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 noncancer effects in male mice of 146.6 mg/kg-day (the highest exposure tested).

NTP (2000a) conducted a chronic bioassay for carcinogenicity in female B6C3F₁ mice. Mice were administered chloral hydrate by gavage in distilled water at 0, 25, 50, or 100 mg/kg 5 days a week for up to 2 years. The calculated exposures are 0, 17.9, 35.7, or 71.4 mg/kg-day. Additional groups were administered chloral hydrate by gavage for 3, 6, or 12 months and held without further dosing for the duration of the study (stop-exposure studies). There was no significant effect on survival, body weight, or organ weights at any exposure. The NOAEL in this study is 71.4 mg/kg-day (the highest exposure tested).

NTP (2000b) conducted a chronic bioassay for carcinogenicity in male B6C3F₁ mice. Groups of 120 male mice received chloral hydrate by gavage in distilled water at 0, 25, 50, or 100 mg/kg for up to 2 years. The calculated exposures are 0, 17.9, 35.7, or 71.4 mg/kg-day. At each exposure 60 mice received feed *ad libitum*; the other 60 mice received feed in measured daily amount calculated to maintain body weight on a previously computed idealized body weight curve. Twelve mice from each diet and dose group were evaluated after 15 months of exposure. The remaining 48 animals from each diet and dose group were evaluated at 2 years. Survival, body weight, organ weights, and serum enzymes in the dosed groups were comparable to the respective vehicle control. Following complete necropsy and histopathological examination, no non-neoplastic changes were found in any organ when compared to the respective vehicle control. The NOAEL for non-neoplastic effects in this study is 71.4 mg/kg-day (the highest exposure tested).

Leuschner and Beuscher (1998) conducted a chronic bioassay 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-day. There was no effect on survival, appearance, behavior, body weight, food and water consumption, and organ weights. There was no evidence of increased incidence of tumors 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 to 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-day and a LOAEL of 135 mg/kg-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, 7.4, 37.4, or 162.6 mg/kg-day. At the termination of the study, a complete necropsy and histopathological examination of liver, kidney, spleen, and testes from all animals was conducted. In addition a complete histopathological examination was conducted on five animals from the high-dose group. There was no change in water consumption, survival, behavior, body weight, or organ weights (liver, kidney, spleen, and testes) at any exposure. There was no indication of liver toxicity at any exposure as shown by the lack of liver necrosis, hyperplasia, increased mitotic index, and only minimal changes in the levels of serum enzymes. The NOAEL in this study is 162.6 mg/kg-day (the highest exposure tested).

Subchronic Bioassays

Sanders et al. (1982) administered chloral hydrate in drinking water to CD-1 mice at 70 or 700 mg/L (equivalent to 16 mg/kg-day or 160 mg/kg-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 (increase in cytochrome b5 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 exposure, respectively). 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 b5 of 12%) 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-day and a NOAEL of 16 mg/kg-day.

Daniel et al. (1992b) exposed male and female Sprague-Dawley rats (10/sex/dose) for 90 days to chloral hydrate in drinking water at a concentration of 300, 600, 1,200, or 2,400 mg/L (equivalent to 24, 48, 96, or 168 mg/kg-day in males and 33, 72, 132, or 288 mg/kg-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-day. The necrotic lesion was minimal at 96 mg/kg-day and was significantly more severe at 168 mg/kg-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-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. Based on the focal hepatocellular necrosis and accompanying serum enzyme changes, the study identifies a LOAEL of 168 mg/kg-day and a NOAEL of 96 mg/kg-day. The 96 mg/kg-day exposure is not considered a LOAEL because the authors reported only minimal microscopic necrosis, there was not a corresponding increase in serum enzymes, and because chronic exposure in Sprague-Dawley rats showed no necrosis at higher exposure (Leuschner and Beuscher, 1998; George et al., 2000).

Reproductive/Developmental Studies

Klinefelter et al. (1995) evaluated sperm morphology and motility in F344 rats administered chloral hydrate in drinking water for 52 weeks at 0, 55, or 188 mg/kg-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-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 to controls. In this study the NOAEL is 55 mg/kg-day; the LOAEL is 188 mg/kg-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-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. There was no change in drinking water consumption or weight gain in the dams. No gross malformations were noted in pups, 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 neurobehavioral tests, except that pups exposed to 204.8 mg/kg-day when tested at 23 days of age showed impaired retention of passive avoidance learning on both the 1-hour and 24-hour retention tests ($p < 0.05$). This study identified a NOAEL for neurodevelopmental toxicity of 21.3 mg/kg-day and a LOAEL of 204.8 mg/kg-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-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 22 at an average exposure of 151 mg/kg-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. At necropsy there was no evidence of cardiac anomalies. Based on this study, the NOAEL for developmental toxicity is 151 mg/kg-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-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-day, developmental toxicity was observed. The effects included a statistically significant ($p < 0.05$) increase in total cardiac defects per litter and an increased number of implantation and resorption sites. The results with trichloroacetic acid are generally consistent with those reported by Smith et al. (1989), who reported adverse developmental effects (levocardia) from trichloroacetic acid at an exposure of 330 mg/kg-day and above.

Saillenfait et al. (1995) tested the potential of chloral hydrate to cause developmental toxicity in vitro using a rat whole-embryo culture system. Embryos 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 mM (20 embryos/dose) for 46 hours. At 2.5 mM 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 mM. Decreases in crown-rump length, somite (embryonic segment) number, and the protein or DNA content of embryos were seen at 1 mM and above. At 1, 1.5, and 2 mM chloral hydrate, 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 mM. 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 et al., 1994), it did cause adverse effects in vitro when a synchronized population of oocytes was exposed prior to resumption of maturation (Eichenlaub-Ritter and 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 hours of maturation. Spindle aberrations (lagging chromosomes and a short interpolar space) were observed when oocytes were treated with trichloroethanol (Eichenlaub-Ritter et al., 1996).

Neurological Study

Kallman et al. (1984) exposed groups of 12 male 5-week-old CD-1 mice to drinking water containing chloral hydrate at 70 or 700 mg/L (equivalent to 15.7 or 160 mg/kg-day) for 90 days. When measured 24 hours after the 90-day exposure was terminated, no treatment-related

effects were observed on mortality, body weight, physical appearance, behavior, locomotor activity, learning in repetitive tests of coordination, response to painful stimuli, strength, endurance, or passive avoidance learning. 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 tenfold increase in exposure and because hypothermia has not been reported as a side effect of chloral hydrate in humans, the decrease in body temperature is not considered an adverse effect. This study identifies a NOAEL for neurobehavioral toxicity of 160 mg/kg-day (the highest exposure tested).

Immunological Study

Kauffmann et al. (1982) administered chloral hydrate to male and female 4-week-old CD-1 mice in drinking water at 70 or 700 mg/L (equivalent to 16 or 160 mg/kg-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 hemagglutination titers (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 exposure, 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 hemagglutination titers or on spleen cell response to the B-cell mitogen at either exposure.

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 defense 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 hemagglutination titer, there might be no significant depression in the ability of the host to mount a protective antibody response. EPA, however, considers the decrease in antibody-forming cells per million spleen cells at the higher exposure in female

mice an adverse response. Accordingly, the NOAEL for immunotoxicity is 16 mg/kg-day; the LOAEL is 160 mg/kg-day.

For more detail on Synthesis and Evaluation of Major Noncancer Effects and Mode of Action, exit to [the toxicological review, Section 4.5 \(PDF\)](#).

I.A.5. Confidence in the Oral RfD

Study — medium

Database — high

RfD -- high

The overall confidence in this RfD assessment is high. 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 chloral hydrate's long use in human medicine, there is no published information on toxicity in controlled studies in humans following extended exposure, and no study clearly establishing a NOAEL in humans. Therefore, confidence in the principal study is medium-to-high.

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 behavioral changes or other neurotoxicity. Chronic studies in rats and mice show no evidence of behavioral changes and no evidence of histopathological changes in nervous tissue. There is some evidence of mild liver toxicity in rodents. These effects are generally observed in males at lower exposures than in females. The effects in the liver in male mice may be associated with enzyme induction and precancerous lesions. A slight decrement in humoral immunity was observed in female mice following exposure for 90 days. 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 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. All of the studies in laboratory animals show noncancer health effects at an exposure far in excess of the

exposure that is effective for sedation in humans. Therefore, confidence in the database is high.

For more detail on Characterization of Hazard and Dose Response, exit to [the toxicological review, Section 6 \(PDF\)](#).

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 2000.

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these comments is included as an appendix to U.S. EPA (2000). [To review this appendix, exit to the toxicological review, Appendix A, Summary of and Response to External Peer Review Comments \(PDF\)](#).

Other EPA Documentation - Final draft for the drinking water criteria document on chlorinated acids/aldehydes/ketone/alcohols. U.S. EPA, Office of Water, March 1994.

Agency Consensus Date - 9/6/2000

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for Chloral hydrate conducted in November 2001 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (fax), or hotline.iris@epa.gov (Internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Chloral hydrate

CASRN — 302-17-0

Last Revised — 09/15/2000

The inhalation Reference Concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/cu.m. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs were derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) and subsequently, according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

There are no adequate data to derive the RfC.

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfC for Chloral hydrate conducted in November 2001 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Chloral hydrate

CASRN —302-17-0

Last Revised — 09/15/2000

Section II provides information on three aspects of the carcinogenic assessment for the substance in question, the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per $\mu\text{g/L}$ drinking water or risk per $\mu\text{g}/\text{cu.m}$ air breathed. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Under the 1986 cancer guidelines (U.S. EPA, 1986), chloral hydrate is assigned to Group C, possible human carcinogen. Under the 1996 proposed guidelines for carcinogen risk assessment (U.S. EPA, 1996), chloral hydrate shows suggestive evidence of human carcinogenicity by the oral route of exposure. There are no carcinogenicity data from humans. Two bioassays in rats in which chloral hydrate was administered by drinking water show no increase in tumors at any site. Because only minimal toxicity was observed in the livers of the rats in these bioassays, the tests were not conducted at the maximum tolerated dose. A chronic bioassay in female mice showed a slight increase in the severity grade of hyperplasia and a slight increase in the incidence of adenoma in the pituitary gland pars distalis at the highest exposure tested. There is some evidence that chloral hydrate causes hepatocellular tumors in male mice. An earlier study showing an increase in hepatic adenomas or trabecular carcinomas following a single bolus exposure could not be confirmed in a study using more animals and higher exposures. Three separate 2-year bioassays in male mice show an increased incidence of hepatocellular adenoma or carcinoma. There are no data identifying a lesion that is a

precursor to the hepatocellular tumors. The strain of mice used has a very high spontaneous incidence of hepatocellular tumors. Two of the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, have been shown to cause hepatocellular tumors in rodents. Trichloroacetic acid causes hepatocellular tumors only in mice. Dichloroacetic acid causes hepatocellular tumors in both rats and mice.

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. A high concentration of chloral hydrate is 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 from the environment. Collectively, these data provide suggestive evidence of carcinogenicity, but the weight-of-evidence is not sufficient to conduct a risk assessment assuming a linear response at low exposure.

For more detail on Characterization of Hazard and Dose Response, exit to [the toxicological review, Section 6 \(PDF\)](#).

For more detail on Susceptible Populations, exit to [the toxicological review, Section 4.6.1 \(PDF\)](#).

II.A.2. Human Carcinogenicity Data

None.

II.A.3. Animal Carcinogenicity Data

Limited.

NTP (2000a) conducted a chronic bioassay for carcinogenicity in female B6C3F₁ mice. Mice were administered chloral hydrate by gavage in distilled water at 0, 25, 50, or 100 mg/kg 5 days a week for up to 2 years. The calculated exposures are 0, 17.9, 35.7, or 71.4 mg/kg-day. Additional groups were administered chloral hydrate by gavage for 3, 6, or 12 months and held without further dosing for the duration of the study (stop-exposure studies). There was no significant effect on survival, body weight, or organ weights at any exposure. Following complete necropsy and histopathological examination, the only significant findings were in the pituitary gland pars distalis. There were no significant effects in the pituitary in the stop-exposure studies. Following the full exposure regime, the incidence of hyperplasia in the

pituitary gland pars distalis was 4/45, 6/44, 4/50, and 9/50 in the control, 25, 50, and 100 mg/kg group, respectively. The average severity grade for hyperplasia was 1.5, 1.0, 1.0, and 2.2 in the control, 25, 50, and 100 mg/kg group, respectively. Only the average severity grade at 100 mg/kg was statistically different from the control ($p < 0.05$). The incidence of adenoma in the pituitary gland pars distalis was 0/45, 2/44, 0/47, and 5/41 in the control, 25, 50, and 100 mg/kg group, respectively. Only at 100 mg/kg was the incidence significantly greater than the control ($p = 0.0237$). For non-neoplastic effects, the NOAEL in this study is 71.4 mg/kg-day (the highest exposure tested). NTP concluded that this study provided equivocal evidence of carcinogenic activity for chloral hydrate in female mice.

NTP (2000b) conducted a chronic bioassay for carcinogenicity in male B6C3F₁ mice. Groups of 120 male mice received chloral hydrate by gavage in distilled water at 0, 25, 50, or 100 mg/kg for up to 2 years. The calculated exposures are 0, 17.9, 35.7, or 71.4 mg/kg-day. At each exposure 60 mice received feed *ad libitum*; the other 60 mice received feed in measured daily amounts calculated to maintain body weight on a previously computed idealized body weight curve. Twelve mice from each diet and dose group were evaluated after 15 months of exposure. The remaining 48 animals from each diet and dose group were evaluated at 2 years. Survival, body weight, organ weights, and serum enzymes in the dosed groups were comparable to the respective vehicle control. Following complete necropsy and histopathological examination, no changes were found in any organ except the liver when compared to the respective vehicle control. The incidence of hepatocellular adenoma or carcinoma in the *ad libitum* study was 16/48, 25/48, 23/47, and 22/48 in the control, 25, 50, or 100 mg/kg groups, respectively. Only in the 25 mg/kg group was the incidence significantly greater than control ($p = 0.0437$). In the dietary-controlled study, the incidence of hepatocellular adenoma or carcinoma was 11/48, 11/48, 14/48, and 18/48 and the incidence of hepatocellular carcinoma was 2/48, 5/48, 4/48, and 8/48 in the control, 25, 50, or 100 mg/kg group, respectively. The only statistically significant increase in incidence was for hepatocellular carcinoma in the 100 mg/kg group ($p = 0.042$). The NOAEL for non-neoplastic effects in this study is 71.4 mg/kg-day (the highest exposure tested). NTP concluded that this study provided some evidence of carcinogenic activity for chloral hydrate in male mice.

George et al. (2000) conducted a chronic bioassay for carcinogenicity in male B6C3F₁ 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-day. At the termination of the study, a complete necropsy and histopathological examination of liver, kidney, spleen, and testes from all animals was conducted. In addition, a complete histopathological examination was conducted on five animals from the high-dose group. There was no change in water consumption, survival, behavior, body weight, or organ weights (liver, kidney, spleen, and testes) at any exposure. There was no increase in the prevalence of neoplasia at sites other than the liver. The male mice showed an increase of proliferative lesions in the liver (hyperplasia,

adenoma, carcinoma, and combined adenoma and carcinoma) at all exposures. The prevalence of proliferative lesions in the control, 13.5, 65, or 146.6 mg/kg-day groups was as follows: hyperplasia, 3/42, 15/46, 13/39, 12/32; adenoma, 9/42, 20/46, 20/39, 16/32; carcinoma, 23/42, 25/46, 23/39, 27/32; adenoma or carcinoma, 27/42, 36/46, 31/39, 29/32. All of the changes were statistically significant ($p < 0.05$) except for carcinoma at the two lower exposures.

Daniel et al. (1992) exposed 40 male B6C3F₁ mice for 104 weeks to drinking water containing chloral hydrate at 1 g/L (equivalent to 166 mg/kg-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 (5 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. The prevalence of liver tumors at terminal sacrifice was statistically significantly ($p < 0.05$) increased over controls, with hepatocellular carcinomas in 11/24 and hepatocellular adenomas in 7/24; 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 to 0/5 controls. No carcinomas, adenomas, or hyperplastic nodules were reported in animals sacrificed at week 30.

NTP (2000a) investigated the ability of a single exposure to chloral hydrate to induce tumors in female and male B6C3F₁ mice. Groups of 15-day-old or 28-day-old female mice (48 animals per dose group) received a single gavage dose of chloral hydrate in distilled water at 0, 10, 25, or 50 mg/kg. An identical study was conducted in 15-day-old male mice. All animals were sacrificed at 105 weeks of age. No neoplastic or non-neoplastic effects were found in any organ at any exposure.

Rijhsinghani et al. (1986) evaluated carcinogenic potential in male mice (C57BL x C3HF₁). Groups of 15-day-old mice received a single dose of chloral hydrate by gavage in distilled water at 0, 5, or 10 mg/kg (26, 15, and 14 mice per group, respectively). Animals were sacrificed when moribund or at week 78, week 88, or between weeks 89 and 92. Livers were examined histopathologically using light and electron microscopy. In mice sacrificed 48 to 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-day dose groups, respectively, compared to 2/19 in controls. The increase in hepatic nodules was statistically significant ($p < 0.05$) only in the 10 mg/kg group.

II.A.4. Supporting Data for Carcinogenicity

Leuschner and 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-day. There was no effect on survival, appearance, behavior, body weight, food and water consumption, and organ weights. There was no evidence of increased incidence of tumors in any organ.

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, 7.4, 37.4, or 162.6 mg/kg-day. At the termination of the study, a complete necropsy and histopathological examination of liver, kidney, spleen, and testes from all animals was conducted. In addition a complete histopathological examination was conducted on five animals from the high-dose group. There was no change in water consumption, survival, behavior, body weight, or organ weights (liver, kidney, spleen, and testes) at any exposure. There was no indication of liver toxicity at any exposure, as shown by the lack of liver necrosis, hyperplasia, increased 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.

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

Genetic Toxicity

There is an extensive database on the genotoxicity of chloral hydrate and its metabolites. The European Union included chloral hydrate in a collaborative study on aneuploidy (Adler, 1993; Natarajan et al., 1993; Parry, 1993; Parry and Sors, 1993). These data are summarized in U. S. EPA (2000).

Chloral hydrate did not induce mutation in most strains of *Salmonella typhimurium*, but did in some studies with *Salmonella typhimurium* TA 100 and in a single study with *Salmonella typhimurium* TA 104. The latter response was inhibited by free-radical scavengers **a**-tocopherol and menadione (Ni et al., 1994).

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 *Saccharomyces 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 crosslinks 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 labeled 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:Wg3h 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 lymphocytes 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. 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 were exposed in vitro prior to the resumption of maturation.

Trichloroethanol, a reduction product of chloral hydrate, did not induce λ prophage in *Escherichia coli* or mutation in *Salmonella typhimurium* TA 100. Trichloroethanol caused spindle aberrations when mouse oocytes were treated in vitro.

Trichloroacetic acid did not induce λ prophage in *Escherichia coli* and was not mutagenic to *Salmonella 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 *Salmonella typhimurium* but did induce λ prophage in *Escherichia coli*. Dichloroacetic acid gave equivocal results for gene mutation in *Salmonella 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 B6C3F₁ mouse (Big Blue[®] mouse) in vivo at an exposure that induces liver tumors in male mice. It is unclear whether dichloroacetic acid can induce primary DNA damage, as some assays are positive and others negative.

Cell Proliferation

The acute effects of chloral hydrate on liver cell proliferation were evaluated by Rijhsinghani et al. (1986) in 15-day-old mice (C57BL x C3HF₁). Mice were given 0, 5, or 10 mg/kg chloral hydrate by gavage in distilled water (9, 10, and 6 mice per group, respectively) and sacrificed after 24 hours. Cell proliferation was evaluated by calculating the mitotic index (number of mitoses/100 nuclei) from liver sections. The mitotic index in liver cells was significantly increased (0.9235) in mice receiving 5 mg/kg when compared to the control value (0.3382),

and elevated (0.7433) (although not statistically significantly) in mice receiving 10 mg/kg. 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 F344 rats and B6C3F₁ mice. Exposures are given in Sections II.A.3 and II.A.4. 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. Labeled nuclei were identified by chromogen pigment over the nuclei and the labeling index was calculated. Outside of the areas with tumors in the livers of male mice, there was no significant evidence of increased hepatocyte proliferation in rats or mice.

Oncogene Activation

The induction of the *H-ras* proto-oncogene in rodents by chloral hydrate was investigated by Velazquez (1994). DNA from normal liver and tumor tissue was obtained from male B6C3F₁ mice administered 1 g/L (166 mg/kg-day) chloral hydrate in drinking water for 2 years. *H-ras* mutations were present in one out of seven (14%) tumors. The spectrum of mutations was the same as that of spontaneous liver tumors. Based on these data, it is unlikely that *H-ras* activation is a mechanism of carcinogenicity relevant to chloral hydrate.

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 B6C3F₁ 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 tumors 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 Cl₃CCO₂• and/or Cl₃C•. 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 loci. These data implicate CYP2E1 as the primary cytochrome subfamily involved in the metabolism of chloral hydrate to reactive intermediates.

Cell Communication

The effects of 1-, 4-, 6-, 24-, 48-, and 168-hour exposures to chloral hydrate (0, 1, 5, or 10 mM) 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 mM at 1, 4, and 6 hours 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 was in the group treated with 1 mM for 24 hours.

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 mice treated with chloral hydrate for 26 weeks. As part of the chronic bioassay for carcinogenicity in male mice, NTP (2000b) found that chloral hydrate in the 100 mg/kg group significantly induced ($p < 0.05$) both lauric acid ω -hydroxylase activity and CYP4A immunoreactive protein in the dietary-controlled study, but not in the *ad libitum* study.

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

II.B.1. Summary of Risk Estimates

Insufficient data are available to calculate an oral slope factor.

II.B.2. Dose-Response Data (Carcinogenicity, Oral Exposure)

None.

II.B.3. Additional Comments (Carcinogenicity, Oral Exposure)

None.

II.B.4. Discussion of Confidence (Carcinogenicity, Oral Exposure)

There are no carcinogenicity data from humans. Two bioassays in rats show no increase in tumors at any site. Because only minimal toxicity was observed in the livers of the rats in these bioassays, the tests were not conducted at the maximum tolerated dose. A chronic bioassay in female mice showed a slight increase in the severity grade of hyperplasia and a slight increase in the incidence of adenoma in the pituitary gland pars distalis at the highest exposure tested. There is some evidence that chloral hydrate causes hepatocellular tumors in male mice. An earlier study showing an increase in hepatic adenomas or trabecular carcinomas following a single bolus exposure could not be confirmed in a study using more animals and higher exposures. Three separate 2-year bioassays in male mice show an increased incidence of hepatocellular adenoma or carcinoma. There are no data identifying a lesion that is a precursor to the hepatocellular tumors. The strain of mice used has a very high spontaneous incidence of hepatocellular tumors. Two of the metabolites of chloral hydrate, trichloroacetic acid and dichloroacetic acid, have been shown to cause hepatocellular tumors in rodents. Trichloroacetic acid causes hepatocellular tumors only in mice. Dichloroacetic acid causes hepatocellular tumors in both rats and mice.

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 *Drosophila 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 *Saccharomyces cerevisiae*, *Aspergillus 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 aneugenic effects of chloral hydrate are exposure-dependent and thought to arise via disruption of the mitotic spindle structure and/or function by inhibition of tubulin and/or microtubule-associated proteins; both substances are components of the spindle apparatus (Brunner et al., 1991; Lee et al., 1987; Wallin and Hartley-Asp, 1993). Some data also suggest that chloral hydrate may act on the spindle apparatus through an increase in the concentration of intracellular free calcium (Lee et al; 1987).

Although chloral hydrate and its metabolites, trichloroacetic acid and dichloroacetic acid, can induce a variety of mutational events, they do so with very low potency. Owing to the high concentration of chloral hydrate and its metabolites required to induce an observable effect in these assays, it is not likely that a genotoxic mode of action can be held responsible for the pituitary adenomas found in female mice or the hepatocellular tumors found in male mice.

Several other mechanisms may play a role in the induction of tumors 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 concerns exposure-dependent 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 tumor. Some data suggest a role for peroxisomal proliferation in the liver of male mice. Another potentially contributing mechanism of carcinogenesis is disruption of intercellular communication, which has been shown in one experiment to be influenced by chloral hydrate.

Although the mechanism of chloral hydrate-induced carcinogenicity in mice is unclear, one mechanism that appears less likely to be responsible is *H-ras* proto-oncogene activation.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

No data are available to calculate an inhalation unit risk.

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA, 2000.

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these

comments is included as an appendix to U.S. EPA (2000). [*To review this appendix, exit to the toxicological review, Appendix A, Summary of and Response to External Peer Review Comments \(PDF\)*](#).

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Consensus Date — 09/06/2000

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for Chloral hydrate conducted in November 2001 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (fax), or hotline.iris@epa.gov (Internet address).

III. [reserved]

IV. [reserved]

V. [reserved]

VI. Bibliography

Substance Name — Chloral hydrate
CASRN — 302-17-0

VI.A. Oral RfD References

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VI.B. Inhalation RfC References

None.

VI.C. Carcinogenicity Assessment References

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VII. Revision History

Substance Name — Chloral hydrate
CASRN — 302-17-0

Date	Section	Description
08/22/1988	I.A.	Oral RfD Summary on-line
09/15/2000	I-VIII	New RfD and cancer assessment; name changed from chloral to chloral hydrate
12/03/2002	I.A.6., I.B., II.D.2.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — Chloral hydrate
CASRN — 302-17-0
Last Revised — 09/15/2000

- 302-17-0
- Chloral hydrate
- Chloral monohydrate
- Trichloroacetaldehyde hydrate
- Trichloroacetaldehyde monohydrate
- 1,1,1-trichloro-2,2-dihydroxyethane